Pain Assessment and Analgesia in the Newborn: An Integrated Approach.

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Beoordeling van Pijn en Pijnbestrijding bij Pasgeborenen:

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"All substances are poisons; there is none which is not. The right dose differentiates a poison and remedy." (Paracelsus 1493-1541)

Aan Francis, Tim, Marijne
Aan mijn ouders

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I

Introduction

I.1 General Introduction

In the early 1980's the treatment and prevention of pain in children, especially in newborns and infants, did not have a high priority in daily clinical practice. Following the pioneer work done both outside the Netherlands by Anand, Aynsley-Green, McGrath PA, and McGrath PJ and in our country by Boelen-van der Loo, Huijer-Abu Saad, de Kuiper and various support groups the scope has changed.

Infants, even prematurely born ones, are capable of feeling pain.[1,2,3] Cutaneous pain receptors are present over the whole body at 20 weeks post-conceptional age. By 30 weeks myelinization of nerve tracts in the spinal cord, the brain stem and the thalamus is complete and the cerebral cortex is functionally premature in infants as well.[1] Due to the immature organisation of connections within the dorsal horn of the spinal cord,[4] and the not yet fully developed inhibiting processes pain thresholds in neonates are lower than they are in adults.[5] This pain threshold is lower in preterm infants than it is in full-term infants, and is presumed to increase after a number of painful procedures, and with postnatal age.[6] However, contradicting these findings, recent research shows that thresholds remain low after injury to the contralateral leg even in infants who were term taking conceptional age as a reference point.[7] This suggests that exposure to frequent and multiple procedures in the neonatal intensive care unit (NICU), which is very common especially in the very preterm infant,[8] increases pain awareness.[5]

Thus newborn infants in fact are not only capable of feeling pain, but even seem to experience more pain than do older children and adults.

Pain results in stress, and this in its turn results in metabolic instability. Primary stress responses to surgery were found to be significantly greater in infants who did not receive analgesic drugs as compared to those who did receive analgesics, and these infants were more susceptible to circulatory and metabolic complications postoperatively.[9-11]

In two classical papers, published in the Lancet and the New England Journal of Medicine, Anand and colleagues showed that preemptive analgesia following cardiac surgery dramatically decreased mortality and morbidity rates.[11,12]

The first studies of long-term effects of early pain experience show that neonatal pain leads to different responses to subsequent painful events.[13-15]

Despite convincing evidence from basic research, and despite the fact that more and more nurses and doctors realize that preventing and treating pain will improve outcome, many neonates are nowadays still subjected to unnecessary pain.[16-18]

Following new insights into the negative effects of pain and stress in newborns, the prescription of systemic opioids for major surgery in newborn infants increased from 10% of infants in 1988 to 91% in 1995.[18] For neonates, 92% of anaesthesists used opioids in 1995 as compared with 18% in 1988.[18] A survey of the use of analgesics for postoperative and non-operative purposes in NICUs in the United Kingdom showed that 59% of these units used drugs for pain relief more than once a week, and 26% more than once a month.[19] A comparable study in the USA revealed a 28.6-fold variation among NICUs in narcotic administration in very low-birth-weight neonates.[20]

The ten NICUs in the Netherlands give analgesia in 40% of ventilated infants, in 80% after traumatic delivery and before intubation, and in 100% before chest drain insertion or postoperatively.[21]

Despite the increased awareness of the problem of procedural pain in infants,

clinicians generally believed that neither pharmacological agents nor comfort measures were frequently used to manage infant pain.[22] This is partly due to ignorance about the pharmacokinetics and pharmacodynamics of drugs; the lack of validated pain assessment instruments and finally the lack of proper training in pain assessment and management. Lack of readily available information in the paediatric literature is a contributing factor as well.[23]
Several editorials in the past 15 years pointed out that premature and newborn babies do feel pain and that it is time for a change in our attitude towards the prescription of analgesia in this age group, but unfortunately the message had to be repeated over all these years, as attitude changes slowly.[3,5,24-32]

Assessment of pain in neonates is hard to measure objectively. Several parameters have been used to measure pain, but these are mostly used in connection with acute sharp pain (Table 1). Combining several of these parameters in both clinical and research setting has resulted in the development of a range of pain assessment instruments.[33-39]
Several multicenter trials are now in progress to validate and evaluate these instruments for specific conditions in preterm, full-term, and surgical newborns and infants.

