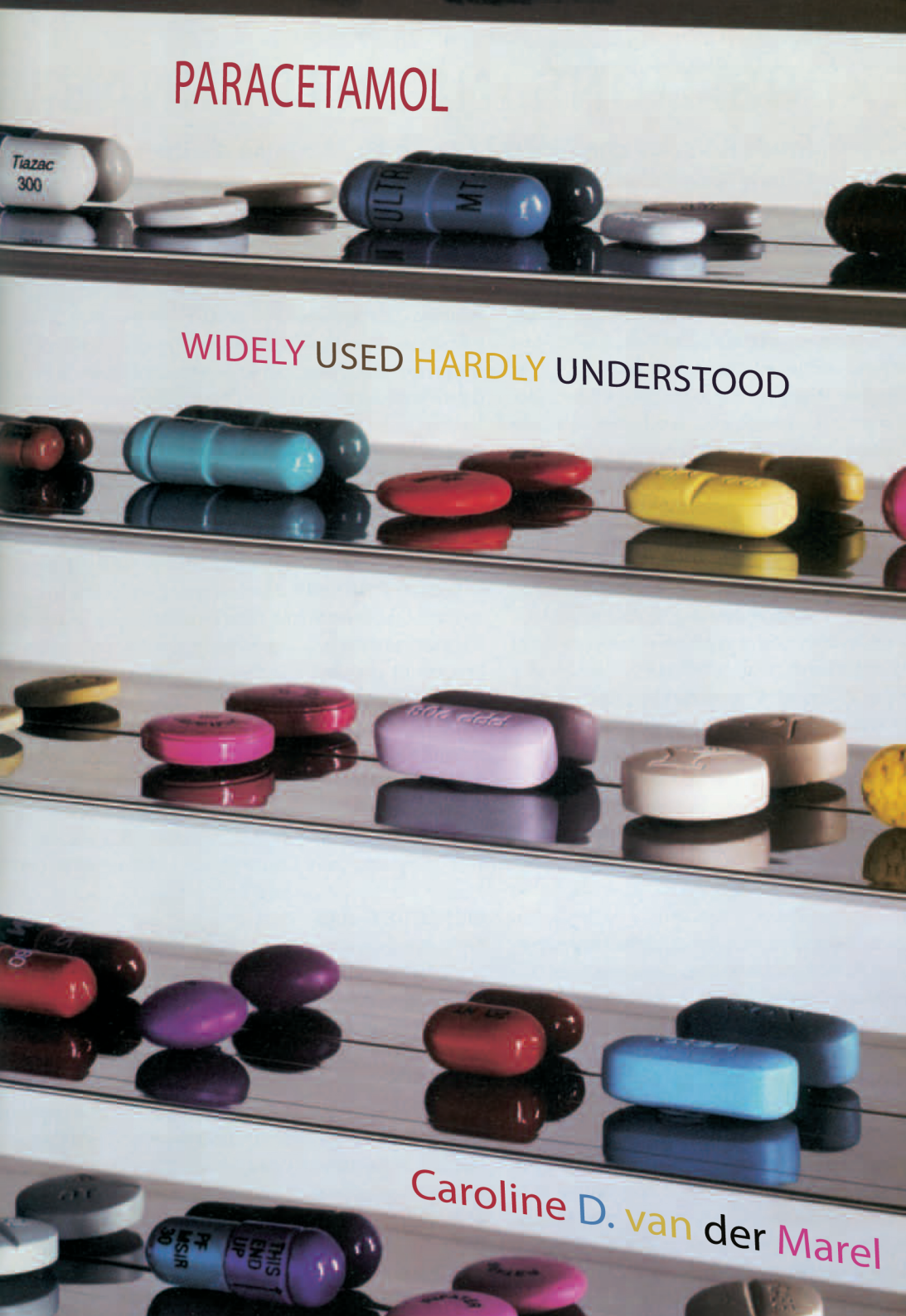
PARACETAMOL



WIDELY USED HARDLY UNDERSTOOD

Caroline D. van der Marel

PARACETAMOL, WIDELY USED HARDLY UNDERSTOOD

De werking van paracetamol bij kinderen: onbekend, maar wel bemind

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De werking van paracetamol bij kinderen: onbekend, maar wel bemind

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aan Colin

Table of contents

Chapter 1

Introduction

Introduction

Paracetamol (APAP), in the USA known as acetaminophen, is widely used both in hospital settings and at home for antipyresis and mild (postoperative) pain. Although APAP is available over the counter and is ranked on the third place, following nystatin and cisapride, when looking at the most commonly prescribed drugs in our Pediatric Surgical Intensive Care Unit (PSICU),[1] it is surprising that there are still little data available concerning the pharmacokinetics (i.e. absorption, distribution, metabolism and elimination) and pharmacodynamics (i.e. effects) of APAP in children. Off label prescription of APAP is common, since prescription is often outside the terms of the product license with regards to the age of the patient, the indication and the dose and frequency of administration. Product license does not account for children < 3 months of age and prolonged use in children < 4 years of age for more than 2 days is advised against,[2] since studies investigating the effects of prolonged use in children are lacking.
Although APAP is considered as a safe drug, when administered within the therapeutic dosing range, concern exists about the maximum daily doses and the prolonged use of APAP, as overdoses of APAP (> 150 mg/kg in children[2]) might be associated with severe toxic effects, i.e. liver necrosis, which might lead to death if not treated adequately or in time with acetylcysteine.

Pharmacokinetics

Studies have been performed evaluating the pharmacokinetics of APAP after oral and rectal administration in children of different ages. These studies showed that rectal administration of APAP is associated with a delayed and erratic absorption as compared to absorption after oral ingestion.[3] Furthermore, higher peak plasma concentrations are obtained following oral APAP administration as compared to rectal APAP administration.[4-6] Consequent to this observation, rectal APAP loading doses should be high, approximately 40 mg/kg.[7] A bioavailability of 80% is

reported comparing suppositories to tablets,[4] while Anderson et al report a relative bioavailabilty of 54%.[8]
An alternative route of APAP administration is intravenous administration of propacetamol. However data considering APAP pharmacokinetics in neonates following intravenous administration of propacetamol are lacking.

Anderson et al report a mean maximum concentration (Cmax) of 17.4 (SD 7.4) mg/l in children (12 months-17 yrs) after major orthopedic surgery, receiving 40 mg/kg APAP rectally.[9] Peak plasma concentration (Cmax) occurred at 2.3 (SD 1.2) hours (Tmax), while mean plasma concentration was 10.6 (SD 4.5) mg/l at six hours.[9] In neonates and infants, a total body clearance of APAP and a volume of distribution at birth of respectively 62% and 174% compared to older children have been reported.[10] A target concentration > 10 mg/l in approximately 50%of the subjects can be achieved by a dose of 45 mg/kg/day at birth, up to a dose of 90 mg/kg/day in 5-year-old children.[10] However a reduced dose of 75 mg/kg/day in an 8-year-old child is sufficient, as clearance is a nonlinear function of weight.[10]

Piletta et al reported that APAP-induced analgesia might be centrally mediated,[11] in which the time-course of APAP in cerebrospinal fluid (CSF) may parallel that of analgesic effect.[12] The CSF equilibration half time suggests that CSF kinetics approximate more closely to the effect compartment than plasma.[13] Since the effect site concentrations equilibrate slowly with plasma, APAP should be given 1-2 h before anticipated pain or fever in children.[13]

Pharmacodynamics

Several studies investigated the effect of APAP following oral or rectal administration. Plasma concentrations of APAP associated with analgesic effects in children are unknown, but antipyretic effects are seen in the range of 10.0-19.7mg/l.[8] APAP suppositories 40 mg/kg given peroperatively achieve effective therapeutic antipyretic plasma concentrations within 1-2 h.[9] An equilibration half-time of an effect compartment of 1.6 hours is

reported.[9]
Cullen et al compared plasma concentrations and the effect on temperature and reported a significant correlation between peak plasma concentrations and maximum drop in temperature.[14] Hopkins et al showed that there was no difference in antipyretic effect between oral and rectal administration.[6]

The analgesic effect of APAP following (adeno)tonsillectomy has been evaluated in a limited number of studies. Anderson et al reported an effect compartment concentration of 10 mg/l to achieve analgesia,[9] whereas Gaudreault et al concluded that the rectal administration of APAP at the induction of anesthesia results in incomplete and delayed absorption and does not prevent the occurrence of immediate postoperative pain in children undergoing adeno-tonsillectomy.[15] Diclofenac is suggested as an alternative for postoperative analgesia following (adeno)tonsillectomy.[16] Rømsing et al compared oral diclofenac (1-2 mg/kg) to oral APAP (22.5 mg/kg) the day after (adeno)tonsillectomy and reported no difference in analgesic effect and no significant reduction in pain scores following diclofenac or APAP ingestion.[17]
The use of APAP in addition to continuous morphine infusion (CMI) has increased in recent years despite the fact that the safety and additional value of this combined treatment has never been studied in newborns and young infants. In adults, combinations of opioids with APAP or non-steroidal anti-inflammatory drugs (NSAIDs) have resulted in a reduced morphine and fentanyl consumption as well as in reduced postoperative pain, without increased adverse effects.[18-23] Morton et al demonstrated reduced morphine requirements in postoperative children 3 to 15 years of age given diclofenac 1 mg/kg 8 hourly, but no effect attributable to APAP 15 mg/kg 6 hourly was shown.[24]

Metabolism and pharmacogenetics

Considering APAP metabolism, there are four pathways through which APAP is metabolized: glucuronidation, sulphation, oxidation and

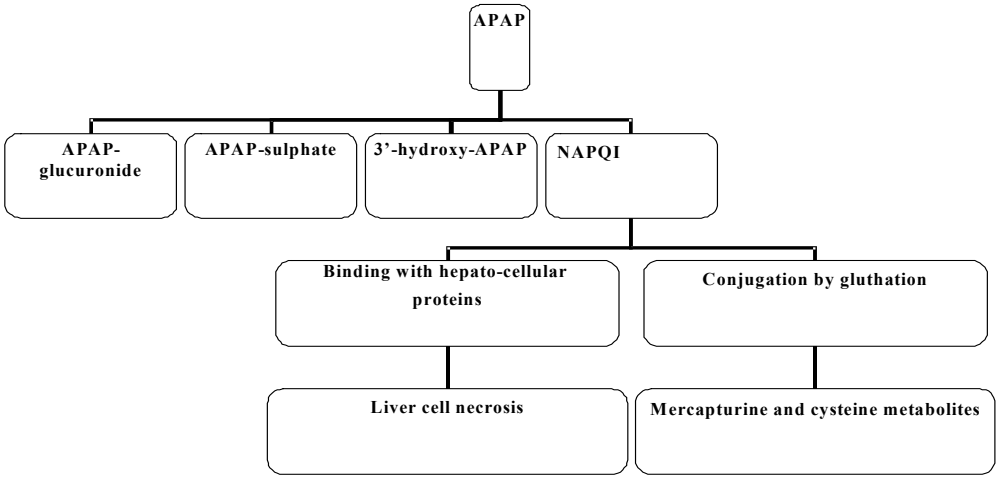

Figure 1 *APAP metabolism*

hydroxylation, in which APAP is metabolized into respectively APAP-glucuronide, APAP-sulphate, N-acetyl-p-benzoquinone- imine (NAPQI) and 3'-hydroxy-APAP (Figure 1).[25,26]
In adults APAP is mainly metabolized through glucuronidation, whereas in children sulphation is the major pathway.[27] This is represented in mean (SE) glucuronidation to sulphation ratios increasing from 0.12 (0.09) in preterm neonates 28-32 weeks gestational age,[28] to 0.28 (0.35) in preterm neonates 32-36 weeks gestational age,[28] 0.34 (0.08) in newborns,[27] 0.75 (0.10) in 3-9 year old children,[27, 29] and 1.61 (0.21) in 12 year old children,[27] with an adult ratio of 1.80 (0.32) (Figure 2).[27] Data considering APAP-glucuronide to APAP-sulphate ratio in infants are lacking. The oxidation pathway is minor in the therapeutic dosing range, but becomes more important when plasma concentrations are reaching toxic levels. Subsequently more NAPQI is formed at toxic plasma concentrations when both the glucuronidation and sulphation pathway are rate limiting, leading to liver necrosis if the amount of gluthation available is not sufficient to conjugate all NAPQI.[25] The enzymes involved in the formation of NAPQI are CYP2E1, CYP3A4, CYP1A2 and possibly CYP3A5.[25, 30, 31] Genotype of these enzymes may alter the expression and/ or the activity of these enzymes and may result in an increased or decreased formation of NAPQI and thus an increased or decreased propensity to APAP toxicity effects. APAP metabolism through hydroxylation into 3'-hydroxy-APAP is negligible.[25]

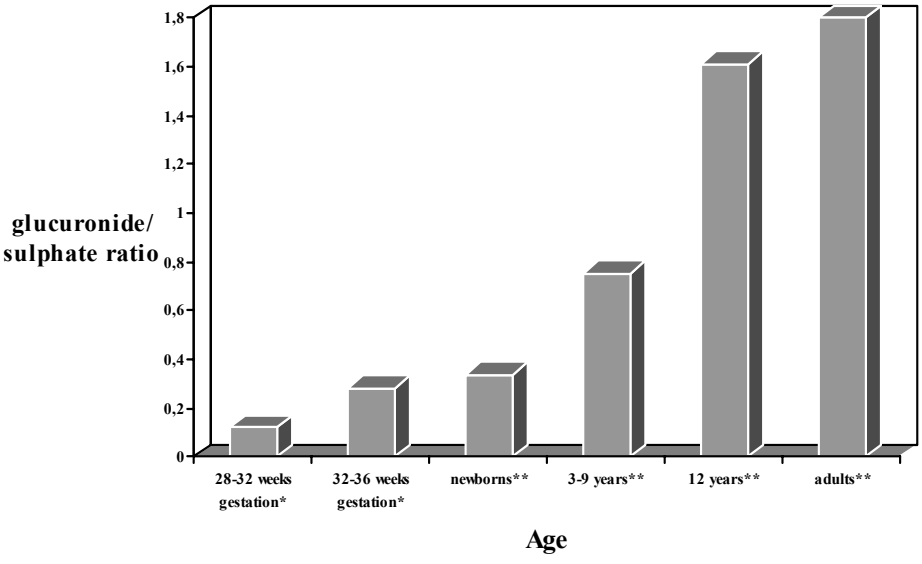

Figure 2 * *van Lingen, 1999*
** *Miller, 1976*

Toxicity

According to international guidelines APAP can be used safely in doses up to 90 mg/kg/day, whereas in the Netherlands a maximum daily dose of 60 mg/kg is recommended.[2,32,33] Doses > 150 mg/kg/day might be associated with toxicity, resulting in liver necrosis.[2,34-36] However children experience less toxicity after high APAP doses compared to adults.[35,37,38] APAP plasma concentrations > 200 mg/l at 4 hours after ingestion and > 50 mg/l at 12 hours after ingestion are associated with toxic effects.[34,35] In case of the occurrence of toxic effects, SGOT becomes elevated and irreversible liver damage may be the consequence.[34-36] Recently data have been published that not only ingestion of toxic APAP doses may impair liver function, but also the chronic use of high APAP doses within the therapeutic ranging dose may impair liver function.[39]

Scope of this thesis

Although APAP is available over the counter and has potential toxic effects, data concerning the pharmacokinetics and pharmacodynamics of APAP in children are limited. To gain a better insight into the

pharmacokinetics, the pharmacodynamics and the pharmacogenetics of APAP in children, we conducted several studies.

Pharmacokinetic studies

A report of age-related changes in the plasma to CSF equilibration half-time (Teq) of APAP in neonates to adolescents, undergoing (semi) elective surgery for placement or revision of a ventriculo-peritoneal shunt or insertion of a temporary external ventricular drain (**chapter 2.1**).
A description of the clearances of APAP to glucuronide and sulphate metabolites as well as the urinary clearance of unmetabolized APAP in infants undergoing major craniofacial surgery, using non-linear mixed effects models (**chapter 2.2**).
A description of pharmacokinetics and pharmacodynamics after administration of a single dose of propacetamol in preterm and term infants on the first day of life, undergoing minor, painful procedures or as additional treatment in infants on opioids (**chapter 2.3**).
A description of diclofenac, 4'-hydroxy-diclofenac (D4OH) and 5'-hydroxy-diclofenac (D5OH) pharmacokinetics after rectal administration in children 2-8 years of age, undergoing (adeno)tonsillectomy (**chapter 2.4**).

Pharmacodynamic studies

A comparison of APAP plasma concentrations and effects between children receiving either multiple doses of APAP rectally or equal doses of oral APAP after an initial rectal loading dose in infants following elective major craniofacial surgery. Furthermore the dose-plasma concentration and the plasma concentration-effect relation of both orally and rectally administered APAP are evaluated (**chapter 3.1**).
A comparison of total postoperative morphine consumption in neonates and infants receiving either placebo or APAP in addition to continuous morphine infusion, following major abdominal or thoracic surgery (**chapter 3.2**).

A comparison of the analgesic effect of rectally administered APAP and diclofenac in children 3-8 years of age undergoing (adeno)tonsillectomy during ambulatory surgery and assessment of the relation between APAP, diclofenac and D4OH plasma concentrations and postoperative pain scores. Furthermore the safety of diclofenac by monitoring postoperative bleeding is assessed (**chapter 3.3**).

Pharmacogenetic studies

A description of a pilot study evaluating the relation between APAP clearance and CYP2E1, CYP3A4 and CYP3A5 genotype in neonates, infants and children (**chapter 4.1**).
A description of a pilot study evaluating the relation between diclofenac clearance and CYP2C9, CYP3A4 and CYP3A5 genotype, between D4OH formation clearance and CYP2C9 genotype and between D5OH formation clearance and CYP3A4 and CYP3A5 genotype in children 3-8 years of age (**chapter 4.2**).

General discussion

The results of the studies described in this thesis and directions for future research are discussed (**chapter 5**).

Future directions

Assessment of the value of EEG and SSR registration for postoperative pain assessment in neonates and infants during the first 48 hours after major abdominal or thoracic surgery (**chapter 6**).

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2

Pharmacokinetic studies

Chapter 2.1

Paracetamol in cerebrospinal fluid in children

Caroline D van der Marel, Brian J Anderson, Marien AL Pluim , TH Rob de Jong, Antonia Gonzalez, Dick Tibboel.
European Journal for Clinical Pharmacology, 2003

Abstract

Background

There are few studies describing paracetamol (APAP) cerebrospinal fluid (CSF) concentrations in children. This current study was undertaken in children from neonates to adolescents in order to investigate age-related changes in the plasma to CSF equilibration half-time (Teq) of APAP.

Methods

Children (n = 41) 1 week-18 years of age undergoing (semi) elective surgery for placement or revision of a ventriculo-peritoneal shunt or insertion of a temporary external ventricular drain, received a loading dose of 30-40 mg/kg APAP 1 hour before scheduled surgery. Blood and CSF samples for APAP concentration analysis were collected during surgery. In those children with a temporary external drain blood and CSF sampling were extended into the postoperative period. APAP and CSF pharmacokinetics were estimated using non-linear mixed effects models. Size was standardized to a 70 kg person using allometric "¼ power models".

Results

Median (25-75th percentile) age and weight of the patients included in this study were 12 (3-78) months and 9.8 (5.7-20.5) kg. Median (25-75th percentile) time between APAP loading dose administration and collection of blood samples and median time (25-75th percentile) between APAP loading dose and collection of CSF were respectively 125 (95-210) and 133 (33-202) minutes. The population mean Teq, standardized to a 70 kg person, was 1.93 (CV 43%) h, an estimate similar to that described in adults (2.1 h). There was no relationship between age and Teq other than that predicted by size. APAP plasma concentrations ranged from 0.0-33.0 mg/l, APAP CSF concentrations ranged from 0.0-21.0 mg/l.

Conclusion
Size rather than blood-brain-barrier maturation determines Teq changes with age in children. We predict a neonate (3.5 kg), 1 year old child (10 kg), 5 year old child (20 kg), 10 year old child (30 kg) and an adult (70 kg) to have a Teq of 0.9, 1, 1.4, 1.6 and 1.93 h respectively.

Introduction

The mechanism of action of paracetamol (APAP) analgesia is multi factorial. It is known to be a potent inhibitor of prostaglandin synthesis within the central nervous system but also acts peripherally by blocking impulse generation within the bradykinin-sensitive chemo receptors responsible for the generation of nociceptive impulses. APAP is also thought to have an analgesic effect by antagonizing N-methyl-D-aspartate (NMDA) and substance P in the spinal cord.[1,2] Analgesic effect involves an inhibitory action on spinal nitric oxide (NO) mechanisms.[3] APAP's antipyretic effect is mediated through inhibition of prostaglandin E_2 in the brain.[4] These temporal disequilibriums have been modeled using delayed effects with an effect compartment. The equilibration half-time between plasma and effect compartment is reported as 53 min (CV 217%) for analgesia and 71 min (CV 10%) for anti-pyresis in children [mean (SD): 9.0 (3.0)].[5,6]

Reduction of CSF prostaglandin concentrations in animals after APAP administration might be responsible for APAP's analgesic effect.[7,8] Consequently the amount of APAP reaching the central nervous system (CNS) and causing inhibition of prostaglandin synthesis may mirror its analgesic effect. APAP CSF concentrations are dependent of APAP plasma concentrations and the permeability of the blood-brain barrier (BBB) to this molecule. Debate exists concerning BBB permeability changes with age.[9] There are few APAP CSF pharmacokinetic studies in adult humans[10,11] and only one study in children earlier reported by Anderson et al.[12] However

seven of the nine children studied by Anderson et al,[12] suffered of traumatic brain injury that may have influenced BBB permeability.

This current study was undertaken in children from neonates to adolescents in order to investigate age-related changes. The results were compared to the results of the study performed by Anderson et al,[12] in order to examine the influence of traumatic brain injury and its possible disruption of the BBB on the plasma-CSF Teq.

Methods

Patients and methods

After approval of the Medical Ethical Committee of the Erasmus MC Rotterdam, informed consent was obtained from the parents of children participating in this study. Children aged between 0 to 18 years undergoing placement or revision of a ventriculo-peritoneal (VP) shunt or insertion of a temporary external ventricular drain were considered for enrolment. Exclusion criteria were pre-existent liver- or kidney disorders, known allergy to APAP and traumatic brain injury.

All children (n = 41) were given a rectal loading dose of 30-40 mg/kg APAP 1 hour before scheduled surgery. Anesthesia was induced using thiopentone, propofol, etomidate or sevoflurane. Before tracheal intubation children received 2-5 μg/kg fentanyl. Tracheal intubation was facilitated with vecuronium or suxamethonium. Breathing was controlled and anesthesia was maintained using O_2/N_2O or O_2/air and isoflurane 0.5-1%. Before incision children were given a further 2-5 μg/kg fentanyl. Extra doses of 2 μg/kg fentanyl were administered if heart rate and/ or mean arterial blood pressure were higher than 10% above baseline values measured 10 minutes after tracheal intubation. A blood sample for APAP plasma concentration analysis was collected directly after induction. A CSF sample for APAP CSF concentration analysis was collected during shunt insertion or revision.

Postoperatively patients received APAP suppositories according to hospital standard dosing schedules. CSF sampling was extended to the postoperative period in patients undergoing insertion of a temporary external ventricular drain (n = 5). The sampling schedule in these patients was adjusted to the clinical circumstances of the individual patients and based on pain scores validated for this population.[13]
APAP suppositories contained 60 mg, 120 mg, 240 mg, 500 mg or 1000 mg APAP in a triglyceride base (Pharmachemie, Haarlem, The Netherlands). Loading dose (30-40 mg/kg) was dependent on available suppository size.

CSF sampling
In patients with VP shunts it was only possible to collect a single CSF and blood sample, taken during surgery. Further CSF sampling was possible in 5 patients with external ventricular drains. These patients were given 45.5-108.0 mg/kg/24h APAP rectally in divided doses, including the pre-operatively administered rectal loading dose. Plasma and CSF sampling was intermittent and varied from 0.5 hourly for 4 h to 2-5 hourly for 68 hours.

Paracetamol assay
Plasma and CSF samples were stored at 4 °C until analysis. APAP plasma and CSF concentrations were determined using fluorescence polarization immunoassay (ADX systems, Abbott Laboratories, North Chicago, IL) (Erasmus MC Rotterdam). The APAP plasma determination limit was 1.0 mg/l, which was defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Precision was measured at APAP plasma concentrations of 15, 35 and 150 mg/l; 55 samples of each concentration were assayed to determine coefficients of variation at these concentrations (CV = SD/mean; RSD = CV*100%). RSD was at these concentrations were 7.22%, 3.37% and 3.11% respectively. The APAP plasma concentration range in which accuracy was measured was 10-150 mg/l.

Modeling

Population parameter estimates were obtained using a non-linear mixed effects model (NONMEM).[14] This model accounts for population parameter variability (between and within subjects) and residual variability (random effects) as well as parameter differences predicted by covariates (fixed effects). The population parameter variability in model parameters was modeled by a proportional variance model. Additive terms characterized the residual unknown variability for plasma and CSF concentrations. This error model assumes that the residual variability is of the same order of magnitude over the whole range of measurements. The population mean parameters, between subject variance and residual variance were estimated using NONMEM version V release 1.1. Estimation used the first order conditional estimate method with the interaction option and ADVAN 6 with Tol = 5. Convergence criterion was 3 significant digits. A FORTRAN F77 compiler (Watcom version 10.6) was used with an Intel Celeron 333 MHz CPU under MS Windows 98.
A first order input, first order elimination, two compartment link model was used to describe the time course of plasma and CSF drug concentrations. The model is shown schematically in Figure 1.

The relevant differential equations were:

$$\begin{aligned} dAgut/dt &= -Ka \times Agut \\ dC/dt &= (Agut \times Ka - C \times CL) / V \\ dCcsf/dt &= ln(2)/Teq \times (C \times PC - Ccsf) \end{aligned}$$

Agut is the amount of drug in the gut at any one time. This amount is assumed equal to the dose at time zero. Ka is the absorption rate constant (h^{-1}); V is the central compartment volume (l); C is the plasma concentration (mg/l); CL is the clearance from the central compartment (l/h); Ccsf is the cerebrospinal fluid concentration (mg/l);

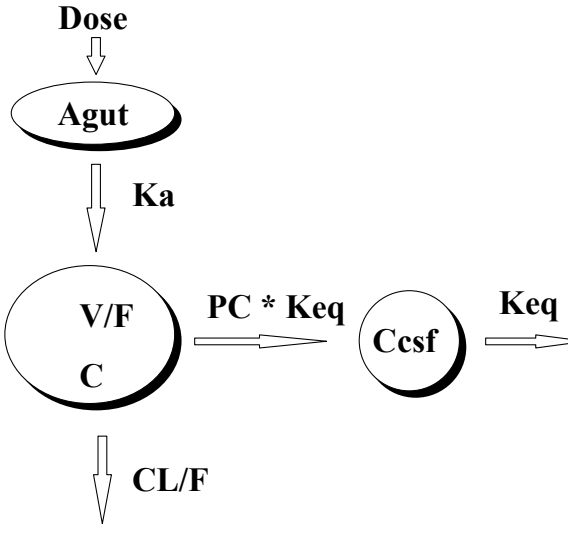

Figure 1 *Diagram of CSF Pharmacokinetic Model. Ka, absorption rate constant (h^{-1}); V/F, central compartment volume (l); C, plasma concentration (mg/l); CL/F clearance from central compartment (l/h); Ccsf, cerebrospinal fluid concentration (mg/l); PC, csf/plasma partition coefficient; Keq, equilibration rate constant (/h). The Keq can be expressed as Ln2/Teq*

APAP is not bound to plasma proteins. Concentrations were measured in serum (containing protein that contributes to total volume) and CSF (without protein). Consequently a partition coefficient was required to model the data. The partition coefficient estimate is similar to the alcohol partition coefficient between csf and serum measured in rats[15] and would be predicted for drugs that distribute in plasma water but which do not bind to plasma proteins[16] because plasma water occupies 90% of plasma by volume. PC is the CSF/ plasma partition coefficient and accounts for protein concentration differences between plasma and CSF;
Teq is the equilibration half time (h) between plasma and CSF.

APAP was administered as an extra vascular dose and both clearance and distribution volume is confounded by bioavailability. Frectal/oral is used to refer to the relative bioavailability of the suppository compared to the oral formulation.

Children with traumatic brain injury

Plasma and CSF time-concentration data from 9 children with external ventricular shunts, originating from the study published by Anderson et al,[12] were included in the analysis of the data from this study in order to examine the influence of traumatic brain injury and its possible disruption of the BBB on the plasma-CSF Teq. Seven of these 9 children suffered traumatic brain injury. All 9 children were ventilator dependent and required external ventricular drains for the management of raised intracranial pressure. These children were given APAP elixir (40 mg/kg, 250 mg/5ml, SmithKline Beecham (NZ) Ltd, Auckland, NZ), instilled down a nasogastric feeding tube and both arterial blood and cerebrospinal fluid sampled at hourly intervals for the first four hours and then two hourly for the subsequent six hours.

A separate additive term was used to characterize the residual unknown variability for plasma and CSF concentrations from each study.

Quality of fit

The quality of fit of the pharmacokinetic model to the data was assessed by visual examination of plots of observed versus predicted concentrations. Models were nested and an improvement in the objective function was referred to the Chi-squared distribution to assess significance e.g. an objective function change (ΔOBJ) of 3.84 is significant at $\alpha = 0.05$.

Covariate Analysis

The parameter values were standardized for a body weight of 70-kg using an allometric model,[17]

$$P_i = P_{std} \, x \, (W_i / W_{std})^{PWR}$$

where P_i is the parameter in the i_{th} individual, W_i is the weight in the i_{th} individual and P_{std} is the parameter in an individual with a weight W_{std} of 70 kg. The *PWR* exponent was 0.75 for clearance, 1 for distribution volumes and 0.25 for time related indices.[18-21]

Interpreting sparse pharmacokinetic data

It was not possible to collect data to obtain time-concentration profiles on current study patients - often only a single plasma APAP sample was collected from each patient. Consequently a larger data set from a previous study reported by Anderson et al investigating age-related APAP pharmacokinetics (n = 221)[22] was included to perform the population analysis.

Results

Population demographics

Forty-one children participated in this current study, 21 boys and 20 girls. The eligible number of patients was 47. Six children were excluded because informed consent was not obtained due to either language difficulties (n = 1) or to parental belief that there was no advantage gained for their child to participate in this study (n = 5). Median age (25-75th percentile) and weight of the children participating were 14 (3-74) months and 10.0 (5.7-20.4) kg. Median (25-75th percentile) APAP loading dose was 32.3 (25.3-42.5) mg/kg. Median (25-75th percentile) time between APAP loading dose administration and collection of blood samples and median time (25-75th percentile) between APAP loading dose and collection of CSF samples were respectively 125 (95-210) and 133 (33-202) minutes. Twenty-three children underwent VP shunt insertion, 13 children had a VP shunt revision and 5 children underwent insertion of a temporary external ventricular drain.

There were 114 observations. APAP plasma concentrations ranged from 0.0-33.0 mg/l, APAP CSF concentrations ranged from 0.0-21.0 mg/l.

Parameter estimates

Parameter estimates with covariate analyses are shown in Table 1a & 1b. Pharmacokinetic estimates and maturational half-lives of V/Foral and CL/Foral were similar to those reported previously.[23] The population mean Teq, standardized to a 70 kg person, was 1.35 (CV 108%) h. Figure 2

shows individual Bayesian Teq predictions and their relationship to age for the complete pooled data set. These predictions are based on maximum *a posteriori* Bayesian estimates of the parameters for each specific individual using their observed data. No relationship between age and Teq was determined. The mean Teq from the earlier data from Anderson et al,[12] was lower (Teq 0.71 h, CV 105%) than that from the current study population (Teq 1.93 h, CV 43%). Two children from the earlier study by Anderson et al,[12] had a Teq greater than 2.5 h (Figure 2). These two children both had posterior fossa pathology, as opposed to the remaining children whom suffered traumatic brain injury.

The type of surgery (VP shunt vs. external ventricular drain) had no effect on individual Bayesian Teq predictions. The covariance of the pharmacokinetic parameters, expressed as the correlation of population parameter variability was low (Table 2).

Table 1a Standardized Population Pharmacokinetic Parameter Estimates

Parameter		Estimate	%CV
Vstd	l/ 70kg	75.7	26
CLstd	l/h/70kg	14.2	49
Felixir		1 fixed	-
Tabs elixir	h	0.426	151
Tlag elixir	h	0.21	68
Frectal/oral	suppository	0.838	
Tabs	suppository h	1.42	65
Tlag	triglyceride base h	0.075	68
Teq	h	1.35	108
PC		1.11	

CLstd = population estimate for CL/Foral (clearance after oral administration l/h/70kg),
Vstd = population estimate for V/Foral (volume of distribution l/70kg),
Tabs = absorption half-life after nasogastric (elixir) or suppository administration (h),
Tlag = absorption lag time after nasogastric (elixir) or suppository administration (h),
Frectal/oral = relative bioavailability of the rectal compared to the oral formulation,
Teq is the serum to CSF equilibration half-time,
PC is the partition coefficient between serum and CSF.

Table 1b Covariate Models and Estimates for Pooled Population Parameters

a) Volume of distribution *V/Foral = (Vstd x (Wt/70)) x (1 + βvol x EXP(-AGE in months * Ln(2)/Tvol)) l*

b) Clearance *CL/Foral = (CLstd x (Wt/70)$^{0.75}$) x (1 + βcl x EXP(-AGE in months * Ln(2)/Tcl)) l/ h*

Parameter	Estimate
βvol	0.781
Tvol	0.0556 months
βcl	-0.597
Tcl	3.11 months

βvol and βcl are parameters estimating the fraction above or below V/Foral and CL/Foral respectively at birth; Tvol and Tcl describe the maturation half-lives of the age-related changes of V/Foral and CL/Foral.

Table 2 Correlation of population pharmacokinetic parameter variability

	CL	V	Tabs elixir	Tabs suppository	Teq
CL	1				
V	0.42	1			
Tabs elixir	0.09	-0.134	1		
Tabs suppository	0.131	-0.172	0.064	1	
Teq	0.043	-0.031	-0.065	0.039	1

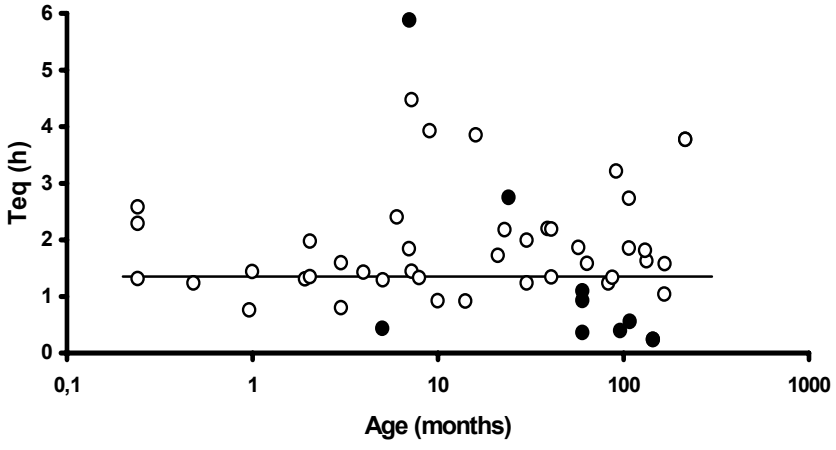

Figure 2 *Individual Bayesian Teq (plasma to CSF equilibration half-time) predictions (standardized to a 70 kg person) and their relationship to age for the complete pooled data set. Predictions from the current data set are shown as x. Predictions from data from Anderson et al are shown as Δ. Standardized Teq does not change with age.*

The individual Bayesian predictions for plasma and CSF concentration are compared to those observed in Figure 3a & 3b. These predictions are based on maximum *a posteriori* Bayesian estimates of the parameters for each specific individual using their observed data. Figure 4a & 4b demonstrate the quality of fit for pharmacokinetic data over the study time period – each subject's data is connected by a line.

Figure 3a

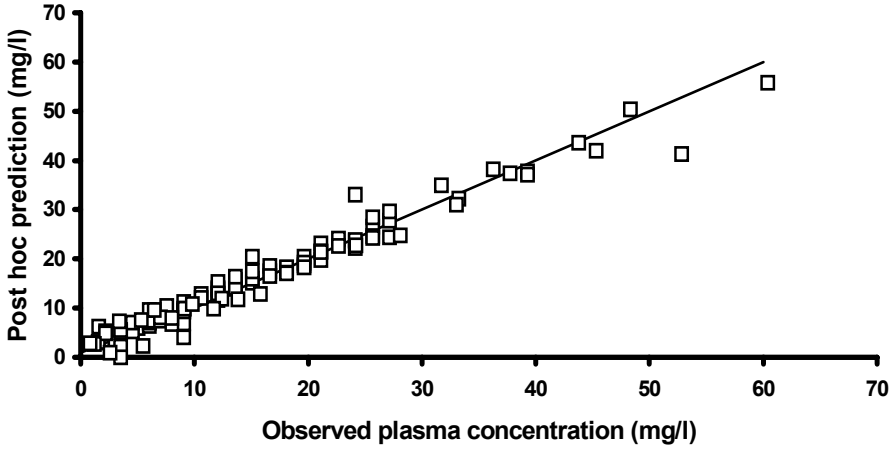

Figure 3b

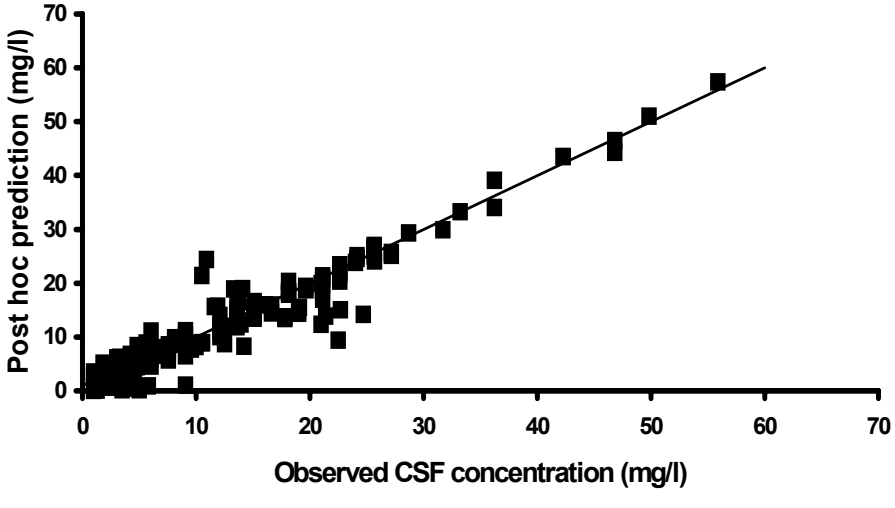

Figure 3 *The individual Bayesian predictions for plasma (Figure 3a) and CSF concentration (Figure 3b) are compared to those observed. The line x = y is the line of identity*

The residual errors (mg/l) for the plasma concentration data were 3.4 and 3.2 for the current data and that from Anderson et al,[12] respectively. The residual errors (mg/l) for the CSF concentration data were 4.3 and 2.0 for the current data and that from Anderson et al,[12] respectively.

Figure 4a

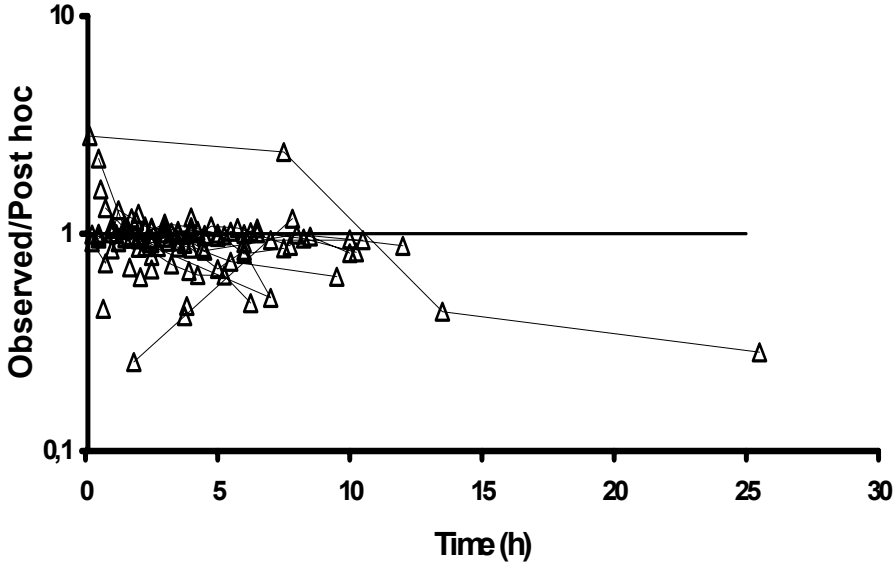

Figure 4b

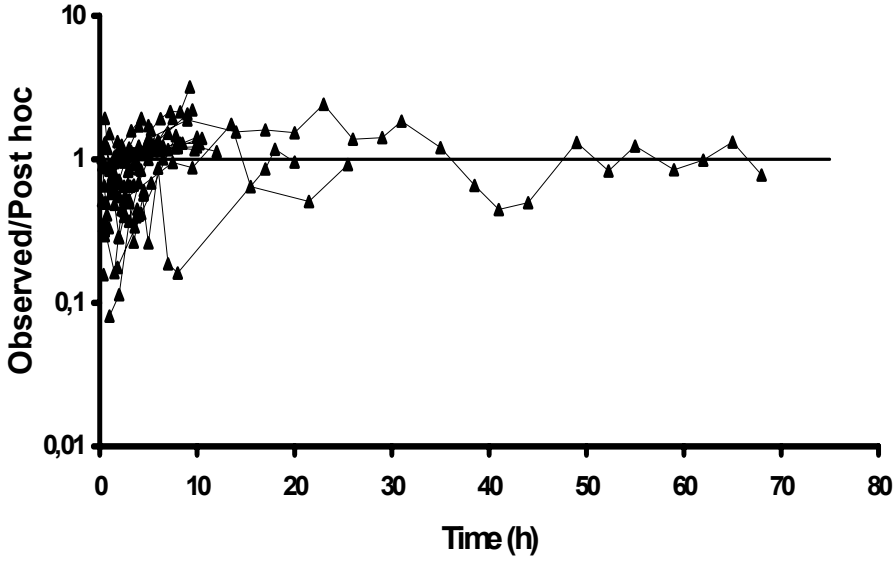

Figure 4 *The quality of fit for pharmacokinetic data over the study time period – each subject's data is connected by a line. Plasma data are shown in Figure 4a. CSF data are shown in Figure 4b.*

Discussion

This study estimates a population plasma to CSF equilibration half-time (Teq) of 1.93 h (standardized to a 70 kg person) for APAP in children of all ages – an estimate similar to the Teq of 2.1 h determined from naïve pooled adult data collected by Bannwarth et al.[10] Patients in that study were adults (n = 43) with rheumatic and nerve root compression pain. They were given an intravenous prodrug of APAP (propacetamol) and a single CSF APAP concentration was measured. Data were modeled with the MKMODEL program[23] using the same equations as in this current study and in the study performed by Anderson et al.[12] Our current data do not support the concept of an increased permeability BBB in early infancy. We have recently expanded this view in relation to opioids.[24] Children with traumatic brain injury, however, did have a lower Teq – consistent with disruption of the BBB.

Size was the first covariate used in our current analysis. This deliberate choice was based on known biological principles. A lot of physiological, structural and time related variables can be predicted within and between species with weight exponents of 0.75, 1 and 0.25 respectively.[20] We have used physiological time, rather than chronological time, to define standardized Teq. The concept of physiological time was developed as a consequence of allometry.[25] For example, most mammals have the same number of heartbeats and breaths in their life span. The difference between small and large animals is that smaller animals have faster physiologic processes and consequently a shorter life span. West et al,[18,19] have used fractional geometry to mathematically explain the allometric power exponents. The "¼ power laws" were derived from a general model that describes how essential materials are transported through space-filled fractional networks of branching tubes.[18] These design principles are independent of detailed dynamics and explicit models and should apply to virtually all organisms.[19] Consequently we might expect a neonate (3.5 kg), 1 year old child (10 kg), 5 year old child (20 kg), 10 year old child (30 kg) and an adult (70 kg) to have a chronological Teq of 0.9, 1, 1.4, 1.6 and 1.93

h respectively (Figure 5), which would result in a more rapid onset of effect in younger children. This is consistent with the speed of onset of other drugs due to distribution to an effect compartment.[26] This covariate should be factored into investigations of APAP effect. We might, for example, expect maximum fever reduction after APAP elixir to occur earlier in 1 year olds than 10 year olds.

The plasma to CSF Teq is longer than the effect compartment Teq estimated for analgesia (53 min, CV 217%)[5] and for anti-pyresis (71 min, CV 10%)[6] in children. These data suggest that the CSF compartment is not the effect compartment responsible for these actions. Anti-pyresis is mediated through prostaglandins in the hypothalamus and analgesia through both prostaglandin synthesis and by antagonizing N-methyl-D-aspartate (NMDA) and substance P in the spinal cord. Our data suggests that analgesic and anti-pyretic effects occur earlier than APAP concentration changes in the CSF.

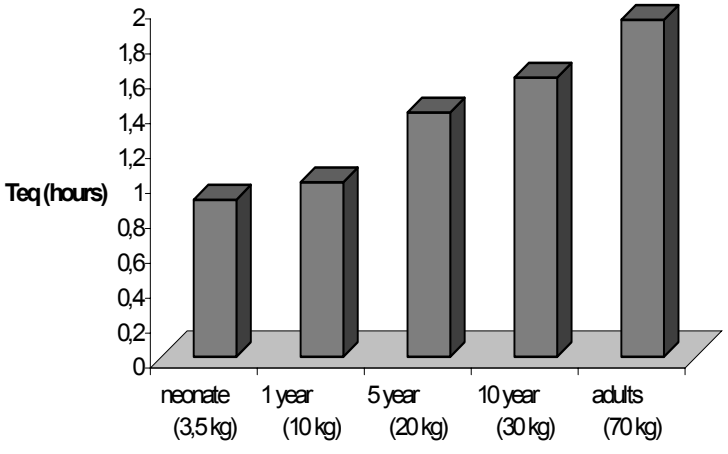

Figure 5 *Teq expected for a neonate (3.5 kg), 1 year old child (10 kg), 5 year old child (20 kg), 10 year old child (30 kg) and an adult (70 kg).*

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Chapter 2.2

Paracetamol and metabolite pharmacokinetics in infants

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Abstract

Background
Data concerning metabolism of paracetamol (APAP) in infants are scant. Previous studies have examined urinary metabolite recovery rates after a single dose of APAP in either neonates (< 6 weeks) or in children (3-9 years). There are no studies investigating infants.

Methods
Infants (n = 47) undergoing major craniofacial surgery were given APAP 19-45 mg/kg 6-, 8-, or 12 hourly as either elixir or suppository formulation for postoperative analgesia, after a loading dose of 33-59 mg/kg rectally during the operation. Serum was assayed for APAP concentration in 40 of these infants at 5, 8, 11, 14, 17 and 20 h postoperatively. Urine samples were collected every 3 h for 24 h in 15 of these infants. The clearances of APAP to glucuronide and sulphate metabolites as well as the urinary clearance of unmetabolized APAP were estimated using non-linear mixed effects models.

Results
Mean (± SD) age and weight of the patients were 11.8 ± 2.5 months and 9.1 ± 1.9 kg. Clearance of APAP to APAP-glucuronide (%CV) and to APAP-sulphate were 6.6 (11.5) l/h and 7.5 (11.5) l/h respectively, standardized to a 70-kg person using allometric '¼ power' models. Glucuronide formation clearance, but not sulphate formation, was related to age and increased with age from a predicted value in a neonate of 2.73 l/h/70 kg to a mature value of 6.6 l/h/70kg with a maturation half-life of 8.09 months. Urine clearance of APAP-glucuronide, APAP-sulphate and unchanged APAP (%CV) were, respectively 2.65, 3.03 and 0.55 (28) l/h/70 kg. The urine clearance of unchanged APAP and metabolites was related to urine volume flow rate. Clearance attributable to pathways other than these measured in urine was not identifiable. The glucuronide/sulphate formation clearance ratio was 0.69 at 12 months age. Sulphate metabolism contributed 50% towards APAP clearance.

Conclusion
Glucuronide formation clearance increases with age in the infant age range but sulphate formation does not. Renal clearance of APAP and its metabolites increases with urine flow rate. This and other studies show that APAP metabolism to glucuronide appears to be similar in infants and children, but in adults is increased in comparison with children. Oxidative pathways were undetectable in this infant study and may explain, in part, the reduced incidence of hepatotoxicity in infants.