Parameters used for pain assessment	
Behavioural	- Changes in facial expression, as brow bulge, nasolabial furrow - Cry - Flexor withdrawal of limb
Physiological	- Increase in heart rate - Increase in respiratory rate - Increase in blood pressure - Decrease in oxygen saturation - Increase in palmar sweating* - Increase in intracranial pressure
Hormonal	- Increased plasma concentrations of catecholamines, cortisol, growth hormone, and glucagon - Decrease in insulin concentration

*Table 1 Parameters used for pain assessment in infants and children. * Not in preterm infants; in term infants present after several days.*

In children, analgesia is generally provided by morphine and paracetamol and to a lesser extent by fentanyl.
On the basis of the concept that neonates indeed can feel pain, several authors studied the effect on stress parameters.[40,41] However, single dose effects were mostly investigated, whereas often data on multiple doses or continuous infusion are needed.
Opiates are widely used, with a wide range of doses, and adverse effects are commonly

reported.[19,42] Paracetamol is the most commonly used non-opioid analgesic drug; of which adverse effects are rarely reported.[19]
In adults and older children pain relief is considered the most important target in the administration of analgesia, and side effects are of secondary importance.[32] The same should apply to infants and newborns.
As they can feel pain, and invasive procedures resulting in pain or distress are frequently performed in infants admitted to the NICU,[8] studies evaluating the effects of morphine and paracetamol in this age group are warranted.
Of the drugs that have been approved by the Food and Drug Administration (FDA) in the past 30 years 80% may not be administered officially to newborns and validated data are lacking.[43,44] In a recent survey in European paediatric hospitals, over half of the patients were given unlicensed or off-label drugs, among which morphine and the most widely prescribed drug and analgesic paracetamol.[45]
Pharmacokinetic and pharmacodynamic data are essential in the first few days after birth, as during transition many metabolic processes undergo rapid changes. Several factors affecting pharmacokinetics and drug metabolism change during the last weeks of gestation,[46,47] and during the first days of life. In view of different body condition and composition in preterm infants there are both arguments for better analgesia and arguments for the opposite (Table 2).[48]
Sensitivity to drugs might be different as the effects of narcotics in infants of mothers who used narcotics during labour, wear off rapidly after hand bagging or low doses of naloxone. An interesting observation relates to infants whose mothers had ingested a paracetamol overdose. They had toxic serum concentrations without apparent hepatic or renal toxicity.[49,50]
As the above mentioned factors are influenced by gestational age, birthweight, the presence of edema, inflammation, or other factors such as severity of illness, studies on both pharmacokinetics and dynamics of regularly used analgetic drugs are warranted, in order to start and adjust analgesics.

Better analgesia	Worse analgesia
- Reduced plasma albumin resulting in more free, active circulating drug - Greater permeability of the blood brain barrier - Immature liver and kidney, resulting in slower elimination	- Larger volume of distribution, leading to lower peak drug levels - Reduced number of receptors

Table 2 Factors that might improve or diminish analgesia in the newborn infant as compared to older children and adults

I.1.1 Scope of the thesis

This thesis described the use of the two most often used analgesic drugs, paracetamol (acetaminophen) and morphine focussing on the pharmacokinetics and pharmacodynamics of these drugs during the first days of life in relation to the use of pain assessment instruments.

The general development of the fetus' senses, touch, pain, hearing, smell and taste, and sight, is discussed in chapter I.2, in the light of the fact that learning abilities of the newborn organism increasingly prove to reflect continuity from prenatal life.
A literature review on paracetamol, a drug known for over a century after its first introduction by Von Mering,[51] is given in chapter II.1, concentrating on the relevance of therapeutic concentrations.
Chapter II.2 describes the method we developed to determine concentrations of paracetamol in serum, and of paracetamol and its metabolites in urine. Because an analgesic for moderate pain is not available for preterm neonates, we were prompted to investigate the pharmacokinetics, metabolism, and pharmacodynamics of a single dose of paracetamol, as described in chapter II.3.
In daily practice, however, paracetamol is given as single dose and multiple dose therapy in case of suspected pain or fever in full term infants, though always off-label, as label instructions are only given for infants over 3 months of age. Consequently in chapter II.4 multiple dose pharmacokinetics and pharmacodynamics on the first day of life are discussed.
Multiple doses of paracetamol after an invasive procedure e.g. vacuum extraction have been given for suspected pain to selected infants. We, therefore, studied the efficacy of multiple doses of paracetamol following vacuum extraction, which is described in chapter II.5
Chapter III.1 describes the validation of a method to determine morphine-concentrations in neonatal serum, and discusses the possibilities to adjust the dose based on morphine concentration and a pain assessment instrument.
As the morphine metabolites, morphine-3-glucuronide and morphine-6-glucuronide, are known to have an important effect on respiration and analgesia, and morphine is used in ventilated preterm infants for several days to weeks, the effects of two different dose regimens on pharmacokinetics and pharmacodynamics were studied as reported in chapter III.2.
Chapter IV contains a general discussion, as well as several options and wishes for future pain management and further studies.

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I.2 The Development of Senses

Based on:

The development of senses.
Birgit Arabin, Richard van Lingen, Wim Baerts, Jim van Eijck.
In: Cervenak AF, Kurjak A (ed). The Fetus as a Patient. Parthenon Publishers, London, New York 1999.

I.2.1 Introduction

The development of senses is a multidimensional process incorporating sensory, emotional, affective, cognitive and evaluative abilities. For a long time it was rather a philosophical than a scientific question how early such abilities are acquired. Aristotle already anticipated the phenomenon that the sensory development is a quiet process of the prenate gradually responding to the extrauterine world. However, negative thinking about prenatal sensory capabilities was more common: Rousseau referred to the fetus as a "witless tadpole" isolated from the agitation of the world. Even first scientific approaches by Preyer in 1885 led to doubtful conclusions about fetal sensory capacities[1]. The advent of technical advances allowed access to the unborn in the womb and the study mainly behavioural responses. Prechtl[2] can be regarded as the pioneer of integrating observations of endogenously evoked behaviour into neonatal and prenatal surveillance. He stressed the clinical importance and provided us with a tool appropriate for the investigation of developmental responses towards internal and external stimuli. Reactive behavioural patterns towards touch, sound (including maternal noise), vibration, light, taste and odor have been observed and may be integrated into a cohesive whole, to describe developmental sensitivity. Besides behavioural reactions, responses may be mediated by physiological, hormonal or metabolic processes. Although the fetal brain can organize and elaborate stimulus information and encode in memory the activation of reflex responses, it is still difficult to define in how far the "memories" are or become conscious, reflect the present or contribute to future sensory development.

This chapter tries to summarize some essential aspects and eventual clinical implications.