Introduction

Paracetamol (APAP) is a popular analgesic for mild postoperative pain in children. The five most commonly prescribed drugs in our Pediatric Surgical Intensive Care Unit (PSICU) are nystatin, cisapride, APAP, cefotaxime and furosemide.[1] Although APAP is one of the most prescribed drugs, there are few data concerning metabolite formation in infants. Prescott has reviewed APAP metabolism in adults; APAP is conjugated to APAP-glucuronide (50-60%) and APAP-sulphate (25-35%).[2] A small part is metabolized through oxidation (2-10%) to the toxic metabolite N-acetyl-p-benzoquinone-imine (NAPQI) and into 3-hydroxy-APAP.[2] Oxidation involves the cytochrome P_{450} system, of which CYP2E1 is the most important.[3] NAPQI is conjugated by glutathione into cysteine and mercapturic acid metabolites.[3] APAP-glucuronide, APAP-sulphate, 3-hydroxy-APAP, cysteine and mercapturic acid metabolites and a small amount of unchanged APAP (2-5%) are excreted in urine.[2]

Hepatotoxicity occurs as a consequence of APAP overdose when an increased amount of APAP is metabolized through oxidation with a consequent increase of NAPQI.[4] Glutathione, required to conjugate NAPQI into the non-toxic cysteine and mercapturic acid metabolites, becomes depleted and NAPQI conjugation with hepatocellular proteins occurs, eventually leading to liver cell necrosis.[4]
APAP developmental pharmacokinetics were studied in premature neonates

and infants by Anderson et al., showing a exponentially decreasing volume of distribution with a maturation half-life of 11.5 weeks and an increasing APAP clearance with a maturation half-life of 11.3 weeks reaching, respectively 72.9 l/70 kg and 10.8 l/h/70 kg at the age of 60 weeks post-conception.[5]

An understanding of the contribution of glucuronide and sulphate metabolite formation is important in order to appreciate the factors that determine the extent of formation of NAPQI. If affinity and capacity of glucuronide and/or sulphate formation is limited in relation to oxidative pathways, then NAPQI formation may increase and with it the risk of hepatotoxicity.

Methods

Patients and methods

The medical ethical committee of the Erasmus MC Rotterdam approved the study, and written informed consent was obtained from the parents of all infants participating in the study. Data were collected from 47 children, of whom 40 participated in a study published previously[6] (see Figure 1) in which we described longitudinal data on plasma APAP levels.

The Erasmus MC-Sophia serves as a level-III referral centre for all pediatric surgical subspecialties. As such, it is the only designated pediatric craniofacial centre in the Netherlands. Around 100 major craniofacial corrections are performed annually. Infants aged between 3 months and 3 years presenting for elective craniosynostosis correction were considered for enrolment. Exclusion criteria were craniotomy for tumors, hydrocephalus or trauma, pre-existent liver or kidney disorders as reflected by abnormal values of liver enzymes, bilirubin, urea and creatinine, severe mental retardation, Glasgow Coma Score < 8, postoperative mechanical ventilation, and known allergy for APAP.

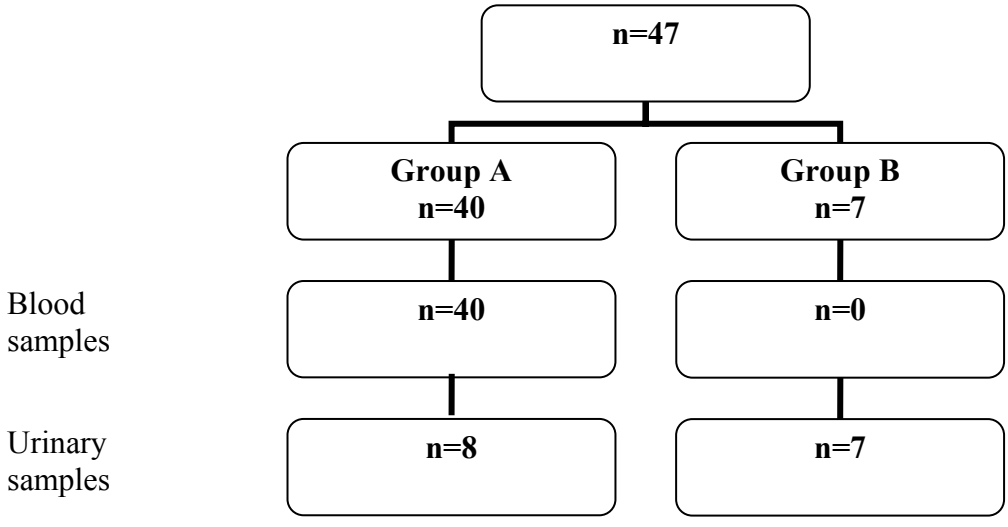

Figure 1 *Flowchart of patients included in this study*

All infants (n = 47) were given a rectal APAP loading dose of approximately 40 mg/kg during craniosynostosis surgery, approximately 2 h before anticipated extubation. At this time, major blood loss had been adequately compensated for and patients were hemodynamically stable. After the operation, patients were admitted to the PSICU for a minimum of 24 hours.

Group A (n = 40) consisted of patients participating in a study comparing the analgesic efficacy of rectal versus oral APAP after craniofacial surgery.[6] Two hours after arrival in the PSICU, patients had a gastric lavage until gastric fluid was clear and patients were then given approximately 20 mg/kg APAP study medication 6-hourly, either orally or rectally. APAP elixir was given through a nasogastric tube. Blood samples were drawn from an indwelling arterial catheter at 30, 60 and 90 min after administration of the rectal loading dose and 1 h before and 2 h after APAP maintenance dose administration. An extra dose of APAP was given if the VAS pain score[7] was more than 4 cm. Blood samples were taken just prior to administration of the extra dose and two h thereafter. The next dose of the study medication was then administered according to the protocol schedule, which has been described previously.[6] From 8 of these 40 patients, urinary samples were also collected for analysis.

Group B (n = 7) consisted of patients meeting the same inclusion criteria as the patients in group A. They received APAP suppositories for maintenance doses, 21-45 mg/kg 6-, 8- or 12-hourly after arrival in the PSICU. From these seven patients urinary samples were also available for analysis.
As a consequence, the total study population for the urinary analysis was 15 (8 from group A and 7 from group B). Urine was collected from the urinary catheter at 3-h intervals starting at 3 h after the arrival in the ICU and finishing at 24 h. After each 3-h collection period, the total amount of urine produced in the previous 3 h was registered and two samples of 3 ml were taken from the urine collected in this 3-h period for APAP, glucuronide and sulphate metabolite analysis. After collection samples were stored at 4°C until the end of the 24-h study period. Samples were then stored at -20°C until analysis.

The formulation for the rectal loading dose and the maintenance doses in the seven children from group B were 60, 120, 240 or 500 mg APAP suppositories contained in a triglyceride base (Pharmachemie, Haarlem, The Netherlands). The loading dose given (32-59 mg/kg) was dependent on available suppository size. Subsequent study medication, both elixir (APAP 24 mg/ml, glycerol 85%, sodium lactate, raspberry essence and sorbitol solution) and APAP suppositories (APAP and Witepsol H15, synthetic saturated triglycerides with a chain length of C12 - C18, as the base), were manufactured in the department of pharmacy. The deviation of the APAP doses in the suppositories was less than 10%. APAP for the study medication was supplied by Bufa b.v., Uitgeest, the Netherlands. The suppositories, the elixir, and all ingredients met with the requirements in the European Pharmacopoeia. APAP suppositories and elixir were manufactured according to the Dutch Pharmacists Formulary. Stability of these preparations is tested by the laboratory of the Royal Dutch Association of Pharmacists.

APAP assay
APAP plasma concentrations were determined using fluorescence polarization immunoassay (Adx system, Abbott Laboratories, North

Chicago IL) (Erasmus MC Rotterdam). The detection limit of this method was 1.0 mg/l. Precision was measured at APAP concentrations of 15, 35 and 150 mg/l. To determine coefficients of variation at these concentrations, 55 samples of each concentration were assayed [CV = (SD/mean); RSD = CVx100%]. RSD was 7.22%, 3.37% and 3.11% at 15, 35 and 150 mg/l, respectively. The APAP concentration range in which the accuracy was determined, is 10-150 mg/l.

APAP-glucuronide, APAP-sulphate and unchanged APAP concentrations in urine were determined using high-performance liquid chromatography (HPLC) (Isala Klinieken, Zwolle). The HPLC system consisted of a pump (ThermoFinnigan, SpectraSYSTEM P2000, flow 1.8 ml/min), an injector (ThermoFinnigan, SpectraSYSTEM AS3000), a Kolomoven (ThermoFinnigan, IGLOO-CIL, 21°C), a detector (ThermoFinnigan, Spectra Focus, 240 nm) and an integrator (ThermoFinnigan, ChromQuest 2.51). The column used was a LiChroCART 125-4, LiChrospher 100 RP-18 (Merck 1.50943). Reagents used were potassium dihydrogen phosphate (Merck 1.04873), formic acid (Merck 1.00264) and isopropanol (Merck 1.01040). The mobile phase of the column consisted of formic acid: isopropanol: 0.1 M potassium dihydrogen phosphate in water (0.1:1.7:98.2, v/v/v). The flow rate was 1.8 ml/min.

Calibration curves were prepared of APAP, APAP-glucuronide and APAP-sulphate by adding standard solutions of APAP, APAP-glucuronide and APAP-sulphate in water to a pooled sample of 'blank' urine of infants, so that the final concentrations of APAP in urine were 5-10-20-40 µg/ml, of APAP-glucuronide 5-50-100-200 µg/ml, and of APAP-sulphate 10-100-500-1000 µg/ml. In a pilot study, APAP-mercapturate and APAP-cysteine were not detected in urine samples of infants; therefore no calibration curves were prepared for APAP-mercapturate and APAP-cysteine. Duplicate urine samples (100µl) were diluted with 900 µl water and mixed, and 20 µl was injected onto the column.
The precision of this method was determined by six replicate assays at two concentrations (of the calibration curve) for APAP, APAP-glucuronide and

APAP-sulphate. The specificity of the method was determined by assay of six independent urine samples of patients who received other drugs (caffeine, gentamicine, carbamazepine, vancomycine, and digoxine). Detection limits (signal/noise ratio: 3) were 0.3 mg/l, 2.3 mg/l and 6.3 mg/l for APAP, APAP-glucuronide, and APAP-sulphate, respectively. Detection limits (signal/noise ratio: 5) were 1.0 mg/l, 3.2 mg/l and 10.1 mg/l for APAP, APAP-glucuronide, and APAP-sulphate, respectively. Precision was 7% for APAP, 3% for APAP-glucuronide and 3% for APAP-sulphate.

Modeling

Population parameter estimates

Urine metabolite data were converted to APAP mg equivalents using a molecular weight of 151.2 mg/mmol for APAP, 328.3 mg/mmol for APAP-glucuronide and 230.2 for APAP-sulphate. Population parameter estimates were obtained using a non-linear, mixed effects model.[8] This model accounts for population parameter variability (between and within subjects) and residual variability (random effects) as well as parameter differences predicted by covariates (fixed effects). The population parameter variability in model parameters was modeled by a proportional variance model. Additive terms characterized the residual unknown variability for serum concentration and the amount of glucuronide, sulphate and unmetabolized APAP in the urine. This error model assumes that the residual variability is of the same order of magnitude over the whole range of measurements. The population mean parameters, between subject variance and residual variance, were estimated using NONMEM version V release 1.1. Estimation used the first-order conditional estimate method with the interaction option and ADVAN 6 with Tol = 5. Convergence criterion was three significant digits. A Compaq Digital Fortran Version 6.5 compiler with Intel Pentium III 1 GHz CPU under Windows 2000 was used. Differential equations were used to describe the pharmacokinetics of APAP and its metabolites.

$$dAgut/dt = -Ka \times Agut$$

Agut is the amount of drug in the gut at any one time.

$$CLT = CLNRP + CLMG + CLMS + CLUP$$
$$dCP/dt = (Agut \times Ka - CP \times CLT) / VP$$
$$dCLMG/dt = (CLMG \times CP - CLUG \times CG) / VMG$$
$$dCLMS/dt = (CLMS \times CP - CLUS \times CS) / VMS$$
$$dCLUP/dt = (CLUP \times CP) / VP$$
$$dCLUS/dt = (CLUS \times CS) / VMS$$
$$dCLUG/dt = (CLUG \times CG) / VMG$$

The model is shown in Figure 2. VP is the volume of distribution for APAP, CP is APAP serum concentration, CLMP is clearance to metabolites of APAP, CLMG is clearance to APAP-glucuronide, CLMS is clearance to APAP-sulphate, VMS is the volume of distribution of sulphate metabolite, VMG is the volume of distribution of glucuronide metabolite, CS is APAP-sulphate serum concentration, CG is APAP-glucuronide serum concentration, CLUG is urine clearance of glucuronide, CLUS is urine clearance of sulphate, CLUP is urine clearance of unmetabolized APAP, CLNRP is clearance attributable to pathways other than these measured in urine in this current analysis and Tabs is the absorption half-life (Ln(2)/Ka). The ratio for the formation of the glucuronide (CLMG) and sulphate (CLMS) metabolites was set the same as the urine clearance ratio for these metabolites i.e.

$$CLMG = CLMP \times (CLUG/(CLUG+CLUS))$$
$$CLMS = CLMP \times (1-CLUG/(CLUG + CLUS))$$

CLMP is the total non-renal clearance through the two metabolites (CLUG + CLUS). The metabolite volumes of distribution (VMS and VMG) cannot be identified using the current study design and were fixed at 17 l/70kg, based on the study by Lowenthal et al.[9] For patients who did not have any urine data, the total clearance of APAP was estimated as a single parameter (CLT) with its own variability.

A model for size and age changes in pharmacokinetics of APAP
Initial models confirmed the previous conclusion by van der Marel et al.[6] that the bioavailability of elixir was low compared to suppository formulation in the 47 infants given APAP for analgesia after craniofacial surgery. In order to gain a better understanding of the elixir bioavailability, data from the 47 craniofacial patients were included with those from a previous study investigating age-related pharmacokinetics (n = 221).[10] A separate additive term was used to characterize the residual unknown variability for serum concentration from each study.

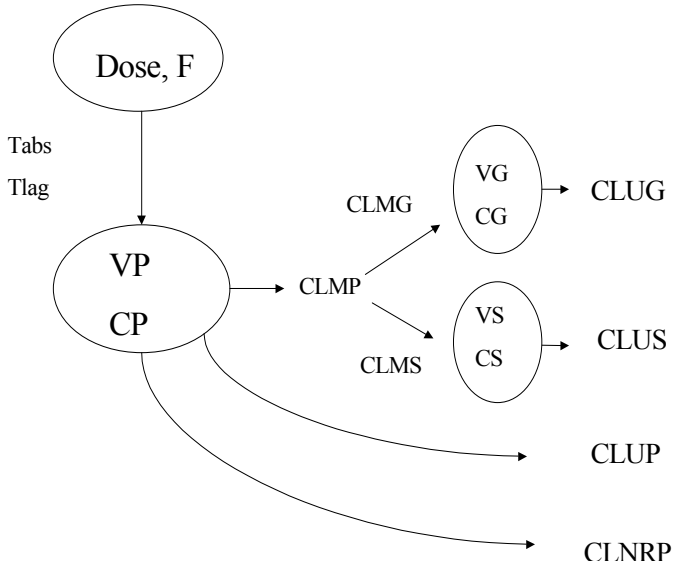

Figure 2 *VP volume of distribution for APAP, CP APAP serum concentration, CLMP the total non-renal clearance through the two metabolites (CLUG + CLUS), CLMG clearance to APAP-glucuronide, CLMS clearance to sulphate, VMS volume of distribution of sulphate metabolite, VMG volume of distribution of glucuronide metabolite, CS APAP-sulphate serum concentration, CG APAP-glucuronide serum concentration, CLUG urine clearance of glucuronide, CLUS urine clearance of sulphate, CLUP urine clearance of unmetabolized APAP, CLNRP non-renal clearance of APAP, Tabs absorption half-life (Ln(2)/Ka), Tlag Lag time*

Covariate Analysis

The parameter values were standardized for a body weight of 70 kg using an allometric model.[11]

$$P_i = P_{std} \times (W_i / W_{std})^{PWR}$$

where P_i is the parameter in the i*th* individual, W_i is the weight in the i*th* individual and P_{std} is the parameter in an individual with a weight W_{std} of 70 kg. The *PWR* exponent was 0.75 for clearance and 1 for distribution volumes.[12-16]

Exponential functions were applied to allow for age-related changes of CLT, CLMG, CLMS, CLUP and VP,[9] e.g.:

$$V/Foral = (VPstd \times (Wt/70)) \times (1 + \beta vol \times EXP(-AGE\ in\ months \times Ln(2)/Tvol))\ l$$

$$CLT/Foral = (CLTstd \times (Wt/70)^{0.75}) \times (1 + \beta cl \times EXP(-AGE\ in\ months \times Ln(2)/Tcl))\ l/h$$

$$CLMG = (CLMGstd \times (Wt/70)^{0.75}) \times (1 + \beta cl \times EXP(-AGE\ in\ months \times Ln(2)/Tcl))\ l/h$$

βvol and βcl are parameters estimating the fraction above or below VP/Foral and CLT/Foral respectively, at birth; *Tvol* and *Tcl* describe the maturation half-lives of the age-related changes of VP/Foral and CLT/Foral.

The influence of urine flow rate (URATE, l/3h) on CLMG, CLMS and CLUP was modeled with an exponential function with a scaling constant (USCALE) e.g.

$$CLUP = CLUPstd \times EXP(USCALE \times URATE)$$

Scaling factors were applied to Foral (elixir bioavailability) for infants who vomited (Fvom) or suffered nasogastric loss (F nasogastric) after oral elixir APAP administration.

The quality of fit of the pharmacokinetic model to the data was assessed by visual examination of plots of observed versus predicted concentrations. Models were nested and an improvement in the objective function was referred to the Chi-squared distribution to assess significance, e.g. an objective function change (ΔOBJ) of 3.84 is significant at $\alpha = 0.05$.

Results

Population Demographics

Mean (±SD) age and weight of the 47 infants undergoing craniofacial surgery were 11.5 ± 2.5 months and 9.2 ± 1.4 kg**.** There were 32 boys and 15 girls. APAP elixir was used as maintenance medication in 20 infants and suppositories were used in the other 27. The 15 patients from whom urine data were collected consisted of 8 boys and 7 girls with a mean age of 11.8 ± 2.5 months and a mean weight of 9.1 ± 1.9 kg . There were no significant differences between patients receiving oral APAP and patients receiving rectal APAP with respect to age, weight, sex, extent of operation procedure, blood loss, duration of operation and underlying diagnosis. The total APAP dose over the 24-h study period was 114 ± 18 mg/kg.

Urine recovery of APAP at steady state (18- to 24-h dose interval) was 70% (CV 38%) of the given dose. This is comparable to the estimated relative bioavailability of the triglyceride suppository (0.72, CV 42%) and suggests almost complete recovery.

Parameter estimates

Formation clearance of APAP-glucuronide (%CV) and APAP-sulphate were 6.5 (11.5) l/h/70 kg and 7.5 (11.5) l/h/70kg, respectively. Final parameter estimates with covariate analyses are shown in Table 1 & Table 2. Pharmacokinetic estimates and maturational half-lives of VP/F and CLT/F were similar to those reported previously.[10] Figure 3 shows clearance changes with age for the complete pooled data set. The clearance estimates for infants undergoing craniofacial surgery (symbol *x*) enrich the data set and are consistent with the other data used in the pooled analysis.

Table 1 Pharmacokinetic parameter estimates. These estimates are standardized to a 70-kg person using an allometric size model; %CV is the coefficient of variation for the population parameter estimate; S.E. is the standard error of the structural parameter estimate. SE% is the S.E. expressed as a percentage of the population parameter estimate. CLT population estimate for CL/Foral (clearance after oral administration l/h/70kg) in infants with no urine data, VPstd population estimate for V/Foral (volume of distribution l/70kg), CLUG, CLUS, CLUP std are urinary clearances of glucuronide, sulphate and unmetabolized APAP before effect of urine flow , Tabs absorption half-life after nasogastric (elixir) in infants out of the neonatal period, triglyceride base suppository and capsule suppository administration (h), Tlag absorption lag time after nasogastric (elixir), triglyceride base suppository and capsule suppository administration (h), Frectal/oral relative bioavailability of the rectal compared to the oral formulation.

Parameter	Estimate	CV %	S.E. %
CLTstd	14.1 l/h/70kg	35.8	6.6
No urine data			
VPstd	78.7 l/70kg	10.3	4.4
CLMGstd	6.6 l/h/70kg	11.5	10.6
VMG	17 l/70kg FIX		
CLMSstd	7.5 l/h/70kg	11.5	10.6
VMS	17 l/70kg FIX		
CLUG std	2.65 l/h/70kg		26.6
CLUS std	3.03 l/h/70kg		15.9
CLUP std	0.55 l/h/70kg	28.0	12.5
F elixir	1 FIX	30.2	
Tabs elixir	0.138 h	140	13.7
Tlag elixir	0.383 h	29.4	6.0
F triglyceride/oral	0.718	42.4	7.1
Tabs triglyceride	2.7 h	64.4	13.9
Tlag triglyceride	0.201 h	29.4	28.3
F capsule/oral	0.727	34.9	6.3
Tabs capsule	0.724 h	64.4	10.1
Tlag capsule	0.513 h	29.4	6.1

Table 2 Covariate models and estimates for pooled population parameters. βvol and βcl are parameters estimating the fraction above or below VP/Foral and CLT/Foral respectively at birth; Tvol and Tcl describe the maturation half-lives of the age-related changes of VP/Foral and CLT/Foral. Fvom is the proportion of drug lost from vomiting after elixir administration; F nasogastric is that proportion lost from nasogastric drainage.

Parameter	Estimate	S.E. %
USCALE[c]	5.5	3.7
Fvom	0.65	31.0
F nasogastric	0.425	14.8
βvol[a]	0.615	48.0
Tvol[a]	1.7 days	30.5
βcl[b]	-0.587	7.1
Tcl[b]	8.09 months	49.1

[a] Volume of distribution
V/Foral = (VPstd x (Wt/70)) x (1 + βvol x EXP(-AGE in months x Ln(2)/Tvol)) l
[b] Clearance
CLT/Foral = (CLTstd x $(Wt/70)^{0.75}$) x (1 + βcl x EXP(-AGE in months x Ln(2)/Tcl)) l h^{-1}
CLMG = (CLMGstd x $(Wt/70)^{0.75}$) x (1 + βcl x EXP(-AGE in months x Ln(2)/Tcl)) l h^{-1}
[c] Relationship of unmetabolized APAP clearance to 3-hourly urine volume (URATE, l/3h)
CLUS = CLUS baseline x EXP(USCALE x URATE)
CLUG = CLUG baseline x EXP(USCALE x URATE)
CLUP = CLUP baseline x EXP(USCALE x URATE)

Clearance attributable to pathways other than these measured in urine was not identifiable in this study (i.e. CLNRP = 0). The urinary clearance ratio APAP-glucuronide to APAP-sulphate was 0.87. The exponential function applied to allow for age-related changes of CLT was also applied to glucuronide metabolite formation. Consequently, the formation clearance ratio of APAP-glucuronide to APAP-sulphate varied with age and was 0.69 at 12 months age and 0.81 at 2 years. Sulphate and glucuronide conjugation contributed equally towards APAP metabolism. The individual Bayesian predictions for serum concentration, 3-hourly urine APAP, glucuronide and sulphate excretion are compared with those observed in Figure 4a, 4b, 4c & 4d, respectively. These predictions are based on maximum *a posteriori* Bayesian estimates of the parameters for each specific individual using their

observed data.
Glucuronide formation clearance, but not sulphate formation, was related to age and increased with age from a predicted value in a neonate of 2.73 l/h/70kg to a mature value of 6.6 l/h/70kg with a maturation half-life of 8.09 months. The objective function did not improve when clearance of unmetabolized APAP and sulphate conjugation was assumed to be age related. These metabolite age relationships are shown graphically in Figure 5.

The clearance of unmetabolized APAP, glucuronide and sulphate metabolites were all related to the volume collected in the 3-hourly urine (i.e. the urine flow rate measured over 3 h) using an exponential function (Figure 6). The objective function was significantly worse (ΔOBJ 35.8) when urine flow rate was only used to account for differences in unchanged urinary APAP clearance. The mean urine clearance of unchanged APAP was 0.55 l/h/70kg (CV 28%).
The residual errors for 3-hourly urine glucuronide, sulphate and APAP were 15.6 , 24.2 and 3.2 mg, respectively. The residual errors for serum concentrations from infants undergoing craniofacial surgery was 3.1 mg/l and from Anderson et al.[10] was 1.8 mg/l.

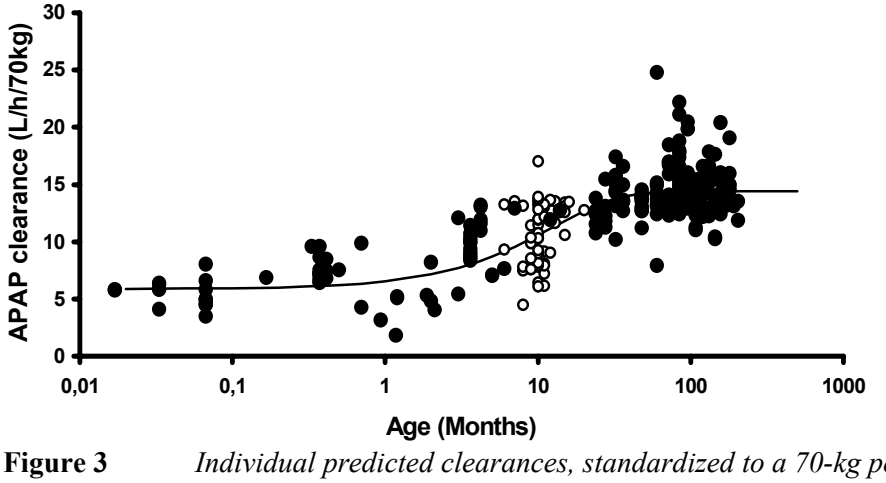

Figure 3 *Individual predicted clearances, standardized to a 70-kg person, from NONMEMs post-hoc step are plotted against Log age. The solid line demonstrates the non-linear relationship between clearance and age. Infants (n = 47) studied after craniofacial surgery are shown as open circles. Infants from the reference data set (n = 221) are shown as closed circles*

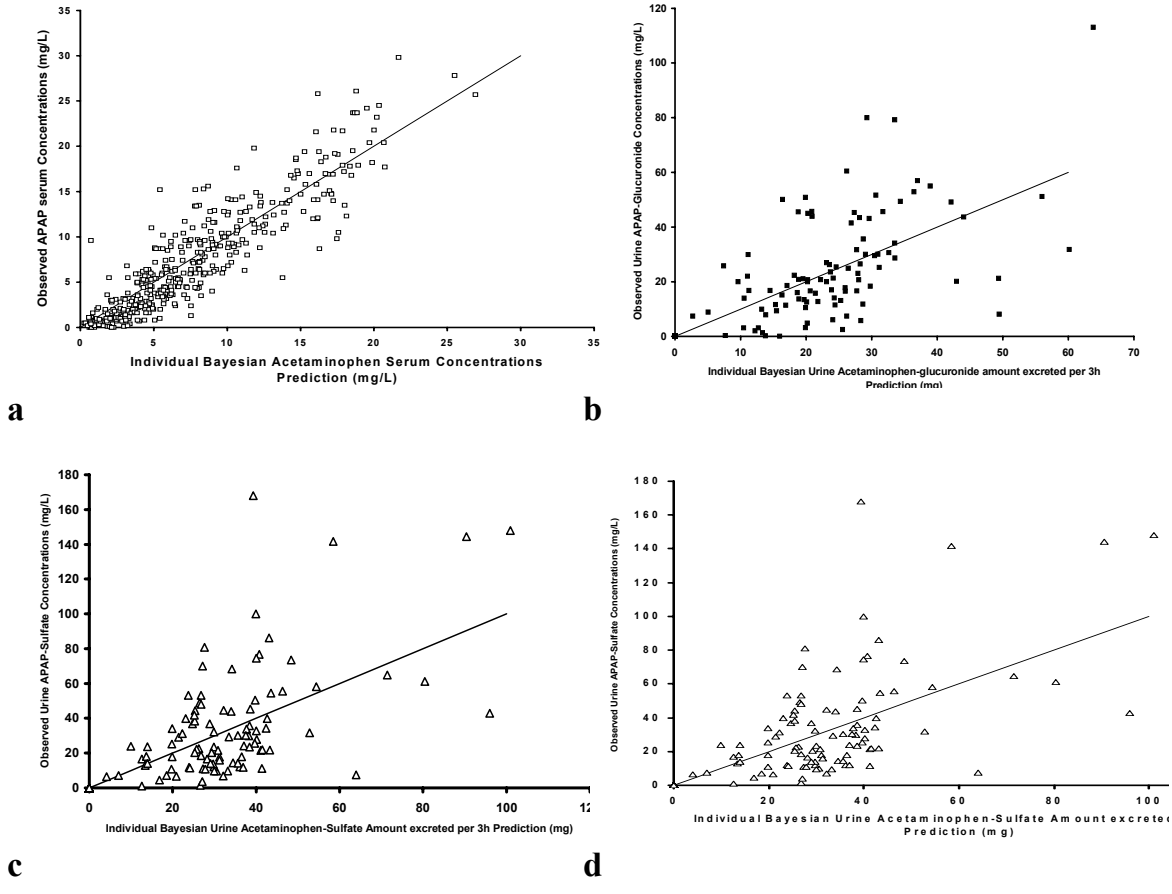

Figure 4 *Quality of fit of pharmacokinetic data. Individual Bayesian concentration predictions based on values of the parameters for the specific individual are compared with observed. The line x = y is the line of identity.* ***a*** *Serum concentration data from craniofacial infants (n = 47) and from the reference data set (n = 221)* ***b*** *urine APAP-glucuronide metabolite data (n = 15)* ***c*** *urine APAP-sulphate metabolite data (n = 15)* ***d*** *urine unmetabolized APAP data (n = 15).*

Discussion

The mean (±SEM) glucuronide to sulphate ratio increases from 0.12 ± 0.09 in preterm neonates 28-32 weeks gestational age,[17] to 0.28 ± 0.35 in preterm neonates 32-36 weeks gestational age,[17] 0.34 ± 0.08 in newborns,[18] 0.75 ± 0.10 in 3- to 9-year-old children[18,19] and 1.61 ± 0.21 in 12- year-old children,[18] with an adult ratio of 1.80 ± 0.32.[18] As Miller et al. studied only seven children,[18] and since there are no studies in infants out of the neonatal age group reported in the literature, we evaluated 15 patients of 6-16 months of age (mean ±SD; 11.8 ± 2.5 months) following major craniofacial surgery.

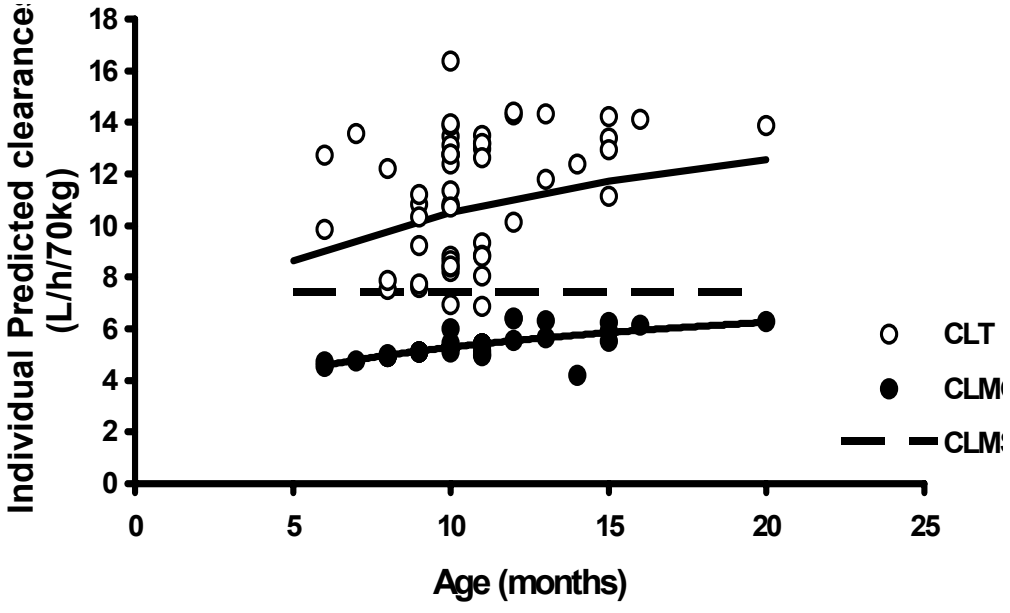

Figure 5 *Individual predicted metabolite clearances of children with urine data (n = 15), standardized to a 70-kg person, from NONMEMs post-hoc step are plotted against age. The total body clearance and clearance to the glucuronide metabolite increase with age, while clearance to the sulphate metabolite was not age-related in the studied infants. CLT total metabolite formation clearance, CLMG glucuronide formation clearance, CLMS sulphate formation clearance*

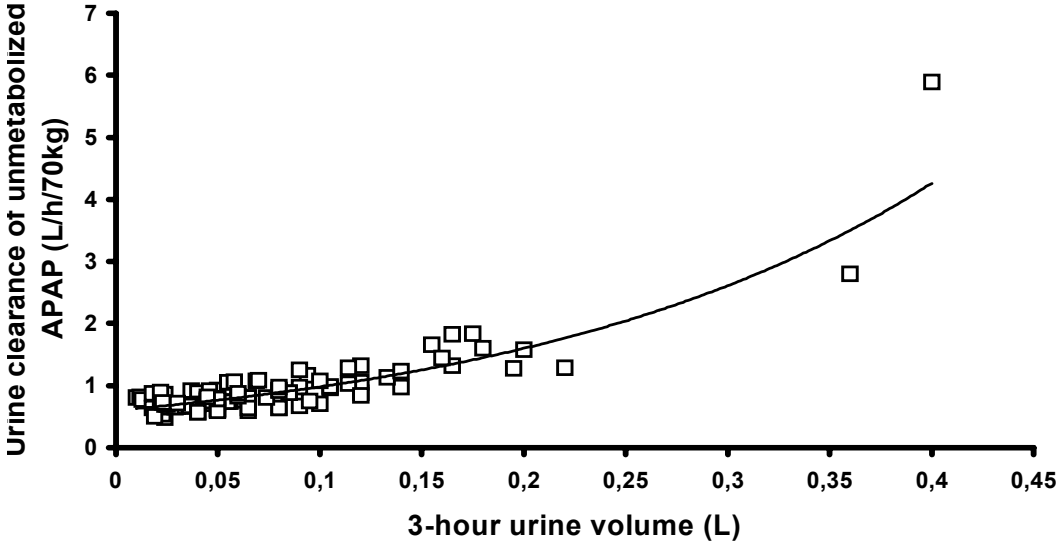

Figure 6 *The relationship between urine clearance of unmetabolised APAP and 3-hourly urine volume collection (URATE) in children with urine data (n = 15)*

In this current study, we have demonstrated a glucuronide to sulphate metabolite ratio increase in infancy. Clearance was to glucuronide and sulphate metabolites as well as renal elimination of unmetabolised APAP. Other routes of elimination were not detectable. Size was the first covariate used in our analysis. By choosing weight as the primary covariate and applying allometric size models, the secondary effects of age could be investigated and parameter estimates at different ages standardized to a 70-kg person. The sulphate metabolite clearance of 7.5 l/h/kg is similar to those described in adults (mean ± SD 6.8 ± 1.2 l/h/70kg) (Table 3) and there was no age-related change detected in this cohort of infants. The objective function (ΔOBJ) improved by only 2, when age related sulphate clearance was added. Urine glucuronide/sulphate ratios of 0.34 in neonates rising to 0.75 in children 3-9 years have been reported.[17,18] Our current data suggests that the ratio reported reaches a constant value by 3 years (glucuronide maturation half-life of 8.09 months). Several studies have shown that the rate constant for APAP-glucuronide formation in neonates is considerably smaller than in adults but the rate constant.[5,17,20] The increase relative to adults may be an artefact of a non-biological based size model and has been observed when applied to clearance.[21]

Our estimate for glucuronide formation clearance 6.6 l/h/70kg (CV 11.5%) is approximately half that of adult estimates (11.8 ± 3.7 l/h/70kg) (Table 3). We estimated a total APAP clearance of 14.6 l/h/70kg in infants who had urine data and 14.1 l/h/70kg in the other infants. These estimates are lower than that reported in adults; the mean total clearance in adults from Table 3, for example, is 21.6 ± 4.3 l/h/70kg. Miller et al.[17] report a glucuronide/sulphate ratio in urine of 1.61 ± 0.21 in 12-year-old children and 1.8 ± 0.32 in adults. These data are suggestive of a further increase in glucuronide metabolism during the teenage years. But this increase might be due to other age-related factors, e.g hormone changes, rather than exclusively to maturation of the enzymes involved in glucuronidation. APAP is metabolized principally by the UGT1A6 enzyme. Although the UGT1A6 enzyme for APAP has been identified, little about its maturation profile is known. In vivo studies reveal that glucuronidation during fetal life

is 1-10% of adult levels. There is an increase slowly after birth and at 6 months it is 50% of adult activity, findings similar to those reported in this current study where we estimate a maturation half-life of 8.09 months. Enzyme activity is not only determined by postnatal age, but might also be regulated by both ontogenic and genetic processes and these have not yet been elucidated.[22]

Table 3 Metabolite clearance (S.D. or range) (l/h/70kg) in adults given a single dose APAP. Estimates are based on urine metabolite analyses *po* oral, *iv* intravenous administration

Reference	CL glucuronide	CL sulphate	CL total	
Mitchell[31]	9.76 (0.38)	5.64 (0.25)	18.2 (0.82)	n = 7, po
Sonne[32]	9.83 (3.17)	4.93 (1.84)	18.9 (3.2)	n = 9, po
Mitchell[33]	15.9 (2.34)	7.62 (1.32)	26.5 (3.7)	n = 9, po
Miners[34]	14.5 (3.2)	7.9 (1.6)	26.1 (4.7)	n = 8, po
Wynne[35]	10.5 (0.42)	7.56 (0.42)	19.7 (0.84)	n = 19, iv
Hoffman[36]	15.6 (1.7)	7.79 (2.59)	22.9 (6.9)	n = 7, po
Baraka[37]	14.8 (5.6)	5.6 (1.2)	22.3 (5.6)	n = 10, po
Rumble[38]	5.4 (1.8)	6.3 (2.4)	21 (5.9)	n = 8, po
Miners[39]	10.7 (1.8)	5.1 (0.73)	18.5 (2.73)	n = 8, po
Miners[40]	14.4 (0.68)	6.2 (0.47)	24.4 (0.85)	n = 12, po
Ismail[41]	6.6 (1.6)	3.7 (1.2)	16.07 (5.3)	n = 8, po
Kamali[42]	7.42 (1.19)	6.02 (1.19)	14.9 (1.47)	n = 9, iv
Haderslev[43]	13.46 (10.95-15.13)	5.51 (5.37-7.06)	22.72 (19.61-24.79)	n = 10, po
Manyike[3]	16.18 (5.18)	8.02 (2.34)	30.54 (7.39)	n = 8, po
Average	11.8 (3.7)	6.8 (1.2)	21.6 (4.3)	

The clearance of unmetabolized APAP normally contributes 2-5% of total urine clearance.[2,3] We estimate the renal clearance of unchanged drug to be 3.7% of total APAP clearance. There was almost complete recovery of administered APAP in the urine at steady state. The metabolites of APAP are thought to be stable in urine. The clearance of APAP and its metabolites in the urine was related to 3-hourly urine volume (i.e. flow) using an exponential function. Miners et al.[23] have shown a 2.5-fold increase in the renal clearance of unchanged APAP in adults as urine flow increased from 0.81 ml/min to 6.00 ml/min (0.7-5.2 ml/h/kg). We were able to define a relationship between urine flow rate and clearance in infants with urine

flows of 0.46-13.6 ml/h/kg. As APAP is a weak acid, with a pKa value of 9.5, it is essentially unionized at all urine pH values, and pH is unlikely to have an effect on urine clearance. APAP is passively reabsorbed throughout the entire nephron, and clearance is expected to increase with increasing urine flow.[24] There was no effect of age on unchanged APAP clearance because glomerular filtration rate and renal blood flow, corrected for body size, are mature by 6 months of age.[25]

Clearance of glucuronide and sulphate conjugates in dogs is similar to clearance of inulin but there is active tubular transport probably localized to the distal nephron; clearance of these metabolites is reported to be independent of urine flow.[26] We observed an improved fit to the urine excretion data when we assumed that clearance of these two metabolites changed similarly to APAP with increased urine flow. This has not been previously reported in humans.

Pharmacokinetic estimates were similar to those previously published. Data from the previous pediatric analysis[10] performed by Anderson et al. were included in this current analysis in order to estimate that portion of dose lost to nasogastric drainage (42.6%) and vomiting (65%) in infants undergoing craniofacial surgery. Gastric emptying after major surgery is delayed and the reduced bioavailability of the oral formulation was noted but not quantified in the previous paper investigating pharmacodynamics in infants undergoing craniofacial surgery.[19] The earlier pooled analysis[10] had fewer infants about the 12-month age bracket and inclusion of these children who had craniofacial surgery in this analysis revealed a longer clearance maturation half-life of 8.09 months (S.E. % 49.1), as opposed to an earlier estimate of 3.25 months (S.E.% 43). However, both of these covariate models predict that total APAP clearance is 94% that of older children by 1 year of age.

We were surprised to be unable to detect oxidative metabolites (cysteine and mercapturic acid conjugates) in our pharmacokinetic analysis (i.e. CLRNP, clearance attributable to pathways other than those measured in

the urine in this current analysis). Hepatotoxicity has been reported in neonates given APAP, but NAPQI was not measured. There is a relatively low level of activity of the cytochrome P_{450} CYP2E1 enzyme system[27,28] in infants and this may contribute to the wider margin of safety reported in infants. However, the contribution of this metabolic pathway, even in adults, can be low (2%) and may be missed if urine collection after APAP administration is incomplete. Although Al-Obaidy et al.[29] report that these metabolites contribute 12.6% of drug excreted in children with chronic liver disease, there are no data available concerning these urinary metabolites in either healthy or sick infants, except for neonates after maternal overdose.[30] The lack of urinary metabolites derived from NAPQI formation may mean that NAPQI formation is negligible in infants and thus explain the apparent lack of APAP hepatotoxicity in this age group.

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Chapter 2.3

Pharmacokinetics of single dose intravenous propacetamol in neonates: effect of gestational age

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Abstract

Aim

To investigate pharmacokinetics and –dynamics after single dose administration of propacetamol in preterm and term infants on the first day of life.

Methods

Neonates were stratified by gestational age (GA). Preterm (< 37 weeks) and term (37-41 weeks) infants received a single dose of propacetamol in the first 24 hours of life when they underwent minor, painful procedures or as additional treatment in infants on opioids. Blood samples were taken from an arterial line and pain was evaluated by a multidimensional pain scale. Results were reported by mean and standard deviation (SD). Student t and Wilcoxon test were used to compare both groups.

Results

Thirty neonates were included, of which 10 were term infants. Mean serum half life (t ½) in preterm infants was 277 (SD 143) minutes and was 172 (SD 59) minutes in term infants ($P < 0.05$) while clearance (CL) was 0.116 (SD 0.08) l/kg/h in preterm and 0.170 (SD 0.06) in term infants ($P < 0.05$). A correlation of GA with t ½ was found (r = -0.46). An effect of gender or administration of prenatal steroids on pharmacokinetics of paracetamol could not be documented. In neonates who only received propacetamol (n = 15), the level of analgesia seems to be associated with a therapeutic (> 5 mg/l) level.

Conclusions

A correlation between GA and T ½ for propacetamol was documented. The maturational trend of CL and T ½ in preterm and term neonates is in line with data on pharmacokinetics of propacetamol beyond the newborn period.

Abbreviations

T ½	serum half life
GA	gestational age
V_d	distribution volume
CL	total body clearance
SD	standard deviation
CV	conventional ventilation
HFO	high frequency oscillation
CPAP	continuous positive airway pressure
HELLP	hemolysis, elevated liver functions, low platelets

Introduction

Adequate management of pain in neonates is a major issue in neonatal care since Anand demonstrated the positive effect of opioids on mortality and morbidity in neonates who underwent cardiac surgery.[1] Although the NOPAIN preliminary trial only documented a non-significant trend in reduction of poor neurologic outcome in preterms on morphine during ventilation, pre-emptive analgesia in ventilated infants is considered standard of care in most neonatal units.[2-5]

Pharmacokinetics and –dynamics of most drugs prescribed in contemporary neonatal intensive care are still rare or even lacking, leading to frequent unlicensed and off-label use of drugs.[6] To a certain extent, this also is true for paracetamol (APAP).
APAP, N-acetyl-p-aminophenol, is a readily available antipyretic and analgesic agent. Although less potent, this drug might have fewer side effects when compared with opioids. It is the most often prescribed drug for treatment of mild to moderate pain in infants, including neonates. This drug can be administered by oral, rectal but also by intravenous route.

The intravenous prodrug administration might improve prediction of concentration and consequent effect compared to rectal and/or oral

formulations by elimination of plasma variability due to absorption kinetics and relative bioavailibity.[7-13] The combined use of opioids and APAP might reduce the need for opioids and reduce the side-effects, especially hypoventilation, in neonates. Although some believe that APAP itself is harmless in neonates, there is potential hepatotoxity.[14] Propacetamol is a pro-drug of APAP and is hydrolysed by plasma esterases after intravenous administration in which one gram of propacetamol liberates 0.5 g of APAP, assuming adequate esterase activity in neonates, in line with documented cholinesterase activity.[10,15] To document pharmacokinetics and pharmacodynamics of propacetamol in neonates of different gestational age (GA), a single dose study in the first 24 hours of life was performed.

Patients and methods

All neonates admitted within the first 24 hours of life in the neonatal intensive care unit and with an arterial line in place were considered for inclusion if propacetamol was administered. The decision to prescribe propacetamol or any other analgesic was made by the attending neonatologist. Propacetamol was administered when infants underwent minor, painful procedures (i.e. insertion of peripheral arterial or venous line, insertion of central venous line or placement of a chest tube) or as additional therapy in infants on opioids. Exclusion criteria were major congenital malformations or severe birth asphyxia (Apgar < 4 at 5 minutes) in line with other studies performed in neonates. The initial dose, i.e. 20 mg (10 mg APAP)/kg dose was based on literature data, with the intention to change this dose if interim analysis of the APAP levels in the first 15 infants was inadequate (plasma levels < 5mg/l within 8-10 hours after administration).[10-13] Maternal use of analgesics (besides APAP) were no reason for exclusion in this single dose pharmacokinetic study.

As part of standard nursing care within the neonatal intensive care unit, a multi-dimensional pain scale was used to document pain/comfort in these neonates. With this pain scale (Leuven neonatal pain scale), three different levels (level 1: < 4/14, no pain; level 2: 4-6/14 mild discomfort; level 3: >

6/14 pain) can be discriminated.[16] An algorithm is used within the unit to administer and to adapt analgesics based on this pain scale.[17]

The number and dose of other analgesics or sedatives prescribed in the first day of life were recorded. Birth weight was documented on admission in the unit. Gestational age (GA) was estimated by routine ultrasound examination before 20 weeks of gestation if available, or was based on the mother last month's period and postnatal physical characteristics. Propacetamol was administered as a 15-minutes infusion to avoid local discomfort.[18] Blood samples (0,2 ml) were taken from an arterial line 30, 60, 90, 120, 180, 240 and 600 minutes after initiation of intravenous administration. The maximal total allowed amount of blood collected in a single neonate was 1 ml/kg. After centrifugation, samples were stored at –20°C until analysis. APAP plasma concentrations were determined using fluorescence polarization immunoassay (Adx system, Abbott Laboratories, North Chicago IL). Determination limit was 1 mg/l and precision was 7%.

Pharmacokinetics were calculated assuming a linear one-compartment model with instantaneous input and first-order output. For every single patient a logarithmic trend line ($y = a \ln(x) + b$) was calculated, based on at least three plasma samples. Relative distribution volume (l/kg) (V_d) and concentration at t = 0 ($C\ max_0$) were calculated. The slope of the curve {slope = (log Cp2 – log Cp1) (t2-t1)} was used to calculate the time constant K (slope x 2.303), elimination half life (0,693/K) (T ½) and total clearance (K x Vd) (CL). Results are expressed by mean, standard deviation (SD) and range.

Student t test (normal distribution) or Wilcoxon test were used to compare clinical and pharmacokinetic findings in preterm (< 37 weeks GA) and term (≥ 37 weeks GA) infants. Linear regression analysis of GA and birth weight on T ½ were calculated (MedCalc®). The protocol was approved by the local ethics committee (Gasthuisberg, Leuven, Belgium) and infants were only included after informed consent of the parents.

Results

Thirty neonates of variable GA were included in this single dose study. Fifteen infants received the 20 mg (10mg APAP)/kg dose and 15 infants received a 40 mg (20 mg APAP)/kg dose. Clinical characteristics are summarised in Table 1.

Table 1 Clinical characteristics of the population. Results are reported by mean, standard deviation and range or by absolute numbers in term (37-41 weeks) and in preterm (< 37 weeks) infants. (HELLP: hemolysis, elevated liver enzymes, low platelets, CV: conventional ventilation, HFO: high frequency oscillation, CPAP: continuous positive airway pressure). Three infants received surgery on the first day of life (esophageal atresia)

		Term	**Preterm**
Number of infants		10	20
Birth weight (g)	mean	3323 (538)	1456 (592)
	range	1980-4000	505-2440
GA (wk)	mean	38.5 (1.2)	31.4 (2.5)
	range	37-40	27-35
Maternal disease	Preeclampsia/HELLP	3	4
	Solutio placentae	1	2
	Other medical conditions	0	2
Delivery	Caesarean	2	12
Diagnosis	Respiratory distress	--	9
	Wet Lung Disease	5	--
	Meconium aspiration	2	--
	Pneumothorax	1	--
	Congenital heart disease	1	--
	Surgical conditions	1	2
	Prematurity	--	9
Ventilation	CV/HFO	8	8
	Nasal CPAP	--	10
Prenatal betamethasone		--	13

Overall mean birth weight was 2111g (SD 1094g) and mean GA at inclusion was 33.8 (SD 3.9 weeks). Mean postnatal age (hours) at inclusion was 12.7 (SD 6.4). Ten infants had a GA ≥ 37 weeks. Twenty infants were preterm (< 37 weeks GA), of whom 10 were younger then 32 weeks GA. Twenty-six infants received respiratory support, of whom 16 (53%) infants

were ventilated. Fifteen (50%) received other analgesics in the first 24 hours of life.

In total 213 blood samples were collected and analyzed. Results of all plasma samples are available in Figure 1a & 1b.

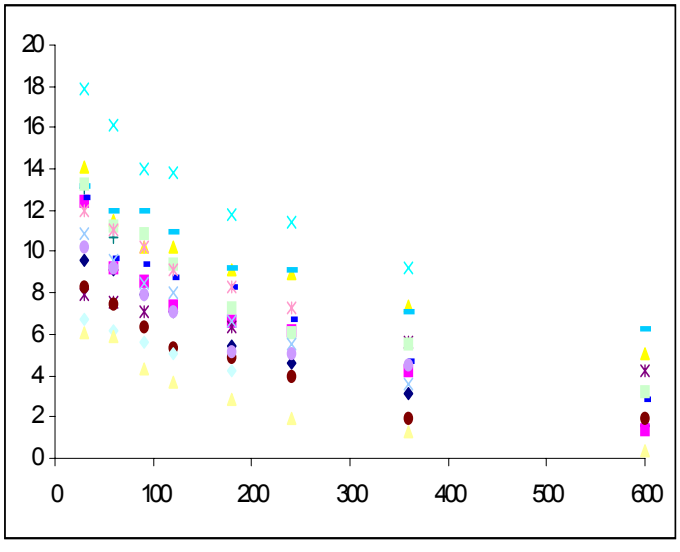

Figure 1a

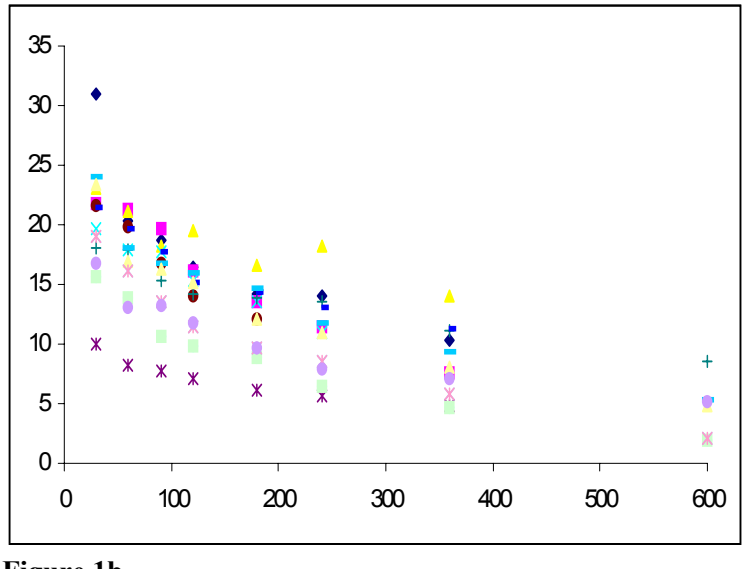

Figure 1b

Figure 1 a-b *Plasma levels of APAP (n = 213) (mg/l) in all infants following single intravenous administration of 20 mg/kg (Figure 1a) or 40 mg/kg (Figure 1b) of propacetamol (X-axis: time in minutes), hereby illustrating major variability.*

Table 2 Pharmacokinetics of propacetamol in preterm (< 37 weeks) and term (37-41 weeks) infants.

		Preterm	**Term**
Number of infants		20	10
Relative V_d (l/kg)	mean	0.61 (0.15)	0.64 (0.25)
	range	0.44-1	0.46-1.3
T ½ (min)	mean	277 (143)	172 (59)
	range	87-680	100-269
Clearance (l/kg/h)	mean	0.116 (0.08)	0.170 (0.06)
	range	0.004-0.24	0.08–0.29

Pharmacokinetic characteristics are summarized in Table 2. No significant difference in relative V_d (l/kg) between preterm and term infants could be documented. T ½ and CL are significantly different (both $P < 0.05$) between preterm and term infants (both $P < 0.05$). Mean T ½ in preterm infants was 277 minutes. In term infants mean T ½ was 172 minutes. Mean CL was significantly lower in preterm infants when compared with term (0.116 versus 0.170 l/kg/h) infants. In < 32 weeks GA infants, mean T ½ was 290 while in more mature infants (32-36 weeks GA), mean T ½ was 265 minutes. Correlation of GA with T ½ (r = - 0.46) was stronger than (birth) weight with T ½ (r = - 0.39). Linear regression analysis of GA on T ½ with 95% confidence intervals is available in Figure 2. We could not document a significant difference in T ½ nor in other clinical characteristics (birth weight, GA) between preterms (< 35 weeks GA) who received (n = 13) and preterms (n = 6) who did not receive prenatal steroids (betamethasone) for lung maturation. Neither could we document any gender-related differences in pharmacokinetics in this study.

Pharmacodynamics: since this is a single dose study, other analgesics were allowed. 15/30 infants received at least one other analgesic [(fentanyl (11), tramadol (6), ibuprofen-lysine (1)] during the first 24 hours based on the standardized evaluation by pain score (Leuven neonatal pain scale). In the hours before APAP was administered, stage 1 (pain scale < 4) was documented in 26/30 infants, during the period when a therapeutic level (> 5 mg/l) was reached, it was 30/30 and thereafter it was 24/30. If we

consider only infants (n = 15) who did not receive any analgesic besides APAP in the first 24 hours, level 1 of analgesia was documented in 14/15 infants before administration, in 15/15 infants in the period when a therapeutic level (> 5 mg/l) was reached and in 12/15 infants after this period.

Figure 2 *Linear regression analysis with 95% confidence intervals of gestational age (GA) on serum half life (T ½) (r= -0.46).*

Discussion

Mean serum half life (T ½) in preterm infants was 277 (SD 143) and was 172 (SD 59) in term infants ($P < 0.05$) while clearance (CL) was 0.116 (SD 0.08) l/kg/h in preterm and 0.170 (SD 0.06) in term infants ($P < 0.05$) in this single dose study. Pharmacokinetics and –dynamics of propacetamol are documented in adults and children but there is only one single study on pharmacokinetics in infants younger than 1 year (n = 12, of which 5 infants < 10 postnatal days) and there are no data of propacetamol in preterm neonates. Pharmacokinetics of propacetamol in this study were compared

with pharmacokinetics of APAP and propacetamol in other cohorts described in literature.

Term neonates

Findings in term infants are in line with the single study on intravenous propacetamol.[10] Autret documented pharmacokinetics in 12 infants, of whom 5 neonates younger than 10 days. Serum half life in these 5 neonates was 210 (SD 30) minutes, CL was 0,149 (SD 0,067) l/kg/h while V_d was 0,7 (SD 0,2).[10] (Table 3) Pharmacokinetics after rectal administration of APAP in term neonates were studied by Van Lingen and Hopkins.[11,18] Van Lingen (n = 10) documented a mean T ½ of 162 (SD 84) minutes and the mean T ½ in neonates studied by Hopkins (n = 9) was 228 minutes. There are no studies available on pharmacokinetics of APAP after nasogastric administration in the first day of life. Studies in neonates by Hopkins (n = 3) and Anderson (n = 16) after nasogastric administration documented serum T ½ of 168 minutes and 576 minutes.[9,19] Co-administration of opioids and its effect on gastric motility might at least partially explain these differences. There is a recent report on unintentional intramuscular administration of propacetamol in one term neonate.
In that single case, calculated serum half life (T ½) was 210 minutes.[20]

Table 3 Maturational trend (mean) of serum half life (T ½) and relative distribution volume (relative V_d) in the first year of life after intravenous administration of propacetamol, based on this population[#] and on the study of Autret[10]. (GA: gestational age).

	Preterm[#]	**Preterm[#]**	**Term[#]**	**Term[10]**	**< 1 year[10]**
	< 32 GA	32-36 GA	day1	< 10days	10-365 days
Number infants	10	10	10	5	7
T ½ (min)	290	265	172	210	126
Relative V_d (l/kg)	0.66	0.56	0.61	0.7	0.9

Preterm infants

Mean T ½ (< 37 GA) (n = 20) and CL after single dose administration were 277 minutes and 0,116 l/kg/h in preterm infants while mean relative V_d was 0.61 l/kg in this study. In < 32 weeks GA infants, mean T ½ was 290 (SD 161), while in more mature infants (32-36 weeks GA) mean T ½ was 265 (SD 121). Data on pharmacokinetics of APAP in preterm neonates are only available after rectal administration. Van Lingen studied pharmacokinetics after rectal administration of APAP in 28 preterm neonates in the first day of life (28-36 weeks GA).[11] T ½ in the 28-32 weeks GA group was 660 (SD 342) minutes and was 450 (SD 240) minutes in 32-36 weeks GA group. Mean maximal concentration was 12.5 and 7.5 mg/l and mean time to reach maximal concentration was 234 and 306 minutes (28-32 and 32-36 weeks).[11] Lin documented a maximal concentration of 8,38 (SD 3,9) mg/l and a time to reach maximal concentration of 78 (SD 40) minutes after rectal administration of 20 mg/kg in 5 preterm neonates.[12]

These findings (T max and C max) are formulation specific but might be relevant in clinical care since therapeutic drug concentration after intravenous administration will be reached sooner.

Combining pharmacokinetic data in term and preterm neonates in our population with the findings of Autret in neonates and infants, a maturational trend during the first year of life is documented (Table 3). This is in line with the developmental pharmacokinetics described after oral or rectal administration of APAP.[7,9,10]

Although we could document a maturational trend in the pharmacokinetics of APAP after intravenous administration, overall correlation (r = -0.46) between GA and T ½ is still weak. In contrast to rectal and oral administration, differences in bio-availability (venous rectal drainage, gastro-intestinal motility) can not explain this variability. Further study is needed to document other variables potentially involved in this variability. Prenatal administration of betamethasone for lung maturation had no maturational effect on T ½ in this study. We could not document any gender-related differences in this study, in contrast to the findings reported in infants after rectal administration.[11]

Pharmacodynamic data suggest an analgesic effect of intravenous APAP in this population. The design (not blinded, other analgesics accepted) of this study does not allow to draw conclusions other than that multiple dose administration of intravenous APAP should be adjusted for GA. Based on the longer T ½ in preterm infants, either the interval should be longer or the dose should be lower, in line with reported regimens on rectal or oral administration.[7,9,11]

Based on the major interindividual variability of PK in preterm infants, we believe it is too early to make any multiple dose recommendations in preterm infants. In term infants, a loading dose of 30 mg/kg propacetamol (i.e. 15 mg APAP), followed by 20 mg/kg every 6 hours might be considered. Since accumulation might still occur in the individual neonate, it is safe and feasible to determine plasma concentrations until additional data are available.

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Chapter 2.4

Diclofenac and Metabolite Pharmacokinetics in Children

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Submitted

Abstract

Background
Data concerning metabolism of diclofenac in children are limited to intravenous and enteric coated oral formulations. There are no data examining diclofenac or its hydroxyl metabolite pharmacokinetics after rectal administration in children.

Methods
Infants (n = 26) undergoing tonsillectomy were given diclofenac 2 mg/kg followed by 1 mg/kg 8-hourly as suppository formulation for postoperative analgesia. Serum was assayed for diclofenac, 4'-hyroxy-diclofenac (D4OH) and 5'-hydroxy-diclofenac (D5OH) concentrations during the procedure and 1, 2 and 3 h postoperatively. The formation clearances of diclofenac to hydroxyl metabolites were estimated using non-linear mixed effects models. A single compartment, first order absorption and first order elimination model was used to describe diclofenac pharmacokinetics.

Results
Mean (SD) age and weight of the patients were 4.5 (1.5) years and 20.5 (4.1) kg. The formation clearance to D4OH (% CV) and to D5OH were 8.41 (8.1) and 3.41 (113) l/h respectively, standardized to a 70 kg person using allometric '¼ power' models. Clearance by other routes contributed 33.0 (64) l/h/70kg. Elimination clearance of hydroxyl metabolites was fixed at 27.5 l/h/70kg. The volumes of distribution of parent diclofenac and its hydroxyl metabolite were 22.8 (19.0) and 45.3 l/70kg. The suppository formulation had an absorption half-life of 0.613 (33.2) h with a lag time of 0.188 (24.9) h. Inter-occasion variability of formation clearance to D4OH, diclofenac volume of distribution, absorption half-time and lag time for the suppository was 36%, 55%, 14% and 119% respectively. The relative bioavailability of the suppository compared to an enteric-coated tablet was 1.26.

Conclusion
The formation clearance of the active metabolite D4OH contributed 19% of total clearance (44.82 l/h/70kg). The rectum is a suitable route for administration of diclofenac in children 2-8 years of age and was associated with a higher relative bioavailabilty than enteric-coated tablets and an earlier maximum concentration (50 vs. 108 min). This pharmacokinetic profile renders diclofenac suppositories a suitable formulation for short duration surgery.

Introduction

Diclofenac, 2-[(2,6-dichlorophenyl) amino] benzene acetic acid, is a non-steroidal anti-inflammatory drug with an approximate relative COX-1/COX-2 specificity ratio of one.[1] Diclofenac has reversible anti-platelet effects that are attributable to the inhibition of thromboxane synthesis. It has been used after tonsillectomy[2-4] and is believed to be associated with a decreased propensity to postoperative bleeding than ketorolac.[5-10] Formulations may be administered orally, topically, intraocularly, intra-articular, intravenously, intramuscularly and rectally. Diclofenac pharmacokinetic parameters have been estimated after intravenous[11] and oral formulations[3] in children, but there are no estimates after rectal administration and metabolite data in children are scant.[12,13] Diclofenac is metabolized by P450 (CYP2C9, CYP3A4 and possibly CYP3A5) phase I hydroxylation and phase II conjugation.[14-16] The principal metabolite in humans is the 4'-hydroxyl derivative of diclofenac (D4OH), metabolized by CYP2C9. D4OH has 30% of the anti-inflammatory and antipyretic activity of diclofenac in animal models.[17]

We had the opportunity to examine diclofenac and metabolite serum concentrations in children 2-8 years old given diclofenac for management of tonsillectomy pain. Data from these patients were combined with those from a published study of children given diclofenac enteric-coated tablets after tonsillectomy.[3] These pooled data were investigated using a

population-based approach that included size as the primary covariate. A concentration-response relationship for diclofenac has not been described and this current study investigates pharmacokinetics only.

Methods

Patients and methods

The study was approved by the Medical Ethical Committee of the Erasmus MC, Rotterdam and written informed consent was obtained from parents. Children (ASA 1 and 2, 2-8 years) undergoing elective tonsillectomy with or without adenoidectomy were enrolled into the study. Exclusion criteria were a known coagulation defects, diclofenac administration within 24 hours of surgery, diclofenac allergy, hepatic disease or abnormal renal function.

Children were given diclofenac suppository 2 mg/kg 30 minutes before scheduled surgery. Blood samples (1 ml) for diclofenac, D4OH and 5'-hydroxydiclofenac (D5OH) concentration assays were obtained through a peripheral venous catheter directly after induction, at the end of the procedure and 1, 2 and 3 hours after awakening.

Suppository formulation

The diclofenac sodium suppositories (with witepsol H15, synthetic saturated triglycerides with a chain length of C12-C18, as base) were manufactured in the hospital pharmacy. The diclofenac dose in the suppository never deviated more than 10%. The ingredients were obtained through regular commercial suppliers. Suppositories and ingredients conformed to the quality requirements in the European Pharmacopoeia.

Diclofenac assay

Diclofenac sodium was purchased from Sigma Aldrich (Saint Quentin Falavier, France), D4OH and D5OH were obtained from Novartis (Novartis International AG, Basel, Switzerland). All the solvents used were analytical grade.

The HPLC system consisted of a P quaternary P 1000 XR pump (ThermoQuest- TQ, Florida, USA), a TQ autosampler, a TQ UV 6000 detector (280 nm) linked to TQ Spectranet for recording and storing throughout the analysis. A LC_8 5µm particle size Supelcosil column (150 x 4.6 mm, Supelco Bellafonte, USA) was used. The mobile phase was a mixture of acetonitrile /sodium acetate 50 mM (70/30, v/v) adjusted to pH 5 by phosphoric acid and the flow rate 1.2 ml/min.
Stock solutions of diclofenac (1 mg/ml), 4- and 5-hydroxymetabolites (500 µg/ml) were prepared in methanol and stored at –20°C. Calibration standards (10-1000 ng/ml) and plasma controls (40, 200, 750 ng/ml) were prepared by appropriate dilutions of the stock solutions in drug-free plasma. Briefly, 500 µl of plasma sample, 100 µl of internal standard and 1500 µl H3PO4 (1M) were extracted in 8 ml of diethylether. After centrifugation, the organic layer was evaporated to dryness at 40°C under nitrogen. The residue is dissolved in a mixture of 50 mM sodium acetate and-methanol (v/v 50/50).

Under the chromatographic conditions used, the retention times were 6.1, 6.9, and 18.5 min for D5OH, D4OH, and diclofenac respectively. Recovery from extraction was over 90% for the three compounds. Calibration curves were linear over the range of 10 to 1000 ng/ml and coefficients of variation of the slope were between 2.8 and 5.8% (n = 5). The limit of quantification was 5 ng/ml for the three compounds. The intra and inter-assay coefficients of variation, determined from three quality controls (40, 200 and 750 ng/ml) were lower than 7%.

Modeling

Population parameter estimates
Diclofenac metabolite data were converted to diclofenac mg equivalents using a molecular weight of 318 for diclofenac sodium and 312.7 for the D4OH and D5OH. Population parameter estimates were obtained using a non-linear mixed effects model. This model accounts for random between subject parameter variability and residual variability (random effects) as

well as between subject parameter differences predicted by covariates (fixed effects). The population parameter variability in model parameters was modeled by an exponential variance model. The covariance between clearance, distribution volume, lag time and absorption half-life was incorporated into the model. An additive and a proportional term characterized the residual unknown variability for diclofenac and the hydroxyl metabolite concentrations. Estimation used the first order conditional estimate method with the interaction option and ADVAN 6 with Tol = 5. Convergence criterion was 3 significant digits. A Compaq Digital Fortran Version 6.6A compiler with Intel Celeron 333 MHz CPU (Intel Corp., Santa Clara, CA) under MS Windows XP (Microsoft Corp., Seattle, WA) was used to compile NONMEM.
Differential equations were used to describe the pharmacokinetics of diclofenac and its metabolites.

$$
\begin{aligned}
CLT &= CL2D4OH + CL2D5OH + CLEX \\
dCS/dt &= (Ka \times dose - CS \times CLT) / V \\
dD4OH/dt &= (CL2D4OH \times CS - CLDOH \times CD4OH) / VOH \\
dD5OH/dt &= (CL2D5OH \times CS - CLDOH \times CD5OH) / VOH
\end{aligned}
$$

This model is shown in Figure 1. CLT is total diclofenac clearance, V is the volume of distribution for diclofenac, CS is diclofenac serum concentration, CD4OH is D4OH concentration, CD5OH is D5OH concentration, CL2D4OH is formation clearance to D4OH, CL2D5OH is formation clearance to D5OH, CLDOH is the elimination clearance of the hydroxyl metabolite, VOH is the volume of distribution of the hydroxyl metabolites, CLEX is diclofenac clearance by other routes, Ka is the absorption rate constant (= Ln(2)/Tabs), Tabs is the absorption half-life.

The elimination clearance of the hydroxyl metabolites (CLDOH) can not be identified with the current study design, but a value of 27.5 l/h in adults has been reported by Landsorp et al.[18]

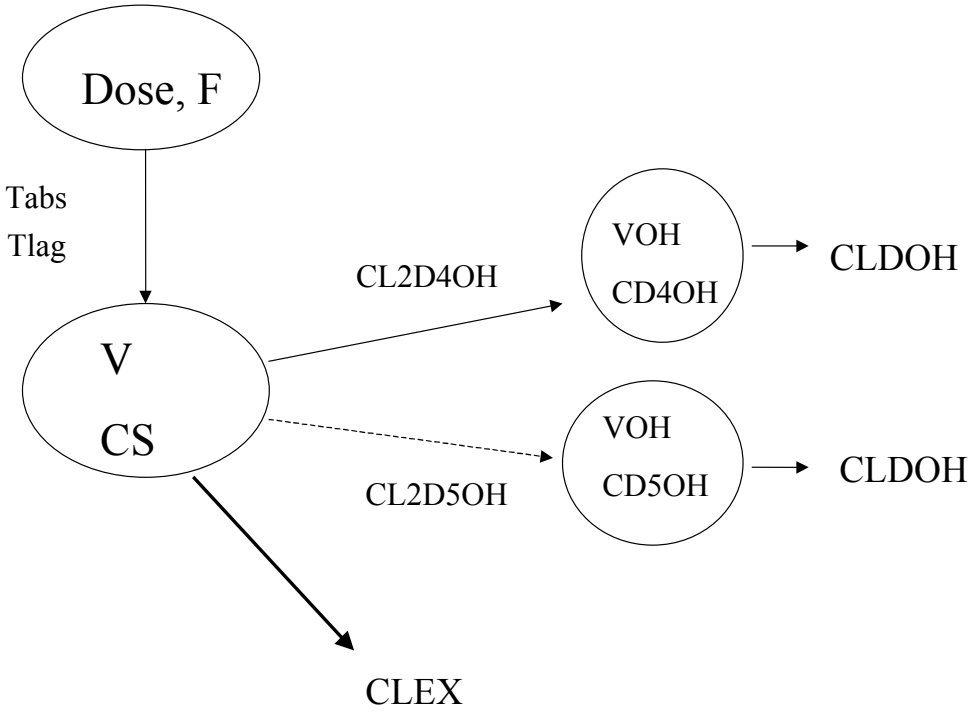

Figure 1 *V = volume of distribution for diclofenac, VOH = volume of distribution for hydroxyl metabolites, CS = diclofenac serum concentration, CL2D4OH = formation clearance to D4OH, CL2D5OH = formation clearance to D5OH, CLDOH = elimination clearance of hydroxyl metabolites, CD4OH = D4OH serum concentration, CD5OH = D5OH serum concentration CLEX = clearance attributable to other pathways, Tabs = absorption half-life (Ln(2)/Ka), Tlag = Lag time*

Diclofenac was administered as an extravascular dose and both clearance and distribution volumes are confounded by bioavailability. Frectal is used to refer to the relative bioavailability of the suppository compared to the oral formulation.

The quality of fit of the pharmacokinetic model to the data was assessed by visual examination of plots of observed versus predicted concentrations. Models were nested and an improvement in the objective function was referred to the Chi-squared distribution to assess significance e.g. an objective function change (ΔOBJ) of 3.84 is significant at $\alpha = 0.05$.

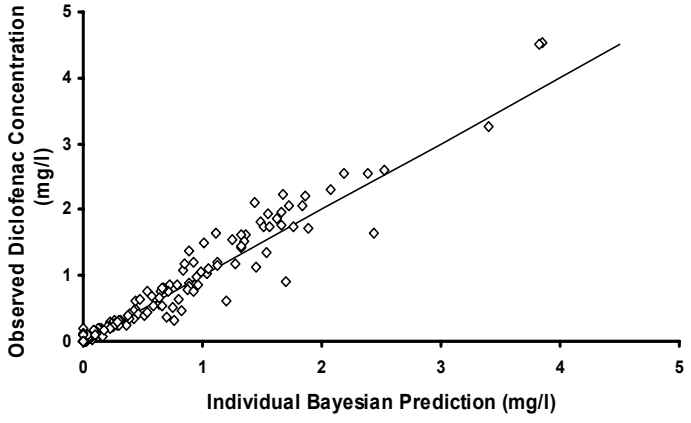

Figure 2a

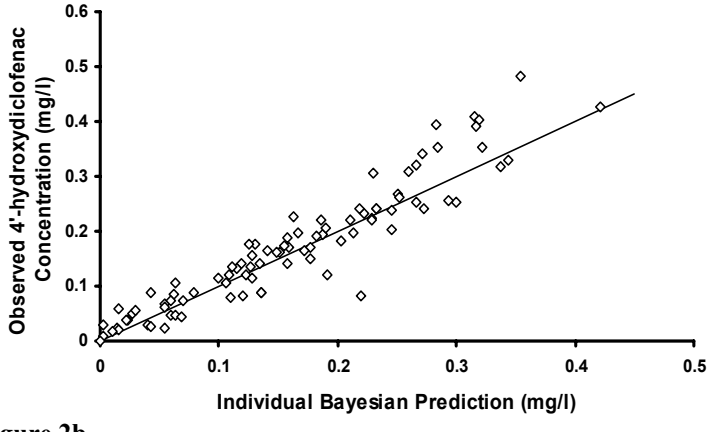

Figure 2b

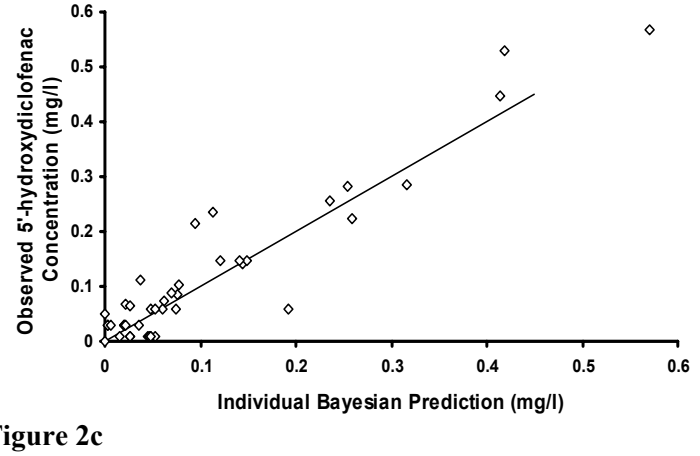

Figure 2c

Figure 2 *Quality of fit of pharmacokinetic data. Individual Bayesian concentration predictions based on values of the parameters for the specific individual are compared to observed. The line x = y is the line of identity. 2a) serum diclofenac concentration data 2b) serum D4OH data 2c) serum D5OH data.*

Covariate Analysis

The parameter values were standardized for a body weight of 70-kg using an allometric model[19]

$$P_i = P_{std} \, x \, (W_i / W_{std})^{PWR}$$

where P_i is the parameter in the ith individual, W_i is the weight in the ith individual and P_{std} is the parameter in an individual with a weight W_{std} of 70 kg. The *PWR* exponent was 0.75 for clearance and 1 for distribution volumes.[20-23]

Inter-occasion variability

The between subject variability in NONMEM is modeled in terms of ETA (η) variables. Each of these variables is assumed to have mean 0 and a variance denoted by ω^2, which is estimated. A covariance between two elements in η (e.g. CL and V) is a measure of statistical association between these two variables. Their covariance is related to their correlation (*R*) i.e.

$$R = covariance/\sqrt{(\omega_{CL} \, x \, \omega_V)}$$

Between occasion variability for the structural parameters of clearance, apparent volume, rectal absorption half-life and lag time were added to the model because some children were given diclofenac on two different occasions. The between occasion covariance for clearance of D4OH, volume, absorption and lag time was also estimated. This covariance is effected by factors that affect these parameters together (e.g. protein binding, total body water), but variability of Frectal is the major contributor to this estimate.

Determining relative rectal bioavailability

In order to gain a better understanding of the suppository bioavailability relative to oral (Frectal), time-concentration profiles from 11 children (mean 11 y, range 5-15 y; mean 50 kg, range 22-67 kg) given enteric-coated

diclofenac tablets 25 or 50 mg (Diclon®, DuraScan Medical Products AS, Odense S, Denmark) after tonsillectomy from a published study were included in the analysis.[3] These children were given diclofenac 1-2 mg/kg the morning after surgery. Blood samples for diclofenac assays were drawn at 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 h after administration. Blood was centrifuged and serum stored at –20^{o}C until assay.

Diclofenac concentrations in that study were measured using HPLC. The calibration curve was linear in the range up to 6 mg/l, with a minimal quantifiable concentration (MQC) of 0.06 mg/l. Intra-assay variations were 2.9% for concentration of 0.6 mg/l and 1.8% for a concentration of 4.5 mg/l. The inter-assay variation was 4.6% at both these concentrations.
A first order process was initially used to estimate enteric-coated diclofenac absorption. However, a zero order model proved superior (ΔOBJ 88.535) and the final model estimated a zero order absorption time (TK0) rather than an absorption half-life (Tabs). The variability between this published study by Rømsing et al[3] and the current study was accounted for by giving each study separate residual errors.

Results

Population Demographics
Mean (SD) age and weight of the patients were 4.5 (1.5) years and 20.5 (4.1) kg. There were 14 boys and 12 girls.

Parameter estimates
The total analysis used 314 concentration observations from 37 subjects. Parameter estimates, standardized to a 70-kg person are shown in Table 1. The covariance of the pharmacokinetic parameters, expressed as the correlation of population parameter variability was low, except that between clearance and absorption (Table 2). Figure 2a, 2b & 2c show pharmacokinetic data analysis fits.

Table 1 Pharmacokinetic parameter estimates.
These estimates are standardized to a 70-kg person using an allometric size model (%CV is the coefficient of variation for the population parameter estimate, except for V, CL2D4OH, Tabs and Tlag where between subject (BSV) and between occasion variability (BOV) were estimated.

Parameter	Estimate	CV %
CLT	44.82 l/h/70kg	
V	22.8 l/70kg	BSV 19.0
		BOV 54.9
CL2D4OH	8.41 l/h/70kg	BSV 8.1
		BOV 35.8
CL2D5OH	3.41 l/h/70kg	113.1
CLEX	33.0 l/h/70kg	64.0
CLDOH	27.5 l/h/70kg FIXED	
Tabs rectal	0.613 h	BSV 33.2
		BOV 13.9
Tlag rectal	0.188 h	BSV 24.9
		BOV 119.2
TK0 oral	0.563 h	-
Tlag oral	1.5 h	24.9
F rectal	1.26	-

CLT = population estimate for CL/Foral (clearance after oral administration L/h/70kg), V = volume of distribution for diclofenac and hydroxy metabolites, CL2D4OH = formation clearance to D4OH, CL2D5OH = formation clearance to D5OH, CLDOH = elimination clearance of hydroxy metabolites, CLEX = clearance attributable to other pathways, Tabs = absorption half-life (Ln(2)/Ka), TK0 = zero order infusion time, Tlag = Lag time, Frectal is the relative bioavailability of the rectal compared to the oral formulation.

Table 2. The covariance of the pharmacokinetic parameters, expressed as the correlation of population parameter variability

	CL2D4OH	Tabs	Tlag	V
CL2D4OH	1			
Tabs	0.875	1		
Tlag	-0.014	0.021	1	
V	-0.054	-0.149	-0.50	1

The formation clearance to D4OH (CL2D4OH) and to D5OH (CL2D5OH) were 8.41 (8.1) and 3.41 (CV, 113%) l/h respectively, standardized to a 70 kg person using allometric '¼ power' models. Clearance by other routes (CLEX) contributed 33.0 (64) l/h/70kg. Elimination clearance of hydroxyl metabolites (CLDOH) was fixed at 27.5 l/h/70kg. The volumes of distribution of parent diclofenac (V) and its hydroxyl metabolite (VOH) were 22.8 (19.0) and 45.3 l/70kg. The suppository formulation had an absorption half-life (Tabs) of 0.613 (33.2) h with a lag time (Tlag) of 0.188 (24.9) h. Enteric-coated tablets had a TK0 of 0.563 and a Tlag of 1.5 (24.9). Inter-occasion variabilities of formation clearance to D4OH, diclofenac volume of distribution, absorption half-time and lag time for the suppository were 36%, 55%, 14% and 119% respectively (Table 1). The ω^2 estimates for the different components contributing to variability are shown in Table 3. The relative bioavailability of the suppository compared to an enteric-coated tablet was 1.26. Figure 3 shows typical time-concentration profiles for diclofenac and the hydroxyl metabolites for an individual.

Residual errors for diclofenac concentrations were similar for both data sets. The current data additive and proportional errors were 0.009 mg/l and 0.349%, while those from Rømsing et al's data were 0.053 mg/l and 0.204%. The additive and proportional errors for 4'-hyroxydiclofenac were 0.023 mg/l and 0.273%. An additive error for 5'-hyroxydiclofenac was 0.063 mg/l.

Table 3. The between-occasion covariance for clearance of D4OH, volume, absorption and lag time.

	CL2D4OH	Tabs	Tlag	V
CL2D4OH	1			
Tabs	0.999	1		
Tlag	0.122	0.071	1	
V	-0.110	-0.124	-0.011	1

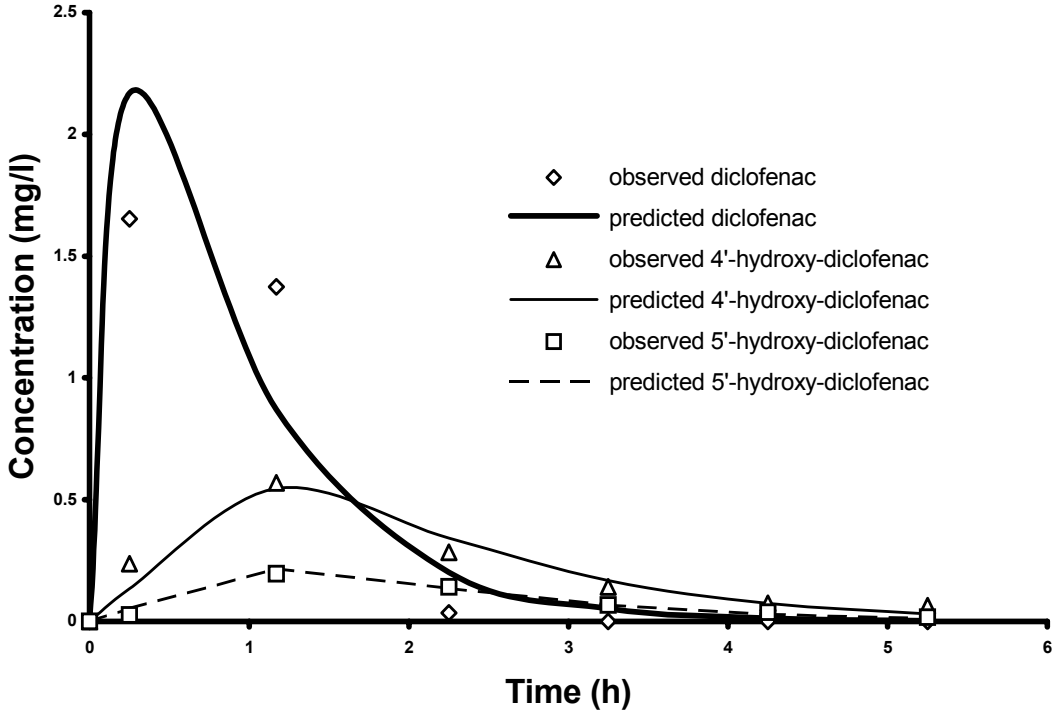

Figure 3 *Diclofenac and hydroxyl metabolite time-concentration profiles for a 4 year old, 19 kg boy given diclofenac suppository 37.5 mg.*

Discussion

We report a clearance, standardized to a 70-kg person using allometric size models, of 44.82 l/h/70kg and volume of distribution of 22.8 l/70kg in children given oral diclofenac tablets. Allometric size models[23] are based on fractal geometry[21,22] and have been used to estimate pediatric pharmacokinetics of other NSAIDs e.g. ibuprofen in children 4-16 year.[24] These models disentangle age-related factors from those related to size. The clearance of ketorolac in children, for example, is reported to be similar to adult values when data from different age groups are pooled.[25] However, a child 1-3 y has a higher clearance than a child 12-16 y and this difference is attributable to size (Table 4a). Similar age-related clearance changes (l/h/kg), predicted using the current data for diclofenac are shown in Table 4b.

Rømsing et al[3] reported a standardized clearance of 48.5 l/h/70kg (assuming a mean weight of 50 kg). A clearance of 0.462 (SD 90) l/kg/h, however,

was reported in children 4.3-6.8 years given 0.5 mg/kg of intravenous diclofenac using a standard two-stage population approach.[11] This standardized clearance of 23.6 l/h/70kg (assuming a mean weight of 20 kg) is less to our current estimate. The standard two-stage approach used in these earlier studies is incapable of investigating covariates such as weight, does not account for the imprecision of individual estimates and may require weighting if estimates are skewed. It is also possible to have strong correlation between parameter variability, so that both clearance and volume estimates are affected by each other.

The volume of distribution of diclofenac in adults is 7-14 l/70kg.[12] This small volume is due to high plasma protein binding (>99.7%) and minimal tissue binding. Although reduced protein binding has been reported for some NSAIDs in children,[26] the magnitude of protein binding differences is small and is unable to explain reported volumes of distribution of 50-63 l/70kg for diclofenac in children.[3,11] Our current estimate of 22.8 in children 2-15 y is reasonable.

Table 4a Ketorolac age related pharmacokinetic changes.
Data from Dsida et al. Anesth Analg 2002;94: 266-70 (n = 36)[25]

Age years	Weight kg	Vss (S.D.) l/kg	CL (S.D.) l/min/kg	CLstd (S.D.) l/min/70kg
1-3	12	0.111 (0.0 25)	0.6 (0.2)	27.0 (9.0)
4-7	20	0.128 (0.047)	0.61 (0.22)	31.2 (11.3)
8-12	30	0.099 (0.014)	0.54 (0.15)	30.6 (8.5)
12-16	50	0.116 (0.040)	0.51 (0.12)	32.8 (7.7)
Adult[38]	70	0.11	0.3-0.55	21-38.5

CLstd is total body clearance standardized to a 70 kg person using an allometric ¾ power model. Weight is estimated

Table 4b Predicted diclofenac age related pharmacokinetic changes
(based on current study)

Age years	Weight kg	CL l/h/kg	CLstd l/h/70kg
1-3	12	1.00	44.82
4-7	20	0.88	44.82
8-12	30	0.79	44.82
12-16	50	0.70	44.82

Suppositories have a reduced relative bioavailability compared to the oral formulations for most drugs.[27,28] attributable to erratic absorption and loss through the anal sphincter. Diclofenac suppository in this study had a relative bioavailability of 1.26. Diclofenac is rapidly and well absorbed by the intestinal tract from suppositories[18] and we report an absorption half-life of 0.613 with a lag time of 0.188 h. The relative bioavailability from the colon in adults was 0.78 (range 0.54-1.09).[29] The absolute bioavailability is 90% (SD 11.6%) after oral administration in adults[30,31] but there are reports that diclofenac may undergo first pass metabolism with 60% of the drug reaching the systemic circulation as intact diclofenac.[32,33] The rectal route may be better than oral for drugs destroyed by gastric acidity or by enzymes in the intestinal wall and microflora. Venous drainage from the rectum through the inferior and middle haemorrhoidal veins may bypass the hepatic portal circulation and result in a rectal relative bioavailabilty (Frectal) greater than 1. Other NSAIDs have also reported high relative rectal bioavailability. The relative bioavailability of suppository compared to oral flurbiprofen was 0.998 in children 6-12 years of age.[34]

Diclofenac metabolites in man have been described in urine and plasma after single dose administration.[16,18,35]. These comprise of D4OH, D5OH, 3'-hydroxy, 4',5'-hydroxy and 3'-hydroxy-4'-hydroxy-methoxy diclofenac in adult volunteers. The amount of D4OH excreted in urine accounts for 30% of the dose and 10-20% of the dose in bile in adults.[14,15,30] The metabolite proportions are unknown in children and we were unable to estimate these from the current study design. Landsdorp et al[18] estimate an apparent total body clearance for D4OH of 27.5 (SD 10.9) l/h in adult volunteers and we assumed this value for the current study. The volume of distribution of this metabolite is unknown. We estimate an hydroxyl metabolite volume of 45.3 l/70kg. The greater volume of the hydroxyl metabolites, compared to the parent diclofenac, could be due to decreased protein binding, but this is currently unknown. D4OH contained in serum samples may be further oxidized to 4',5-dihydroxydiclofenac, increasing the error of the estimate.[18]

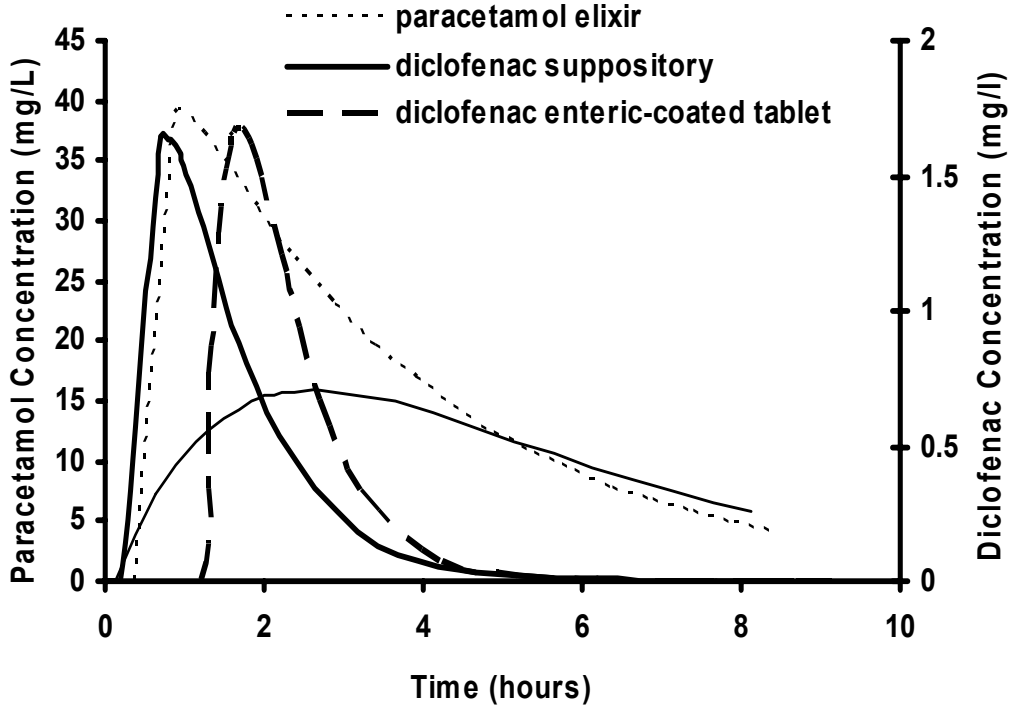

Figure 4 *Simulated time-concentration profiles for a typical 20-kg individual given diclofenac suppository 2mg/kg, diclofenac enteric-coated tablet 2 mg/kg, APAP suppository 40 mg/kg and APAP elixir 40 mg/kg. APAP parameter estimates are from Anderson et al.[39] Diclofenac parameter estimates are from current study.*

The analysis of the 5'-hydroxyl metabolite was less satisfying. Most serum sample concentrations were below the MQC (0.05 mg/l) and only three children had complete time-concentration profiles above MQC. Consequently we have less confidence in the elimination clearance of this metabolite.

Figure 4 shows simulated time-concentration profiles for a diclofenac suppository and enteric-coated tablet in a 20-kg child compared to profiles for paracetamol (APAP) suppository and elixir. Peak concentrations are reached earlier after diclofenac suppository than APAP elixir. Diclofenac absorption is rapid by the rectal route. The effect compartment equilibration half-time (Teq) of diclofenac analgesia is unknown, but that for analgesia in adults with bone pain given ketorolac (25 min)[36] is less than that for children suffering tonsillectomy given APAP (52 min).[37] We might predict that the Teq for diclofenac analgesia is also less than that for APAP

analgesia and that the diclofenac suppository is a suitable formulation for short duration surgery because effect onset is quicker than APAP.

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3

Pharmacodynamic studies

Chapter 3.1

Analgesic efficacy of rectal versus oral paracetamol in children after major craniofacial surgery

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Abstract

Background

Analgesic paracetamol (APAP) plasma concentrations after major surgery in neonates and infants have not yet been established in the literature. We therefore conducted a study in our intensive care unit.

Methods

Forty children, mean (SD) age, 10.3 (2.3) months, received 20 mg/kg APAP either orally (n = 20) or rectally (n = 20) 6-hourly after a rectal loading dose (40 mg/kg) during elective craniofacial correction. Blood samples were taken 1 hour before and 2 hours after administration of APAP maintenance doses; pain scores were obtained every 3 hours.

Results

APAP plasma concentrations were higher in patients receiving rectal APAP (mean area under the concentration-time curve [AUC], 171.2 mg.h/l) than in patients receiving oral APAP (mean AUC, 111.9 mg.h/l). Pain scores were higher in patients receiving oral APAP. However, after exclusion of the patients who vomited from the patients receiving oral APAP, APAP plasma concentrations and pain scores did not differ between the groups. There was no relation between APAP plasma concentrations and pain scores. Although 9 of all 40 patients (22.5%) did not reach the expected analgesic APAP plasma concentrations of 10 to 20 mg/l, < 7.5% of the VAS pain scores exceeded 4 cm, which was considered as a cut off point.

Conclusion

These are the first data showing that the analgesic APAP plasma concentration after major surgery in this age group, does not always reach the 10 to 20 mg/l level. These data also show that, after a rectal loading dose of 40 mg/kg has been given during surgery, the best way of administering APAP after craniofacial surgery is the rectal route. (Clin Pharmacol Ther 2001;70:82-90.)

Introduction

Paracetamol (APAP) is widely used for postoperative analgesia in patients who experience moderate pain. Dose-effect relationships,[1] dose-concentration relationships,[2] and effects of APAP on body temperature[3] in children have been reported in the literature. Findings in newborns receiving APAP after vacuum extraction have been published recently by our group.[4] However, there are no data on the analgesic APAP plasma concentrations in neonates and infants after major surgery.

In children aged from 2 to 15 years undergoing tonsillectomy, an APAP plasma concentration of 10 mg/l is considered necessary to achieve satisfactory pain relief.[5] The optimal plasma concentration to obtain analgesia is usually considered to be 10 to 20 mg/l,[6,7] a level that is extrapolated from findings in adults.

Because the absorption rate and bioavailability of orally administered APAP differ from those in rectally administered APAP [6,8,9] and oral medication may cause nausea and vomiting, it is important to obtain information about both routes of administration.

The delayed and erratic absorption after rectal administration leads to unpredictable APAP plasma concentrations and does not consistently produce rapid onset of analgesia.[10] An APAP rectal loading dose of at least 30 to 40 mg/kg is therefore recommended to achieve satisfactory pain relief.[11] The advised maximum daily dose of APAP is 90 to 100 mg/kg, which then allows for 3 doses of 20 mg/kg after an initial loading dose of 40 mg/kg.
In a randomized controlled trial we compared the efficacy of both oral and rectal APAP administration in young children undergoing major craniofacial surgery by comparing APAP plasma concentrations with changes in scores for two validated pain measurement instruments for this age group.[12]

We primarily aimed at determining the differences in APAP plasma concentrations and effects between children receiving either multiple doses of APAP rectally or equal doses of oral APAP after an initial rectal loading dose. The secondary aim of the study was to determine a dose-plasma concentration and a plasma concentration-effect relation of both orally and rectally administered APAP in children aged 3 months to 3 years after elective major craniofacial surgery.

Methods

Patients and methods

The Sophia Children's Hospital serves as a level III referral centre for all pediatric surgical subspecialties. As such, it is the only designated pediatric craniofacial center in the Netherlands. Approximately 100 major craniofacial corrections are performed annually.

After approval of the study by the Medical Ethical Committee of the University Hospital Rotterdam and after written informed consent was obtained from the parents, 45 children were enrolled consecutively during the period from March 1999 through March 2000. Inclusion criteria were age between 3 months and 3 years and elective craniofacial correction for different forms of craniosynostosis. Exclusion criteria were craniotomy of tumors, hydrocephalus, or trauma, pre-existent liver or kidney disorders as reflected by abnormal values of liver enzymes, bilirubine, urea, and creatinin, severe mental retardation, Glasgow Coma Score < 8, postoperative mechanical ventilation, and known allergy for APAP.

Participant flow and follow-up

The eligible number of patients was 54. Because the parents of 9 children did not give informed consent, 45 of them were included in this study. Five of the 45 included patients were later excluded from analysis for various reasons as follows: logistic problems with the delivery of the study medication (2 patients), returning from the operating room with mechanical

ventilation (1 patient), and withdrawal of the parental informed consent during the study (2 patients). These 5 patients were found not to differ from the 40 included patients with regards to operation procedure, blood loss, and pain scores.
Hence the data of 40 patients were analyzed for this study, 20 receiving oral APAP and 20 receiving rectal APAP.