I.2.2 Sensitivity and experience of pain

Anatomy, sensory physiology and responsiveness

Cutaneous sensory receptors appear in the perioral area of the human fetus in the 7th week, spread to the face, hands and feet at around 11 weeks, to the trunk, arms and legs at around 15 weeks and to all cutaneous and mucous surfaces at around 20 weeks. The spread of cutaneous receptors is accompanied by the development of synapses between sensory fibers in the dorsal horn of the spinal cord, which first appear at around 6 weeks. Sensory nerves first grow into the spinal cord at 14 weeks' gestation. Development of the fetal neocortex begins at 8 weeks. Most sensory pathways to the neocortex have synapses in the thalamus. Between 20 and 24 weeks thalamocortical fibers establish synaptic connections with dentritic processes of neocortical neurons. After that time it is more likely that the fetus might not only react towards but also "experience" touch and pain. *In vivo* measurements of glucose utilization have shown that maximal metabolic activity is located in sensory areas of the neonatal brain, suggesting the functional maturity. The existence of neurotransmitters, endogenous opioids released pre- and perinatally in response to fetal stress and of stereospecific opiate receptors at spinal and supraspinal levels as well as behavioural reactions have further increased our knowledge of sensory or painful sensation and offered implications for treatment.[3]

As supposed for motor activities[4] sensitivity to touch may develop before the response to relevant biological or psychological sensory input. Hooker was the first to describe reactions towards cutaneous stimulation of embryos after therapeutic terminations of pregnancy by hysterotomy, maintaining them in an isotonic fluid bath.[5] With the introduction of real-time ultrasound, it became possible to observe early prenatal movements and behaviour.[6] To study reactions of single fetuses towards touch *in utero* would be combined with ethical and practical restraints. However, twins are naturally exposed to cutaneous stimulations of the co-twin, enabling us to study the onset of reactions towards touch *in vivo*.

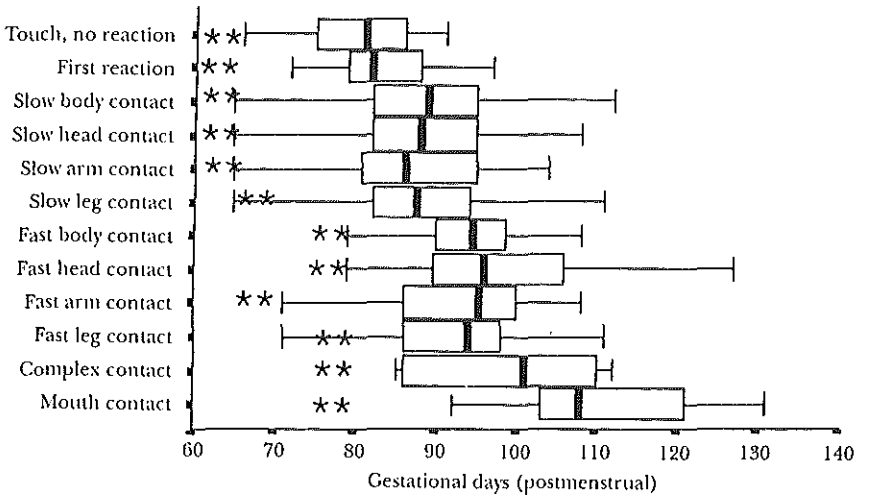

Figure 1 Onset of prenatal contacts in 20 dichorionic and 5 monochorionic diamniotic twins twin pairs (Box-Whisker plots) compared to two cases of monoamniotic twins (•) , ultrasound observations at 1 week-intervals

Multiple pregnancies to study reactions to cutaneous stimulation *in vivo*

The onset of reactions towards touch can ideally be investigated in monoamniotic multiplets, since first reactions towards touch are observed earlier than in monochorionic diamniotic multiplets and again earlier than in dichorionic multiplets (Figure 1). As described *in vitro*[5] we found *in vivo* that monoamniotic multiplets responded to tactile stimulation between 8 and 9 weeks.[6] Early contacts in monoamniotic and monochorionic twin pregnancies are also more numerous compared to dichorionic pregnancies because the membranes may prevent early reaching and touch *in utero*.[7] The development of reactions towards cutaneous stimulations of a co-twin in utero reveals different qualities. Up to 16 weeks, we have meanwhile analyzed several contact patterns according to the speed of initiatives and reactions or part of the body involved.[7] In advanced pregnancy, it is more difficult to differentiate fetal reactions to touch by conventional ultrasound methods. Three-dimensional real-time ultrasound may facilitate observations in the future. From early fetal heart rate (FHR)/fetal movement analysis of singletons compared to twins, one can conclude that inter-twin reactions contribute to an increased number of simultaneous FHR accelerations.