Procedure
By protocol, anesthesia was induced with the patients under either sevoflurane or intravenous thiopental. After administration of vecuronium and 3 μg/kg fentanyl intravenously, the patients were intubated and ventilated with air, oxygen, and isoflurane. An arterial catheter, central venous line, nasogastric tube, and urinary catheter were inserted while the patients were under anesthesia. The scalp was infiltrated with bupivacaine 0.25% and epinephrine 1:200,000 up to a maximum of 1 ml/kg. Before the skin incision was made, fentanyl 15 to 25 μg/ kg was administered intravenously. Approximately 2 hours before anticipated extubation, a loading dose of APAP (40 mg/kg) was administered rectally. Usually at this time major blood loss had been adequately compensated and the patient was hemodynamically stable. After the operation patients were admitted to the Pediatric Surgical Intensive Care Unit (PSICU) for a minimum of 24 hours, depending on the clinical condition.

Two hours (t = 2) after arrival in the PSICU, patients underwent a gastric lavage until the gastric fluid was clear on aspiration. The 20-mg/kg APAP maintenance doses were given at t = 6, t = 12 and t = 18 hours. Oral medication was given via the nasogastric tube. If the nasogastric tube had been accidentally removed, the medication was given by mouth.

Blood samples were taken from the arterial catheter at 30, 60 and 90 minutes after the rectal loading dose of APAP and at t = 5, t = 8, t = 11, t = 14, t = 17, and t = 20 hours. Samples were taken 1 hour before (minimum APAP plasma concentration) and 2 hours after (maximum APAP plasma concentration) the administration of an APAP maintenance dose.[13]

Trained intensive care nurses obtained pain scores every 3 hours (t = 3, t = 6, t = 9, t = 12, t = 15, t = 18, t = 21 and t = 24 hours), using the visual analogue scale (VAS) and the COMFORT score.[12,14,15] From the original COMFORT score the behavioral items were summed as published recently.[12] With regard to the VAS used as an observational instrument, there is evidence that, first the interrater reliability is good between nurses or between nurses and physicians in judging postoperative pain in pediatric samples.[16-21] Second, the concurrent validity with other observational pain instruments has also been established by different authors.[12,16 22]

An extra dose of the study medication was given if the VAS score was ≥ 4 cm. Blood samples were taken just before administration of the extra dose and 2 hours thereafter. The next dose of the study medication was then administered according to the protocol schedule. APAP plasma concentrations were determined using fluorescence polarization immunoassay (Adx system; Abbott Laboratories, North Chicago Ill).[11] The study design is represented schematically in Figure 1a & 1b.

	2 h before anticipated extubation	1.5 h before anticipated extubation	1 h before anticipated extubation	0.5 h before anticipated extubation
APAP loading dose	x			
Bloodsample		x	x	x

Figure 1a

T	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Arrival in PSICU	x																								
Gastric lavage			x																						
Bloodsample						x			x			x			x			x			X				
Maintenance dose APAP							x						x						x						
Pain scores				x			x			x			x			x			x			x			x

Figure 1b

The study protocol was stopped if the parents withdrew their informed consent during the study or if the patient returned from the operating room with mechanical ventilation. In the former case all data of the patient involved were excluded from further analysis and the randomization number was not used again.

Statistical methods

The area under the concentration-time curve (AUC) of the APAP plasma concentrations and of the pain scores of patients receiving either oral APAP or rectal APAP were compared per group using the Mann-Whitney *U* test (both the pain scores and the APAP plasma concentrations had nonnormal distributions; $P = 0.05$).[23]

After logarithmic transformation of the mean APAP plasma concentrations and of the mean pain scores, multiple regression analysis was performed to establish the relation between APAP plasma concentrations and pain scores. Corrections were made for receiving oral or rectal APAP, for receiving an extra dose of APAP, and for vomiting in patients receiving oral APAP.

Assignment

The randomization schedule for oral or rectal administration had been made before the study by computer block randomization. After inclusion of a patient, the hospital's pharmacist prepared the study medication according to the randomization schedule. The schedule was kept solely by the pharmacist to ensure blinding until the end of the study.

Masking (blinding)

The study medication of patients receiving rectal APAP consisted of APAP suppositories and placebo elixir, that of patients receiving oral APAP consisted of APAP elixir and placebo suppositories. Since all children received both an elixir and a suppository and since APAP suppositories (with APAP and witepsol H15, synthetic saturated triglycerides with a chain length of carbon 12 to 18 as the basis), elixir (with APAP 24 mg/ml, glycerol 85%, sodium lactate, raspberry essence and sorbitol solution), and placebos were all manufactured in the department of the hospital pharmacy,

patients, nurses and investigators were blinded for the route of the actual APAP administration.

The composition of the placebo suppositories and placebo elixir was equal, except for the APAP. The APAP doses in the suppositories never deviated more than 10% (18-20 mg/kg). APAP was supplied by Bufa bv (Uitgeest, the Netherlands). The suppositories, the elixir, and all ingredients met the requirements in the European Pharmacopoeia. APAP suppositories and elixir were manufactured according to the Dutch Pharmacists Formulary. Stability of these preparations is tested by the laboratory of the Royal Dutch Association of Pharmacists.

Results

Analysis

The mean (standard deviation [SD]) age of the patients receiving oral APAP was 10.0 (2.7) months, and their mean (SD) weight was 9.2 (1.1) kg. Patients receiving rectal APAP had a mean (SD) age of 10.6 (1.9) months and a mean (SD) weight of 9.4 (1.5) kg.

There were no significant differences between patients receiving oral APAP and patients receiving rectal APAP with respect to age, weight, gender, baseline heart rate, baseline mean arterial pressure, duration of the operation, amount of blood loss during the operation, and the operative procedures (Table 1).

Confounders

In both groups 3 patients required an extra dose of APAP (rectal APAP: t = 5, t = 14, and t = 4 hours; oral APAP: t = 3, t = 4 and t = 9 hours).

Eleven patients receiving rectal APAP and 12 patients receiving oral APAP vomited once or twice. Of the latter, 5 required metoclopramide and 1 required domperidon; of the former, 3 required metoclopramide and 2 required ondansetron.

Because of restlessness or presumed fear, midazolam was given to 3 patients receiving oral APAP and to 2 patients receiving rectal APAP.

Table 1 Characteristics of patients

	Rectal group (n = 20)	*Oral group (n = 20)*
Age (mo)		
Mean (SD)	10.6 (1.9)	10.0 (2.7)
Range	8-15	6-20
Weight (kg)		
Mean (SD)	9.4 (1.6)	9.2 (1.1)
Range	5.4-12.2	6.7-11.0
Sex		
Male	15	14
Female	5	6
Condition requiring operative correction		
Scaphocephaly	10	10
Trigonocephaly	6	1
Plagiocephaly	2	7
Brachycephaly	2	2
Duration of operation(min)		
Mean (SD)	221 (40)	214 (26)
Range	175-315	170-270
Amount of blood loss (ml)		
Mean (SD)	983 (560)	741 (448)
Range	400-2800	300-2000
Baseline heart rate (beats/min)		
Mean (SD)	141 (26)	148 (23)
Range	75-195	105-195
Baseline mean arterial pressure (mm Hg)		
Mean (SD)	78 (15)	73 (12)
Range	53-106	48-93

APAP plasma levels

The rectal loading dose of 40 mg/kg resulted in widely varying APAP plasma concentrations (mean values [SD] at 30, 60 and 90 minutes after the rectal loading dose: 2.0 [2.8] mg/l, 4.2 [5.4] mg/l, and 6.3 [7.4] mg/l respectively) (Figure 2a). There was no difference between the rectal and the oral group in mean APAP plasma concentrations after the rectal loading dose. The AUC of the APAP plasma concentrations (t = 5 to t = 20 hours) was significantly higher in patients receiving rectal APAP (mean AUC, 171.2 mg.h/l) than in the patients receiving oral APAP (mean AUC, 111.9 mg.h/l) ($P = 0.004$) (individual values of APAP plasma concentrations are depicted in Figure 2b). After exclusion of those patients who vomited in the oral group, there was no longer a significant difference anymore between the AUCs in the two groups (mean AUC rectal group: 171.2 mg.h/l and

mean AUC oral group after exclusion of patients who vomited: 144.3 mg.h/l) ($P = 0.3$).

The APAP plasma concentrations at the time of the extra APAP administration were 5.5, 13.5 and 0.9 mg/l in the patients receiving rectal APAP and 15.8, 7.5 and 4.9 mg/l in the patients receiving oral APAP.

In all patients, both in the oral and in the rectal group, APAP plasma concentrations ≥ 5 mg/l were reached at least at one sampling point. Among all patients 22.5% showed APAP plasma concentrations of 5 to 10 mg/l (oral group, 35%, rectal group 10%). APAP plasma concentrations of 10 to 15 mg/l were reached by 27.5% of all patients (oral group 20%; rectal group, 35%); 20% reached APAP plasma concentrations of 15 to 20 mg/l (both in the oral and in the rectal group, 20%); APAP plasma concentrations ≥ 20 mg/l were reached in 30% of all patients (oral group, 25%, rectal group, 35%).

Pain scores

The AUC of the COMFORT scores (mean AUC rectal group: 265.4; mean AUC oral group: 286.2) and the AUC of the VAS scores (mean AUC rectal group: 16.1 cm.h; mean AUC oral group: 22.5 cm.h) were significantly higher in patients receiving oral APAP ($P = 0.02$ and $P = 0.04$ respectively) (individual VAS and COMFORT scores and means are depicted in Figure 3a & 3b). However, there was no significant difference after exclusion of the patients who vomited in the oral group (mean AUC COMFORT scores and mean AUC VAS scores oral group after exclusion of the patients who vomited: 279.9 and 22.5 cm.h, respectively) ($P = 0.2$ and $P = 0.2$ respectively).

Multiple regression analysis with corrections for receiving oral or rectal APAP, for receiving an extra dose of APAP, and for vomiting in patients receiving oral APAP showed no significant relation between the APAP plasma concentrations and the pain scores (ie, the VAS score and the COMFORT score [$P = 0.2$ and $P = 0.2$ respectively]).

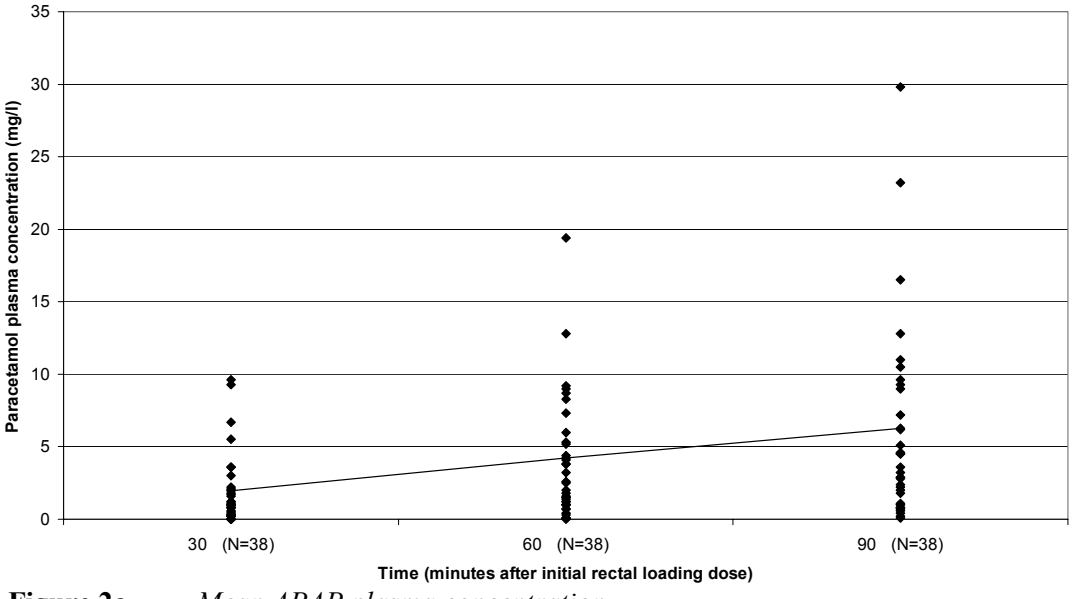

Figure 2a *Mean APAP plasma concentration*

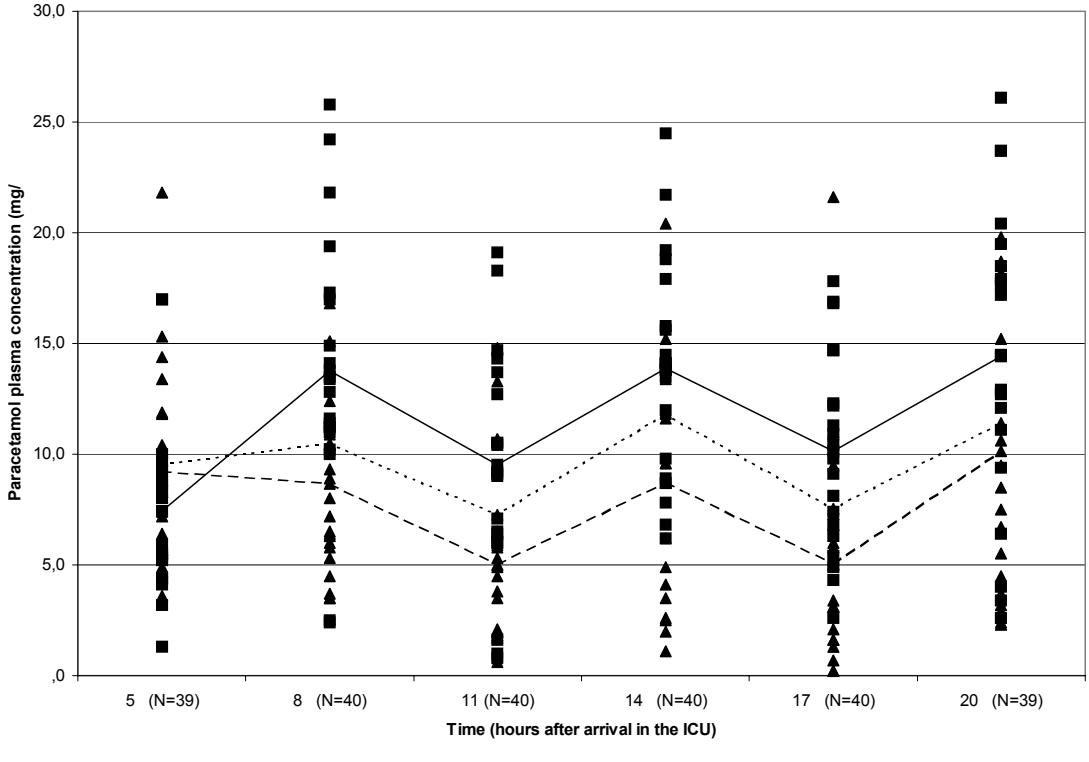

Figure 2b *Solid line: Mean APAP plasma concentration rectal group;*
dashed line: Mean APAP plasma concentration oral group;
dotted line: Mean APAP plasma concentration in oral group after exclusion of patients who vomited;
triangles: patient receiving oral APAP;
squares: patient receiving rectal APAP

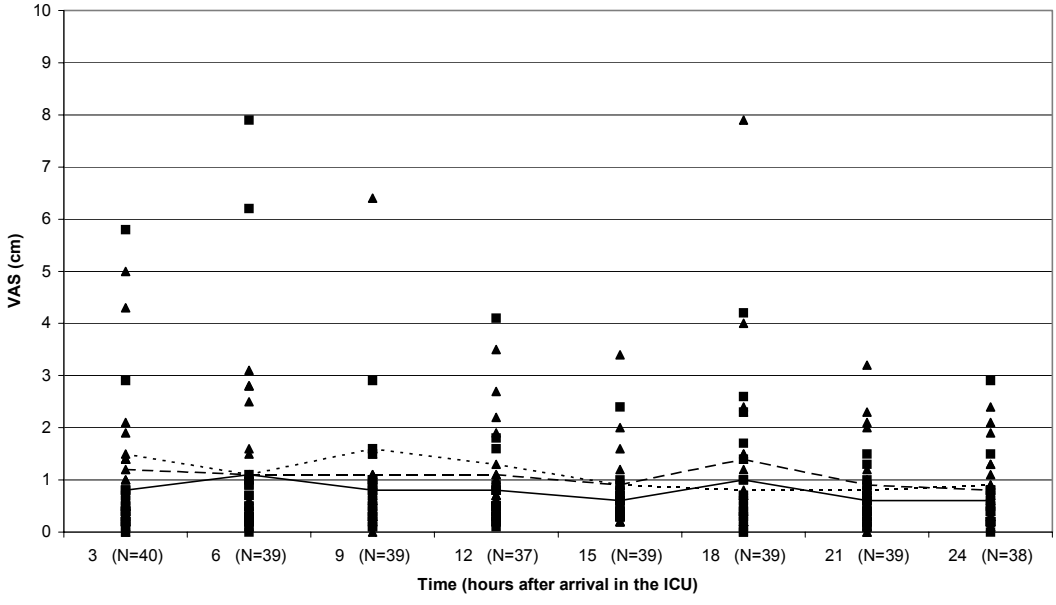

Figure 3a *Solid line: Mean VAS score in rectal group;*
Dashed line: Mean VAS score oral group;
Dotted line: Mean VAS score oral group after exclusion of patients who vomited;
Triangles: patient receiving oral APAP;
Squares: patient receiving rectal APAP

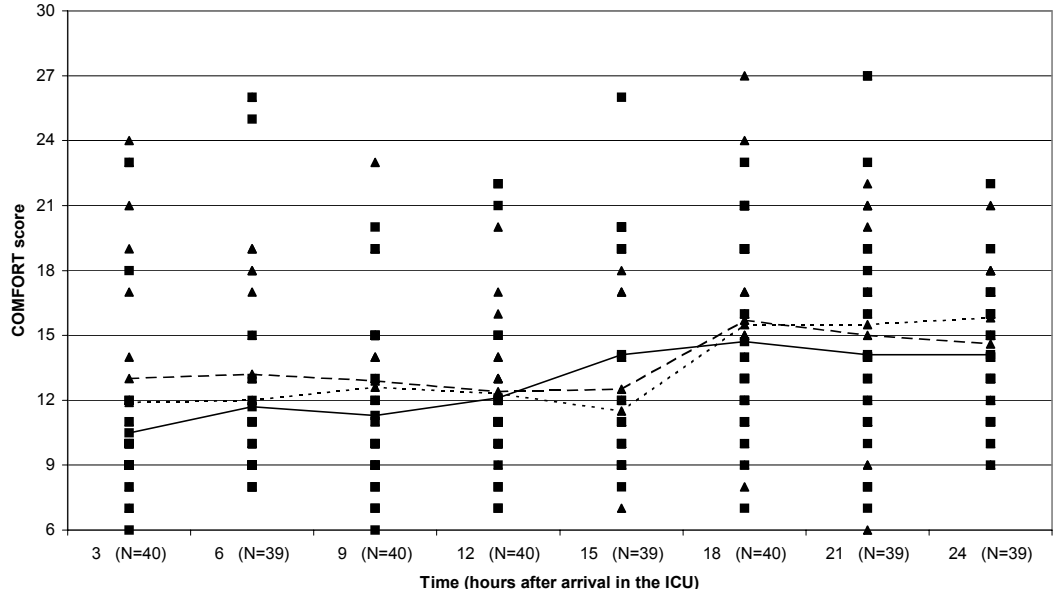

Figure 3b *Solid line: Mean COMFORT score in rectal group***;**
Dashed line: Mean COMFORT score oral group;
Dotted line: Mean COMFORT score oral group after exclusion of patients who vomited;
Triangles: patient receiving oral APAP;
Squares: patient receiving rectal APAP

The percentage of VAS scores < 4 cm was 93.6% at APAP plasma concentrations of 0 to 5 mg/l, 97,8% at 5 to 10 mg/l, 100%, at 10 to 15 mg/l, 92.5% at 15 to 20 mg/l, and 100% at concentrations > 20 mg/l. Although the VAS scores decreased slightly over time, with an increase at t = 18 hours, the COMFORT scores increased slightly over time.

The percentage of VAS scores ≥ 4 cm declined over time. The median APAP plasma concentration at VAS scores ≥ 4 cm was 5.6 mg/l (25th percentile, 2.3 mg/l; 75th percentile, 9.0 mg/l).

Discussion

The APAP plasma concentrations in patients receiving rectal APAP were significantly higher than those in patients receiving oral APAP. This is surprising, since previous studies showed lower concentrations after rectal administration compared with oral administration at the same doses.[7,9] A possible explanation for the lower concentrations after oral administration is that patients in the oral group who vomited, received little or no APAP at all at a given time point. After exclusion of these patients, there was indeed no significant difference in APAP plasma concentrations, although the rectal concentrations were still higher than the oral concentrations. Another explanation is that the peak APAP plasma concentration after oral administration was missed as a result of sampling 2 hours after the maintenance dose was administered. Normally, the peak plasma concentration after oral APAP administration is expected within 30 to 60 minutes.[24] However the slower gastric emptying in patients after general anesthesia results in a peak plasma concentration after 90 to 120 minutes,[24] which makes it less probable that the peak plasma concentration is missed. Also normal adult rate of gastric emptying may not be reached until the age of 6 to 8 months.[9] Before normal adult rates are reached, gastric emptying is slow and erratic,[9,25] and gastric emptying and drug absorption is inhibited by narcotic analgesics.[26]

Pain scores were significantly higher in those patients receiving oral APAP and thus inversely related to the lower APAP plasma concentrations in these patients.
However, a significant relation between APAP plasma concentrations and pain scores could not be established. Other studies in infants delivered by vacuum extraction at term also report absence of such a relation.[4]

Since ondansetron has no psychoactive properties and domperidon and metoclopramide have only weak psychoactive properties, it is very unlikely that a single dose of these antiemetics had influence on the pain scores. On the other hand, midazolam, which does not have significant analgesic properties, might have had influence on the pain scores, especially on the COMFORT score, because this rating scale is a measurement instrument for distress. However, the VAS score should not be influenced by midazolam.

The required analgesic APAP plasma concentrations of 10 to 20 mg/l[6,7] were not reached by 22.5% of all patients during the 24-hour observation period. However, still more than 92.5% of the VAS scores in these patients did not exceed 4 cm. Studies in adults showed that APAP plasma concentrations of 6 to 24 mg/l, after intravenous administration, produce adequate analgesia.[27,28] Since APAP plasma concentrations $\geq$ 5 mg/l were reached in all the children in our study and more than 92.5% of VAS scores in all children were under 4 cm, the analgesic range for this age group could well be < 10 to 20 mg/l.

The slight decrease in VAS scores over time is explained by the fact that the most intense pain usually occurs in the first hours after the operation and then slowly decreases. At t = 18 hours there was a sudden increase in VAS scores preceded by a low APAP plasma concentration at t = 17 hours. Taking into account this low value and the absence of a relation between APAP plasma concentrations and pain scores are, the low plasma concentrations at t = 17 hours cannot (fully) explain the sudden increase in VAS scores at t = 18 hours. A possible explanation is that t = 18 hours is approximately the time the children wake up the morning after the

operation and find themselves in a stressful environment. Also, in view of the low mean age of the study population, fear and anxiety could partly be responsible for this phenomenon. This would also explain the increase in Comfort scores at t = 18 hours.
The fact that after the operation the faces of the children become swollen and that they are scarcely able to open their eyes after several hours, provides an argument for distress.
Another factor to explain the widely varying APAP plasma concentrations are differences in the activities of the enzymes involved in the APAP metabolism. These differences mainly result from differences in the patients' genetic background.[29] Since all our patients were approximately the same age, age-related maturation[29] of enzyme activity cannot be held responsible for the differences in activity.

Further research should aim at detecting differences in enzyme activity and at correlating the widely varying APAP plasma concentrations with the genetic background.
We conclude that these are the first data showing that the analgesic APAP plasma concentration after major surgery in this age group does not always reach the 10 to 20-mg/l level. However, a relation between APAP plasma concentrations and pain scores was absent. These data also show that, after a rectal loading dose of 40 mg/kg has been given during surgery, the best way of administering APAP after craniofacial surgery is the rectal route. As a result of this study rectal administration of APAP after craniofacial surgery is now standard policy in our PSICU.

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Chapter 3.2

Rectal paracetamol versus diclofenac in children following (adeno)tonsillectomy

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Abstract

Background

Paracetamol (APAP) is commonly prescribed for pain relief after (adeno)tonsillectomy [(A)TE], but reports of effectiveness are inconsistent. Diclofenac has been suggested as an alternative, although concerns about possible postoperative bleeding have tempered the use of diclofenac following (A)TE. We designed and conducted this study to compare the analgesic effect of rectally administered APAP and diclofenac in children undergoing (A)TE during ambulatory surgery and to assess the relation between APAP, diclofenac and 4'hydroxy-diclofenac plasma concentrations and postoperative pain scores. Furthermore we assessed the safety of diclofenac by monitoring postoperative bleeding and by registering the use of bipolar diathermy at the end of the procedure to arrest further bleeding.

Methods

A randomized controlled trial was performed in 65 children given either APAP (40 mg/kg loading dose, 30 mg/kg 8-hourly maintenance dose) or diclofenac (2 mg/kg loading dose, 1 mg/kg 8-hourly maintenance dose) rectally. APAP loading dose was administered 90 minutes before surgery, diclofenac loading dose was administered 30 minutes before surgery. Analgesic effect was assessed every 15 minutes during the first hour postoperatively, using validated pain scores (VAS and POCIS). After the first hour, analgesic affect was assessed 1-hourly until discharge. Morphine (5 μg/kg) was administered intravenously if VAS $\geq$ 4 cm or POCIS $\geq$ 4. Blood samples were collected at the start and at the end of surgery and 1, 2 and 3 hours postoperatively Furthermore we registered whether children had primary or secondary bleeding following the procedure and whether bipolar diathermy was performed at the end of the procedure to arrest further bleeding. Following discharge, parents of the children performed a daily VAS score for the first postoperative week.

Results
Data of 60 patients were analyzed. Median (25th-75th percentile) age was 4 (3-5) years. Both the APAP and the diclofenac group consisted of 30 patients. There was no difference in analgesic effect between APAP and diclofenac and there was no relationship between plasma concentrations and pain scores. Two children had primary bleeding, both given APAP. Bipolar diathermy was performed in 14 patients, 8 versus 6 in respectively the APAP and the diclofenac group.

Conclusion
Rectal diclofenac (2 mg/kg loading dose, 1 mg/kg 8 hourly) does not provide better pain relief than rectal APAP (40 mg/kg loading dose, 30 mg/kg 8-hourly) following (A)TE. Our data showed no relationship between plasma concentrations and pain scores and no increase in incidence of primary or secondary bleeding due to the administration of diclofenac was shown, despite its effect on platelet aggregation.

Introduction

(Adeno)tonsillectomy [(A)TE] is generally considered as a very traumatic and painful procedure. Adequate treatment to reduce this traumatic and painful experience is important, since insufficient analgesia might lead to behavioral changes postoperatively.[1] Rectal paracetamol (APAP) 15-20 mg/kg 6-8-hourly is used frequently, but this often does not provide adequate postoperative analgesia.[2,3] For adequate analgesia a rectal loading dose of 40 mg/kg approximately 2 hours before the procedure has been suggested.[4,5] However, with a recommended daily dose of 75 mg/kg and a loading dose of 40 mg/kg, the ability to give extra doses of APAP if pain persists, is reduced.[6] Despite dosing equivalence, APAP plasma concentrations are widely varying and unpredictable,[7,8] due to triglyceride base suppository absorption, relative bioavailability and clearance parameters, which are associated with considerable variability (CV 90%, 30%, 41% respectively.[9] Considering the dose-effect relationship, Anderson

et al. reported that a target effect compartment concentration of 10 mg/l was associated with a mean pain score (VAS 0-10) reduction of 2.6 following (adeno)tonsillectomy.[10] But this reduction may be inadequate if pain scores are high directly following the procedure. The large pharmacodynamic variability (EC50 CV 107%) also means that pain may be poorly controlled in some individuals and might explain why other studies in neonates and infants have been unable to establish a relationship between APAP plasma concentrations and analgesic effect.[8,10,11]

Rectal diclofenac 1-2 mg/kg approximately 1 hour prior to the procedure is considered as an alternative for APAP for postoperative analgesia following (A)TE,[12] but the concerns about possible postoperative bleeding have tempered the use of diclofenac following (A)TE.[13] A retrospective evaluation of the incidence of postoperative bleeding following (A)TE did not seem to confirm the increased risk of postoperative bleeding.[14] In contrast to APAP, there are few pharmacokinetic-pharmacodynamic data for diclofenac in children. Rømsing et al. were unable to establish a relationship between diclofenac dose and effect.[15] However, diclofenac has an active metabolite, 4'-hydroxy-diclofenac (D4OH), and this metabolite may contribute to the analgesia observed after diclofenac dosing.

We designed and conducted a double blind, randomized study, to compare the analgesic effect of rectally administered APAP and diclofenac in children undergoing (A)TE during ambulatory surgery and to assess the relation between APAP, diclofenac and D4OH plasma concentrations and postoperative pain scores. Furthermore we assessed the safety of diclofenac for this procedure by monitoring postoperative bleeding.

Patients and methods

Patients and methods

Following approval of the study by the Medical Ethical Committee of the Erasmus MC Rotterdam and after written informed consent was obtained from the parents, 65 children, undergoing elective (A)TE during ambulatory surgery, were included consecutively during the period from July 2000 till November 2001.

Inclusion criteria were: age between 3 and 12 years, elective (A)TE, ASA status 1 or 2 and the presence of at least one Dutch speaking parent at the day of surgery. Exclusion criteria were: coagulopathy, diclofenac or APAP < 24 hours prior to surgery, known allergy for diclofenac or APAP, hepatic diseases interfering with drug metabolism and abnormal renal function.

Procedure

Patients were randomly assigned to receive a rectal loading dose of either 40 mg/kg APAP 90 minutes before scheduled surgery or 2 mg/kg diclofenac 30 minutes before scheduled surgery.[4,5,12] Patients receiving APAP 90 minutes before scheduled surgery received a placebo suppository 30 minutes before scheduled surgery, while patients receiving diclofenac 30 minutes before scheduled surgery received a placebo suppository 90 minutes before scheduled surgery (Figure 1a).

Anesthesia was performed according to standardized protocols. Anesthesia was induced using intravenously propofol 3-4 mg/kg, sufentanyl 0.2 μg/kg and mivacurium 0.2 μg/kg or by inhalation, with sevoflurane 8%. After tracheal intubation anesthesia was maintained with isoflurane 0.5 MAC.

During the procedure when heart rate was 10% or more above baseline value, as described in our earlier randomized controlled trial,[8] 10 μg/kg alfentanyl was administered. If heart rate was still 10% or more above baseline value, 10 minutes after the administration of 10 μg/kg alfentanyl, another dose of 10 μg/kg alfentanyl was administered. Heart rate baseline value was obtained 10 minutes after intubation.

(A)TE was performed using blunt dissection. Bleeding was stopped with packing. If bleeding persisted, bipolar diathermy was used to arrest further bleeding.
Postoperatively analgesic effect was assessed using validated pain scores, performed by trained investigators. POCIS (consisting of face, crying, breathing, body, arms, legs and agitation), and Visual Analogue Scale (VAS) scores were obtained every 15 minutes during the first hour postoperatively and then every hour until discharge of the child at the end of the day.[16,17] In addition we asked the parents of the children to score the VAS 1-hourly postoperatively until discharge (Figure 1b).
Depending on the POCIS and VAS scores extra pain medication could be administered.

When POCIS ≥ 4 or VAS ≥ 4 cm, 5 μg/kg morphine was administered intravenously until the child was in minimal pain as indicated by POCIS < 4 or VAS score < 4 cm. Ten minutes after the administration of morphine, pain was reassessed. If the child was still in pain, a second dose of 5 μg/kg morphine was administered.
Blood samples (1.0 ml) for APAP or diclofenac and D4OH plasma concentration analysis were taken through a peripheral intravenous canule at the start and at the end of surgery and 1, 2 and 3 hours postoperatively (Figure 1b).
We documented whether children had experienced primary or secondary bleeding and whether bipolar diathermy had been performed at the end of the procedure. Primary bleeding was defined as postoperative bleeding, which made re-operating necessary.[18,19] Secondary bleeding was defined as postoperative bleeding on day 2-day 7 following surgery, which made re-operating necessary.[18,19]
Before discharge parents received 10 blinded suppositories, each consisting of either 30 mg/kg APAP or 1 mg/kg diclofenac, to take home as maintenance doses. Parents were advised to administer them at 8-hourly intervals, starting 8 hours after administration of the first suppository of study medication before surgery, which was usually before discharge. Parents were advised to administer APAP suppositories if their child was

still in pain after finishing the blinded suppositories supplied by the hospital. We asked parents to fill in a pain diary at home, consisting of a daily VAS score, during the first week after the procedure and to return this by mail. We also asked them to record the number of blinded suppositories their child used in this diary and extra APAP required after finishing the 10 blinded suppositories (Figure 1c).

APAP assay

APAP plasma concentrations were determined using fluorescence polarization immunoassay (Adx system, Abbott Laboratories, North Chicago IL) (ErasmusMC, Rotterdam).[8] Detection limit of this method is 1.0 mg/l. Precision was measured at APAP concentrations of 15, 35 and 150 mg/l. To determine coefficients of variation at these concentrations 55 samples of each concentration were assayed [CV = (SD/mean); RSD = CV*100%]. RSD was 7.22%, 3.37% and 3.11% at 15, 35 and 150 mg/l respectively. The APAP concentration range, in which the accuracy was determined, is 10-150 mg/l.

Diclofenac and D4OH assay

Diclofenac sodium and naproxen were purchased from SIGMA ALDRICH (Saint Quentin Falavier, France), D4OH was provided by Novartis, USA. All solvants used were analytical grade.

The HPLC system consisted of a Quatemary P 1000 XR pump (ThermoQuest-TQ, Florida, USA), a TQ autosampler, a TQ UV 6000 detector (280nm) linked to TQ Spectranet for recording and storing throughout analysis. We used a LC_8 5 µm particle size Supelcosil column (150x4.6 nm, Supelco Bellafonte, USA). The mobile phase was a mixture of acetonitrile/ sodium acetate 50 mV (70/30, v/v) adjusted to pH 5 by phosphoric acid and the flow rate 1.2 ml/min.

Stock solutions of diclofenac (1000 mg/l) , D4OH (500 mg/l) and naproxen (1000 mg/l) were prepared in methanol and stored at –20°C. Calibration standards (0.01-1 mg/l) and plasma controls (0.04, 0.2, 0.750 mg/l) were prepared by appropriate dilutions of the stock solutions in drug-free plasma. Naproxen (10 mg/l) was used as internal standard.

	90 minutes before scheduled surgery		*30 minutes before scheduled surgery*	
	APAP *40 mg/kg*	*Placebo*	*Diclofenac* *2 mg/kg*	Placebo
APAP group	X			*X*
diclofenac group		*X*	*X*	

a administration loading doses

	Start of procedure	End of procedure	Arrival recovery = 0h	0.25h	0.50h	0.75h	1h	2h	3h	4h	5h	6h
Blood sample	X	X					X	X		X		
POCIS			X	X	X	X	X	X				
VAS investigator			X	X	X	X	X	X	X	X	X	
VAS parents			X				X	X	X	X	X	
Study medication												X

b during procedure and postoperatively until discharge

	Day 1		Day 2			Day 3			Day 4		Day 5	Day 6	Day 7
		23.00	7.00	15.00	23.00	7.00	15.00	23.00	7.00	15.00			
Study medication		X	X	X	X	X	X	X	X	X			
VAS parents	HOSPITAL			X			X			X	X	X	X

c during first week postoperatively at home

Figure 1 Study flow chart

Under the chromatographic conditions used, the retention times were 18.5, 6.9 and 7.9 min for diclofenac, D4OH and naproxen respectively. Recovery from extraction was over 90% for the two compounds. Calibration curves were linear over the range of 0.01 to 1 mg/l and coefficients of variation of the slope were between 2.8 and 5.8% (n = 5). The limit of quantification was 5 μg/l for the two compounds. The intra and inter-assay coefficients of variation, determined from three quality controls (0.04, 0.2, 0.750 mg/l) were lower than 7%.

Data analysis

The Mann Whitney U-test was used to assess whether APAP and diclofenac differed in analgesic effect. For this purpose the summary measurement approach was performed;[20] all pain scores (i.e. VAS observer, VAS parents, and POCIS scores) were averaged into one single pain score. Chi-square tests were used to assess differences in prevalence in primary- and secondary bleeding, and bipolar diathermy.

Power analysis

It was expected that 40% of the patients in the APAP group would need extra morphine,[21,22] versus 16 % in the diclofenac group.[23-25] For a power of 0.80 and $\alpha = 0.05$, 50 patients were required in each group.[24] Interim analysis was performed after inclusion of 60 patients.

Assignment and blinding

A block randomization schedule was used to allocate APAP or diclofenac. The schedule was kept solely by the pharmacist to ensure blinding until the end of the study.

The APAP and diclofenac sodium suppositories (with witepsol H15, synthetic saturated triglycerides with a chain length of C12-C18, as the base) were manufactured in the hospital pharmacy. As all children received suppositories (either APAP and placebo or diclofenac and placebo) manufactured in the department of the hospital pharmacy, patients and parents, nurses and investigators were blinded for the actual content of the suppositories.

The composition of the placebo suppositories was equal to the composition of the APAP and diclofenac suppositories, except for the APAP and the diclofenac. The APAP and diclofenac doses in the suppositories never deviated more than 10%. The ingredients were obtained via regular commercial suppliers. Suppositories and ingredients conformed with the quality requirements in the European Pharmacopoeia.

Results

Participant flow and follow up

The eligible number of patients was 113. The parents of 48 patients refused informed consent for varying reasons: to much extra handling of their child (8), language difficulties (6), not familiar with diclofenac (5), child has suffered enough (4), no reason for not participating (25). As a result we included 65 patients in this study, of which 5 patients were excluded for analysis because they did not receive standard intervention as allocated (4) or due to withdrawal of parental informed consent during the study(1). Characteristics of these 5 patients did not differ from the characteristics of the 60 patients used for analysis. Both the APAP and the diclofenac group consisted of 30 patients.

Participants' flow is represented graphically in Figure 2.

Analysis

Median (25th-75th percentile) age and weight of patients receiving APAP (11 boys, 18 girls) and diclofenac (16 boys, 13 girls) were respectively: 4.0 (3.0-5.5) years, 19 (16.0-22.0) kg and 4.0 (3.5-5.0) years, 20.0 (17.3-23.5) kg.

Age, weight, gender, operative procedure, duration of the operative procedure, postoperative bleeding, bipolar diathermy, extra anesthesia, extra morphine, postoperative administration of metoclopramide of the APAP and diclofenac group are shown in Table 1.

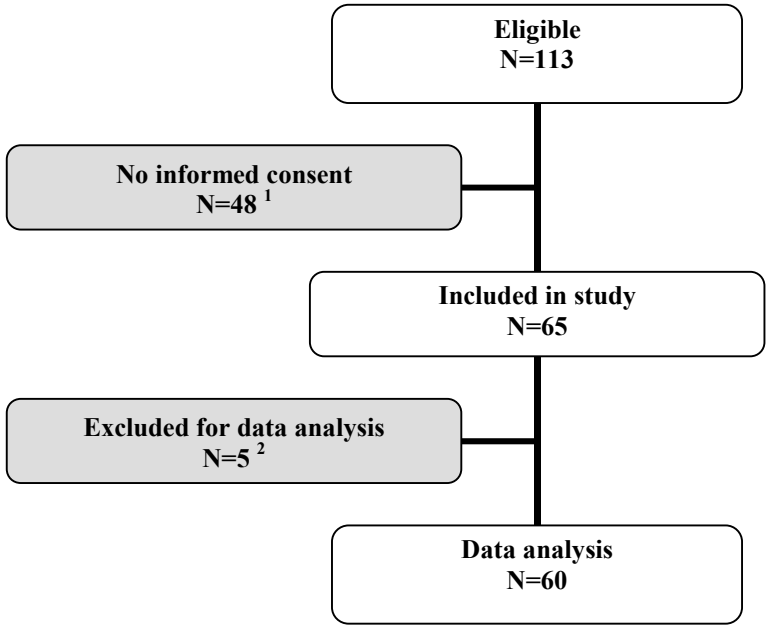

Figure 2 *Patients' flowchart*
1. reasons: to much extra handling of their child (8), language difficulties (6), not familiar with diclofenac (5), child has suffered enough (4), no reason for not participating (25)
2. reasons: not receiving standard intervention as allocated (4), withdrawal of parental informed consent during the study (1)

Pain scores

VAS scores obtained by the investigator were highest directly after the procedure and then decreased. Approximately 3-4 hours after the procedure, which was respectively 5-6 hours after loading dose for the APAP group and 4-5 hours after loading dose for the diclofenac group, VAS scores were slightly increasing. However these VAS scores stayed below 4 cm (Figure 3). There was no significant difference in VAS scores between children receiving APAP and children receiving diclofenac (Figure 3). The number of children receiving extra morphine was not significantly different between both groups. Two children in the APAP group and three children in the diclofenac group had a VAS $\geq$ 4 cm during the first hour postoperatively and received extra morphine (5 μg/kg).

Table 1 Patients' characteristics

		APAP (n = 29)	**Diclofenac (n = 29)**
Age (years)	Mean (SD)	4.2 (1.4)	4.5 (1.4)
	Range	2-7	2-8
Weight (kg)	Mean (SD)	19.4 (5.0)	20.4 (4.5)
	Range	14-33	13-30
Gender	boys	11	16
	girls	18	13
Operative procedure	ATE	28	26
	TE	1	3
Duration of procedure (min)	Mean (SD)	52 (17)	47 (13)
	Range	30-110	30-80
Bipolar diathermy		8	6
Postoperative bleeding		2	0
Extra fentanyl during anesthesia		14	13
Extra morphine frequency	0	27	25
	1	2	3
	2	0	1
Postoperative metoclopramide		5	2

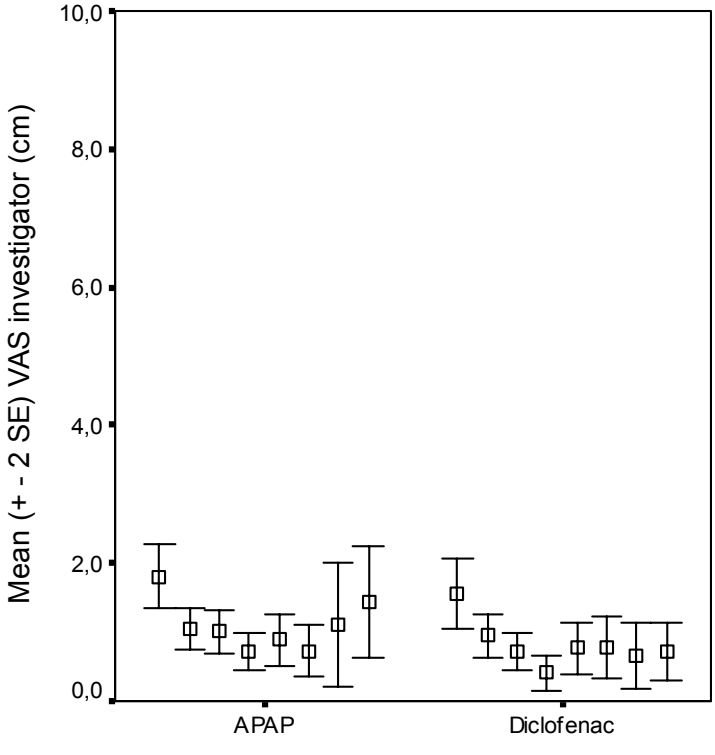

Figure 3 *VAS scores obtained by investigator*

VAS scores obtained by the parents of the children were significantly higher ($p = 0.045$) and more varying compared to the VAS scores obtained by the investigator. There was no significant difference in VAS scores obtained by the parents between the APAP group and the diclofenac group (Figure 4) and postoperative course of the VAS scores obtained by the parents was comparable to the postoperative course of VAS scores obtained by the investigator, being highest directly after the procedure and then decreasing (Figure 4).
POCIS scores were highest directly following the procedure and decreasing during the first hours postoperatively. There was no significant difference in POCIS scores between both groups (Figure 5).

APAP plasma levels
Patients were sampled at the start and at the end of the procedure and 1, 2 and 3 hours postoperatively, resulting in a total of 95 samples collected from 25 patients assayed for APAP plasma concentrations. APAP plasma concentrations varied widely and ranged from 4.8-27.0 mg/l. Concentrations were highest at the start of surgery, which was approximately 90 minutes after loading dose, and decreased slowly postoperatively (Figure 6).

Diclofenac and D4OH
Patients were sampled at the start and at the end of the procedure and 1, 2 and 3 hours postoperatively, resulting in a total of 97 samples collected from 25 patients assayed for diclofenac and D4OH. Diclofenac plasma concentrations varied, ranging from 0-2.6 µg/ml and being highest at the start of surgery, approximately 30 minutes after loading dose, and decreasing slowly postoperatively (Figure 7a). D4OH concentrations varied from 0-.5 µg/ml. Highest plasma concentrations were measured at the start of surgery, but there was only a slight decrease during the postoperative period (Figure 7b).

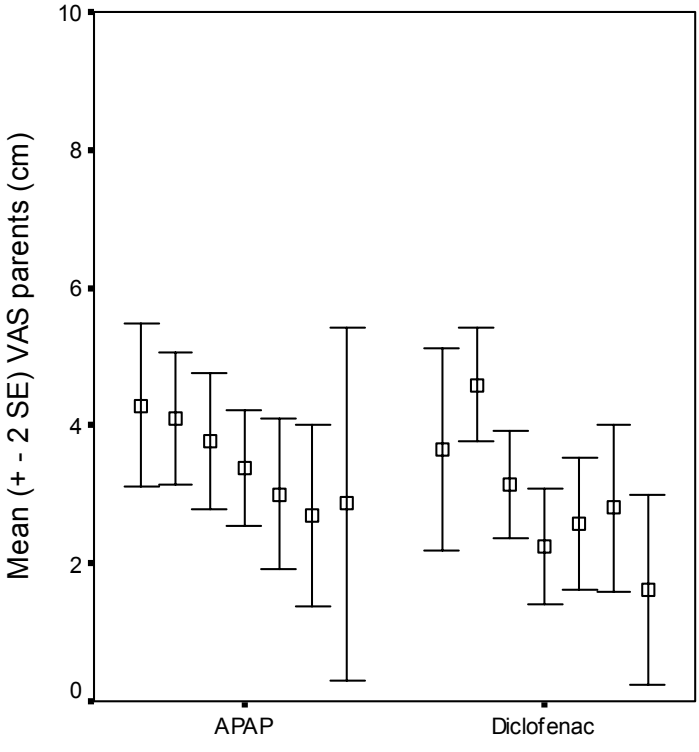

Figure 4 *VAS scores obtained by parents*

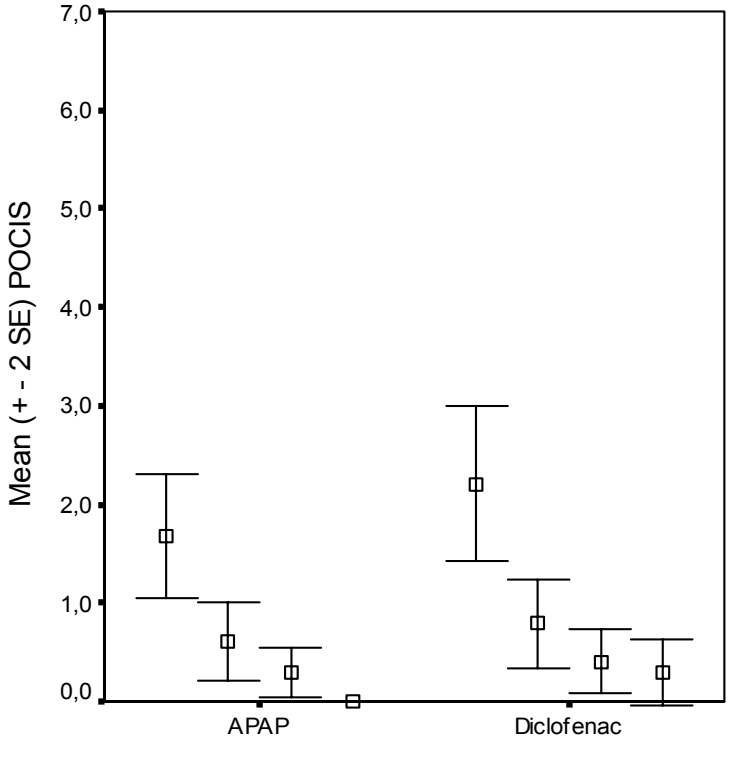

Figure 5 *POCIS scores*

Pain scores and plasma concentrations

All children receiving APAP had APAP plasma concentrations > 10 mg/l at least at two or three sampling points and analgesia was assessed adequate.[10] Two children receiving APAP had a VAS > 4 cm during the first hour postoperatively, but APAP plasma concentrations were > 10 mg/l in both children. Diclofenac and D4OH plasma concentrations in the 3 children with VAS ≥ 4 during the first hour postoperatively were comparable to the diclofenac and D4OH plasma concentrations in children with VAS < 4 as well.

Safety of diclofenac

Primary bleeding was experienced in only 2 patients both receiving APAP, respectively 65 and 110 minutes postoperatively. There were no children experiencing secondary bleeding.

Bipolar diathermy was performed at the end of the procedure to arrest further bleeding in 14 patients, respectively 8 versus 6 in the APAP group and in the diclofenac group, which was not significantly different.

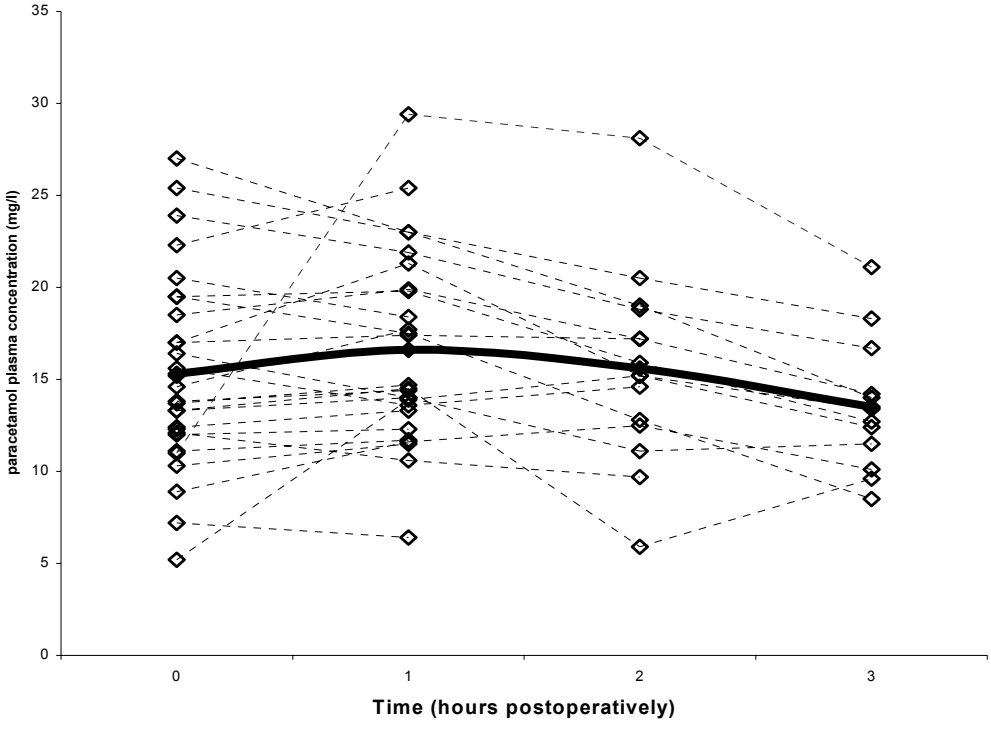

Figure 6 *APAP plasma concentrations*

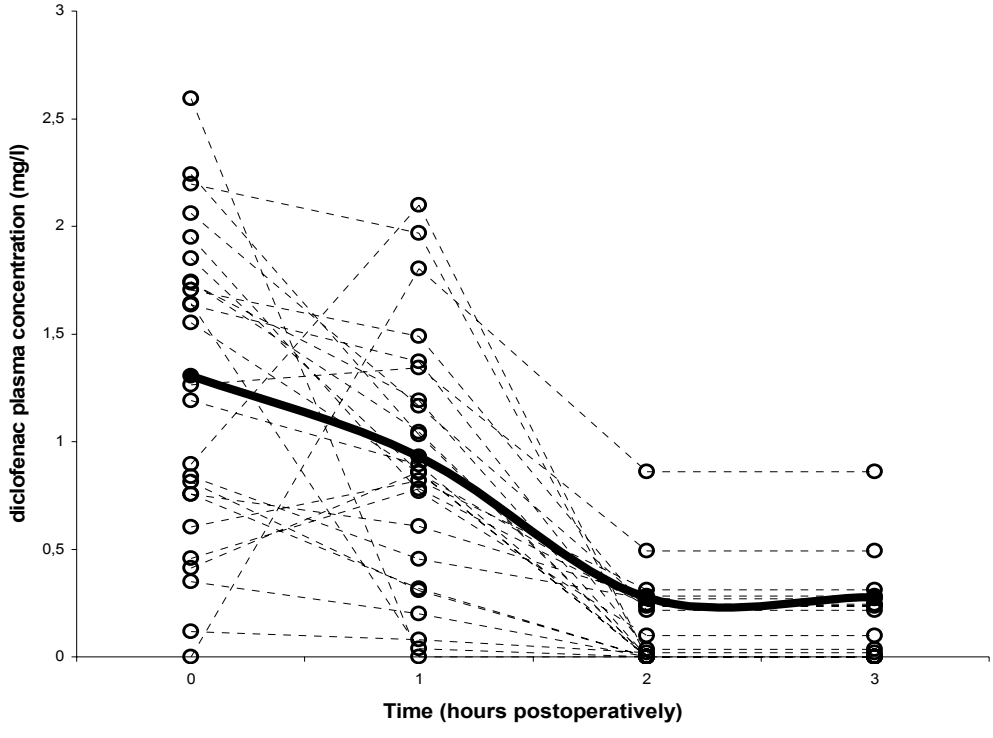

Figure 7a *Diclofenac plasma concentrations*

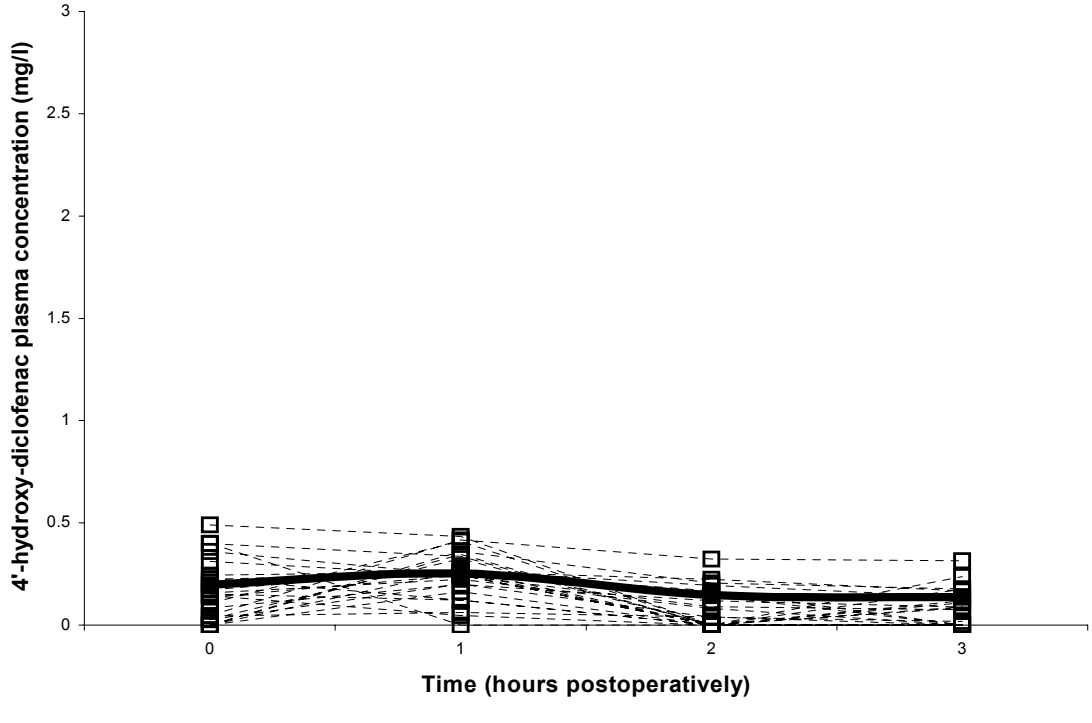

Figure 7b *D4OH plasma concentrations*

Postoperative course at home

72% (n = 42: APAP n = 20, diclofenac n = 22) of the parents returned the pain diary with VAS scores obtained daily at home during the first week postoperatively. VAS scores varied widely, with a slight decrease in VAS scores during the week. There was no significant difference in VAS scores between children receiving APAP and children receiving diclofenac (Figure 8).

Parents of 7 children explicitly reported in the pain diary how many suppositories of the supplied study medication they had administered to their children: 1 child (diclofenac) did not receive any study medication after leaving the hospital, 2 children (both diclofenac) received study medication during the first 2 days postoperatively and 2 children (both diclofenac) received study medication during the first 3 days postoperatively. The parents of 2 children (1 diclofenac, 1 APAP) reported their child needed extra APAP until 6 days postoperatively after the supplied study medication was administered.

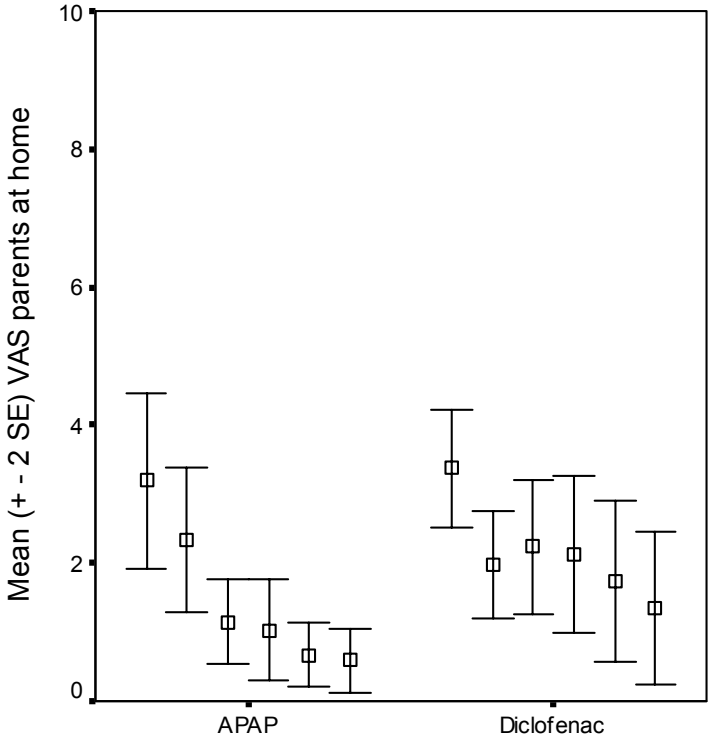

Figure 8 *VAS scores obtained by parents at home*

Discussion

The results of our study comparing the analgesic effect of rectally administered APAP and diclofenac in children undergoing (A)TE during ambulatory surgery, showed no significant difference in analgesic effect between APAP 40 mg/kg 90 minutes before surgery and diclofenac 2 mg/kg 30 minutes before surgery. Both VAS scores and POCIS scores did not differ significantly between APAP and diclofenac group. Since analgesia was adequate in both groups and we could not find a difference in analgesic effect between both groups after data of 60 patients were analyzed in the interim analysis, we decided not to perform further inclusion of patients.

We chose time of loading doses respectively 90 minutes prior to the procedure for APAP,[4,5] and 30 minutes prior to the procedure for diclofenac.[7] Drugs were given prior to the surgical procedure in the expectation that we would achieve maximal effect compartment concentrations at the conclusion of surgery. Both time of loading dose and amount of loading dose are of direct importance for the analgesic effect postoperatively and therefore have a direct influence on the postoperative course. If children experience less pain, i.e. if postoperative analgesia is adequate, children will be more motivated to increase their fluid intake, which has a beneficial effect on their postoperative course.[1,27]

As expected, pain scores were highest directly after the procedure, decreasing slowly during the first hours postoperatively. There was a slight increase in pain scores approximately 4 hours postoperatively, respectively 6 and 5 hours after APAP and diclofenac loading dose. This increase approximately 4 hours postoperatively has also been noted by Anderson et al. and can be attributable to both placebo effect and decreasing effect compartment concentrations.[10] Maintenance doses according to the protocol were administered 8 hours after administration of the first suppository of study medication, respectively 8 and 7 hours after APAP and diclofenac loading dose, which was approximately 6 hours postoperatively. Although

VAS scores stayed below 4 cm, postoperative course, as reflected in the pain scores, might suggest that the first maintenance dose should be administered at 4 hours instead of 6 hours postoperatively. This way an increase in pain scores can be prevented, resulting in a better overall analgesia. Next maintenance doses should then be administered according to protocol at 8-hourly intervals.

Pain scores were obtained by investigators and by the parents of the children, resulting in a comparable postoperative course. Although there was no significant difference in VAS scores obtained by the parents between APAP and diclofenac group, parents scored significantly higher compared to the investigator.

As a result of adequate analgesia there was little variability in pain scores, which makes assessment of the relationship between pain scores and plasma concentrations difficult. Furthermore postoperative natural pain resolution, behavioral coping mechanisms and residual effects of anesthesia have an effect on postoperative pain scores and might have obscured our assessment of the analgesic effect of both APAP and diclofenac.[14,22] Lavy reported that the postoperative pain resolution was faster in children < 10 years of age compared to children > 10 years of age.[28] According to Anderson, size is an important contributor in this: smaller children have a higher metabolism, resulting in a higher healing speed.[10] This higher healing speed in smaller children might have contributed to the postoperative pain resolution course and therefore to the assessment of the analgesic effect of APAP and diclofenac
APAP plasma concentrations varied widely, which has been reported previously in other age groups.[8,29,30]

Diclofenac concentrations were comparable to the concentrations measured by Rømsing et al. and by Haaspasaari et al. Rømsing et al. measured diclofenac plasma concentrations in children 5-15 years of age following (A)TE after administration of a single dose of 1-2 mg/kg diclofenac orally.[14] Haaspasaari et al. measured diclofenac concentrations in children

2-7 years of age with rheumatoid arthritis after administration of 1.5 mg/kg diclofenac orally.[31]

Anderson reported that 84% of the children included in his study had adequate analgesia with APAP plasma concentrations > 10 mg/l.[10] In our study all children receiving APAP had APAP plasma concentrations > 10 mg/l at least two or three sampling points. Analgesia was assessed inadequate in 2 children receiving APAP, which had a VAS > 4 cm during the first hour postoperatively. However APAP plasma concentrations were > 10 mg/l in both children. This supports the lack of a relationship between APAP plasma concentrations and pain scores, as has been previously described in other age groups.[8,10,11] Arendt-Nielsen et al. and Nielsen et al. reported a delay of 1 hour in maximum analgesia and peak APAP plasma concentrations in adults,[32,33] supporting the absence of a direct relationship between APAP plasma concentrations in this age group as well.

Also diclofenac and D4OH plasma concentrations in the 3 children with VAS ≥ 4 during the first hour postoperatively were comparable to the diclofenac and D4OH plasma concentrations in children with VAS < 4. The absence of a relationship between diclofenac plasma concentrations and pain scores has previously been reported by Rømsing et al.[14]

To evaluate the alleged increased risk on postoperative bleeding associated with diclofenac,[13] we monitored the incidence of primary and secondary bleeding. Although the number of patients receiving diclofenac in this study was low, no primary or secondary bleeding in children receiving diclofenac was observed in our study. Furthermore the number of children, in whom bipolar diathermy was performed at the end of the procedure to arrest further bleeding, was comparable in both groups, indicating there was no increased peroperative bleeding in children receiving diclofenac.

Conclusion

Rectal diclofenac (2 mg/kg loading dose, 1 mg/kg 8-hourly) does not provide better pain relief than rectal APAP (40 mg/kg loading dose, 30 mg/kg 8-hourly) following (A)TE. Our data showed no relationship between plasma concentrations and pain scores and no increase in incidence of primary or secondary bleeding due to the administration of diclofenac was shown, despite its effect on platelet aggregation.

Acknowledgements

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Chapter 3.3

Does paracetamol decrease morphine consumption after major surgery in young infants?

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Submitted

Abstract

Background

The use of paracetamol (APAP) in addition to continuous morphine infusion (CMI) has increased in recent years despite the fact that the safety and additional value of this combined treatment has never been studied in newborns and young infants. We therefore investigated if addition of APAP decreased morphine consumption in postoperative newborns and infants after major thoracic or abdominal surgery.

Methods

A randomized controlled trial (RCT) was performed in 71 patients given either APAP 90-100 mg/kg/day or placebo rectally. Children received a morphine loading dose of 100 μg/kg and then 5 μg/kg/h CMI if < 45 weeks post conceptional age (PCA) or 10 μg/kg/h if ≥ 45 weeks PCA. Analgesic effect was assessed 2-3-hourly during the first 48 hours using VAS and COMFORT pain scores, validated for this age group. Extra morphine was administered or CMI was increased if the VAS was ≥ 4. The infusion rate was decreased in the second 24 hours if the VAS was < 4. Blood samples were collected for APAP plasma concentration analysis. Data were analyzed using Mann Whitney U test, ordinal regression, and multivariate logistic regression.

Results

We were able to analyze the data of 54 patients; 17 patients were excluded. Median (25th -75th percentile) age was 0 (0-2) months. APAP was administered to 29 patients and 25 received placebo. Additional morphine bolus requirements and increases in CMI were similar in both groups. Furthermore there was no significant difference in total morphine consumption. COMFORT and VAS sores did not differ between APAP and placebo group. Postoperative total morphine consumption was not related to age, although children < 45 weeks PCA needed less additional morphine boluses ($p < 0.01$) and needed less increases in CMI ($p < 0.01$). Mean APAP plasma concentrations ranged from 9.5 to 27.6 mg/l.

Conclusion
APAP as adjuvant to CMI does not have an additional analgesic effect and should not be considered as standard of care in young infants, 0-2 months of age, following major thoracic or abdominal surgery.

Introduction

In many hospitals around the world continuous morphine infusion (CMI) is considered as standard for postoperative analgesia after major surgical procedures in young infants.[1] Neonates and infants have an increased risk of respiratory depression with CMI because clearance is both reduced and associated with large inter-individual variability, resulting in higher plasma concentrations than older children given similar doses.[2,3] The additional use of paracetamol (APAP) has become more and more popular, since it may be associated with less morphine use, reduction of stress responses and a lower incidence of side effects.[4] In adults, combinations of opioids with APAP or non-steroidal anti-inflammatory drugs (NSAIDs) have resulted in a reduced morphine and fentanyl consumption as well as in reduced postoperative pain, without increased adverse effects.[5-10] Morton has demonstrated reduced morphine requirements in postoperative children 3 to 15 years of age given diclofenac 1 mg/kg 8 hourly, but no effect attributable to APAP 15 mg/kg 6-hourly was shown.[11]
We conducted a randomized controlled trial to test the hypothesis that morphine consumption is reduced in postoperative patients receiving the combination of morphine and APAP compared to patients receiving morphine alone.

Methods

Patients and methods
Approval for the study was granted by the Medical Ethical Committee of the Erasmus MC Rotterdam and written informed consent was obtained

from parents. Children were enrolled consecutively during the period from January 2001 till May 2002.
Inclusion criteria were: neonates and infants aged 0-1 year, ≥ 36 weeks post conceptional age (PCA), weight ≥ 1500 grams and abdominal, including urological, or thoracic surgery. Exclusion criteria were: received opioids, APAP, or other analgesics, sedative drugs or muscle relaxants < 12 hours prior to surgery, hepatic diseases interfering with drug metabolism, abnormal renal function (creatinine > 2 SD for age), neurological damage (posthypoxic encephalopathy or major congenital anomalies of the central nervous system) and severe spasticity or hypotonia.

Procedure
Patients were randomly assigned to receive rectal APAP (30 mg/kg loading dose for children < 4 kg and 40 mg/kg loading dose for children > 4 kg, followed by 20 mg/kg 6-hourly) in group A or placebo as adjuvant to continuous morphine infusion (CMI) in group B.[12]
Anesthesia was induced using intravenous thiopentone 3-5 mg/kg or by inhalation with sevoflurane in a nitrous oxide/ oxygen mixture. Fentanyl 5 μg/kg was given intravenously before tracheal intubation to all children. Tracheal intubation was facilitated with atracurium 0.5-1 mg/kg or suxamethonium 2 mg/kg. Ventilation was controlled and anesthesia was maintained with oxygen/nitrous oxide or oxygen/air, isoflurane 0.5-1 MAC, dose corrected for age.[13,14]

Monitoring consisted of ECG, non-invasive blood pressure (NIBP), oxy-haemoglobin saturation (SpO2), end-tidal carbon dioxide levels (PetCO2) and temperature. The obtained NIBP and heart rate (HR) at 10 minutes after intubation were used as peroperative baseline values as described earlier.[13,14]
Before incision a dose of 5 μg/kg of fentanyl was given. Extra doses of fentanyl (2 μg/kg) were given when the HR and/ or the mean arterial blood pressure were 10 % or more above baseline values.
Peroperative fluids were given in a standardized way, to maintain a glucose infusion rate between 4-6 mg/kg/min. Body temperature was kept within

normal ranges. At the end of surgery the neuromuscular block was antagonized and the patients were extubated, unless the anesthesiologist and surgeon decided to continue mechanical ventilation.

The rectal loading dose (APAP or placebo) was administered directly after induction of anesthesia. At the end of surgery all patients received an intravenous loading dose of morphine HCL 100 μg/kg. After surgery all children received CMI with a background of 5 μg/kg/h for children < 45 weeks post conceptional age (PCA) and 10 μg/kg/h for children ≥ 45 weeks PCA.
Pain assessment was performed by Intensive Care Unit (ICU) nurses and investigators, using pain scores validated for this age group and these circumstances. Visual Analogue Scale (VAS, 0-10) and COMFORT (0-30) scores were obtained every 2 hours during the first 24 hours postoperatively and every 3 hours during the second 24 hours after surgery, as part of the routine nursing observations during handling of the child.[15-17]
CMI was routinely decreased in the second 24 hours depending on the VAS score.

When VAS ≥ 4, extra amounts of morphine 5 μg/kg were administered until the child was in minimal pain as indicated by a VAS score < 4. Ten minutes after each extra dose of morphine, pain was reassessed. When the child needed an extra morphine bolus ≥ 3 times / hour, CMI was increased with 5 μg/kg/h, which could be increased if the child still needed extra doses of morphine > 3 times/hour (the maximum morphine background for children < 45 weeks PCA was 15 μg/kg/h and 30 μg/kg/h for children ≥ 45 weeks PCA).
Distress other than that originating from pain was assessed by COMFORT scores ≥17 and VAS < 4. Children then received midazolam for extra sedation (Figure 1).
Blood samples (0.2 ml) for APAP plasma concentrations analysis were taken through the arterial catheter, which was inserted after induction, at 30 and 90 minutes after loading dose, at the end of surgery and 5, 8, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, 47 hours after the arrival in the ICU.

Algorithm PSICU

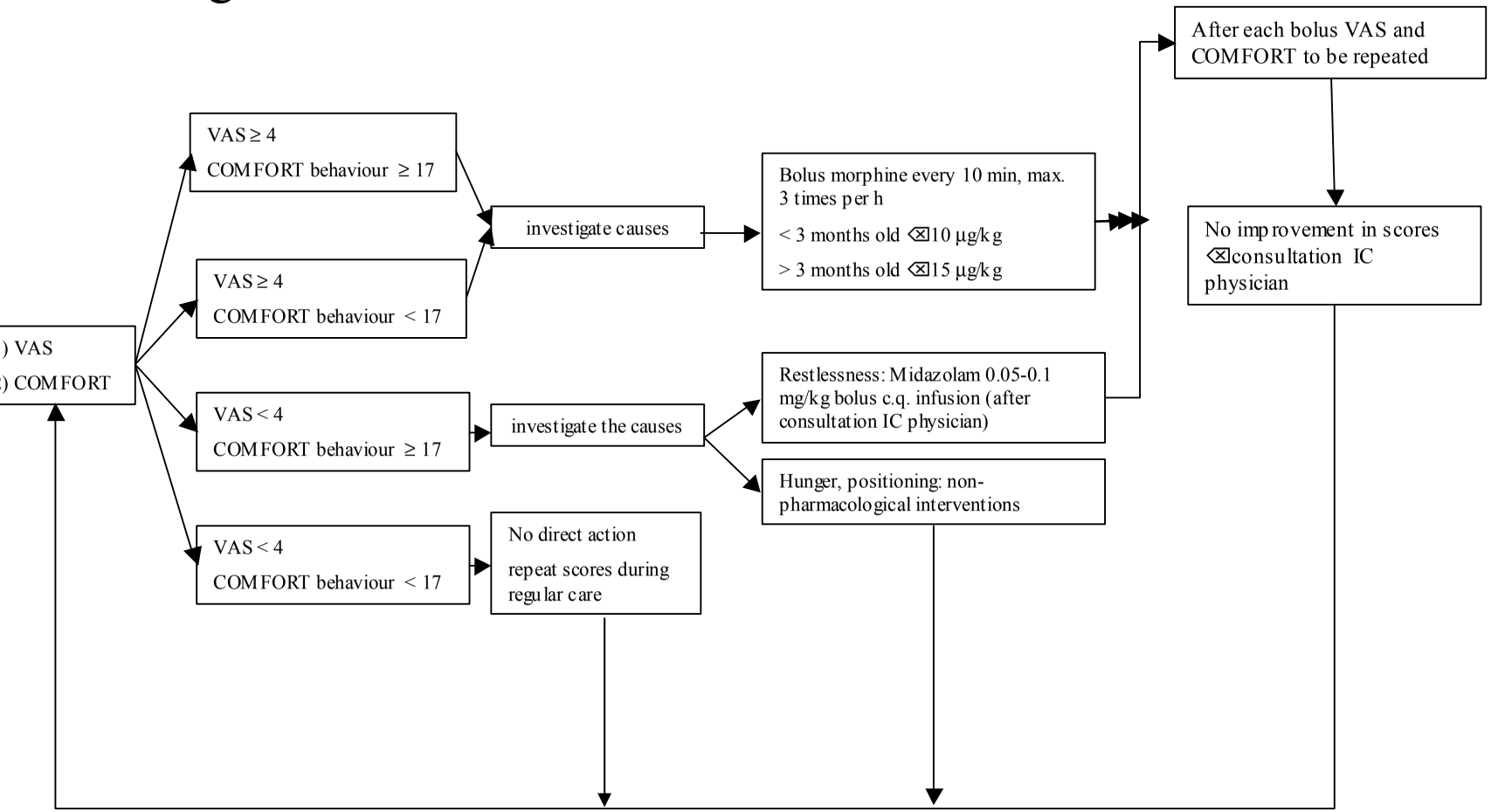

Figure 1 *Algorithm for receiving extra morphine or midazolam. Bouwmeester et al, 2002*[18]

Participant flow and follow up

The eligible number of patients was 110. The parents of 39 patients refused informed consent for varying reasons: too much extra handling of their child (n = 30), dislike of clinical trials (n = 6), no direct advantage to their child (n = 2) and language difficulties (n = 1). As a result we included 71 patients in this study, of which 17 patients were excluded from analysis. Reasons for exclusion for data analysis were: not receiving standard intervention as allocated (n = 10), withdrawal of parental informed consent during the study (n = 1), logistical problems (n = 3) and canceling or rescheduling of the procedure after inclusion (n = 3). 29 of these 54 patients received APAP, 25 received placebo as adjuvant to intravenous morphine (Figure 2).

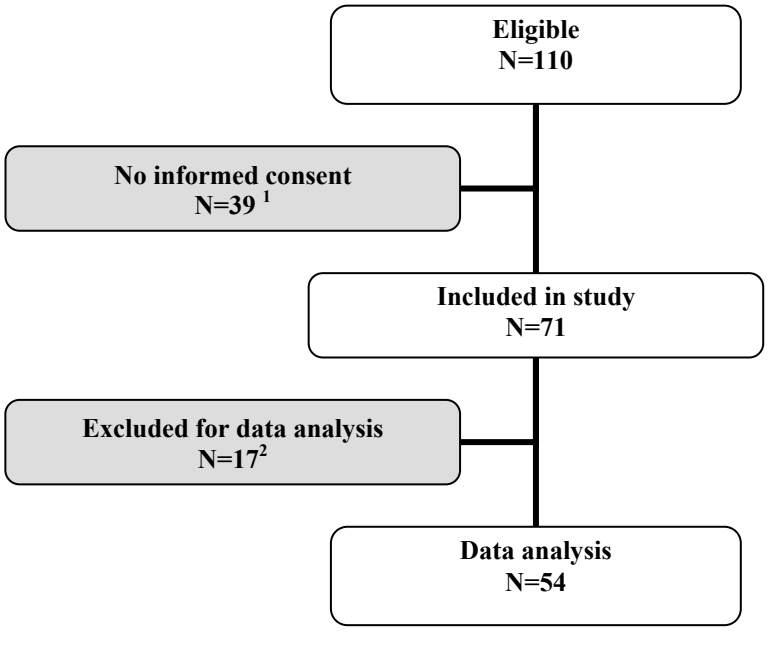

Figure 2 *Patients' flowchart*

1. Reasons for not obtaining informed consent: to much extra handling (n = 30), dislike of clinical trials (n = 6), no direct advantage (n = 2), language problems (n = 1)
2. Reasons for exclusion for data analysis: not receiving standard intervention as allocated (n = 10), withdrawal of parental informed consent during the study (n = 1), logistical problems (n = 3), OK canceling or rescheduling of the procedure after inclusion (n = 3)

Data analysis

Differences in morphine requirements were analyzed using the summary measurement approach.[18] Since data were too skewed and could not be transformed to normality, ordinal regression analysis was performed. For this purpose we calculated the average morphine requirements during the first 48 hours, followed by discretizing the average morphine requirements into four categories, using the 25th, 50th, and 75th percentile as cut off scores. Age of the infants, loading dose of morphine at the end of surgery and number of postoperative rescue doses of morphine were added as covariates. Differences in number of children needing postoperative rescue doses of morphine and increases in CMI were analyzed using Logistic regression analysis. Age was entered as covariate. In all analyses age was entered as a dummy variable, i.e. < 1 month versus ≥ 1 month of age; coded as 1 and 0, respectively.

Power analysis

It was expected that 40% of the patients in the APAP group would need extra morphine versus 80% in the placebo group. For a power of 80% [α = 0.05, (two-sided), Fisher's exact test] 27 patients were required in each group.[19] Seventy patients had to be included to compensate for dropouts.

Assignment and blinding

The randomization schedule, for APAP or placebo, was made prior to the study by random permuted blocks of four patients' assignment, to guarantee equal group sizes. The schedule was kept solely by the pharmacist to ensure blinding until the end of the study.

The study medication of patients receiving APAP consisted of APAP suppositories (with APAP and Witepsol H15, synthetic saturated triglycerides with a chain length of C12 - C18, as base). Study medication of patients receiving placebo consisted of placebo suppositories. As all children received suppositories (either APAP or placebo) manufactured in the department of the hospital pharmacy, patients and parents, nurses and investigators were blinded for the actual content of the suppositories.[20]

The composition of the placebo suppositories was equal to the composition of the APAP suppositories, except for the APAP. The APAP doses in the suppositories never deviated more than 10%. APAP was supplied by Bufa b.v., Uitgeest, the Netherlands. The suppositories and all ingredients met with the requirements in the European Pharmacopoeia. APAP suppositories were manufactured according to the Dutch Pharmacists Formulary. Stability of these preparations is tested by the laboratory of the Royal Dutch Association of Pharmacists.

Results

Analysis

Median (25-75th percentile) age and weight of patients receiving APAP (16 boys, 13 girls) and placebo (13 boys, 12 girls) as adjuvant to intravenous morphine were respectively: 0.0 (0.0-1.0) months, 3.1 (2.6-3.6) kg and 0.0 (0.0-2.5) months, 3.1 (3.8-5.2) kg.

Age, weight, gender, baseline heart rate (HR), baseline mean arterial pressure (MAP), operative procedure, peroperative blood loss, duration of the operative procedure, extra medication and mean duration of postoperative mechanical ventilation of the APAP, the placebo group and the group of patients excluded from analysis are shown in Table 1.
Most frequent performed abdominal, urological and thoracic surgical procedures were respectively: closure of diaphragmatic hernia, intestinal atresia, nefrectomy and esophageal atresia repair,

Pain scores

There were no significant differences between patients receiving APAP and placebo as adjuvant to intravenous morphine with respect to COMFORT scores and VAS scores.
VAS scores were low and showed a decline after the first 4 hours postoperatively (Figure 3a). In the APAP group lowest en highest median (25th–75th percentile) VAS scores in the first 4 hours postoperatively were

respectively 0.0 (0.0-0.3) and .2 (0.0-0.5). After the first 4 hours postoperatively lowest en highest median (25th–75th percentile) VAS scores were respectively 0.0 (0.0-0.1) and 0.1 (0.0-0.4). In the placebo group lowest en highest median (25th –75th percentile) VAS scores in the first 4 hours postoperatively were respectively 0.2 (0.0-1.2) and 0.3 (0.0-2.2). After the first 4 hours postoperatively lowest en highest median (25th –75th percentile) VAS scores were 0.0 (0.0-0.0) and 0.2 (0.0-1.0) respectively. VAS scores ranged from 0.0-6.6.

Although individual Comfort scores exceeded 17, cut off point for patients being in distress, median COMFORT scores were low (Figure 3b).[22] In the APAP group lowest and highest median (25th –75th percentile) COMFORT score were respectively 9.0 (8.0-11.0) and 12.0 (9.0-13.0). In the placebo group lowest and highest median (25th-75th percentile) COMFORT score were respectively 10.0 (9.0-12.0) and 12.0 (10.0-16.0). COMFORT scores ranged from 6-26.

Morphine consumption

Ordinal regression analysis, using the complementary logit link function, showed no significant differences between the APAP and placebo group in median (25th -75th percentile) total morphine consumption, respectively 7.91 (6.59-14.02) µg/kg/h and 7.19 (5.45-12.06) µg/kg/h for the APAP and the placebo group ($p = 0.60$). Age was not related to total morphine consumption ($p = 0.38$). However total morphine consumption was related to the amount of morphine loading dose and to additional morphine requirements postoperatively, being higher when infants had received a higher morphine loading dose ($p < 0.01$) or when additional morphine boluses or increases in CMI were needed ($p < 0.01$); pseudo R-square (Nagelkerke) was 0.66. Logistic regression showed no difference in additional morphine boluses, increases in CMI and decreases in CMI between APAP and placebo group ($p = 0.36$, $p = 0.06$ and $p = 0.51$ respectively).

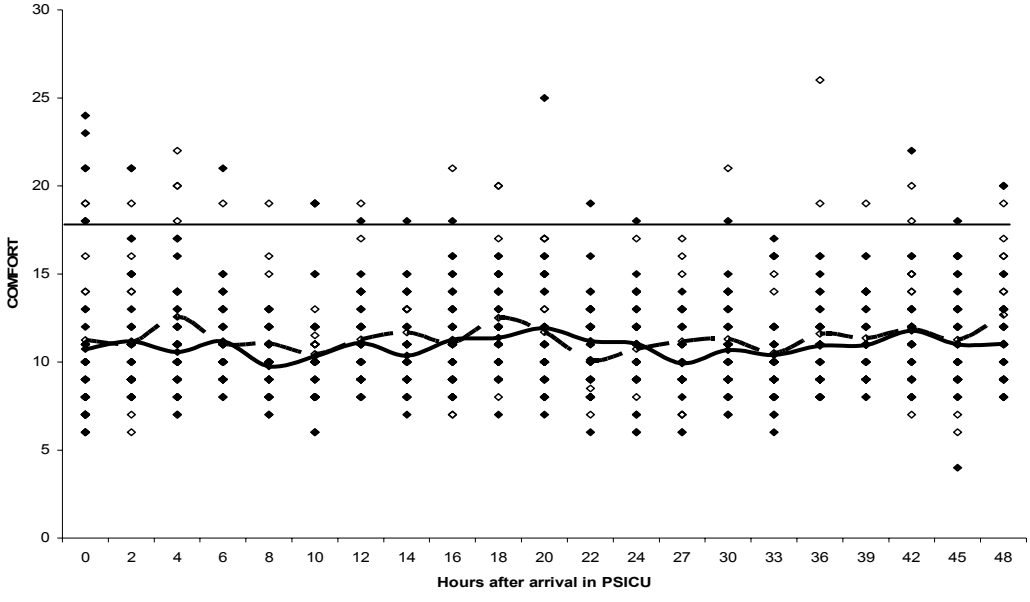

Figure 3a *COMFORT scores*

♦ *children receiving APAP as adjuvant to intravenous morphine*

——— *mean*

◊ *children receiving placebo as adjuvant to intravenous morphine,*

- - - - - - - *mean*

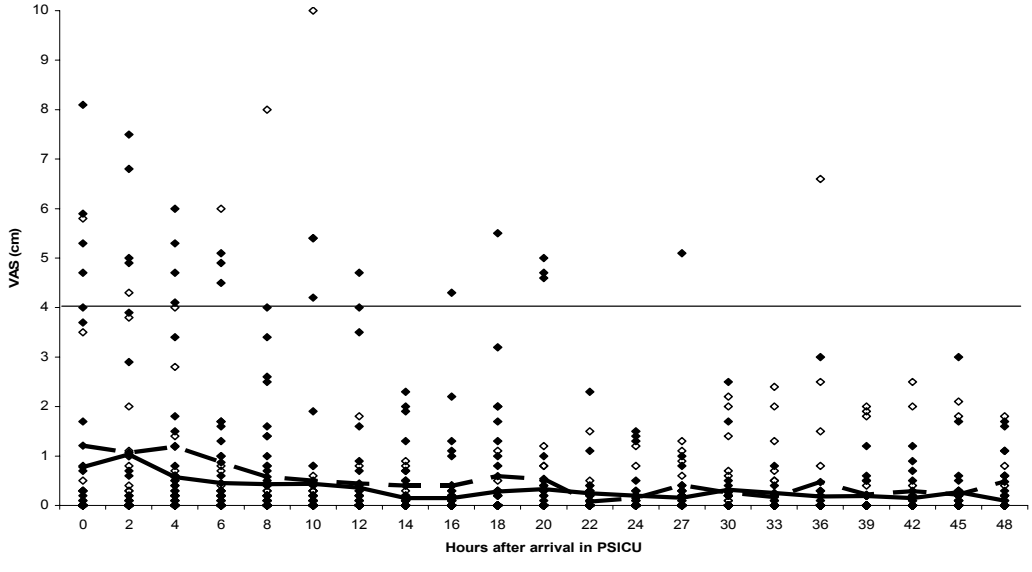

Figure 3b *VAS scores*

♦ *children receiving APAP as adjuvant to intravenous morphine*

——— *mean*

◊ *children receiving placebo as adjuvant to intravenous morphine,*

- - - - - - - *mean*

Table 1 Patients' characteristics

	Included for analysis (n = 54)		Excluded for analysis (n = 17)
	Placebo (n = 25)	APAP (n = 29)	(n = 17)
Age (months)*	0 (0-3) 0-9	(0-1) 0-10	3 (0-7) 0-8
Weight (kg)*	3.1 (2.8-5.2) 1.8-9.3	3.1 (2.6-3.6) 1.7-9.2	5.3 (3.5-6.5) 2.3-8.5
Gender			
Male	13	16	10
Female	12	13	7
Baseline heart rate (beats/min)*	132 (126-152) 118-169	136 (125-152) 99-167	144 (140-147) 138-150
Baseline mean arterial pressure (mm Hg)*	51 (49-64) 27-115	53 (46-61) 38-81	52 (45-56) 33-75
Operative procedures*			
Thoracic	7	7	2
Abdominal	18	19	11
Urological	0	3	4
Peroperative blood loss (ml)*	15 (10-30) 5-120	20 (5-40) 0-85	25 (10-70) 5-80
Duration of operative procedure (min)*	150 (135-233) 85-365	160 (120-210) 60-300	135 (120-205) 90-285
Extra medication			
Midazolam	8	10	-
Norcuron	3	1	-
Mean duration of postoperative mechanical ventilation (h)*	12.0 (0.0-25.5) 0.0-48.0	20.0 (2.0-42.0) 0.0-48.0	-

*Median (25th-75th percentile); Range

Regarding age, infants <1 month of age needed significantly less additional morphine boluses (n = 1/30) compared with infants ≥ 1 month of age (n = 10/24; $p < 0.01$); pseudo R-square (Nagelkerke) was 0.44. The number of

children needing an increase in CMI was significantly lower in infants < 1 month of age compared with infants ≥1 month of age (n = 3/30 versus (n = 16/24; p < 0.01); pseudo R-square (Nagelkerke) was 0.43. There was no significant difference in CMI decreases in the second 24 hours postoperatively between infants < 1 month (n = 18/30) and ≥ 1 month of age (n = 10/24; p = 0.69); pseudo R-square (Nagelkerke) was 0.06.

Paracetamol plasma concentrations

APAP plasma levels increased during the first hours postoperatively and then reached a steady state concentration. Individual APAP plasma levels varied widely (0.8-59.9 mg/l) (Figure 4).

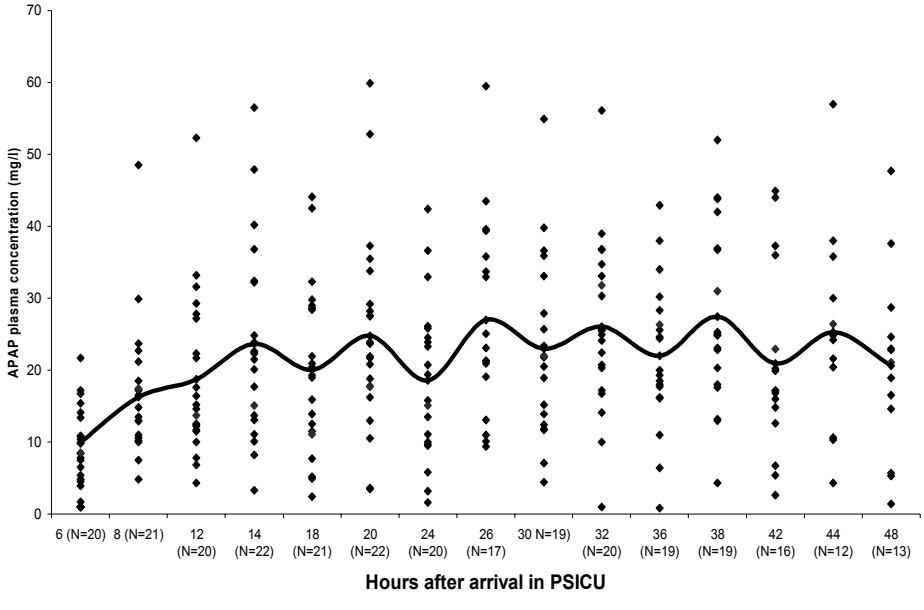

Figure 4 *APAP plasma levels*
♦ *Individual APAP plasma levels,*
——— *mean*

Discussion

Despite concerns about the adverse effects of morphine in neonates and infants, such as respiratory depression, morphine is the standard analgesic after major surgical procedures in young infants.[3,1] Combined analgesic regimens, leading to adequate analgesia with lower doses of opiods and subsequently reduced side effects have been proposed.[4]

Our study assessed the potential morphine sparing effect of APAP in infants 0-2 months of age after major thoracic or abdominal surgery. Patients receiving APAP and patients receiving placebo as adjuvant to intravenous morphine did not differ significantly with regards to additional morphine boluses (VAS ≥ 4), incidence of increases in CMI or incidence of CMI decreases in the second 24 hours postoperatively when VAS < 4. There was no significant difference in total morphine consumption between APAP and placebo group [median (25th-75th percentile) total morphine consumption respectively 7.91 (6.59-14.02) μg/kg/h and 7.19 (5.45-12.06) μg/kg/h]. The result that VAS scores were not significantly different between both groups supports the absence of a morphine sparing effect of APAP, since according to the study protocol extra morphine was administered when VAS ≥ 4. These findings are in line with the results of Morton, showing no morphine sparing effect of APAP in children 3-15 years of age following appendectomy.[11] In contrast studies in adults have reported a decrease in postoperative morphine consumption when morphine was combined with APAP.[5] Possibly the fact that the studies in adults were performed using self report, which was not possible in our study due to the age of our patients, might have been of influence on these results. Furthermore a placebo effect in adults can not be ruled out, reporting lower pain and using less morphine due to the placebo effect.

Patients ≥ 45 weeks PCA (n = 18) received a loading dose of 100 μg/kg followed by CMI of 10 μg/kg/h, based on a study showing that morphine infusion at a dose of 10.9-12.3 μg/kg/h provided adequate analgesia in children 0 to 3 years of age after major abdominal surgery. [14] Based on the

study of Kart et al, advising a CMI of 7 μg/kg/h, and the results of Bouwmeester et al, indicating that neonates had lower morphine requirements, children < 45 weeks PCA (n = 36) received a loading dose of 100 μg/kg, followed by a CMI of 5 μg/kg/h [22,23] Looking at the incidence of additional morphine requirements and increases in CMI we found them to be lower in patients < 1month of age, regardless of APAP or placebo group. This is consistent with the results of Bouwmeester et al, indicating that neonates had lower morphine requirements.[23] Based on our results 5 μg/kg/h CMI was sufficient for children < 1 month of age.

Although there was no relation between age and total morphine consumption, we did show that children ≥ 1 month of age had a higher incidence of additional morphine boluses and increases in CMI. The extra amount of morphine administered with additional morphine boluses and increases in CMI did not result in a statistical significant difference in total morphine consumption. Also the fact that the group of children < 45 weeks PCA consisted of 36 children, whereas the group of children ≥ 45 weeks PCA consisted of only 18 children, might have affected the results.

Most of the children included in this study had adequate analgesia, which makes it difficult to assess a dose-effect relationship. Pharmacodynamic variability might result from variability in distribution from the blood to the site of action and from variability in the sensitivity receptors. The contribution of variability in distribution from the blood to the site of action will depend largely on changes in perfusion of target tissue (5-60%). The sensitivity of receptors, defined in terms of affinity for binding or potency relative to another agent, may be an important source of variability in response when typical concentrations produce effects that are less than 80% maximal. Typical values are 5% (effect > 80% of Emax) and 50% (effect > 20% of Emax). The imprecision and bias in the EC_{50} is increased if observed effect intensity is low compared to predicted Emax. A typical value for inter-individual variability in efficacy is 30%. The observed response may not be a direct consequence of drug-receptor binding, but rather through intermediate physiological mechanisms (e.g. antipyretics,

angiotensin converting enzyme inhibitors). A typical value for this variability is 30%.[24]

Individual APAP plasma concentrations showed wide variability (range 0.8-59.9 mg/l), despite dosing equivalence. This is well recognized by others and is attributable, in part, to absorption variability, size effects and genetic polymorphisms interfering with APAP metabolism, such as from CYP2E1 and CYP3A4.[20,25-28]

Population pharmacokinetic parameter estimates are associated with considerable variability. Factors affecting this variability are absorption, tissue distribution, metabolic elimination and renal elimination. Holford and Peck report typical values of 30% for absorption, 10% for tissue distribution, 50% for metabolic elimination and 20% for renal elimination.[24] They also report a value of 50% for compliance with medication regimens.[24] This however was not an issue in our study. These parameter variabilities contribute to the large concentration ranges seen after a rectal APAP dosing.[20,25,27] The use of concentration to link dose and effect allows PK variability to be separated from PD variability. The reduction in total variability produced by the removal of the PK component has been estimated to be 50% or greater.

Many clinical studies in which APAP is compared to another analgesic are destined either to fail showing a difference between the two analgesic treatments or to have inadequate power because pain score reporting methods, the pain stimulus and pharmacokinetic and pharmacodynamic parameters all have large variability.[27]

Conclusion

APAP as adjuvant to CMI does not have an additional analgesic effect and should not be considered as standard of care in young infants, 0-2 months of age, following major thoracic or abdominal surgery.

Acknowledgements

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4

Pharmacogenetic studies

Chapter 4.1

The impact of pharmacogenetics on the pharmacokinetics and metabolism of paracetamol in children

Caroline D van der Marel, Jeroen WB Peters, Ron H van Schaik, Marien AL Pluim, John N van den Anker, Dick Tibboel

Abstract

Introduction

Paracetamol (APAP) is metabolized through glucuronidation, sulphation and oxidation. A negligible part of APAP is hydroxylated into 3'-hydroxy-APAP. In the glucuronidation UGT1A6 and to a far lesser extend UGT1A9 are involved in the formation of APAP-glucuronide, whereas in the formation of N-acetyl-p-benzoquinone-imine (NAPQI) through oxidation CYP2E1, CYP3A4, CYP1A2 and possibly CYP3A5 are involved. Since NAPQI is the APAP metabolite responsible for the toxic effects and CYP2E1, CYP3A4 and possibly CYP3A5 are the enzymes responsible for the majority of the oxidation, we conducted a pilot study to analyze the relation between APAP clearance and CYP3A4, CYP3A5 and CYP2E1 genotype in children receiving APAP during participation in previously reported studies. Furthermore we evaluated the relation between age and clearance to account for developmental changes.

Methods

Children received an APAP loading dose followed by administration of APAP maintenance doses for postoperative analgesia. Blood samples were obtained for APAP plasma concentration analysis. To obtain genomic DNA, either a blood sample was collected or buccal swabs were used. DNA analysis was performed for CYP3A4, CYP3A5 and CYP2E1 genotype.