Gender differences have been reported for cognition, aggression and sociability in humans. Explanations have focused on neuroanatomic differences and exposure to steroid hormones. We have analyzed twin pairs with different gender combinations between 8 and 16 weeks. In the group of only male twins, fast initiatives combined with fast reactions and the number of complex contacts were significantly increased compared to only female or mixed twin pairs.[7] It is suggested that differences of testosterone levels might have an impact on early development *in utero*.
Limitations in the interpretation of our twin studies include difficulties in differentiating reactions to touch from a parallel onset of endogenously evoked movements, and passive from active reactions.

It was speculated that the tactile stimuli of multiplets might improve their development during early follow-up examinations up to the age of 6 years, when multiplets scored better than singletons,[7] and that not only activity that promotes growth and dendritic branching of individual neurons, but similarly, that sensation from receptor cells might promote the development of the neural system.[8]

I.2.3 Fetal pain

Pain includes feeling, suffering and learning (memory). The increasing number of prenatal techniques and of operations in premature neonates has given rise to controversial discussions how early and in how far fetuses and neonates feel pain. This has implications for professionals who provide abortions. Fetal responses to invasive techniques were observed as blood flow redistribution from 18 weeks[9] and as an increase of cortisol and β-endorphin from at least 23 weeks of gestation.[10] Nevertheless, the most rational approach is to make an informed guess based on knowledge of the development of the nervous system or measurements and observations in preterm infants. Some authors argue for placing the onset of pain sensation at somewhere between 6 and 12 weeks after conception.[11] Others define recorded responses before 26 weeks as reflexes without conscious appreciation, suggesting that, only then, it is likely that the fetus not only reacts towards a stimulus but also experiences pain.[12] It seems to be accepted that the thalamus may assume some functions to contribute to fetal awareness of pain, but it is still not known when the brain is mature enough to register pain.

To avoid suffering and long-term effects, it is considered advisable to provide the fetus with analgesia. Suffering during termination of pregnancy is also a question of concern. The Royal College of Obstetricians and Gynaecologists recommends that practitioners who undertake diagnostic or therapeutic surgical procedures upon the fetus at or after 24 weeks consider the requirements of fetal analgesia and sedation, either by agents given to the mother or directly.[13] Before performing late terminations of pregnancy it is suggested that fetocide be considered. Maternal analgesia which transfers across the placenta is supposed to be sufficient in cases with early terminations of pregnancy. Research is needed to determine how the detection and treatment of pain can be extended to the fetal patient in a direct way.

The preterm infant of 23 weeks' gestation shows reflex responses to noxious stimuli. A variety of physiological, hormonal, metabolic and behavioural changes have been observed after painful procedures; these include cardiovascular variables, palmar sweating, increase of renin, epinephrine (adrenaline), norepinephrine (noradrenaline), catecholamines and glucocorticoids, which are related to the intensity of the stimulus[6]. Stimulation of the cutaneous flexor reflex showed that reflex thresholds were proportionally related to increasing gestational age.[14] Therefore, infants under 32 weeks might need even more analgesia to avoid stress responses and pain experience. In our group we score neonates from 24 weeks onwards according to the neonatal infant pain score (NIPS) looking at cry patterns, breathing, body language and facial expressions.[15] If we suspect that infants experience pain from ventilation or invasive procedures we start treatment with morphine or acetaminophen (APAP). In a recent study we found significant higher concentrations with lower gestational age, two hours after administration of paracetamol.[16] We take advantage of the increased half-life due to decreased clearance of the drug in preterm infants to reach higher concentrations according to the supposed lower pain threshold (Figure 2). Little attention is given to the experience of pain during vacuum or forceps delivery. Newborns born by either of these procedures in our unit receive pain treatment directly after birth.