Results

115 patients were eligible for this study. Informed consent for DNA analysis was obtained from 106 patients. In 57 of these patients both genotyping was performed and a sufficient number of blood samples was collected to allow us to calculate APAP clearance. Therefore we were able to study 57 children in this study. Median (25th-75th percentile) age and weight of the patients were respectively 10 (1-11) months and 8.5 (3.5-10.4) kg. The study group consisted of 32 boys and 25 girls. Median (25th-75th percentile) APAP clearance was 1.1 (0.5-2.6) l/h. APAP plasma concentrations ranged from 0-59.9 mg/l.

We could not establish a relation between APAP clearance and CYP3A4, CYP3A5 and CYP2E1 genotype. APAP clearance increased with age.

Conclusion
This pilot study showed no relation between APAP clearance and CYP3A4, CYP3A5 and CYP2E1 genotype. APAP clearance increased with age. Since population allelic frequencies of the mutations are low, further research in a larger sample of patients is needed for complete analysis of the relation between APAP and CYP3A4, CYP3A5 and CYP2E1 genotype.

Introduction

A wide variability in paracetamol (APAP) plasma concentrations is reported after APAP administration,[1,2,3] resulting from variability in absorption,[1] size effects,[1] and from variability in enzyme activity of the enzymes involved in APAP metabolism. Differences in enzyme activity might result both from developmental changes in enzyme activity as from genetic polymorphisms leading to an altered enzyme activity.[4]
APAP is metabolized through glucuronidation, sulphation and oxidation (Figure 1).[5] A negligible part of APAP is hydroxylated into 3'-hydroxy-APAP.[5] In the glucuronidation UGT1A6 and to a far lesser extend UGT1A9 are involved in the formation of APAP-glucuronide,[6] whereas in the formation of N-acetyl-p-benzoquinone-imine (NAPQI) through oxidation CYP2E1, CYP3A4, CYP1A2 and possibly CYP3A5 are involved.[4,5,7] NAPQI is the APAP metabolite responsible for the toxic effects and is conjugated with glutathion. When there is not enough glutathion present to conjugate all NAPQI, NAPQI will bind with hepato-cellular proteins and will cause liver necrosis.[5] Glucuronidation and sulphation are the major pathways of APAP metabolism, whereas APAP metabolism through oxidation will increase when high or toxic doses of APAP are administered and metabolism through sulphation and glucuronidation is rate limiting. Unchanged APAP and APAP metabolites are all excreted in urine.

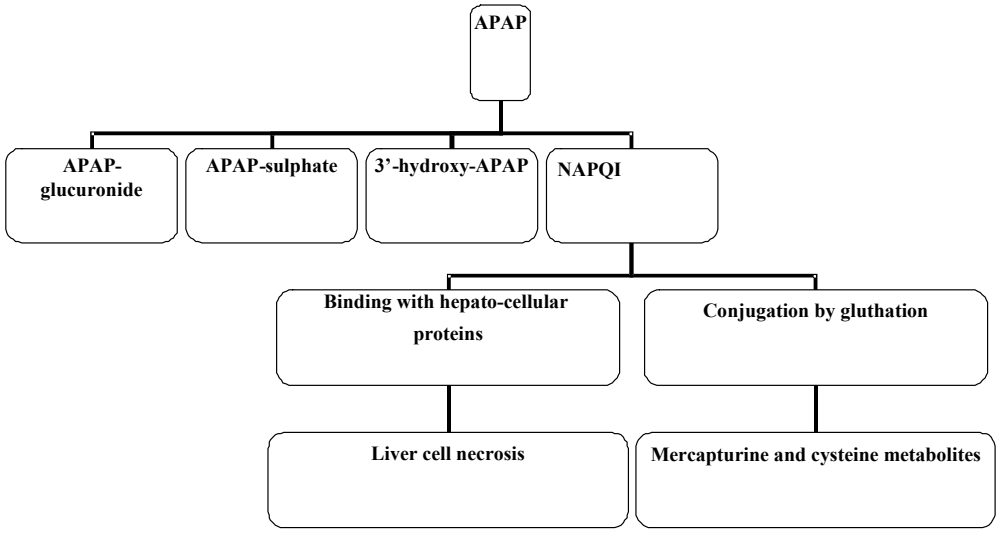

Figure 1 *APAP metabolism*

Miller studied the contribution of the different pathways of APAP metabolism at different ages. In neonates sulphation is the major pathway and contributes 50% towards total APAP metabolism, whereas glucuronidation and oxidation contribute respectively 18 and 28%.[8] Compared to neonates, the contribution of sulphation has slightly decreased to 44% in children 3-9 years of age, whereas the contribution of glucuronidation has increased to 30 %. The contribution of the oxidation at this age is 21%.[8] In children 12 years of age, the contribution of sulphation has been further decreased to 30%, and glucuronidation has been further increased to 45%. The contribution of the oxidation at this age is 20%.[8] In adults, the contribution of sulphation is still 30% and glucuronidation has further increased to 50%. Oxidation accounts for 16%.[8] The percentage of APAP excreted unchanged is equal at the different ages, approximately 4-5%.

Evaluating the ratio between APAP glucuronidation and APAP sulphation, an increase from 0.12 in preterm infants 28 to 32 weeks gestational age[9] to 0.28 in preterm infants 32 to 36 weeks gestational age[9] and 0.34 in newborns[8] is reported. We studied infants with a mean age of 10 months and showed a ratio of 0.69.[10] This is comparable with a ratio of 0.75 found in infants 3-9 years of age.[8,11] In 12 year olds and adults, ratios were

respectively 1.69 and 1.8.[8] The contribution of glucuronidation towards total APAP metabolism increases with age.

Since NAPQI is the APAP metabolite responsible for the toxic effects and CYP3A4, CYP2E1 and possibly CYP3A5 are the enzymes responsible for the majority of the oxidation, we conducted a pilot study to analyze the relation between APAP clearance and CYP3A4, CYP2E1 and CYP3A5 genotype in children receiving APAP during participation in previously reported studies performed at the Erasmus MC Rotterdam.[10, 12, 13] Furthermore we evaluated the relation between age and clearance to account for developmental changes.

Patients and methods

Following approval of the study by the Medical Ethical Committee of the Erasmus MC Rotterdam and after separate written informed consent for DNA analysis was obtained from the parents of the children participating in 3 of our previously reported trials,[10,12,13] in which APAP was administered and APAP plasma concentrations were measured.

I. 45 children were included in a randomized controlled trial comparing the analgesic effect of rectal versus oral APAP following major craniofacial surgery (chapter 3.1). Children received a rectal loading dose of 40 mg/kg directly after induction of anesthesia, followed by 20 mg/kg APAP either rectally or orally postoperatively. APAP plasma concentrations were measured 30, 60 and 90 minutes after loading dose administration, and 1 hour prior to and 2 hours after APAP maintenance dose administration during the first 24 hours postoperatively. Median (25th-75th percentile) age was 10 (9-11) months (Figure 2).[10]
II. 71 children were included in a randomized controlled trial evaluating the potential morphine sparing effect of APAP following major abdominal and thoracic surgery (chapter 3.3). 37 children were eligible for this current study, receiving a rectal APAP loading dose of 30-40 mg/kg directly after induction of anesthesia, followed by 20 mg/kg 6-

hourly postoperatively. APAP plasma concentrations were sampled 30 and 90 minutes after loading dose and just prior to and 2 hours after APAP maintenance dose administration for the first 48 hours postoperatively. Median (25th-75th percentile) age was 0 (0-2) months (Figure 2).[12]

III. 65 children were included in a randomized controlled trial comparing the analgesic effect of rectal APAP versus rectal diclofenac for postoperative analgesia following (adeno)tonsillectomy (chapter 3.2), of which 33 children were eligible for this currents study, receiving APAP 40 mg/kg 90 minutes prior to surgery. APAP plasma concentrations were measured directly after induction of anesthesia, at the end of the procedure and 1, 2 and 3 hours postoperatively. Median (25th-75th percentile) age was 4 (3-5) years (Figure 2).[13]

Procedure

Children received an APAP loading dose according to the study protocol of the study in which they participated, followed by administration of APAP maintenance doses. Blood samples were obtained for APAP plasma concentration analysis according to the study protocols. For DNA analysis, either a blood sample (1 ml) was obtained directly after induction of anesthesia or buccal swaps were used to obtain DNA.

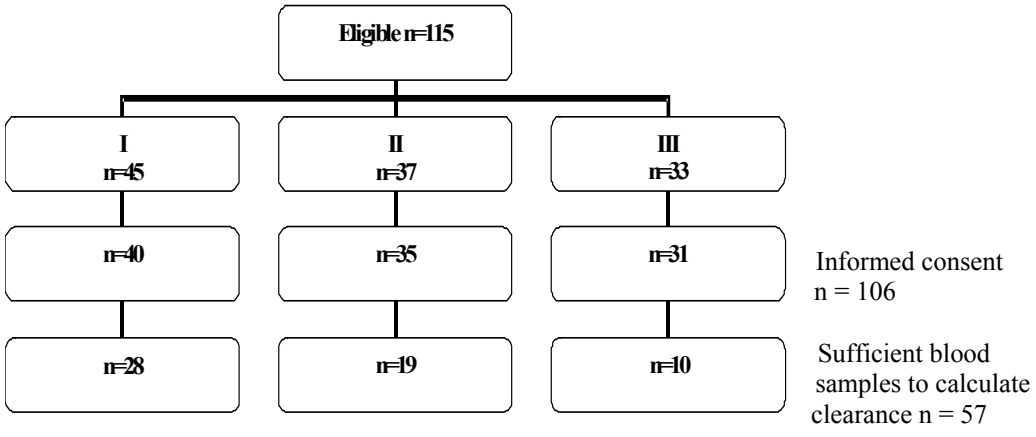

Figure 2 *Patients' flowchart*
I patients undergoing major craniofacial surgery[10]
II patients undergoing major abdominal or thoracic surgery[12]
II patients undergoing (adeno)tonsillectomy[13]

APAP assay

Plasma samples were stored at 4 °C until analysis. APAP plasma concentrations were determined using fluorescence polarization immunoassay (ADX systems, Abbott Laboratories, North Chicago, IL) (Erasmus MC Rotterdam). The APAP plasma determination limit was 1.0 mg/l which was defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Precision was measured at APAP plasma concentrations of 15, 35 and 150 mg/l; 55 samples of each concentration were assayed to determine coefficients of variation at these concentrations (CV = SD/mean; RSD = CV*100%). RSDs at these concentrations were 7.22%, 3.37% and 3.11% respectively. The APAP plasma concentration range in which accuracy was measured was 10-150 mg/l.

DNA analysis

DNA analysis for was performed isolating genomic DNA from blood using GenomicPrep Blood DNA Isolation Kit (Amersham Pharmacia Biotech, Buckinghamshire, UK). DNA samples were analyzed for CYP3A4*1b, CYP3A5*3, CYP2E1*1d, CYP2E1*2, CYP2E1*5 and CYP2E1*7 mutations using PCR-RFLP method developed by van Schaik et al.[14]

Data analysis

APAP clearances were calculated using the Area Under the Curve (AUC).[15]Analysis of the relation between clearance and genotype was performed using cross tabs after discretizing clearances based on the interquartile ranges. For CYP3A4 genotype, we compared patients being wild type (*1/*1) to patients being heterozygous (*1/*1b) and to patients being homozygous (*1b/*1b) for CYP3A4*1b. For CYP3A5 genotype, we compared patients being wild type (*1/*1) or heterozygous (*1/*3) to patients being homozygous (*3/*3), as only patients being wild type or being heterozygous have CYP3A5 activity.[16] For CYP2E1 we compared patients being wild type (*1/*1) to patients being hetero- or homozygous for CYP2E1*1d (*1/*1d; *1d/*1d), CYP2E1*2 (*1/*; *2/*2), CYP2E1*5

(*1/*5; *5/*5), CYP2E1*7 (*1/*7; *7/*7) and for CYP2E1*7 and CYP2E1*1d (*1d/*7).

Results

Participant flow and follow up

115 patients were eligible for this current analysis. Informed consent for DNA analysis was obtained for 106 patients; the parents of 9 children refused informed consent, as they did not see any additional value in DNA analysis for their children. Blood sampling was not sufficient for calculation of clearances in 49 patients. Therefore data of 57 children were available for DNA analysis and plasma concentration analysis (Figure 2).

Patients' characteristics

Median (25th-75th percentile) age and weight of the patients were respectively 10 (1-11) months and 8.5 (3.5-10.4) kg. The study group consisted of 32 boys and 25 girls. Median (25th-75th percentile) APAP clearance was 1.1 (0.5-2.6) l/h (Table 1, Figure 3).

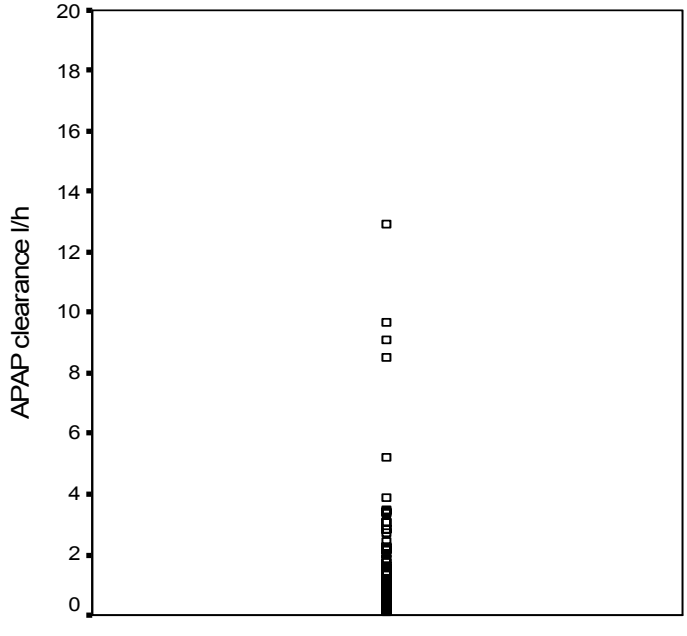

Figure 3 *APAP clearance*

Table 1 Patients' characteristics

Age (years)[1]		10 (1-11)
		0-72
Weight (kg)[1]		8.5 (3.5-10.4)
		1.7-31.0
Gender	Male	32
	Female	25
APAP clearance (l/h)[1]		1.1 (0.5-2.6)
		0.1-12.9

[1] median (25th-75th percentile); range

APAP plasma concentrations

I. Median (25th-75th percentile) APAP plasma concentration in the children undergoing major craniofacial surgery was 9.0 (5.3-13.5) mg/l, ranging from 0.2-26.1 mg/l (Figure 4a).[10]
II. Median (25th-75th percentile) APAP plasma concentration in the children undergoing major abdominal or thoracic surgery was 20.8 (12.4-29.8) mg/l, ranging from 0.0-59.9 mg/l (Figure 4b).[12]
III. Median (25th-75th percentile) APAP plasma concentration in the children undergoing (adeno)tonsillectomy was 14.2 (11.0-18.9) mg/l, ranging from 4.8-29.4 mg/l (Figure 4c).[13]

DNA analyses

DNA analysis was performed for CYP3A4*1b, CYP3A5*3, CYP2E1*1d, CYP2E1*2, CYP2E1*5 and CYP2E1*7. 57 samples were available for analysis. Considering CYP3A4, 65% (n = 37) of the patients were wild type (*1/*1), 4% (n = 2) of patients were heterozygous (*1/*1b) and 4% (n = 2) of patients were homozygous (*1b/*1b) for CYP3A4*1b. Quality of buccal swaps was not sufficient to perform CYP3A4 genotyping in 28% (n = 16) of the patients (Figure 5a).

Considering CYP3A5, 7% (n = 4) of the patients were wild type (*1/*1), 7% (n = 4) of patients were heterozygous (*1/*3) and 54% (n = 31) of patients were homozygous (*3/*3) for CYP3A5*3. Quality of buccal swaps was not sufficient to perform CYP3A5 genotyping in 32% (n = 18) of the patients (Figure 5b).

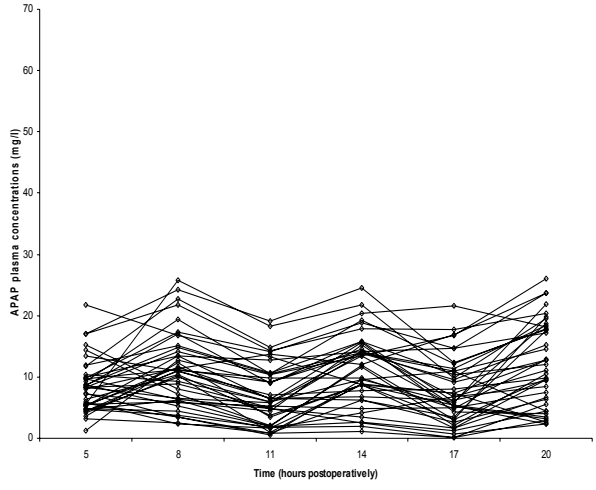

Figure 4a *APAP plasma concentrations in children undergoing major craniofacial surgery*

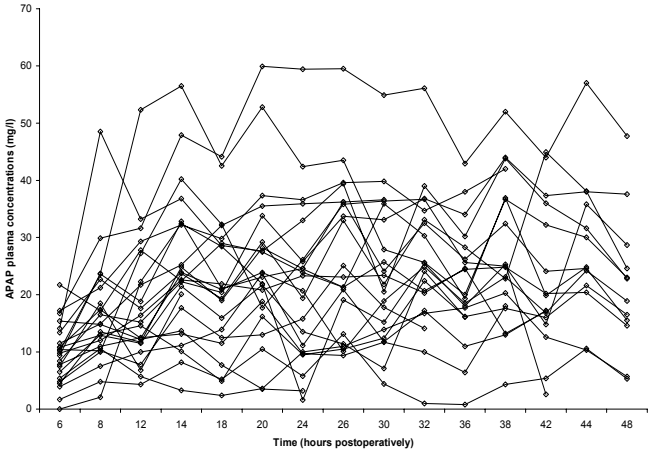

Figure 4b *APAP plasma concentrations in children undergoing major abdominal or thoracic surgery*

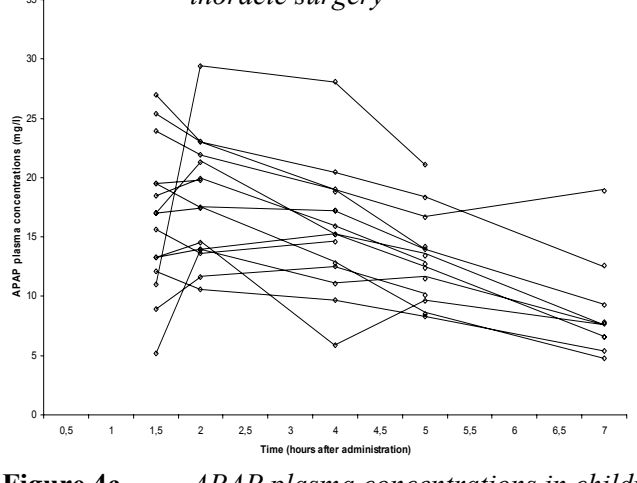

Figure 4c *APAP plasma concentrations in children undergoing (adeno) tonsillectomy*

Analyzing CYP2E1, 55% (n = 31) of the patients were wild type (*1/*1), 2% (n = 1) was heterozygous for CYP2E1*2 (*1/*2), 2% (n = 1) was heterozygous for CYP2E1*5 (*1/*5), 13% (n = 7) was heterozygous for CYP2E1*7 (*1/*7) and 2% (n = 1) was heterozygous for CYP2E1*7 and CYP2E1*1d (*1d/*7). Quality of buccal swaps was not sufficient to perform CYP2E1 genotyping in 26% (n = 12) of the patients (Figure 5c).

Relation APAP clearance, genotyping and age

Genotyping of the outliers in APAP clearance shows CYP3A4 and CYP2E1 wild type (*1/*1) and CYP3A5*3 homozygous (*3/*3) for the patient with an APAP clearance of 8.5 l/h; CYP3A4*1b homozygous (*1b/*1b), CYP3A5 wild type (*1/*1) and CYP2E1*7 heterozygous (*1/*7) for the patient with an APAP clearance of 9.1 l/h; CYP3A4 and CYP2E1 wild type (*1/*1) and CYP3A5*3 homozygous (*3/*3) for the patient with an APAP clearance of 9.7 l/h and CYP3A4 wild type (*1/*1), CYP3A5*3 homozygous (*3/*3) and CYP2E1*7 heterozygous (*1/*7) for the patient with an APAP clearance of 12.9 l/h (Table 2).

There was no relation between APAP clearance and CYP3A4, CYP3A5 and CYP2E1 (Figure 6a, 6b & 6c). Table 3 shows the number of patients being wild type, heterozygous or homozygous for CYP3A4, CYP3A5 and CYP2E9 genotype in the different interquartile ranges of APAP clearance (Table 3). APAP clearance increases with age (Figure 7).

Table 2 Genotyping of outliers in clearances

		CYP3A4	**CYP3A5**	**CYP2E1**
APAP clearance (l/h)				
	8.5	*1/*1	*3/*3	*1/*1
	9.1	*1b/*1b	*1/*1	*1/*7
	9.7	*1/*1	*3/*3	*1/*1
	12.9	*1/*1	*3/*3	*1/*7

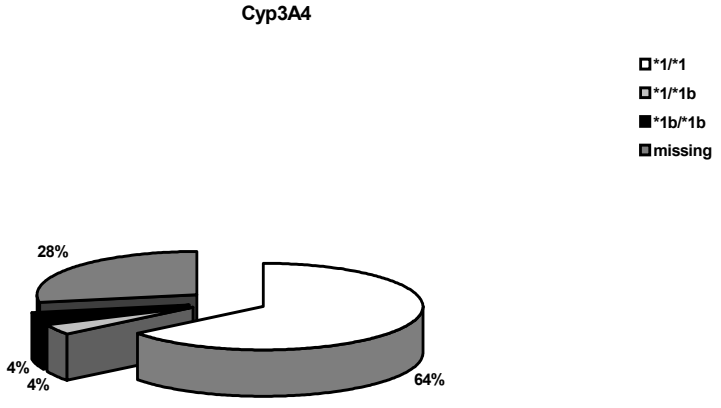

Figure 5a

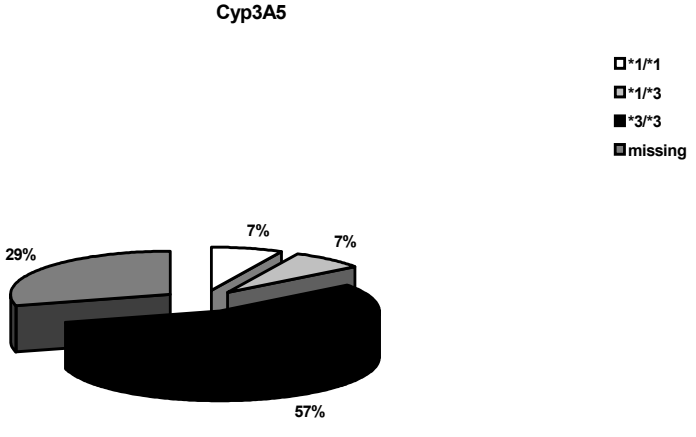

Figure 5b

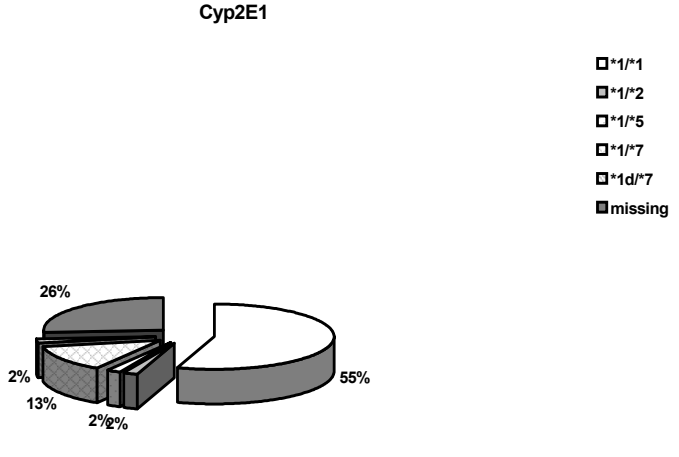

Figure 5c

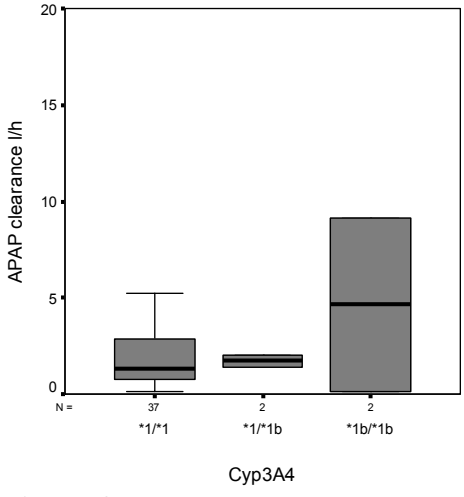

Figure 6a

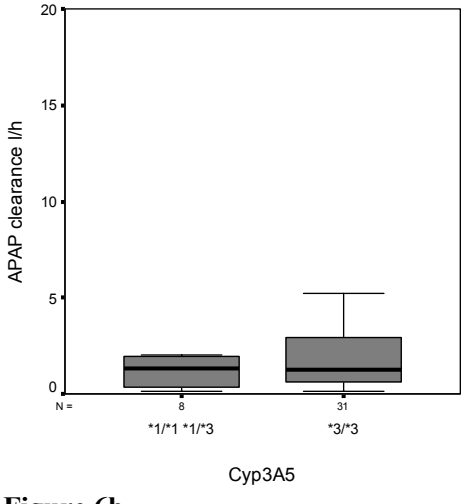

Figure 6b

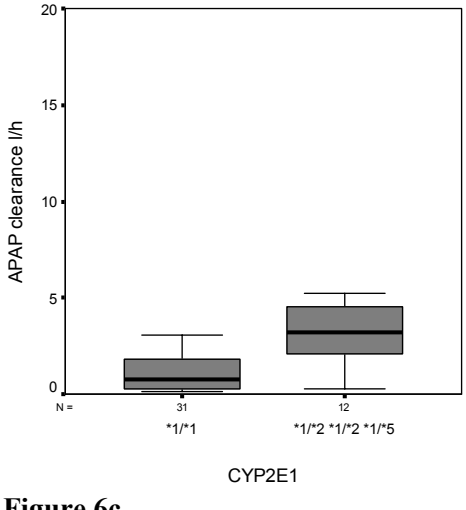

Figure 6c

Table 3 CYP3A4, CYP3A5 and CYP2E1 genotype and APAP clearance

Interquartile ranges of APAP clearance		**1st**	**2nd**	**3rd**	**4th**
CYP3A4	*1/*1	1	1	1	1
	*1/*1b	2	0	2	0
	*1b/*1b	6	9	6	10
CYP3A5	*1/*1	0	0	2	0
	*1/*3	1	0	0	1
	*3/*3	3	14	9	11
CYP2E1	*1/*1	11	8	6	6
	*1/*2	-	-	1	-
	*1/*5	-	-	-	1
	*1/*7	1	1	1	4
	*1d/*7	-	-	-	1

Discussion

This is the first study that investigates the relationship between genotype and APAP clearance in children. In this pilot study we could not establish a relationship between CYP3A4, CYP3A5 or CYP2E1 genotype and APAP clearance. The number of patients in this pilot study is limited and therefore we can not draw definite conclusions with regards to the relationship between CYP3A4, CYP3A5 or CYP2E9 genotype and APAP clearance.

The fact that we could not establish a relation between APAP clearance and CYP3A4, CYP3A5 and CYP2E1 genotype is in line with the fact that only 21-28% of APAP is metabolized through oxidation at this age.[8] The contribution of the oxidation to APAP metabolism is minor compared to the contribution of the glucuronidation and sulphation pathway, and only increases when glucuronidation and sulphation are rate limiting in case high or toxic doses of APAP are administered. Thummel et al reported that CYP3A4 has a higher affinity for APAP, whereas CYP2E1 has a greater capacity to form NAPQI.[7] CYP3A4 is mainly involved in oxidation when APAP doses within the normal dosing range are administered, compared to CYP2E1 being mainly involved in the oxidation when high or toxic doses are administered.[7] APAP doses administered in this study were within the

normal dosing range (90 mg/kg/day). Therefore it was to be expected that APAP metabolism occurred mainly through glucuronidation and sulphation, explaining the absence of a relation between APAP clearance and CYP3A4, CYP3A5 and CYP2E1 genotype.

Furthermore the small number of patients and the relatively low population allelic frequencies (Table 4) of the mutations analyzed in this study could have contributed to the fact that we could not establish a relation between APAP clearances and CYP3A4, CYP3A5 and CYP2E1 genotype. This is in line with the results reported by de Wildt et al,[24] showing no relationship between the presence or absence of CYP3A4*1b alleles and midazolam clearances in preterm born infants 26.0-33.6 weeks gestational age. Although the frequency of CYP3A4*1b in the Caucasian population is 5.3% (van Schaik et al, 2000), the number of patients used by the Wildt et al was low (n = 29), making it difficult to establish a potential relationship between midazolam clearance and the presence or absence of CYP3A4*1b alleles.

Table 4 Allelic frequencies of CYP3A4*1b, CYP3A5*3, CYP2E1*1d, CYP2E1*2, CYP2E1*5, CYP2E1*7

	Population
CYP3A4*1b	
Caucasians[14]	5.3%
US Caucasians[17]	9.6%
African Americans[18]	54.6%
CYP3A5*3	
Caucasians[16]	91%
Japan[19]	71%
African Americans[19]	27%
CYP2E1*1d	
Caucasians[20]	3.5%
China[21]	23%
CYP2E1*2	
China[22]	1.3%
CYP2E1*5	-
CYP2E1*7	
North Europe[23]	20%

When APAP is administered in high or toxic doses, metabolism through oxidation will increase. More NAPQI will subsequently be formed, leading to toxic effects if not enough glutathion is present to detoxify NAPQI. Patients will be more susceptible to toxic APAP effects, when either glutathion stores are reduced or glucuronidation pathway is compromised, for example in situations of acute protein-calorie malnutrition,[25] or when enzyme activity is altered. When altered enzyme activity in the glucuronidation pathway leads to a decreased glucuronidation and sulphation is rate limiting, the contribution of the oxidation pathway increases, possibly leading to more toxic effects. When enzyme activity is altered in the oxidation pathway, this might directly lead to an increased or decreased NAPQI formation and thus affecting the clinical outcome of APAP overdoses.

Conclusion

This pilot study showed no relation between APAP clearance and CYP3A4, CYP3A5 and CYP2E1 genotype. APAP clearance increased with age. Since population allelic frequencies of the mutations are low, further research in a larger sample of patients is needed for complete analysis of the relation between APAP and CYP3A4, CYP3A5 and CYP2E1 genotype.

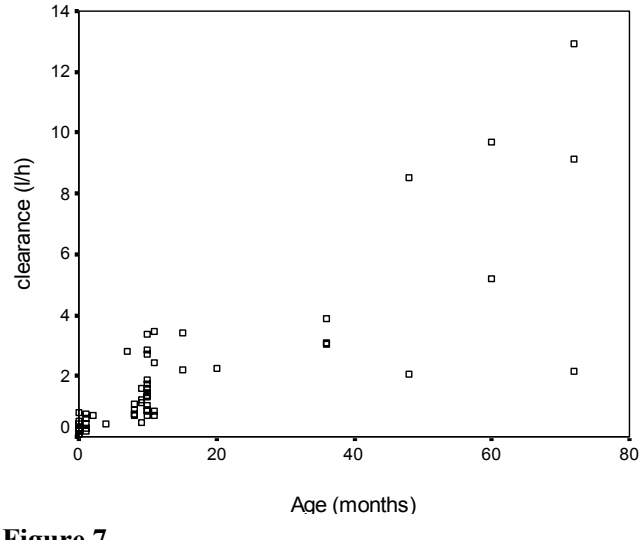

Figure 7

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Chapter 4.2

The impact of pharmacogenetics on the pharmacokinetics and metabolism of diclofenac in children

Caroline D van der Marel, Jeroen WB Peters, Ron H van Schaik, John N van den Anker, Dick Tibboel

Abstract

Introduction

Data considering the pharmacokinetics of diclofenac in children are scant. Studies have mainly focused on measuring diclofenac plasma concentrations. However, one of the diclofenac metabolites, 4'-hydroxy-diclofenac (D4OH), has activity as well. Diclofenac is metabolized into D4OH by CYP2C9, whereas metabolism into 5'-hydroxy-diclofenac (D5OH) occurs through CYP3A4 and possibly through CYP3A5. We conducted a pilot study to investigate the relation between diclofenac clearance and CYP2C9, CYP3A4 and CYP3A5 genotype, between D4OH formation clearance and CYP2C9 genotype and between D5OH formation clearance and CYP3A4 and CYP3A5 genotype.

Methods

Children (n = 32), 3 to 8 years of age, received a loading dose of 2 mg/kg diclofenac rectally 30 minutes before scheduled elective (adeno)tonsillectomy. Blood samples (1 ml) for the analysis of diclofenac, D4OH and D5OH plasma concentrations were obtained directly after induction, at the end of the procedure and 1, 2 and 3 hours after awakening. For DNA analysis, a blood sample (1 ml) was obtained directly after induction of anesthesia. Data analysis was performed using cross tabs after discretizing clearances based on the interquartile ranges.

Results

Blood samples were not available in 6 patients, hence data of 26 patients were analyzed, 14 boys and 12 girls. Median (25th-75th percentile) age and weight of the patients were respectively 4.0 (3.8-5.0) years and 20.0 (17.8-22.7) kg. Diclofenac plasma concentrations ranged from 0-2.6 mg/l, D4OH plasma concentrations ranged from 0-0.5 mg/l and D5OH plasma concentrations ranged from 0-0.6 mg/l. We could not establish a relation between diclofenac clearance and CYP3A4, CYP3A5, CYP2C9 genotype. Nor could we establish a relation between D4OH formation clearance and

CYP2C9 or between D5OH formation clearance and CYP3A4 and CYP3A5.

Conclusion
This pilot study showed no relation between diclofenac clearance and CYP3A4, CYP3A5, CYP2C9 genotype. Nor did it show a relation between D4OH formation clearance and CYP2C9 or between D5OH formation clearance and CYP3A4 and CYP3A5.

Introduction

Data on diclofenac pharmacokinetics in children are scant. Only a few studies have included the analysis of diclofenac plasma concentrations. Rømsing reported diclofenac plasma concentrations in children 5 to 15 years of age following administration of single oral doses (1-2 mg/kg) of diclofenac.[1] Although diclofenac has an active metabolite, 4'-hydroxy-diclofenac (D4OH), no data are available of clinical studies that have investigated diclofenac metabolite plasma concentrations.

Diclofenac belongs to the Non-Steroidal Anti Inflammatory Drugs (NSAIDs) and is almost completely metabolized and eliminated in bile (30-35%) and urine (50-70%). Only a small part (0.5%) is eliminated unchanged in urine. Ten percent of diclofenac is glucuronidated as an intact molecule and eliminated in urine (5-10%) and in bile (< 5%). The largest part of diclofenac is hydroxylated first, after which it is conjugated with glucuronic acid, sulphic acid, taurine or other ligands.[2]

The main metabolite of diclofenac is D4OH, which is formed through hydroxylation by CYP2C9. This hydroxylation can be inhibited by sulfaphenazol, phenytoin and warfarin. After hydroxylation into D4OH, D4OH is glucuronidated and eliminated in urine (20-30%) and in bile (10-20%).[2]
After hydroxylation of diclofenac into 3'-hydroxy-diclofenac (D3OH), D3OH is conjugated with glucuronic acid, whereas hydroxylation of

diclofenac into 5'-hydroxy-diclofenac (D5OH) and into 4',5'-dihydroxy-diclofenac can also be followed by conjugation with the amino acid taurine or with sulphic acid. After conjugation, these metabolites are eliminated in urine (10-20%) and bile (5-6%).[2] The enzymes responsible for hydroxylation into D5OH are CYP3A4 and possibly CYP3A5.[3] CYP3A4 activity is influenced by age, medication, disease state, nutrition and, potentially, genotypic expression.[3-6]

The 3'-hydroxy-4'methoxydiclofenac metabolite ($t_{½}$ 80 h) of diclofenac is eliminated only in small amounts in urine (1.4%) and accumulates, resulting in high concentrations after 6-10 months in case of prolonged use.[2]
Diclofenac has 2 active metabolites, D4OH AND D3OH. D4OH has an anti-inflammatory effect (30% of the activity of diclofenac), whereas D3OH has a weak anti-inflammatory effect as well. Other diclofenac metabolites are pharmacologically inactive.[2] Figure 1 represents diclofenac metabolism schematically.

There are no studies in the literature evaluating the effect of CYP3A4, CYP3A5 and CYP2C9 genotype on diclofenac clearance, D4OH formation clearance and D5OH formation clearance. Genotype might be linked to an increased or decreased enzyme activity in individuals hetero- or homozygous for certain mutations in specific enzymes. Therefore we conducted a pilot study in children receiving rectal diclofenac for postoperative analgesia and determined diclofenac, D4OH and D5OH plasma concentrations and analyzed DNA for CYP3A4*1b, CYP3A5*3, CYP2C9*2 and CYP2C9*3, to evaluate the effect of CYP3A4, CYP3A5 and CYP2C9 genotype on diclofenac clearance, D4OH formation clearance and D5OH formation clearance.

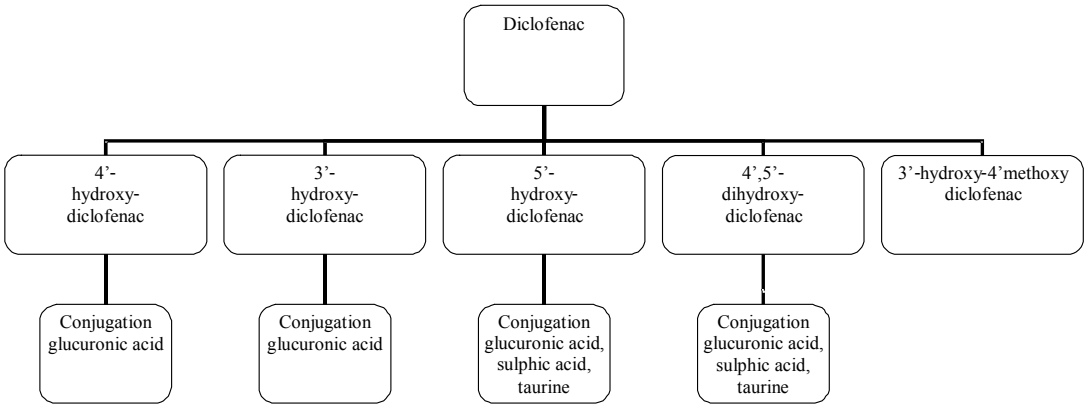

Figure 1 *Diclofenac metabolism*

Patients and methods

Following approval of the study by the Medical Ethical Committee of the Erasmus MC Rotterdam and after written informed consent was obtained from the parents, 65 children were included in a randomized controlled trial comparing the analgesic effect of rectal paracetamol (APAP) versus rectal diclofenac for postoperative analgesia following (adeno)tonsillectomy.[7] Inclusion criteria were: age between 3 and 12 years, elective (A)TE and ASA status 1 or 2. Exclusion criteria were: coagulopathy, diclofenac or APAP < 24 hours prior to surgery, known allergy for diclofenac or APAP, hepatic diseases interfering with drug metabolism and abnormal renal function.

Patients receiving diclofenac (n = 32) were eligible for this current analysis, identifying the relation between genotype and diclofenac clearance, D4OH formation clearance and D5OH formation clearance. Separate informed consent was obtained for DNA analysis. No patients received drugs known to alter the activity of CYP3A4 or CYP2C9.

Procedure

Children received a loading dose of 2 mg/kg diclofenac rectally 30 minutes before scheduled (A)TE. Blood samples (1 ml) for the analysis of diclofenac, D4OH and D5OH plasma concentrations were obtained through a peripheral venous catheter directly after induction of anesthesia, at the end

of the procedure and 1, 2 and 3 hours after awakening. For DNA analysis, a blood sample (1 ml) was obtained directly after induction of anesthesia.

Diclofenac, D4OH and D5OH assay
Diclofenac sodium and naproxen were purchased from SIGMA ALDRICH (Saint Quentin Falavier, France), D4OH and D5OH were provided by Novartis, USA. All solvents used were analytical grade.
The HPLC system consisted of a Quaternary P 1000 XR pump (ThermoQuest-TQ, Florida, USA), a TQ auto sampler, a TQ UV 6000 detector (280nm) linked to TQ Spectranet for recording and storing throughout analysis. We used a LC_8 5 µm particle size Supelcosil column (150 x 4.6 nm, Supelco Bellafonte, USA). The mobile phase was a mixture of acetonitrile/ sodium acetate 50 mV (70/30, v/v) adjusted to pH 5 by phosphoric acid and the flow rate 1.2 ml/min.

Stock solutions of diclofenac (1000 mg/l) , D4OH and D5OH (500 mg/l) and naproxen (1000 mg/l) were prepared in methanol and stored at –20°C. Calibration standards (0.01-1 mg/l) and plasma controls (0.04, 0.2, 0.750 mg/l) were prepared by appropriate dilutions of the stock solutions in drug-free plasma. Naproxen (10 mg/l) was used as internal standard. Briefly, 500 µl of plasma sample, 100 µl of internal standard and 1500 µl H3PO4 (1M) were extracted in 8 ml of diethylether. After centrifugation, the organic layer was evaporated to dryness at 40°C under nitrogen. The residue is dissolved in a mixture of 50 mM sodium acetate and –methanol (50/50, v/v).

Under the chromatographic conditions used, the retention times were 18.5, 6.9, 6.1 and 7.9 min for respectively diclofenac, D4OH, D5OH and naproxen. Recovery from extraction was over 90% for the three compounds. Calibration curves were linear over the range of 0.01 to 1 mg/l and coefficients of variation of the slope were between 2.8 and 5.8% (n = 5). The limit of quantification was 5 µg/l for the three compounds. The intra and inter-assay coefficients of variation, determined from three quality controls (0.04, 0.2, 0.750 mg/l) were lower than 7%.

DNA analysis

DNA analysis for CYP3A4*1b, CYP3A5*3, CYP2C9*2 and CYP2C9*3 mutations was performed isolating genomic DNA from blood using GenomicPrep Blood DNA Isolation Kit (Amersham Pharmacia Biotech, Buckinghamshire, UK). DNA samples were examined for CYP3A4*1b, CYP3A5*3, CYP2C9*2 and CYP2C9*3 mutations using PCR-RFLP method developed by van Schaik et al.[8]

Data analysis

Diclofenac clearance, D4OH formation clearance and D5OH formation clearance were estimated using non-linear mixed effects models. A single compartment, first order absorption and first order elimination model was used to describe diclofenac pharmacokinetics.[9] Analysis of the relation between clearances and genotype was performed using cross tabs after discretizing clearances based on the interquartile ranges. For CYP3A4 genotype, we compared patients being wild type (*1/*1) to patients being hetero- or homozygous for CYP3A4*1b (*1/*1b; *1b/*1b). For CYP3A5 genotype, we compared patients being wild type (*1/*1) or heterozygous (*1/*3) to patients being homozygous (*3/*3), as only patients being wild type or being heterozygous have CYP3A5 activity.[10] For CYP2C9 we compared patients being wild type (*1/*1) to patients being hetero- or homozygous for CYP2C9*2 or CYP2C9*3 (*1/*2; *1/*3; *2/*2; *3/*3), as the latter enzymes are associated with decreased enzyme activity.[10]

Results

Participant flow and follow up

32 patients received diclofenac and were eligible for this current analysis. Blood samples were not available in 6 patients. Therefore data of 26 children were available for DNA analysis and plasma concentration analysis.

Patients' characteristics

Median (25th-75th percentile) age and weight of the patients were respectively 4.0 (3.8-5.0) years and 20.0 (17.8-22.7) kg. The study group consisted of 14 boys and 12 girls. (Table 1)

Diclofenac and metabolite plasma concentrations

Diclofenac plasma concentrations ranged from 0-2.6 mg/l, being highest at the start of surgery, approximately 30 minutes after loading dose, and decreasing slowly postoperatively (Figure 2a). D4OH concentrations varied, ranging from 0-0.5 mg/l. Highest plasma concentrations were measured at the start of surgery, but there was only a slight decrease during the postoperative period (Figure 2b). D5OH concentrations were often not detectable. Therefore it was not possible to plot all concentrations. They ranged from 0-0.6 mg/l (Figure 2c).

Diclofenac clearance and D4OH and D5OH formation

Median (25th-75th percentile; range) diclofenac clearance, D4OH formation clearance and D5OH formation clearance were respectively 18.1 (15.6-20.2; 11.2-66.9), 3.3 (2.8-3.9; 2.0-4.7) and 1.1 (0.7-1.6; 0.5-9.0) l/h. Clearances are represented in Figure 3, showing 2 outliers for diclofenac, respectively with a clearance of 30.8 l/h and 66.8 l/h, and 4 outliers for D5OH formation clearance with a D5OH formation clearance of 5.1 l/h, 5.7 l/h, 9.0 l/h and 15.3 l/h.

Table 1 Patients' characteristics

Age (years)[1]		4.0 (3.8-5.0)
		2-8
Weight (kg)[1]		20.0 (17.8-22.7)
		14.0-30.0
Gender	Male	12 (46.2%)
	Female	14 (53.8%)

[1] median (25^{th}-75^{th} percentile); range

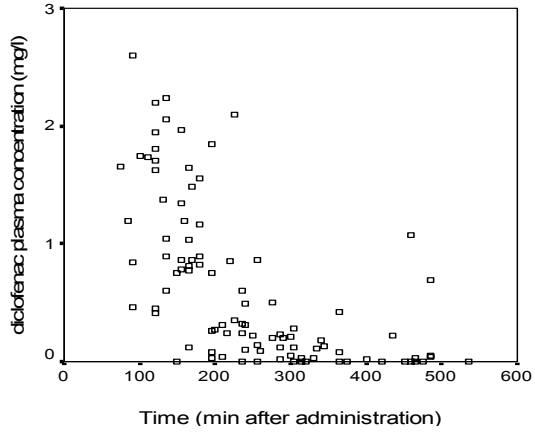

Figure 2a *Diclofenac plasma concentrations*

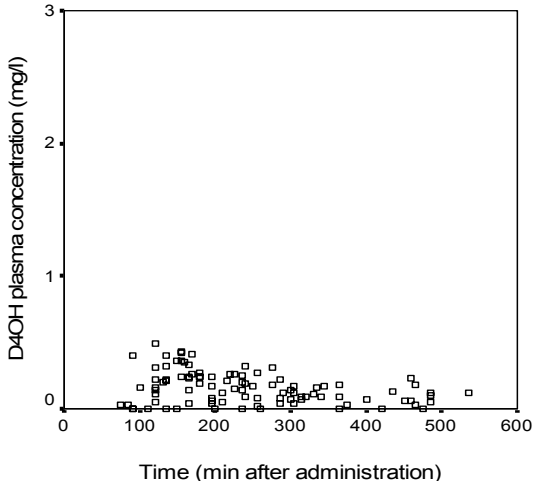

Figure 2b *D4OH plasma concentrations*

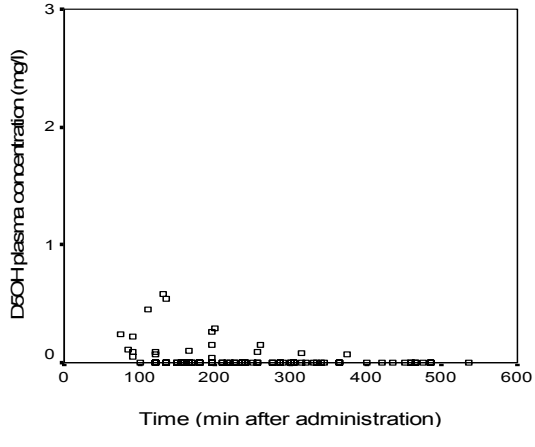

Figure 2c *D5OH plasma concentrations*

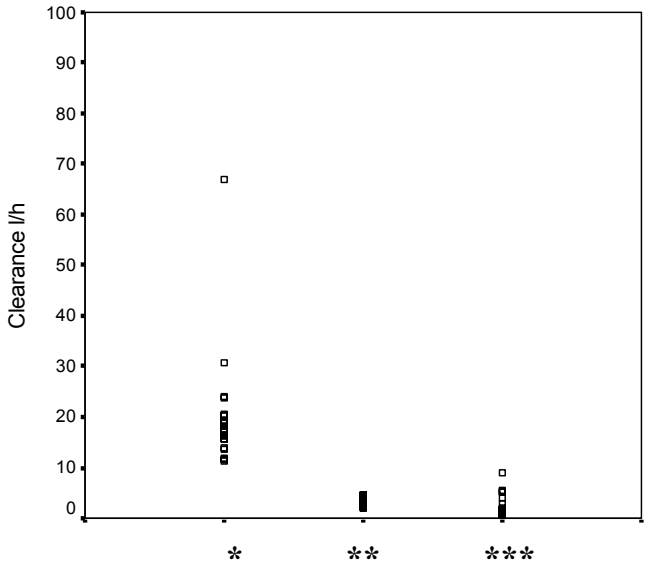

Figure 3 *Clearances*
** Diclofenac clearance*
*** D4OH formation clearance*
**** D5OH formation clearance*

DNA analysis

DNA analysis was performed for CYP3A4*1b, CYP3A5*3, CYP2C9*2 and CYP2C9*3. 25 samples were available for analysis. 1 sample was missing due to logistical problems. Considering CYP3A4, 88% (n = 23) of the patients were wild type (*1/*1) and 8% (n = 2) of patients were heterozygous for CYP3A4*1b (*1/*1b). There were no homozygous patients for CYP3A4*1b (Figure 4a).

Considering CYP3A5 8% (n = 2) of the patients were wild type (*1/*1), 15% (n = 4) were heterozygous for CYP3A5*3 (*1/*3) and 73% (n=19) of the patients were homozygous for CYP3A5*3 (*3/*3) (Figure 4b). Analyzing CYP2C9 69% (n = 18) of the patients were wild type (*1/*1), 15% (n = 4) of the patients were heterozygous for CYP2C9*2 (*1/*2), 12% (n = 3) of the patients were heterozygous for CYP2C9*3 (*1/*3) No patients were homozygous for CYP2C9*2 (*2/*2) or CYP2C9*3 (*3/*3) or heterozygous for both CYP2C9*2 and CYP2C9*3 (*2/*3) (Figure 4c).

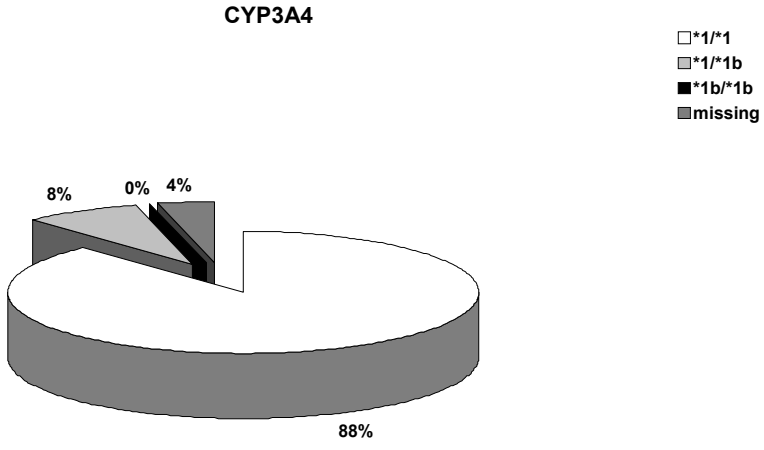

Figure 4a

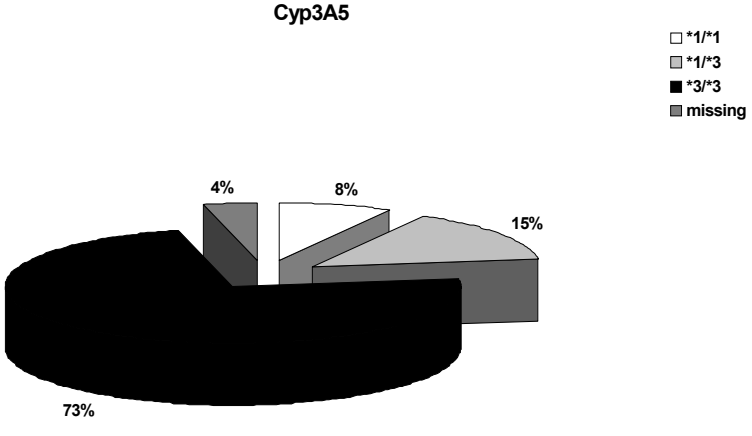

Figure 4b

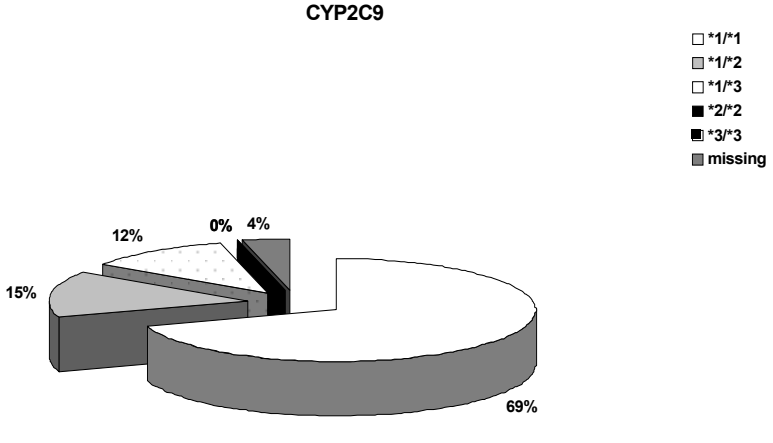

Figure 4c

Table 2 Genotyping of outliers in clearances

		CYP3A4	CYP3A5	CYP2C9
Diclofenac clearance (l/h)				
	30.8	*1/*1	1/*3	*1/*1
	66.8	-	-	-
D5OH formation clearance (l/h)				
	9.0	*1/*1	*3/*3	*1/*1
	5.3	*1/*1	*1/*3	*1/*1
	5.1	*1/*1	*3/*3	*1/*2
	5.7	*1/*1b	*1/*3	*1/*1

Relation between clearances and genotyping

Genotyping of the outliers in diclofenac clearance shows CYP3A4 wild type (*1/*1), Cyp3A5 heterozygous (*1/*3) and CYP2C9 wild type (*1/*1) for the patient with a diclofenac clearance of 30.8 l/h. Genotyping for the patient with a diclofenac clearance of 66.8 l/h is missing (Table 2). Genotyping of the outliers in D5OH formation clearance shows Cyp3A5 homozygous (*3/*3) and CYP3A4 and CYP2C9 wild type (*1/*1) for the patient with a D5OH formation clearance of 9.0 l/h; Cyp3A5 heterozygous (*1/*3) and CYP3A4 and CYP2C9 wild type (*1/*1) for the patient with a D5OH formation clearance of 5.3 l/h; Cyp3A5 homozygous (*3/*3), CYP3A4 wild type (*1/*1) and CYP2C9 heterozygous (*1/*2) for the patient with a D5OH formation clearance of 5.1 l/h; Cyp3A5 heterozygous (*1/*3), CYP3A4 heterozygous (*1/*1b) and CYP2C9 wild type (*1/*1) for the patient with a D5OH formation clearance of 5.7 l/h (Table 2). There was no relation between diclofenac clearance and CYP3A4, CYP3A5 or CYP2C9 genotype (Figure 5a, 5b, 5c). There was also no relation between D4OH formation clearance and CYP2C9 (Figure 6) or between D5OH formation clearance and CYP3A4 and CYP3A5 (Figure 7a, 7b). Table 3 shows the number of patients being wild type, heterozygous or homozygous for CYP3A4, CYP3A5 and CYP2C9 genotype in the different interquartile ranges of the diclofenac clearance (Table 3a), D4OH formation clearance (Table 3b) and D5OH formation clearance (Table 3c).

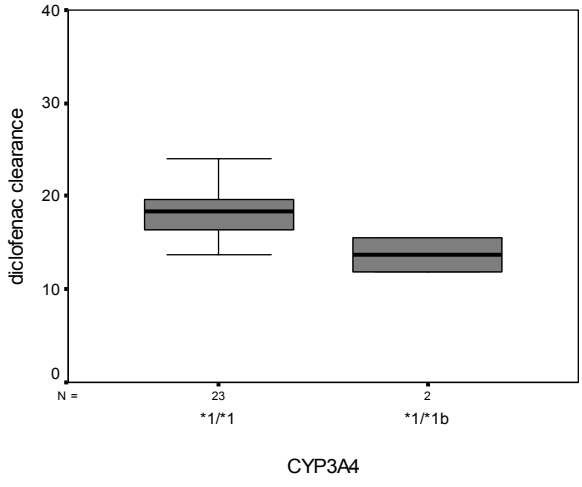

Figure 5a

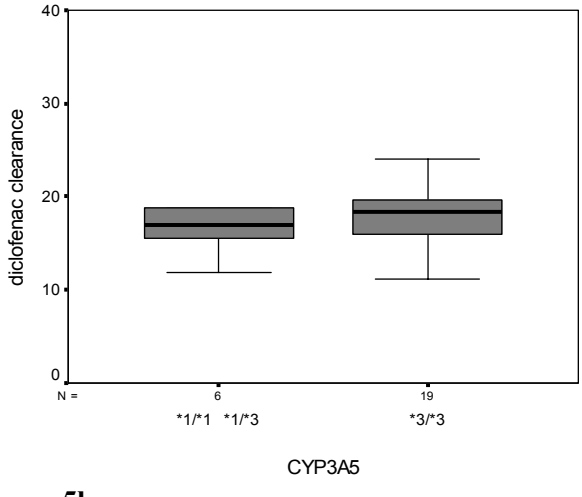

Figure 5b

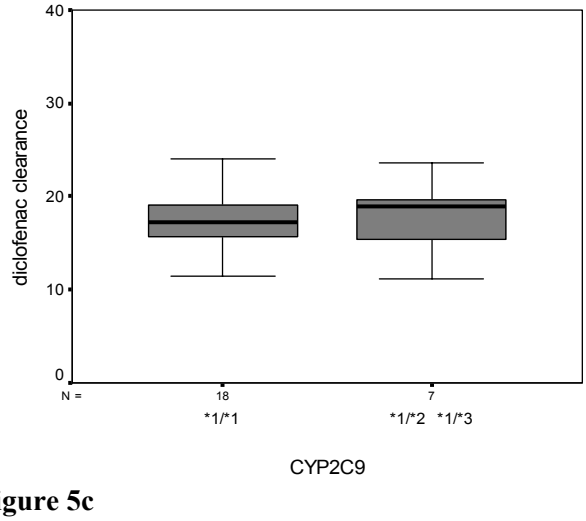

Figure 5c

Table 3a CYP3A4, CYP3A5 and CYP2C9 genotype and diclofenac clearance

Interquartile ranges of diclofenac clearance		**1st**	**2nd**	**3rd**	**4th**
CYP3A4	*1/*1	4	6	7	6
	*1/*1b	2	0	0	1
CYP3A5	*1/*1	1	0	0	0
	*1/*3	1	1	1	2
	*3/*3	4	5	6	4
CYP2C9	*1/*1	4	7	3	4
	*1/*2	0	0	3	1
	*1/*3	2	0	1	0

Table 3b CYP2C9 genotype and D4OH formation clearance

Interquartile range of D4OH formation clearance		**1st**	**2nd**	**3rd**	**4th**
CYP2C9	1/1	5	2	6	5
	1/2	0	0	3	1
	1/3	0	3	1	0

Table 3c CYP3A4 and CYP3A5 genotype and D5OH formation clearance

Interquartile range of D5OH formation clearance		**1st**	**2nd**	**3rd**	**4th**
CYP3A4	*1/*1	6	5	6	5
	*1/*1b	0	2	0	1
		-	-	-	-
CYP3A5	*1/*1	0	1	0	0
	*1/*3	1	3	0	1
	*3/*3	5	3	6	5

Discussion

This is the first study that investigates the relationship between genotype and diclofenac clearance and genotype and D4OH formation clearance and D5OH in children. In this pilot study we could not establish a relationship between CYP3A4, CYP3A5 or CYP2C9 genotype and diclofenac clearance, between D4OH formation clearance and CYP2C9 genotype or between D5OH formation clearance and CYP3A4 and CYP3A5 genotype.

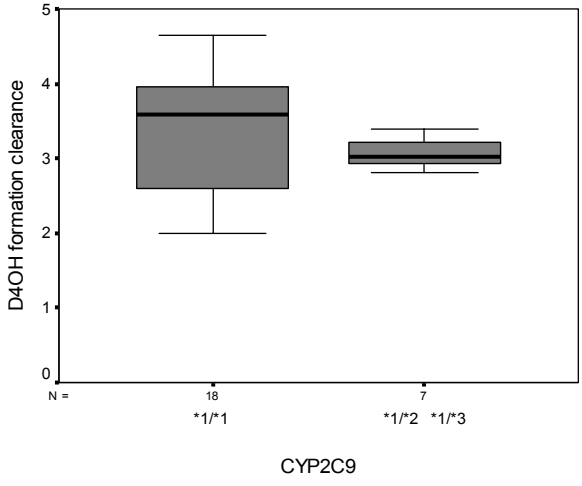

Figure 6

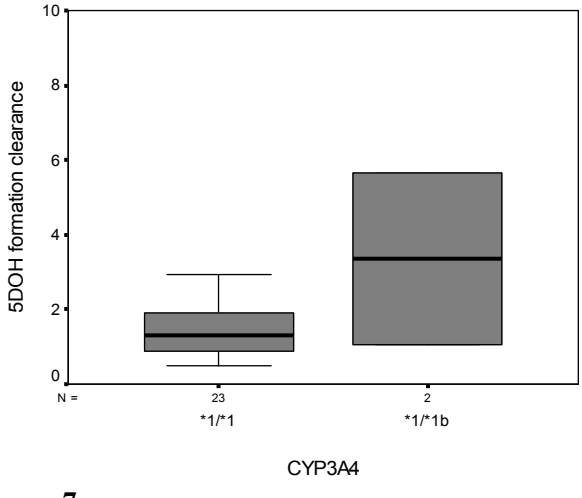

Figure 7a

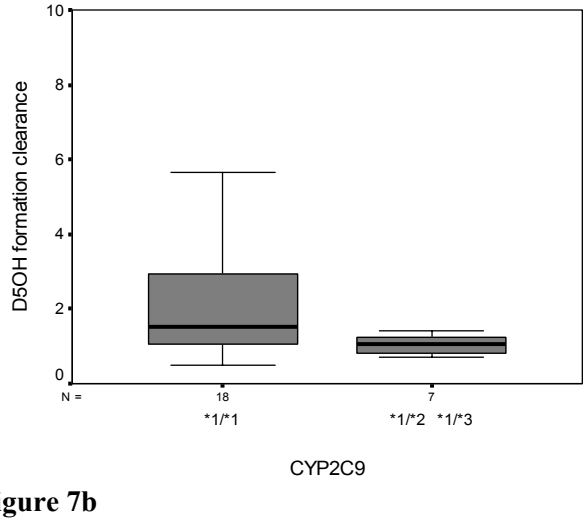

Figure 7b

The number of patients in this pilot study is limited and therefore we can not draw definite conclusions with regards to the relationship between CYP3A4, CYP3A5 or CYP2C9 genotype and diclofenac clearance and D4OH and D5OH formation clearance. Aithal et al described patients having CYP2C9*2 or CYP2C9*3 alleles to require lower doses of warfarin to achieve an anticoagulant effect similar to that in patients being wild type and to be more likely to have an excessive anticoagulant response.[10] Furthermore, bleeding episodes tend to be more common in persons having CYP2C9*2 or CYP2C9*3 alleles.[10]

Looking at the metabolism of diclofenac, a potential effect of decreased CYP2C9 activity in patients being homozygous for CYP2C9*2 or CYP2C9*3, the effect would be higher on diclofenac clearance compared to its influence on D4OH formation clearance, since only 20% of diclofenac is metabolized into D4OH and therefore the amount of D4OH is low, which is shown in Figure 2b.[9]
Table 4 shows the population allelic frequencies of the mutations analyzed in this study.

Table 4 Allelic frequencies of CYP3A4*1b, CYP3A5*3, CYP2C9*2 and CYP2C9*3

	Population frequency
CYP3A4*1b	
Caucasians[8]	5.3%
US Caucasians[11]	9.6%
African Americans[12]	54.6%
CYP3A5*3	
Caucasians[13]	91%
Japan[14]	71%
African Americans[14]	27%
CYP2C9*2	
African Americans[15]	3.6%
Sweden[16]	10.7%
CYP2C9*3	
African Americans[17]	1.2%
Korea[18]	1.1%

Frequencies of the mutations analyzed in this study were comparable between the children included in our study and the normal population. Since population frequencies are relatively low, except for CYP3A5*3, and samples of only 25 patients were available in this study, the number of patients in this study was not sufficient to establish a relationship between clearances and genotype. De Wildt et al showed no relationship between CYP3A4 genotype and midazolam clearances in preterm born infants 26.0-33.6 weeks gestational age.[19] Although the frequency of CYP3A4*1b in the Caucasian population is 5.3%,[8] the number of patients used by the Wildt et al was low (n = 29), making it difficult to establish a potential relationship between midazolam clearance and CYP3A4 genotype.

Furthermore the developmental changes in enzyme activity have an effect on clearance other than that of altered enzyme activity due to differences in genotype. CYP3A4 activity changes with age.[3] Before birth CYP3A4 activity is low, but activity increases rapidly after birth and reaches adult levels of activity at the age of 6 to 12 months.[3] During infancy CYP3A4 activity appears to be increased.[3] Developmental changes of CYP3A5 and CYP2C9 have not been described so far.
Since children included in this study were approximately of the same age, the influence of developmental changes on enzyme activity probably will not have influenced our results.
Besides the influence of developmental changes on enzyme activity, there might also be an influence of expression on the enzyme activity. We do not know to what extent certain mutations are expressed and subsequently to what extent certain mutations lead to altered enzyme activity. Therefore we do not know what the actual influence of these mutations is on enzyme activity. All these factors might have had an effect on our results.

Conclusion

This pilot study showed no relation between diclofenac clearance and CYP3A4, CYP3A5 or CYP2C9 genotype. There was also no relation between D4OH formation clearance and CYP2C9 or between D5OH

formation clearance and CYP3A4 and CYP3A5. Further research in a larger sample of patients is needed to fully analyze the relation between diclofenac clearance, D4OH and D5OH formation clearances and CYP3A4, CYP3A5 and CYP2C9 genotype.

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Chapter 5

General discussion

Pharmacokinetic studies

The pharmacokinetics of paracetamol (APAP) in children have received limited attention in the literature. Most studies have aimed at the differences in APAP pharmacokinetics following oral versus rectal administration. Delayed and erratic absorption, leading to unpredictable APAP plasma concentrations following rectal administration and higher peak plasma concentrations following oral administration have been reported.[1-4] Relative bioavailability of suppositories compared to oral administered APAP varies from 54-80%.[2,5] Alternative routes of administration, such as intravenous administration of propacetamol, have not been investigated in neonates until recently (**chapter 2.3**).[6]

Pharmacokinetic studies have reported APAP plasma concentrations, time to achieve maximum plasma concentrations (Tmax) and maximum APAP plasma concentration (Cmax). Linking APAP plasma concentrations to analgesic effect shows the absence of a relation between analgesic effect and APAP plasma concentrations (**chapter 3.1, 3.2**).[7,8] A delay in maximum analgesic effect and peak plasma concentrations is reported,[9,10] suggesting that the plasma compartment is not the effect compartment. Piletta et al have suggested that APAP-induced analgesia might be centrally mediated,[11] in which the time-course of APAP in CSF may parallel that of analgesic effect.[12] Anderson et al suggest that cerebrospinal fluid (CSF) kinetics approximate more closely the effect compartment than plasma.[13] Based on these reports we measured APAP CSF concentrations and compared these with APAP plasma concentrations (**chapter 2.1**), resulting in a equilibration half life (Teq) of 1.93 (CV 43%) h. Taking into account the effect compartment Teq estimated for analgesia (53 min, CV 217%) and for anti-pyresis (71 min, CV 10%) in children, our results suggest that the CSF compartment is not the effect compartment responsible for these actions since analgesic and anti-pyretic effects occur earlier than APAP concentration changes in the CSF.[14,15]
APAP plasma concentrations are not related to analgesic effect (**chapter 3.1, 3.2**).[7,8] However APAP plasma concentrations are related to APAP

concentrations in the effect compartment and provide information on the amount of APAP absorbed and the amount of APAP available for the effect compartment.
Moreover, measuring APAP plasma concentrations reveals information on APAP metabolism, enabling us to determine APAP clearance. When combined with measurement of APAP metabolites, i.e. APAP-glucuronide, APAP-sulphate and mercapturine and cysteine metabolites, in urine, APAP clearances to APAP-glucuronide, APAP-sulphate and N-acetyl-p-benzoquinone-imine (NAPQI) can be determined. These data provide information about the relative contributions of the different pathways involved in APAP metabolism. This enables us to study potential toxic effects and developmental changes both in clearance and in enzyme activity of the enzymes involved in APAP metabolism.

An increase in mean (SE) APAP-glucuronide to APAP-sulphate ratio from 0.12 (0.09) in preterm neonates 28-32 weeks gestational age,[16] to 0.28 (0.35) in preterm neonates 32-36 weeks gestational age,[16] 0.34 (0.08) in newborns,[17] 0.75 (0.10) in 3-9 year old children[17,18] and 1.61 (0.21) in 12 year old children,[17] with an adult ratio of 1.80 (0.32)[17] has been reported. Our results studying the APAP-glucuronide to APAP-sulphate ratio in infants shows a ratio of 0.69 at the age of 12 months (**chapter 2.2**). The contribution of glucuronidation to total APAP clearance appeared to be similar in infants compared to children, whereas the contribution of the glucuronidation is increased in adults. The fact that we were not able to detect oxidative pathways in our study supports the minor importance of this pathway in the normal dosing range. Furthermore infants and children have a relative enhanced capacity for sulphation.
As glucuronide formation clearance increases with age, a developmental effect of enzyme activity in glucuronidation (UGT1A6 and UGT1A9) might be involved. Developmental changes in glucuronidation have been reviewed recently by the Wildt et al.[19]

As an alternative route of APAP administration we investigated pharmacokinetic and pharmacodynamic actions of propacetamol in preterm

and term neonates (**chapter 2.3**).[6] Intravenous administration of propacetamol enables us to more precise dosing, since bioavailabilty is not compromised by first pass effects. Furthermore APAP peak plasma concentrations are achieved more rapidly. Considering the clearance and half life in preterm and term neonates we found a maturational trend, which was in line with propacetamol pharmacokinetic data reported beyond the neonatal period. However pharmacokinetic data of APAP following administration of propacetamol in children are limited.

Pharmacodynamic studies

Evaluating the pharmacodynamic effects of APAP, all three studies showed no difference in effect between both treatment groups (**chapter 3.1** comparing analgesic effect of oral versus rectal APAP following major craniofacial surgery: 40 patients with a mean (SD) age of 10.3 (2.3) months;[7] **chapter 3.2** comparing analgesic effect of APAP versus diclofenac: 60 children with a median (25th-75th percentile) age of 4 (3-5) years; **chapter 3.3** evaluating the potential morphine sparing effect of APAP: 54 patients with a median (25th-75th percentile) age of 0 (0-2) months). The number of patients requiring extra analgesia was low, indicating that overall analgesia was adequate, making it hard to assess a dose-effect relation. Distribution of patients between both groups was too uniform to determine any differences in analgesic regimens. Furthermore following the concept of pre-emptive analgesia, we administered loading doses prior to or during the operative procedure in order to achieve maximum analgesic effect at the time of awakening, which enhances adequate postoperative analgesia. Most important in performing these studies, is the fact that medical ethical aspects make it impossible to perform studies in which part of the children do not receive adequate analgesia.

Furthermore when assessing pharmacodynamics a large variability in effects is reported, resulting from distribution of the blood to the site of action and from variability in the sensitivity of the receptors.[20] The

contribution of the variability in distribution from the blood to the site of action will depend largely on changes in perfusion of the target tissue (5-60%). The sensitivity of the receptors, which is defined in terms of affinity for binding or potency relative to another agent, might also be an important source of variability in effects.[20]

The primary outcome parameter in these studies is the absence or presence of pain. In our study population we used COMFORT scale and VAS scores to assess the analgesic effects.[21-23] The age of the children in our study population made self-report, which is generally considered as the "gold standard", impossible. Therefore both nurses and investigators were trained to perform COMFORT scale scores to objectively assessing patients' specific pain behavior. However the COMFORT scale not exclusively represents pain, but also distress, which might originate both from pain as well as from other forms of distress, for example the absence of the parents and the ICU environment.

VAS scores represent pain experienced by the patients, but can not be considered as the "gold standard", due to inter-individually varying patterns of pain response, which can not be accounted for in the VAS score. Furthermore there is inter-observer variability.[22,24] Overall the effect on amount of pain as determined by validated pain scores as a "surrogate golden standard" is daily clinical practice and remains the primary outcome parameter.

Pharmacogenetic studies

Studying pharmacogenetics, we investigated the effect of DNA mutations encoding for enzymes involved in diclofenac and APAP metabolism. DNA mutations might lead to an altered enzyme activity or an altered expression of the enzyme involved, subsequently leading to an altered drug metabolism. If there's only one pathway involved in drug metabolism, effects of altered enzyme activity may be extensive. Patients may be slow

or fast metabolizers due to altered enzyme activity and therefore requiring a different drug dosing regimen. However large samples of patients are needed to fully investigate the effect of DNA mutations on drug metabolism, since population allelic frequencies of most mutations are low (for example CYP3A4*1b 5.3-9.6% in Caucasians[25,26] and CYP2E1*1d 3.5% in Caucasians[27] and 23% in Chinese[28]).

Evaluating the effect of DNA mutations in enzymes involved in the oxidative pathway of APAP metabolism, we were not able to detect a relation between genotype and APAP clearance (**chapter 4.1**). This was in line with the fact that the contribution of the oxidative pathway to APAP metabolism is minor within the normal dosing range. However these mutations might have significant relevance when toxic APAP doses are administered and sulphation and glucuronidation are rate limiting, leading to an increased or decreased formation of NAPQI and therefore to increased or decreased toxic effects. This may indeed partly explain the inter individual differences in propensity to APAP toxicity.

When we evaluated the effect of DNA mutations of enzymes involved in diclofenac metabolism on diclofenac and diclofenac metabolite formation clearances in a pilot study (**chapter 4.2**), we could not establish a relation between clearances and genotype.

The relevance of this kind of studies is underlined by Aithal et al, who described patients having CYP2C9*2 or CYP2C9*3 alleles to require lower doses of warfarin to achieve an anticoagulant effect similar to that in patients being wild type and to be more likely to have an excessive anticoagulant response.[29] Furthermore, bleeding episodes tend to be more common in persons having CYP2C9*2 or CYP2C9*3 alleles,[29] outlining the importance of certain mutations on both adverse effects and toxic effects.

Looking at the metabolism of diclofenac, a potential effect of decreased CYP2C9 activity in patients having CYP2C9*2 and/or CYP2C9*3 alleles

would be higher on diclofenac clearance compared to its influence on D4OH formation clearance, since only 20% of diclofenac is metabolized into D4OH and therefore the amount of D4OH is low (**chapter 2.4**).

Future directions

Pharmacokinetics

Most of the pharmacokinetic studies report APAP plasma concentrations, both as a measure for analgesic effect as well as an indicator for potential toxicity. However, the plasma compartment is not the effect compartment and maximum analgesic effect is delayed after peak plasma concentrations. To circumvent this problem an alternative approach is warranted, for example population modeling. When using population modeling, smaller numbers of samples are needed, times on which samples are collected are less strict and pharmacokinetic parameters can be estimated based on smaller numbers of samples.

The use of APAP plasma concentrations as an indicator for potential APAP toxicity may not be the right choice, since there is no direct relation between APAP plasma concentrations and the formation of the oxidative metabolite NAPQI, which is responsible for APAP's toxic effects. It would be better to focus on the clearance through oxidation, i.e. the NAPQI formation clearance, and on the availability of glutathion stores, as glutathion detoxifies NAPQI. Glutathion stores may be compromised in critically ill children with low protein-calorie intake.[30] In addition to the low glutathion stores, resulting from this low protein-calorie intake, the capacity for glucuronidation in these critically ill children might be decreased,[30] leading to an increased oxidation and subsequently to an increased NAPQI formation clearance. On the other hand, Carcillo et al report that cytochrome P450 mediated drug metabolism is reduced in critically ill children with sepsis induced multiple organ failure.[31] This might lead to a decreased potential APAP toxicity, since metabolism through oxidation will then be decreased.

Measuring APAP plasma concentrations following oral or rectal administration in order to determine clearance, one should realize that both volume of distribution as well as clearance are obscured by bioavailabilty. We used relative bioavailability of the rectal compared to the oral formulation to correct for differences in bioavailability between oral and rectal formulations. However, only by comparing APAP plasma concentrations after rectal or oral administration with APAP plasma concentrations following intravenous administration of APAP, in which bioavailability is 100%, we will be able to determine bioavailability in an adequate way.

Furthermore we need to explore developmental changes in APAP clearances, which might partly be due to developmental changes in enzyme activity. This way we will be able to predict the risk of APAP toxicity for children in different age groups, since children experience less toxicity after high APAP doses compared to adults,[32-34] which makes it important to investigate toxicity changes with age.

Pharmacodynamics

The main problem in studying pharmacodynamics is objectively assessing the effects of APAP. Antipyretic effects can be measured objectively. The analgesic effects in neonates, infants and children however are more difficult to assess objectively. In determining the analgesic effect of APAP the most important question that remains is how to assess the analgesic effect of APAP in children. As the majority of the children in our studies was below the age of 1 year, we could not use self-report pain measurement instruments, which are considered to be the “gold standard”. Even in our study comparing the analgesic effect of APAP and diclofenac, in which children were 2-7 years of age, children at the age of 5 years were not always able to report their pain adequately and consistently following (adeno)tonsillectomy in hospital setting.

Using the COMFORT scale and the VAS score, an attempt is made to objectively assess pain and thus analgesic effect. Although both nurses and investigators were trained to perform the COMFORT scale scores, this should not be considered as a surrogate gold standard. Non-invasive methods in combination with validated pain assessment instruments open new possibilities to explore the relation between neurophysiological changes and observed behavior during painful moments. Several methods may reveal the integrity of the pain conducting system (fMRI, PET scan, laser Evoked Potentials).[35] However methods that objectively assess and quantify the reaction to pain are restricted to studies in adult volunteers up till now. Therefore we conducted the study described in **chapter 6**, to assess the value of Electroencephalography (EEG) and Sympathic Skin Response (SSR) for postoperative pain assessment.[36] In this study the clinical role of the SSR could not be unambiguously assessed, most likely because of the homogeneity of the group of children included in this study with respect to adequate and equal pain control. However SSR monitoring is an interesting new variable in future pain studies.

Pharmacogenetics

As we demonstrated in **chapter 4**, evaluating the relation between clearances and genotype, the largest problem in pharmacogenetic studies is the limited number of patients. As population allelic frequencies of most mutations are low, large samples of patients are needed in order to fully investigate the effect of DNA mutations on enzyme activity or expression.

When linking genetic blueprint to toxic APAP effects, developmental changes in enzyme activity and the impact of specific mutations on enzyme activity should be further investigated. However only by administering different doses of APAP to children of different ages and measuring NAPQI formation clearance or mercapturine or cysteine metabolites, one can fully investigate the contribution of the oxidative pathway under different circumstances in different age groups and thus predict potential toxic effects of APAP. Although potentially very important, medical ethical

reasons will prevent the conduction of this kind of studies in the pediatric age group.

Conclusion

Against the background of the increasing knowledge of genetics, these pharmacokinetic and pharmacodynamic studies together with new, non-invasive methods to visualize pain within the central nervous system can be considered as a model for future research of drugs, in particular analgesic drugs.

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Chapter 6

Are Sympathic Skin Response and Electro Encephalogram registration a valuable contribution to postoperative pain assessment in neonates and infants?

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Submitted

Abstract

Background

Although the concept of nociception and the presence of specific pain tracts in the central nervous system have been known for many years, very little clinical research has been done with regards to the central conduction of pain in young infants. Non-invasive methods in combination with validated pain assessment instruments are potential powerful tools to determine a relationship between neurophysiological changes and the observed behavior during painful moments.

Methods

Following major thoracic or abdominal surgery a simultaneous Electro Encephalogram (EEG) and Sympathic Skin Response (SSR) registration of 30 minutes was made on the first and on the second day postoperatively in 31 patients in the age of 0-12 months. These children participated in a randomized clinical trial evaluating morphine and morphine in combination with paracetamol for postoperative pain management. Simultaneous standardized video registrations were made in 15 patients. During the 30-minute registration a painful stimulus, consisting of standardized pressure on 1 of the patient's fingernails, was applied to the patients after 15 and after 20 minutes. Postoperative pain assessment using the validated COMFORT scale and Visual Analogue Scale (VAS) was performed every 2 or 3 hours and during SSR and EEG registration.

Results

Analysis of the frequencies and the amplitudes of the SSRs during the SSR registration showed no relation between age and SSR frequency or between age and SSR amplitude. SSR amplitudes in awake situation were highest compared to drowsy situation and sleep. In addition SSR amplitudes were higher on the second day postoperatively, compared to the first day postoperatively. Analysis of the video registrations showed no correlation between SSR frequency and VAS or COMFORT scale scores. An increase in spontaneous SSR frequency was observed during the 2 minutes

preceding the painful stimulus. Spontaneous SSR frequency decreased during the interval between the first and the second painful stimulus. Visual assessment of the EEG registrations showed that the number of unchanged EEGs expressed as a percentage of the total number of unchanged EEGs and EEGs showing arousal, in sleep, awake and drowsy situation after spontaneous SSRs was respectively 71%, 23% and 62% compared to a percentage of 22% after induced SSRs.

Conclusion
This study describes a method for continuous Sympathic Skin Response monitoring as an objective neurophysiological variable. The clinical role could not be unambiguous assessed in this study, most likely because of the homogeneity of the group of children included in this study with respect to adequate and equal pain control. Also, the ICU environment might encounter non-painful distress, resulting in spontaneous SSRs. However SSR monitoring is an interesting new variable in future pain studies.

Introduction

One of the key issues in pain assessment is the response and conduction of the painful (nociceptive) stimulus in the central nervous system. Very little clinical research has been done with regards to the central conduction of pain, even though the concept of nociception and the neuroanatomy of pain tracts have been known for many years. Non-invasive methods in combination with validated pain assessment instruments open new possibilities to determine a relation between the neurophysiological changes and the observed behavior during painful moments.[1]

Although several methods may reveal the integrity of the pain conducting system (fMRI, PET scan, laser Evoked Potential),[2] methods that objectively assess and quantify the reaction to pain as a normal function are sparse. Also, most methods require a standardized stimulus-response procedure and/ or a co-operative patient to (subjectively) respond to given stimuli and have only been tested in an experimental setting.[3]

Electroencephalography (EEG) and the Sympathic Skin Response (SSR) are neurophysiological methods that have the potential to objectively measure and quantify the reaction to pain. Andersson observed EEG changes to painful stimuli in 28 out of 31 premature babies less than 33 weeks gestational age. Visual assessment of the EEGs showed that the EEG reaction was either a generalized desynchronisation or sudden, generalized, increase in amplitude.[4]

The Sympathic Skin Response (SSR) is a reflection of the activation of the autonomic nervous system in response to a stimulus like pain.[5] The main pathways involved in the SSR are nociceptive afferent pathways, and efferent from the hypothalamus, ventrolateral brainstem reticular formation, down to the spinal cord in the lateral column to sympathic ganglia and finally C-fibers that innervate the sweat glands. Changes in sweat gland production are responsible for the SSR in combination with local changes in the epidermis.[5] Although the SSR is usually studied as a response to a standardized stimulus, it is expected that the response can also be measured as a result of an 'in vivo' pain stimulus.

The use of behavioral pain assessment scores has become standard of care in our ICU.[6,7] The aim of the present study was to assess the value of EEG and SSR registration for postoperative pain assessment. We included neonates and infants at the age of 0 – 12 months for 48 hours following major abdominal or thoracic surgery as part of a randomized clinical trial evaluating the effect of additional paracetamol (APAP) on morphine consumption, using the COMFORT scale[6,8] and the Visual Analogue Scale[9] to assess the effect of the analgesic regimen.

Methods

Patients

Following approval of the study by the Medical Ethical Committee of the Erasmus MC Rotterdam and The Central Committee on Research Involving

Human Subjects and after written informed consent was obtained from the parents, a simultaneous EEG and SSR registration of 30 minutes was made on the first and the second day following major thoracic or abdominal surgery.

Patients included were between 0 and 12 months of age, ≥ 36 weeks gestational age and body weight ≥ 1800 grams. Based on the magnitude of the surgical procedure all patients received morphine postoperatively. After a loading dose of 100 μg/kg morphine at the end of the operative procedure, children < 45 weeks postconceptional age received 5 μg/kg/h and children ≥ 45 weeks postconceptional age received 10 μg/kg/h morphine, based on a randomized clinical trial performed before in our ICU.[7] According to the study protocol patients received either APAP (80-100 mg/kg/24 hours) or placebo in addition to continuous intravenous morphine.

Procedure

Patients were included as part of a study protocol investigating the morphine sparing effect of APAP in neonates and infants. Postoperatively patients were admitted to the Pediatric Surgical Intensive care Unit (PSICU) for at least 48 hours. Patients received either morphine or morphine in combination with APAP for postoperative analgesia. Pain assessment using the COMFORT scale[6,8] and the Visual Analogue Scale (VAS)[9] was performed every 2 hours during the first 24 hours postoperatively and every 3 hours during the second 24 hours postoperatively. Thirty-minute EEG and SSR registrations were made at the ICU during the first and the second day after surgery, preferably between 9.00 and 12.00 am. In order to record a neonatal derivation of the EEG, 14 Ag-AgCl surface electrodes were placed on the patient's head according to the international 10-20 system. To record the SSR, we placed 2 surface electrodes on one of the patient's hands (one on the palm and one on the dorsum as a reference) and 2 surface electrodes on one of the patient's feet (one on the palm and one on the dorsum as a reference). During the 30-minute registration spontaneous occurring SSRs were recorded. In addition SSRs were recorded after a painful stimulus, which was given to the

patients after 15 minutes and after 20 minutes. This stimulus consisted of applied pressure on one of the patient's fingernails performed as standard procedure in the evaluation of the level of consciousness using the pediatric Glasgow Coma Scale in children less than 4 years of age.[10] The exact time of the stimuli was recorded on the computer during EEG and SSR registration. In 15 patients simultaneous video registrations of the patients during the time of EEG and SSR registration were made as well, enabling off line reassessment of clinical signs of pain. These video registrations were used by the investigators to determine the VAS and COMFORT scale. The total number of SSRs recorded during the 30 minute registration, the total number of spontaneous SSRs and the number of induced SSRs after painful stimuli were assessed. In addition the amplitude of the SSR in sleep, drowsy and awake situation was measured.

Statistical analysis

Mann-Whitney U test was used to analyze the relation between age and the total number of SSRs, the number of spontaneous SSRs and the number of induced SSRs; Spearman's rho for the correlation between age and SSR amplitude in sleep, drowsy and awake situation; one-way Anova for the difference in SSR amplitude in sleep, drowsy and awake situation; Friedman test for the difference in SSR frequency in sleep, drowsy and awake situation; Wilcoxon signed ranks test for the difference between SSR frequency (total, induced and spontaneous) on day 1 and day 2 postoperatively and for the difference in amplitude in sleep, drowsy and awake situation on day 1 and day 2 postoperatively.

Results

In the period from January till September 2001 we included 31 patients, of which we made a total of 57 EEG and SSR registrations. EEG and SSR registrations at the second day after surgery were missing in 5 patients: 1 due to logistic problems, 1 due to early discharge from the ICU and 3 due to withdrawal of the parental informed consent for the second EEG and SSR

registration. Median age (25th-75th percentile) was 48 (38-54) weeks postconceptional age. The analgesic regimen, assessed by trained nurses using the COMFORT scale and the VAS, was adequate in all patients at the moment of EEG and SSR registration (VAS < 4cm, COMFORT scale < 17), in which patients received a higher total dose of morphine on the first day postoperatively compared to the second day postoperatively (mean (SD) respectively 325 (104) and 206 (87) μg/kg).

EEG registration

For EEG analysis we focused on the EEG during periods of painful stimuli and periods where spontaneous SSRs were recorded. In a subgroup of 24 consecutive EEGs, the number of unchanged EEGs after spontaneous SSRs, expressed as a percentage of the total number of unchanged EEGs and EEGs showing arousal in sleep, awake and drowsy situation after spontaneous SSRs was respectively 71%, 23% and 62% compared to a percentage of 22% after induced SSRs (Table 1).

Visual assessment of these 24 EEGs revealed 4 different patterns of changes at the same moment of the SSR responses, either spontaneous occurring or provoked after a painful stimulus. Of the EEGs that were not artefact obscured, 27% showed EEG changes during spontaneous SSRs and 77% during induced SSRs. Within the same patient the patterns of EEG reactivity could differ between the various SSR periods. These EEG patterns were an increase in fast activity (Figure 1a), a decrease in the amount of faster activity, a more general slowing of the background activity or a general attenuation in background amplitude (Figure 1b & 1c, Table 1).

Table 1

	Spontaneous SSRs				Induced SSRs
	Total	**Awake**	**Drowsy**	**Sleep**	
Total number of SSRs	334	205	38	91	42
With unchanged EEG	187	110	18	59	8
With EEG changes	68	33	11	24	27
Increase of fast activity	8	3	1	4	5
Decrease of fast activity	14	7	2	5	4
General slowing	12	1	4	7	8
Attenuation	34	22	4	8	10
EEG artefact obscured	79	62	9	8	7

EEG changes after spontaneous and induced SSRs in a subgroup of 24 EEG registrations

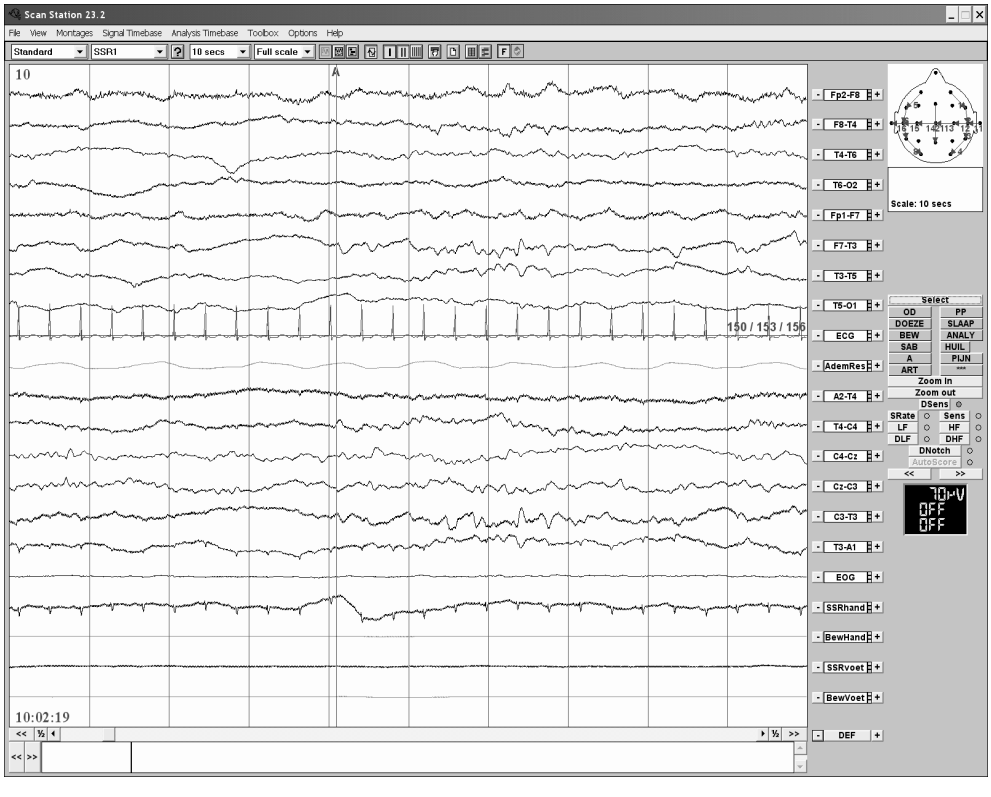

Figure 1a *EEG increase in fast activity acceleration after a spontaneous SSR*

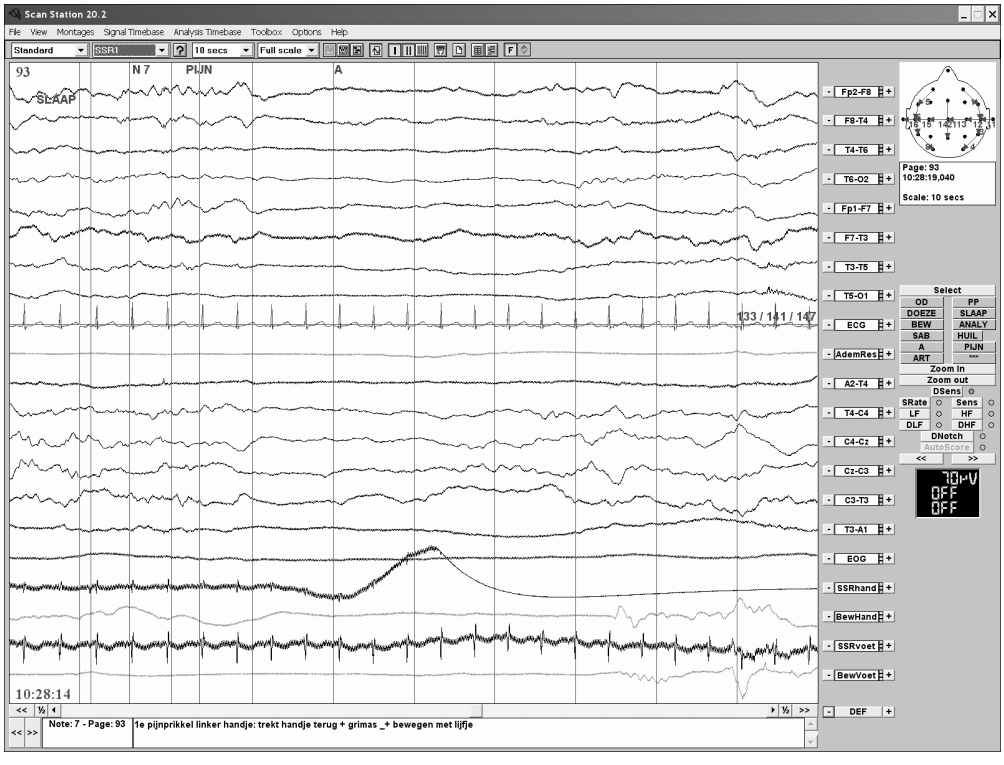

Figure 1b *EEG attenuation in background amplitude deceleration after an induced SSR*

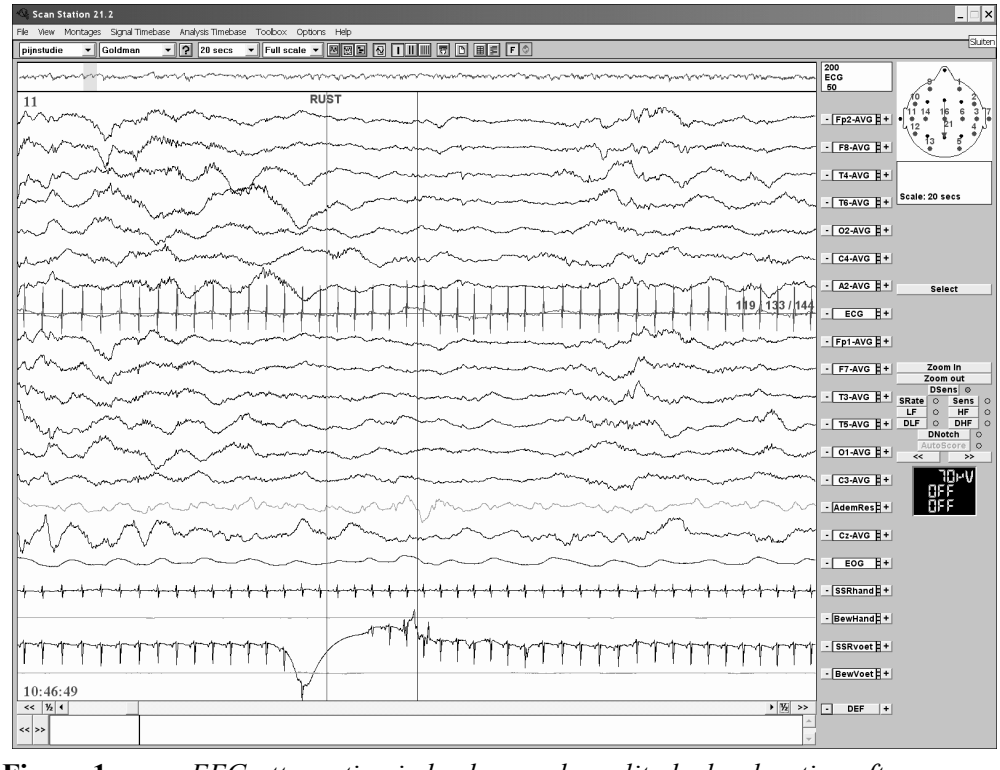

Figure 1c *EEG attenuation in background amplitude deceleration after a spontaneous SSR*

SSR registration
Age was not significantly related to the total number of SSRs, the number of spontaneous SSRs and the number of induced SSRs. Neither was age related to the SSR amplitude in sleep, drowsy and awake situation. However there was a significant difference in SSR amplitude (spontaneous and induced) in sleep, drowsy and awake situation. SSR amplitude was highest in awake situation (Table 2a).

When we compared the SSR registrations made on the first day postoperatively to the SSR registrations made on the second day postoperatively, there were no significant differences in total number of SSRs, number of spontaneous SSRs and number of induced SSRs (Table 2b). The SSR amplitude in sleep, drowsy and awake situation however differed significantly between day 1 and day 2 postoperatively, being higher on the second day postoperatively (Table 2b).
SSR frequency showed an increase in spontaneous SSR frequency during the 2 minutes preceding the painful stimulus. Spontaneous SSR frequency in the interval between the first and the second painful stimuli was decreased compared to spontaneous SSR frequency before the first painful stimulus.

Video registrations
Analysis of the video registrations showed no correlation between SSR frequency and VAS or COMFORT scale score.

Discussion

To our knowledge this is the first study in children, admitted to an ICU, using SSR monitoring for the assessment of pain. Up until now there was no experience with the use of SSR registration other than in an experimental setting. [3]
In this study we were able to continuously monitor the occurrence of SSRs in neonates and infants in the age range of 0-12 months. Other recently developed “pain visualising” methods (PET scan, fMRI) [2] can not be

applied in a daily clinical setting of the ICU and, moreover, these latter methods can not be used for neuromonitoring purposes.

Our data showed no relation between age and SSR frequency or between age and SSR amplitude, which might indicate that sympathic activity resulting in a SSR is comparable for all subjects within this age range. However there was a significant difference in SSR amplitude between sleep, drowsy and awake situation, being highest in awake situations. This might be explained by a higher responsiveness in awake situation.
The number of spontaneous SSRs increased during the 2 minutes preceding the painful stimulus, which might be due to the fact that preparations were made to apply pressure on one of the patient's fingernails by touching the patient's finger and to register the exact time of the painful stimulus.
The SSR amplitude in sleep, drowsy and awake situation however was higher on the second day postoperatively, which could be explained by a more sedated condition on the first day postoperatively as a consequence of the effects of anesthesia or to the higher doses of morphine patients were receiving on the first day postoperatively (mean (SD) morphine consumption on day 1 and 2 respectively 325 (104) µg/kg and 206 (87) µg/kg).

The SSR response is the result of sympathic activity.[5] A stimulus that can provoke a SSR is non specific and includes various stimuli, for example noise stimuli or painful stimuli.[5]
Therefore, for interpretation of SSRs one should keep in mind that not every SSR is provoked by pain and one should be very careful in controlling the circumstances under which SSR registration takes place, before it can be used as an additional tool for pain assessment in non verbal patients. Especially when SSR registrations of different patients are compared, it is very difficult to control these circumstances. Although SSRs were seen after painful stimuli in our study, the presence of SSRs was not always clearly related to the moment of painful stimuli or distress. The tentative question is whether these spontaneous occurring SSRs without visible pain or distress are indicative of internal pain or distress.

Table 2a

	awake	drowsy	sleep
SSR total [1]	11.0	3.5	4.2
	27.5 ; 0-139	7.6 ; 0-33	5.0 ; 0-15
Amplitude SSR [1]	846	670	744
	938 ; 400-6200	890 ; 300-4000	769 ; 300-5300
SSR total [2]	14.2	3.6	6.5
	12.8 ; 0-39	6.5 ; 0-29	12.0 ; 0-49
Amplitude SSR [2]	1311	830	550
	892 ; 300-5000	966 ; 400-4400	893 ; 200-4600

Mean (SD; range) SSR number during 30-minute registration and amplitudes (μV) in sleep, drowsy and awake situation on day 1 and day 2 postoperatively

Table 2b

	day 1	day 2
SSR total	20.8	27.4
	5.4 ; 0-139	4.1 ; 2-72
SSR spontaneous	18.7	24.3
	5.3 ; 0-130	3.7 ; 2-62
SSR induced	2.2	3.1
	0.4 ; 0-9	0.6 ; 0-10

Mean (SD; range) SSR number (total, spontaneous and induced) during 30-minute registration on day 1 and day 2 postoperatively

The fact that we could not establish a correlation between SSR frequency and VAS or COMFORT scale score might be explained by the fact that the group of patients included in this study was to homogenous. The absence of high VAS scores and COMFORT scale scores indicates that the analgesic regimen was adequate in all patients, which makes it more difficult to test the EEG and SSR as suitable instruments for objective assessment of pain. Another explanation could be that in this clinical setting patients might be distressed for other reasons than pain, for example the noisy environment on the ICU and the absence of their parents. Distress other than originating from pain, might provoke SSRs as well. This makes it more difficult to

distinguish the SSRs resulting from the actual painful stimuli, than induced by other reasons.
Visual assessment of the EEG registrations showed EEG changes both after spontaneous and after induced SSRs. However the number of unchanged EEGs expressed as a percentage of the total number of unchanged EEGs and EEGs showing arousal was higher after induced SSRs. This indicates that, although visible signs of pain are absent, the registration of SSRs could well be a measure for activity within the central nervous system resulting from pain or more generally from arousal.

Conclusion

This study describes a method for continuous SSR monitoring as an objective neurophysiological variable. The clinical role could not be unambiguously assessed in this study, most likely because of the homogeneity of the group of children included in this study with respect to adequate and equal pain control. Also, the ICU environment might encounter non-painful distress, resulting in spontaneous SSRs. However SSR monitoring is an interesting new variable in future pain studies.

Acknowledgements

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7

Summary/Samenvatting

Chapter 7.1

Summary

Summary

Paracetamol (APAP), known as acetaminophen in the USA, is widely used both in hospital settings and at home for antipyresis and mild (postoperative) pain. Even though APAP is available over the counter and is the third most prescribed drug in our Pediatric Surgical Intensive Care Unit (PSICU), following nystatin and cisapride, there are still surprisingly few data available concerning the pharmacokinetics and pharmacodynamics of APAP in children.
To further elucidate APAP's pharmacokinetics (i.e. the absorption, distribution, metabolism and elimination of APAP), pharmacodynamics (i.e. the effects encountered by APAP) and pharmacogenetics (i.e. the influence of DNA on APAP metabolism) in children, we conducted the studies summarized below.

Pharmacokinetic studies

APAP-induced analgesia might be centrally mediated, with the time-course of APAP in CSF paralleling that of analgesic effect. There are few studies, however, describing APAP cerebrospinal fluid (CSF) concentrations in children. To investigate age-related changes in the plasma to CSF equilibration half-time (Teq) of APAP, we studied 41 children undergoing (semi) elective surgery for placement or revision of a ventriculo-peritoneal shunt or insertion of a temporary external ventricular drain, and receiving a loading dose of 30-40 mg/kg APAP 1 hour before scheduled surgery. Median (25th-75th percentile) age and weight of the children were 12 (3-62) months and 10.0 (5.8-20.0) kg. Median (25th-75th percentile) time between APAP loading dose administration and collection of blood samples and median time (25th-75th percentile) between APAP loading dose and collection of CSF were 125 (95-210) and 133 (33-202) minutes, respectively. The population mean Teq, standardized to a 70 kg person, was 1.93 (CV 43%) h, an estimate similar to that described in adults (2.1 h). There was no relationship between age and Teq other than that predicted by

size. APAP plasma concentrations ranged from 0.0 – 33.0 mg/l, APAP CSF concentrations ranged from 0.0 – 21.0 mg/l.
Size rather than blood-brain-barrier maturation determines Teq changes with age in children. Based on these results we predict a neonate (3.5 kg), a 1-year-old child (10 kg), a 5-year-old child (20 kg), a 10-year-old child (30 kg) and an adult (70 kg) to have a Teq of 0.9, 1, 1.4, 1.6 and 1.93 h, respectively (**chapter 2.1**)

Data concerning APAP metabolism in infants are scant. Previous studies have examined urinary metabolite recovery rates after a single dose of APAP either in neonates (<6 weeks) or in children (3-9 years). There are no such studies in infants. Therefore we studied 47 infants undergoing major craniofacial surgery receiving APAP 19-45 mg/kg 6-, 8-, or 12 hourly as either elixir or suppository formulation for postoperative analgesia, after a loading dose of 33–59 mg/kg rectally during the operative procedure. Mean (SD) age and weight of the patients were 11.8 (2.5) months and 9.1 (1.9) kg. Clearance of APAP to APAP-glucuronide (%CV) and to APAP-sulphate were 6.6 (11.5) and 7.5 (11.5) l/h, respectively, standardized to a 70 kg person using allometric '¼ power' models. Glucuronide formation clearance, but not sulphate formation clearance, was related to age and increased with age from a predicted value in a neonate of 2.73 l/h/70kg to a mature value of 6.6 l/h/70kg with a maturation half-life of 8.09 months. Urine clearance of APAP-glucuronide, APAP-sulphate and unchanged APAP (%CV) were 2.65, 3.03 and 0.55 (28) l/h/70kg, respectively. The urine clearance of unchanged APAP and metabolites was related to urine volume flow rate. Clearance attributable to pathways other than these measured in urine was not identifiable. The glucuronide/sulphate formation clearance ratio was 0.69 at 12 months age. Sulphate metabolism contributed 50% towards APAP clearance. Glucuronide formation clearance increases with age in the infant age range but sulphate formation does not. Renal clearance of APAP and its metabolites increases with urine flow rate.
This and other studies show that APAP metabolism to glucuronide appears to be similar in infants and children, but is higher in adults in comparison to children. Oxidative pathways were undetectable in this infant study and

may explain, in part, the reduced incidence of hepatotoxicity in infants (**chapter 2.2**).

In order to investigate pharmacokinetic and pharmacodynamic actions of APAP after intravenous administration of a single dose of propacetamol in preterm and term infants on the first day of life, we studied 20 preterm and 10 term infants.
Mean (SD) serum half lifes ($t_{½}$) in preterm and term infants were 277 (143) min and 172 (59) min ($p<0.05$), respectively; mean (SD) clearances (CL) were 0.116 (0.08) l/kg/h and 0.170 (0.06) l/kg/h ($p<0.05$), respectively. A correlation between gestational age and $t_{½}$ was established ($r = -0.46$). We could not show an effect of gender or administration of prenatal steroids on the pharmacokinetics of APAP. The level of analgesia seemed to be associated with a therapeutic (> 5 mg/l) level in neonates receiving no medication other than propacetamol (n=15). The maturational trend of CL and $t_{½}$ in preterm and term neonates was in accordance with propacetamol pharmacokinetic data reported beyond the neonatal period (**chapter 2.3**).

The metabolism of diclofenac in children has only been studied after intravenous administration and with the use of enteric-coated oral formulations. There are no data on diclofenac or its hydroxyl metabolite pharmacokinetics after rectal administration in children. Therefore we studied 26 infants undergoing (adeno) tonsillectomy receiving diclofenac 2 mg/kg loading dose followed by 1 mg/kg 8-hourly as a suppository for postoperative analgesia.
Mean (SD) age and weight of the patients were 4.5 (1.5) years and 20.5 (4.1) kg. The formation clearance to 4'-hydroxy-diclofenac (D4OH) (%CV) and to 5'-hydroxy-diclofenac (D5OH) were 8.41 (8.1) and 3.41 (113) l/h, respectively, standardized to a 70 kg person using allometric '¼ power' models. Clearance by other routes contributed 33.0 (64) l/h/70kg. Elimination clearance of hydroxyl metabolites was fixed at 27.5 l/h/70kg. The volumes of distribution of parent diclofenac and its hydroxyl metabolite were 22.8 (19.0) and 45.3 l/70kg, respectively. Suppositories had an absorption half-life of 0.613 (33.2) h with a lag time of 0.188 (24.9)

h. Inter-occasion variabilities of formation clearance to D4OH, diclofenac volume of distribution, absorption half-time and lag time for the suppository were 36%, 55%, 14% and 119%, respectively. The relative bioavailability of the suppositories compared to the enteric-coated tablets was 1.26.
The formation clearance of the active metabolite D4OH contributed 19% to total clearance (44.82 l/h/70kg). The rectum is a suitable route for administration of diclofenac in children 2-8 years of age and is associated with a higher relative bioavailability than enteric-coated tablets and an earlier maximum concentration (50 vs. 108 min). This pharmacokinetic profile renders diclofenac suppositories a suitable formulation for short duration surgery (**chapter 2.4**).

Pharmacodynamic studies

Analgesic APAP plasma concentrations after major surgery in neonates and infants have not yet been established in the literature. We therefore studied 40 children with a mean (SD) age of 10.3 (2.3) months, receiving 20 mg/kg APAP either orally (N=20) or rectally (N=20) 6-hourly after a rectal loading dose (40 mg/kg) during elective craniofacial surgery.
APAP plasma concentrations and AUC were higher in patients receiving rectal APAP (mean AUC: 171.2 mg.hour/l) than in patients receiving oral APAP (mean AUC: 111.9 mg.hour/l). Pain scores were higher in patients receiving oral APAP. However, after exclusion of the patients who vomited after oral APAP administration, APAP plasma concentrations and pain scores did not differ between both groups. There was no relation between APAP plasma concentrations and pain scores.
Although 9 of all 40 patients (22.5 %) did not reach the expected analgesic APAP plasma concentrations of 10- 20 mg/l, less than 7.5 % of the VAS pain scores exceeded the 4 cm cut-off score. This shows that analgesic APAP plasma concentrations after major surgery in this age group do not always reach the 10-20 mg/l level. Comparing rectal and oral administration, our findings also demonstrate that after craniofacial surgery

the rectal route is the best way of administering APAP , after a rectal loading dose of 40 mg/kg during surgery (**chapter 3.1**).

APAP is commonly prescribed for pain relief after (adeno)tonsillectomy [(A)TE] but reports of effectiveness are inconsistent. Diclofenac has been suggested as an alternative, although concerns about possible postoperative bleeding have tempered the use of diclofenac following (A)TE. We designed and conducted a study to compare the analgesic effect of rectally administered APAP and diclofenac in children undergoing (A)TE during ambulatory surgery and to assess the relations between APAP, diclofenac and D4OH plasma concentrations and postoperative pain scores. Furthermore we assessed the safety of diclofenac by monitoring postoperative bleeding and by registering the use of bipolar diathermy at the end of the procedure to arrest further bleeding.
Data of 60 patients were analyzed. Median (25th-75th percentile) age was 4 (3 – 5) years. Both groups consisted of 30 patients. There was no difference in analgesic effect between APAP and diclofenac and there was no relationship between plasma concentrations and pain scores. Two children from the APAP group had primary bleeding. Bipolar diathermy had been performed in 14 patients, 8 in the APAP group versus 6 in the diclofenac group.
These findings indicate that rectal diclofenac (2 mg/kg loading dose, 1 mg/kg 8 hourly) does not provide better pain relief than rectal APAP (40 mg/kg loading dose, 30 mg/kg 8-hourly) following (A)TE. No relationship was shown between plasma concentrations and pain scores, and no increase was shown in incidence of primary or secondary bleeding due to the administration of diclofenac, despite its effect on platelet aggregation (**chapter 3.2**).

The use of APAP in addition to continuous morphine infusion (CMI) has increased in recent years, despite the fact that safety and additional value of this combined treatment have never been studied in newborns and young infants. We therefore investigated if addition of APAP would decrease

postoperative morphine consumption in newborns and infants after major thoracic or abdominal surgery.
We studied 54 patients with a median (25th -75th percentile) age of 0 (0-2) months. APAP was administered to 29 patients and 25 received placebo. Additional morphine bolus requirements and increases in CMI did not differ between the groups, and nor did total morphine consumption. COMFORT and VAS scores as well did not differ between the groups. Postoperative total morphine consumption was not related to age, although children < 45 weeks PCA needed less additional morphine boluses ($p<0.01$) and needed less increases in CMI ($p<0.01$). Mean APAP plasma concentrations ranged from 9.5 to 27.6 mg/l.
Our findings show that APAP as adjuvant to CMI does not have an additional analgesic effect following major thoracic or abdominal surgery and should not be considered as standard of care in young infants, 0-2 months of age, undergoing such surgery (**chapter 3.3**).

Pharmacogenetic studies

Given that the toxic effects of APAP result from the oxidative metabolite N-acetyl-p-benzoquinone-imine (NAPQI), we conducted a pilot study to analyze the relation between APAP clearance and the genotype of the enzymes involved in the oxidative pathway, CYP3A4, CYP2E1 and possibly CYP3A5, in children receiving APAP during participation in previously reported studies. Furthermore we evaluated the relation between age and clearance to account for developmental changes.
Children received an APAP loading dose followed by APAP maintenance doses. Blood samples were obtained for APAP plasma concentration analysis. DNA was obtained from either a blood sample or a buccal swap. DNA analysis was performed for CYP3A4*1b, CYP3A5*3, CYP2E1*1d, CYP2E1*2, CYP2E1*5 and CYP2E1*7 genotype.
Data of 57 children (32 boys and 25 girls) were available for analysis. Median (25th-75th percentile) age and weight of the patients were 10 (1-11) months and 8.5 (3.5-10.4) kg, respectively. Median (25th-75th percentile)

APAP clearance was 1.1 (0.5-2.6) l/h. APAP plasma concentrations ranged from 0.0-59.9 mg/l.
This pilot study showed no relation between APAP clearance and CYP3A4, CYP3A5 and CYP2E1genotype. APAP clearance increased with age. Further research in a larger sample of patients is needed for complete analysis of the relation between APAP and CYP3A4, CYP3A5 and CYP2E1 genotype (**chapter 4.1**).

Data on the pharmacokinetics of diclofenac in children are scant. Studies mainly concentrated on measuring diclofenac plasma concentrations, notwithstanding the fact that the diclofenac metabolite D4OH has activity as well. Diclofenac is metabolized into D4OH by CYP2C9, whereas metabolism into D5OH occurs through CYP3A4 and possibly through CYP3A5. We conducted a pilot study in 32 children receiving 2 mg/kg diclofenac, analyzing the relations between diclofenac clearance and CYP2C9, CYP3A4 and CYP3A5 genotype, between D4OH formation clearance and CYP2C9 genotype and between D5OH formation clearance and CYP3A4 and CYP3A5 genotype.
As blood samples were not available in 6 patients, we analyzed the data of 26 patients only, 14 boys and 12 girls. Median (25th-75th percentile) age and weight of the patients were 4.0 (3.8-5.0) years and 20.0 (17.8-22.7) kg, respectively. Diclofenac plasma concentrations ranged from 0-2.6 mg/l, D4OH plasma concentrations ranged from 0-0.5 mg/l and D5OH plasma concentrations ranged from 0-0.6 mg/l. DNA was analyzed for CYP3A4*1b, CYP3A5*3, CYP2C9*2 AND CYP2C9*3.
This pilot study showed no relation between diclofenac clearance and CYP3A4, CYP3A5, CYP2C9 genotype. Nor did it show a relation between formation clearance to D4OH and CYP2C9 or between formation clearance to D5OH and CYP3A4 and CYP3A5. Further research in a larger sample of patients is needed for complete analysis of the relation between diclofenac clearance, formation clearances to D4OH and D5OH and CYP3A4, CYP3A5 and CYP2C9 genotype (**chapter 4.2**).

General discussion

Considering APAP pharmacokinetics, one should keep in mind that the plasma compartment is not the effect compartment and that the maximum analgesic effect is delayed after peak plasma concentrations. Measuring APAP plasma concentrations as an indicator for potential APAP toxicity or to determine clearance, one should realize that the oxidative metabolite NAPQI is responsible for APAP's toxic effects and that both volume of distribution as well as clearance are obscured by the variability in bioavailabilty after oral or rectal administration. Furthermore we need to explore developmental changes in APAP clearances, which might partly be due to developmental changes in enzyme activity. Thus we will be able to predict the risk of APAP toxicity for children in different age groups.
The main problem in studying pharmacodynamics in the very young is to objectively assess the analgesic effect of APAP, because self-report is not possible in these patients. Using the COMFORT scale and the Visual Analogue Scale (VAS) scores – as assigned by the nurses who take care of the children – as the basis for an analgesic algorithm, an attempt is made to objectively assess pain and hence the analgesic effect. Non-invasive methods in combination with validated pain assessment instruments open new ways to explore the relation between neurophysiological changes and observed behavior during painful moments. Several methods – fMRI, PET scan, laser Evoked Potentials – may serve to reveal the integrity of the pain conducting system). The value of Electroencephalography (EEG) and Sympathic Skin Response (SSR) for postoperative pain assessment is described in **chapter 6**.
Evaluating the relation between clearances and genotype, the main problem in pharmacogenetic studies is the number of patients needed to draw conclusions. As population allelic frequencies of most mutations are low, large samples of patients are needed in order to fully investigate the effect of DNA mutations on enzyme activity or expression. When linking genetic blueprint to toxic APAP effects, developmental changes in enzyme activity and the impact of specific mutations on enzyme activity should be further investigated. However, only the administration of different APAP doses to

children of different ages and the measuring of NAPQI formation clearance or mercapturine or cysteine metabolites, will enable to fully investigate the contribution of the oxidative pathway under different circumstances in different age groups, and thus to predict potential toxic effects of APAP. From a health care perspective this is the most important issue (**chapter 5**).

Future perspectives

Although the concept of nociception and the presence of specific pain tracts in the central nervous system have been known for many years, very little clinical research has been performed on the central conduction of pain in young infants. Non-invasive methods in combination with validated pain assessment instruments are potentially powerful tools to determine a relationship between neurophysiological changes and the observed behavior during painful moments. Following major thoracic or abdominal surgery a 30-minutes simultaneous EEG and SSR registration of was made on the first and second days postoperatively in 31 patients aged 0-12 months. During registration a painful stimulus, consisting of standardized pressure on a fingernail, was applied after 15 and after 20 minutes. Postoperative pain assessment using the validated COMFORT scale and the Visual Analogue Scale (VAS) was performed every 2 or 3 hours and during SSR and EEG registration.

Analysis of the frequencies and the amplitudes of the SSRs during the SSR registration showed no relation between age and SSR frequency or between age and SSR amplitude. The SSR amplitudes in awake situation were highest compared to drowsy situation and sleep. In addition, SSR amplitudes on the second day postoperatively were higher than those on the first day postoperatively. Analysis of the video registrations showed no correlation between SSR frequency and VAS or COMFORT scale scores. An increase in spontaneous SSR frequency was observed during the 2 minutes preceding the painful stimulus. Spontaneous SSR frequency decreased over the interval between the first and the second painful stimulus. Visual assessment of the EEG registrations showed that the

number of unchanged EEGs expressed as a percentage of the total number of unchanged EEGs and EEGs showing arousal, in sleep, awake and drowsy situation after spontaneous SSRs was 71%, 23% and 62%, respectively, compared to a percentage of 22% after induced SSRs.

The clinical role of the SSR could not be assessed unambiguously in this study, most likely because of the homogeneity of the group of children included in this study with respect to adequate and equal pain control. Also, the ICU environment might induce non-painful distress, resulting in spontaneous SSRs. Nevertheless, SSR monitoring promises to be an interesting new variable in future pain studies (**chapter 6**).

Conclusion

Against the background of the increasing knowledge of genetics, these pharmacokinetic and pharmacodynamic studies together with new, non-invasive methods to visualize pain within the central nervous system can be considered as a model for future research of drugs, in particular analgesic drugs.

Chapter 7.2

Samenvatting

Samenvatting

Paracetamol (APAP), in de Verenigde Staten bekend als acetaminophen, wordt zowel in ziekenhuizen als thuis, gebruikt als middel tegen koorts en lichte en postoperatieve pijn. Alhoewel APAP zonder recept verkrijgbaar is, en na nystatine en cisapride het meest gebruikte geneesmiddel is in onze kinderchirurgische intensive care unit, is er verrassend weinig bekend over de farmacokinetische en farmacodynamische eigenschappen van APAP bij kinderen.
Om in deze lacune te voorzien hebben we onderzoek verricht bij jonge kinderen naar de farmacokinetiek (d.w.z. de absorptie, distributie, het metabolisme en de eliminatie) van APAP, de farmacodynamiek (d.w.z. het veroorzaakte effect) door APAP en de farmacogenetische eigenschapen (d.w.z. de invloed van DNA op het metabolisme) van APAP. De verschillende onderzoeken zijn onderstaand samengevat.

Farmacokinetisch onderzoek

De door APAP geïnduceerde analgesie wordt wellicht centraal tot stand gebracht, met het tijdsverloop van APAP in de liquor parallel aan dat van het pijnstillende effect. Er zijn echter maar enkele onderzoeken gepubliceerd naar de concentraties van APAP in liquor bij kinderen. Wij onderzochten leeftijdsafhankelijke veranderingen in de plasma-tot-liquor evenwichtshalfwaardetijd (Teq) van APAP bij 41 kinderen, die een (semi-)electieve ingreep ondergingen – het aanbrengen of vernieuwen van een ventriculo-peritoneale shunt of het inbrengen van een tijdelijke externe drain – en die een uur voor de ingreep een oplaaddosis van 30-40 mg/kg APAP kregen toegediend.
De mediane (25^{e}-75^{e} percentiel) leeftijd en het gewicht van de kinderen waren respectievelijk 12 (3-62) maanden en 10,0 (5,8-20,0) kg. De mediane (25^{e}-75^{e} percentiel) tijd tussen het toedienen van de oplaaddosis en het afnemen van bloed bedroeg 125 (95-210) minuten, die tussen het toedienen van de oplaaddosis en het afnemen van liquor 133 (33-202) minuten. De mediane Teq in de groep, gestandaardiseerd naar een persoon van 70 kg,

was 1,93 (CV 43%) uur, een schatting die overeenkomt met die beschreven in volwassenen (2,1 uur). Afgezien van het verband voorspeld door lichaamsgrootte werd geen verband aangetoond tussen leeftijd en Teq. De APAP plasmaconcentraties liepen uiteen van 0,0-33,0 mg/l, de APAP liquorconcentraties van 0,0-21,0 mg/l.
Het is bij kinderen eerder de lichaamsgrootte dan de ontwikkeling van de bloed-hersenen-barriere die de veranderingen in Teq met de leeftijd bepaalt. Op grond van deze bevindingen voorspellen we dat bij een neonaat (3,5 kg), een eenjarig kind (10 kg), een vijfjarig kind (20 kg), een tienjarig kind (30 kg) en een volwassene (70 kg) de Teq respectievelijk 0,9, 1, 1,4, 1,6 en 1,93 uur bedraagt (**hoofdstuk 2.1**)

Er is ook weinig bekend over het metabolisme van APAP bij jonge kinderen. Eerder is onderzoek gedaan naar de urinemetaboliet terugwin-ratio's na een eenmalige dosis APAP aan neonaten (< 6 weken) en oudere kinderen (3-9 jaar). Er is nog geen onderzoek gedaan bij jonge kinderen. Om in deze lacune te voorzien hebben we onderzoek gedaan bij 47 jonge kinderen die een grote craniofaciale ingreep moesten ondergaan. Als postoperatieve pijnstilling kregen ze om de zes, acht of twaalf uur 19-45 mg/kg APAP toegediend in de vorm van een elixir of een zetpil. Tijdens de operatie hadden ze rectaal een oplaaddosis van 33–59 mg/kg gekregen.
De mediane (SD) leeftijd en het gewicht van de patiënten waren respectievelijk 11,8 (2,5) maanden en 9,1 (1,9) kg. De klaring van APAP naar APAP-glucuronide (%CV) en naar APAP-sulfaat bedroeg respectievelijk 6,6 (11,5) en 7,5 (11,5) l/h, gestandaardiseerd naar een persoon van 70 kg met behulp van allometrische '¼ power' modellen. De klaring naar glucuronide, maar echter niet die naar sulfaat, bleek leeftijdsafhankelijk te zijn, en nam toe van een voorspelde waarde van 2,73 l/h/70kg voor een neonaat, tot een waarde van 6,6 l/h/70kg voor een volwassene, met een ontwikkelingshalfwaardetijd van 8,09 maanden. De urineklaring van APAP-glucuronide, APAP-sulfaat en onveranderde APAP (%CV) bedroeg respectievelijk 2,65, 3,03 en 0,55 (28) l/h/70kg. De urineklaring van onveranderde APAP en zijn metabolieten was gerelateerd aan de geproduceerde hoeveelheid urine. Klaring toe te schrijven aan

andere routes dan deze gemeten in urine kon niet worden geïdentificeerd. De glucuronide/sulfaat ratio bedroeg 0,69 op de leeftijd van 12 maanden. Sulfaat metabolisme droeg 50% bij aan de APAP-klaring. Klaring naar glucuronide neemt bij jonge kinderen toe met de leeftijd, in tegenstelling tot de klaring naar sulfaat. De renale klaring van APAP en zijn metabolieten neemt toe met de hoeveelheid geproduceerde urine.
Uit zowel dit onderzoek als uit andere publicaties blijkt dat het metabolisme van APAP via de glucuronidering hetzelfde is bij jonge en oudere kinderen, maar bij volwassenen hoger is in vergelijking met kinderen. Oxidatieve routes kwamen niet naar voren uit dit onderzoek bij jonge kinderen en dit zou gedeeltelijk de lagere incidentie van hepatotoxiciteit bij jonge kinderen kunnen verklaren (**hoofdstuk 2.2**).

We hebben ook onderzoek gedaan naar de farmacokinetische en farmacodynamische werking van APAP na intraveneuze toediening van een enkele dosis propacetamol aan twintig prematuur geborenen en tien ‘a terme’ geborenen op hun eerste levensdag.
De gemiddelde (SD) serum halfwaardetijd ($t_{½}$) in de prematuur geborenen en de ‘a terme’ geborenen bedroeg respectievelijk 277 (143) min en 172 (59) min ($p < 0,05$), en de gemiddelde (SD) klaringen (CL) waren respectievelijk 0,116 (0,08) l/kg/h en 0,170 (0,06) l/kg/h ($p < 0,05$). Er bleek een correlatie te bestaan tussen duur van de zwangerschap en $t_{½}$ ($r = -0,46$). Invloed van geslacht of prenatale toediening van steroïden op de farmacokinetische werking van APAP, was niet aantoonbaar. De mate van pijnstilling bij vijftien andere neonaten die geen andere medicatie kregen dan propacetamol leek op een therapeutisch niveau te liggen (> 5 mg/l). De ontwikkelingstrend van CL en $t_{½}$ bij zowel de prematuur geborenen als de ‘a terme’ geborenen kwam overeen met de eerder gerapporteerde farmacokinetische gegevens van propacetamol voor een periode later dan de neonatale periode (**hoofdstuk 2.3**).

Het metabolisme van diclofenac in kinderen is alleen nog maar onderzocht na intraveneuze toediening en na het gebruik van tabletten met een coating. Er zijn geen gegevens bekend over de farmacokinetiek van diclofenac of

zijn hydroxyl-metaboliet na rectale toediening bij kinderen. Daarom deden we onderzoek bij 26 jonge kinderen van wie de amandelen werden geknipt en die een oplaaddosis diclofenac van 2 mg/kg kregen toegediend, gevolgd door 1 mg/kg om de acht uur als zetpil voor postoperatieve pijnstilling. De gemiddelde (SD) leeftijd en het gewicht van de patiënten waren respectievelijk 4,5 (1,5) jaar en 20,5 (4,1) kg. De klaring naar 4'-hydroxy-diclofenac (D4OH) (%CV) en naar 5'-hydroxy-diclofenac (D5OH) bedroeg respectievelijk 8,41 (8,1) en 3,41 (113) l/h, gestandaardiseerd naar een persoon van 70 kg m.b.v. allometrische '¼ power' modellen. Klaring langs andere routes droeg 33,0 (64) l/h/70kg bij. De eliminatieklaring van hydroxyl-metabolieten werd vastgesteld op 27,5 l/h/70kg. Het verdelingsvolume van diclofenac en zijn hydroxyl-metaboliet bedroeg respectievelijk 22,8 (19,0) en 45,3 l/70kg. De zetpillen hadden een absorptie halfwaardetijd van 0.613 (33.2) uur met een vertragingstijd (lag time) van 0,188 (24,9) uur. De inter-occasion variabiliteit van klaring naar D4OH, diclofenac verdelingsvolume, absorptie-halfwaardetijd en lag time voor de zetpillen was respectievelijk 36%, 55%, 14% en 119%. De relatieve biologische beschikbaarheid van de zetpillen in vergelijking met de tabletten met coating bedroeg 1,26.
De klaring naar de actieve metaboliet D4OH droeg 19% bij aan de totale klaring (44,82 l/h/70kg). De rectale route is een geschikte route voor de toediening van diclofenac bij kinderen van 2-8 jaar, en geeft een hogere relatieve biologische beschikbaarheid dan tabletten met een coating en een eerder-optredende maximale concentratie (50 vs. 108 min). Dit farmacokinetische profiel maakt diclofenac zetpillen geschikt bij kortdurende ingrepen (**hoofdstuk 2.4**).

Farmacodynamisch onderzoek

Er zijn nog geen publicaties verschenen waarin pijnstillende APAP-concentraties in plasma, na grote ingrepen bij neonaten en jonge kinderen werden gepresenteerd. Wij deden daarom onderzoek bij 40 kinderen met een gemiddelde (SD) leeftijd van 10,3 (2,3) maanden, die na een electieve

craniofaciale operatie, om de zes uur 20 mg/kg APAP kregen toegediend, hetzij oraal (n = 20) hetzij rectaal (n = 20), allen na een rectale oplaaddosis van 40 mg/kg tijdens de operatie.
De APAP-concentraties in plasma en AUC bleken bij de patiënten die rectaal APAP kregen toegediend hoger te zijn (gemiddelde AUC: 171,2 mg.uur/l) dan bij degenen die APAP oraal kregen toegediend (gemiddelde AUC: 111,9 mg.uur/l). Bij de laatste groep waren de pijnscores hoger. Er was geen verschil in APAP-concentraties en pijnscores in beide groepen als de patiënten uit de orale groep die moesten overgeven na toediening van APAP niet werden meegeteld. Er werd geen verband aangetoond tussen de APAP-concentraties en de pijnscores.
Alhoewel bij 9 van de 40 patiënten (22,5 %) de verwachte pijnstillende APAP-concentraties in plasma van 10-20 mg/l niet werden bereikt, waren slechts 7,5 % van de VAS pijnscores hoger dan het afkappunt van 4 cm. Na zware ingrepen worden bij kinderen van deze leeftijd dus niet altijd de veronderstelde pijnstillende APAP-concentraties in plasma van 10-20 mg/l bereikt. Tevens blijkt dat na een craniofaciale operatie, waarbij een rectale oplaaddosis van 40 mg/kg is toegediend, de rectale route voor APAP de voorkeur geniet boven de orale route (**hoofdstuk 3.1**).

Algemeen wordt APAP voorgeschreven als pijnbestrijding na het knippen van de amandelen, maar de publicaties over de effectiviteit daarvan zijn niet consistent. Een alternatief is diclofenac, maar het gebruik hiervan wordt geremd door angst voor postoperatieve bloedingen. We hebben een onderzoek opgezet, waarin we de pijnstillende werking van rectaal toegediende APAP en diclofenac konden vergelijken bij kinderen waarbij in dagbehandeling de amandelen werden geknipt, en tevens de relaties konden vaststellen tussen APAP, diclofenac, D4OH-concentraties in plasma, en postoperatieve pijnscores. De veiligheid van diclofenac en het gebruik van bipolaire diathermie aan het eind van de ingreep, beoordeelden wij door het registreren van postoperatieve bloedingen. De gegevens van 60 patiënten, met een mediane (25^{e}-75^{e} percentiel) leeftijd van 4 (3-5) jaar, werden geanalyseerd. De helft daarvan kreeg APAP toegediend, de andere helft diclofenac. We vonden geen verschil in pijnstillende werking tussen APAP

en diclofenac, en ook geen relatie tussen concentraties in plasma en pijnscores. Bij twee kinderen, die beiden APAP hadden gekregen, ontstonden primaire bloedingen. Bipolaire diathermie werd bij 14 patiënten toegepast, en wel bij 8 die APAP, en 6 die diclofenac hadden gekregen. Uit onze bevindingen leiden we af dat rectaal toegediende diclofenac (2 mg/kg oplaaddosis, 1 mg/kg om de acht uur) na het knippen van de amandelen geen betere pijnbestrijding geeft dan rectaal toegediende APAP (40 mg/kg oplaaddosis, 30 mg/kg om de acht uur). Er werd geen relatie aangetoond tussen concentraties in plasma en pijnscores, noch een toename van primaire of secundaire bloedingen als gevolg van de toediening van diclofenac, dat bekend staat om de verstoring van de aggregatie van bloedplaatjes (**hoofdstuk 3.2**).

In de afgelopen jaren kan er een toename worden gezien van het gebruik van APAP in aanvulling op continue morfine infusie (CMI), ondanks het feit dat de veiligheid en de toegevoegde waarde van deze combinatiebehandeling nog niet is onderzocht bij pasgeborenen en jonge kinderen. Daarom onderzochten we of bij pasgeborenen en jonge kinderen die extra APAP kregen na een grote thorax- of buikoperatie, de behoefte aan morfine daalde.
Van een totaal van 54 patiënten met een mediane (25^{e}-75^{e} percentiel) leeftijd van 0 (0-2) maanden kregen er 29 APAP, en 25 placebo. De behoefte aan aanvullende morfine in de vorm van bolussen en de toenamen in CMI verschilden niet in beide groepen. Er bleek ook geen significant verschil in het totale morfineverbruik te zijn. De COMFORT en VAS scores verschilden niet tussen beide groepen. Het postoperatieve totale morfineverbruik was niet gerelateerd aan de leeftijd, alhoewel kinderen jonger dan 45 weken post conceptie minder aanvullende morfinebolussen nodig hadden ($p < 0.01$) en konden volstaan met geringere toenamen in CMI ($p < 0.01$). De gemiddelde APAP-concentraties in plasma liepen uiteen van 9,5 tot 27,6 mg/l.
Onze bevindingen geven aan dat APAP ter ondersteuning van CMI geen extra pijnstillende uitwerking heeft, en dat deze combinatie niet als

standaardbehandeling moet worden beschouwd voor jonge kinderen van 0-2 maanden oud na een grote thorax- of buikoperatie (**hoofdstuk 3.3**).

Farmacogenetisch onderzoek

Aangezien de toxische werking van APAP wordt veroorzaakt door de oxidatieve metaboliet N-acetyl-p-benzoquinone-imine (NAPQI), deden we een pilot studie om de relatie te analyseren tussen APAP-klaring en het genotype van de enzymen die betrokken zijn bij de oxidatieve route, CYP3A4, CYP2E1 en mogelijk CYP3A5. De studiegroep bestond uit kinderen die APAP kregen toegediend in het kader van eerdere onderzoeken. Tevens hebben we de relatie tussen leeftijd en klaring geanalyseerd om een verklaring te vinden voor veranderingen tijdens de ontwikkeling.
De kinderen kregen een oplaaddosis APAP gevolgd door 'onderhouds'-doseringen APAP. Er werd bloed afgenomen voor de bepaling van de APAP-concentratie in plasma. DNA werd verkregen hetzij uit het bloed hetzij uit het wangslijmvlies, en gescreend op de CYP3A4*1b, CYP3A5*3, CYP2E1*1d, CYP2E1*2, CYP2E1*5 en CYP2E1*7.
Uiteindelijk waren de gegevens van 57 kinderen, 32 jongetjes en 25 meisjes, beschikbaar voor analyse. Hun mediane (25^e-75^e percentiel) leeftijd en gewicht waren respectievelijk 10 (1-11) maanden en 8,5 (3,5-10,4) kg. De mediane (25^e-75^e percentiel) APAP-klaring bedroeg 1,1 (0,5-2,6) l/h, en de APAP-concentraties in plasma liepen uiteen van 0.0-59.9 mg/l.
Deze pilot studie toonde geen verband aan tussen APAP-klaring en de CYP3A4, CYP3A5 en CYP2E1 genotypen. De APAP-klaring nam toe met de leeftijd. Aanvullend onderzoek in een grotere groep patiënten is noodzakelijk om de relaties tussen APAP en de CYP3A4, CYP3A5 en CYP2E1 genotypen volledig te kunnen analyseren (**hoofdstuk 4.1**).

Er is weinig bekend over de farmacokinetische eigenschappen van diclofenac bij kinderen; eerder onderzoek richtte zich vooral op het meten

van de concentraties diclofenac in plasma. Diclofenac heeft echter ook een werkzame metaboliet, D4OH. Diclofenac wordt gemetaboliseerd tot D4OH door CYP2C9, of tot D5OH door CYP3A4 en wellicht CYP3A5. We hebben een pilot studie verricht bij 32 kinderen die 2 mg/kg diclofenac kregen toegediend, en analyseerden de relaties tussen diclofenac-klaring van en de CYP2C9, CYP3A4 en CYP3A5 genotypen, tussen de klaring naar D4OH en het CYP2C9 genotype, en tussen de klaring naar D5OH en de CYP3A4 en CYP3A5 genotypen.
Doordat van 6 patiënten geen bloed beschikbaar was, werden uiteindelijk de gegevens van 26 patiënten geanalyseerd, 14 jongens en 12 meisjes. De mediane (25^e-75^e percentiel) leeftijd en het gewicht waren respectievelijk 4,0 (3,8-5,0) jaar en 20,0 (17,8-22,7) kg. De concentraties diclofenac in plasma liepen uiteen van 0-2,6 mg/l, die van D4OH van 0-0,5 mg/l, en die van D5OH van 0-0,6 mg/l. Het DNA van de kinderen werd gescreend op CYP3A4*1b, CYP3A5*3, CYP2C9*2 en CYP2C9*3.
We vonden geen relatie tussen diclofenac-klaring en de CYP3A4, CYP3A5, en CYP2C9 genotypen, en evenmin tussen de klaring naar D4OH en CYP2C9, of de klaring naar D5OH en CYP3A4 en CYP3A5.
Vervolgonderzoek in een grotere groep patiënten is nodig om de relaties tussen de klaring van diclofenac, de klaring naar D4OH en naar D5OH, en de CYP3A4, CYP3A5 en CYP2C9 genotypen volledig te kunnen analyseren (**hoofdstuk 4.2**).

Algemene beschouwing

Wat betreft de farmacokinetische eigenschappen van APAP is van belang dat het plasma-compartment niet het effect-compartment is, en dat de maximale pijnstillende werking vertraagd volgt na piekconcentraties in plasma. Als men de APAP-concentratie in plasma wil gebruiken als indicator voor een mogelijke toxische werking van APAP of om de klaring te bepalen, dient men zich te realiseren dat de oxidatieve metaboliet NAPQI verantwoordelijk is voor de toxische werking van APAP, en dat zowel het

distributievolume als de klaring worden gemaskeerd door de variabiliteit in biologische beschikbaarheid na orale of rectale toediening.
Voorts dienen we nader inzicht te krijgen in de ontwikkelingsveranderingen in APAP-klaring, die wellicht gedeeltelijk samenhangen met ontwikkelingsveranderingen in de activiteit van enzymen. Dit zal het mogelijk maken het risico van een toxische werking van APAP voor kinderen in verschillende leeftijdsgroepen te bepalen.
Het grootste probleem bij het bestuderen van de farmacodynamiek is het objectief vaststellen van de pijnstillende werking van APAP, aangezien de kinderen van de leeftijd die we in onze onderzoeken hebben betrokken niet zelf hun pijngevoel kunnen rapporteren. Het toepassen van de COMFORT schaal en de Visuele Analoge Schaal (VAS) door de verpleegkundigen die de kinderen verzorgen, als basis voor een analgetica algoritme, is een stap in de goede richting om pijn – en daarmee de pijnstillende werking van het middel – objectief vast te stellen. Niet-invasieve methoden in combinatie met gevalideerde pijnmeetinstrumenten bieden nieuwe mogelijkheden om de relatie tussen neurofysiologische veranderingen en geobserveerd gedrag tijdens pijnmomenten te onderzoeken. Met verschillende methoden – fMRI, PET scan, laser Evoked Potentials – zou de integriteit van het pijngeleidingssysteem kunnen worden achterhaald. De bruikbaarheid van Electro-Encefalo-Grafie (EEG) en Sympathic Skin Response (SSR) voor postoperatieve pijnbeoordeling wordt beschreven in **hoofdstuk 6**.
Het probleem bij farmacogenetisch onderzoek is het grote aantal patiënten dat nodig is om verbanden te kunnen leggen tussen klaring en genotype, zodat de juiste conclusies kunnen worden getrokken. Omdat de allelen van de meeste mutaties weinig voorkomen in de bevolking, zijn grote cohorten patiënten nodig om de effecten van DNA-mutaties op de activiteit of expressie van enzymen volledig te kunnen onderzoeken. Teneinde de genetische blauwdruk in verband te kunnen brengen met de toxische werking van APAP, dienen de ontwikkelingsveranderingen in enzymactiviteit en de impact van specifieke mutaties op de activiteit van enzymen nader onderzocht te worden. Echter, slechts door het toedienen van verschillende doseringen APAP aan kinderen van verschillende leeftijd en door het meten van de klaring van NAPQI-vorming of mercapturine of

cysteine metabolieten, kan men de bijdrage van de oxidatieve route onder verschillende omstandigheden in verschillende leeftijdsgroepen volledig vaststellen, en daarmee de mogelijke toxische uitwerking van APAP voorspellen. Gezien vanuit het gezichtspunt van de gezondheidszorg is dit het allerbelangrijkste thema (**hoofdstuk 5**).

Toekomstperspectieven

Alhoewel het begrip nociceptie en het bestaan van specifieke pijnkanalen in het centrale zenuwstelsel al vele jaren bekend zijn, is er nog maar weinig klinisch onderzoek gedaan naar de centrale geleiding van pijn bij jonge kinderen. Niet-invasieve methoden in combinatie met gevalideerde pijnmeetinstrumenten zijn in aanleg krachtige middelen om verbanden te leggen tussen neurofysiologische veranderingen en het waargenomen gedrag tijdens pijnmomenten. Bij 31 patiënten van 0-12 maanden oud werden gedurende 30 minuten simultaan een EEG- en een SSR- registratie gedaan op de eerste en de tweede dag na een grote thorax- of buikoperatie. Tijdens die registratie kregen de patiënten na 15 en na 20 minuten een pijnprikkel in de vorm van gestandaardiseerde druk, uitgeoefend op een van de vingernagels. Om de twee of drie uur en tijdens de EEG- en SSR-registraties werd de postoperatieve pijn gemeten m.b.v. de gevalideerde COMFORT schaal en de Visuele Analoge Schaal (VAS).
Bij analyse van de frequenties en de amplitudes van de SSR's bleek er geen verband te zijn tussen leeftijd en SSR-frequentie of tussen leeftijd en SSR-amplitude. De SSR-amplituden in wakkere toestand bleken hoger te zijn dan die bij slaperigheid of slaap. Voorts waren de SSR-amplitudes gemeten op de tweede dag hoger dan die op de eerste dag na de operatie. De analyse van de videoregistraties toonde geen verband aan tussen SSR-frequentie en scores op de VAS of COMFORT schaal. Een toename in spontane SSR-frequentie werd waargenomen gedurende de 2 minuten voorafgaand aan de pijnprikkel. De spontane SSR-frequentie nam af gedurende het interval tussen de eerste en tweede pijnprikkel. De visuele beoordeling van de EEG-registraties gaf als uitkomst dat het aantal ongewijzigde EEG's uitgedrukt

als percentage van het totale aantal ongewijzigde EEG's en de EEG's die 'in slaap', 'in wakkere toestand' en 'in slaperige toestand' lieten zien na spontane SSR's, respectievelijk 71%, 23% en 62% bedroeg, in vergelijking met een percentage van 22% na geïnduceerde SSR's.
De klinische rol van de SSR kon bij dit onderzoek niet ondubbelzinnig worden vastgesteld, hoogstwaarschijnlijk vanwege de homogeniteit van de onderzochte groep kinderen wat betreft adequate en gelijke mate van pijnbestrijding. Het verblijf op de intensive care unit zou een kind "zonder pijn" ook overstuur kunnen maken, met spontane SSR's als gevolg. Niettemin is SSR-monitoring een interessante nieuwe optie bij toekomstig pijnonderzoek (**hoofdstuk 6**).

Conclusie

Tegen de achtergrond van onze groeiende kennis van de genetica, kunnen deze farmacokinetische en farmacodynamische onderzoeken in combinatie met nieuwe methoden om pijn in het centrale zenuwstelsel op een niet-invasieve wijze te visualiseren, worden beschouwd als een model voor nader onderzoek naar geneesmiddelen, en pijnstillende middelen in het bijzonder.

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Curriculum Vitae

Caroline van der Marel werd op 28 januari 1977 geboren te Voorburg. In 1995 deed ze eindexamen in 10 vakken aan het Marnix Gymnasium te Rotterdam en behaalde ze haar gymnasium diploma cum laude. In de periode van 1995 tot 1999 studeerde ze geneeskunde aan de Erasmus Universiteit Rotterdam. Haar afstudeeronderzoek deed ze op de afdeling Kinderchirurgie in het Erasmus MC-Sophia onder leiding van professor Tibboel. Na het behalen van haar doctoraal in 1999 heeft ze tot 2002 promotie onderzoek gedaan op de afdeling kinderchirurgie in het Erasmus MC-Sophia, waarvan de resultaten in dit proefschrift zijn beschreven. In de periode van 1997 tot 2002 is ze tevens werkzaam geweest als teamleidster van het studententeam cardiologie in het thoraxcentrum in het Erasmus MC. In 2002 is ze begonnen aan haar co-schappen, die ze in maart 2003 af zal ronden.