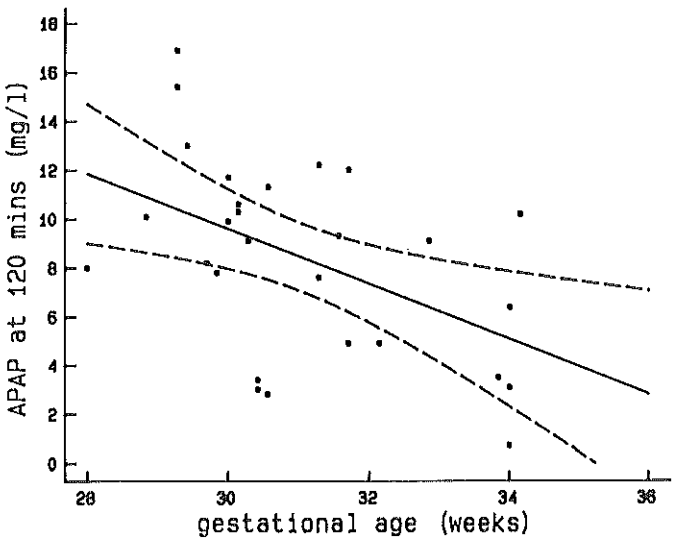

Figure 2 Linear regression analysis with confidence limits of gestational age on acetaminophen (APAP), concentrations at 120 minutes (16).

I.2.4 Hearing

The onset and development of fetal hearing are dated by extrapolation from animal studies or from measurements in premature babies and by studying prenatal reactions to acoustic stimuli. References to the extensive detailed literature on fetal hearing development can be obtained in a previous publication.[17]

Anatomy, acoustic physiology

From 10 weeks onwards the external ear and later even the tympanic membrane are visualized by ultrasound. The outer ear collects sound energy and shapes it towards the tympanic membrane. The ossicles of the middle ear develop between 4 and 6 gestational weeks and reach full size by 18 weeks. Only then they become ossified; they are of adult size by 8 months of gestation. At 7 weeks, the Eustachian tube and the tympanic cavity are formed. In the middle ear, acoustic energy is transduced into mechanical energy. The inner ear consists of a membranous labyrinth inside a bony labyrinth. Ossification does not occur until each portion has attained adult size. Hair cell differentiation, synaptogenesis and ciliogenesis of the membranous labyrinth are completed at around 24 weeks. The basis of the frequency-related regional displacement of the entire cochlear partition was revealed by the classic Nobel laureate von Bekesy. With the entry of the acoustic nerve into the brainstem, auditory neurons multiply and project the information to the auditory cortex. Many auditory abilities are attributable to subcortical processing. Therefore, decorticated animals are capable of detecting intensity and frequency of sounds, and anencephalic fetuses demonstrate behavioural reactions to external stimuli.

Sound is created by a vibratory source, causing molecules to be displaced. The amplitude is measured in pascals (Pa). Sound pressure level and frequencies is given in decibels (dB) and hertz (Hz), respectively. The range of 20-20000 Hz is the bandwidth of human hearing. Information about intrauterine hearing conditions are obtained from sheep experiments or from pregnancies with ruptured membranes: a bone conduction route is assumed, whereby sound energy is diminished by 10-20 dB for frequencies <250 Hz and by 40-50 dB for >500 Hz.

A second reason for fetal "sound isolation" is the sound pressure attenuation. Thereby, transmission losses range from 39 dB at 500 Hz to 85 dB at 5000 Hz. It has been shown that sound attenuation decreases during late gestation. In humans, sound of at least 65-70 dB is transmitted with attenuation of 30 dB in sound pressure level for tones of up to 12 kHz. Frequencies of 200 Hz are even enhanced. The sound environment *in utero* is dominated by frequencies <500 Hz and mean sound pressure levels of 90 dB caused by vessel pulsations, intervillous injections and maternal digestion or breathing. It might have an "imprinting" and a masking effect for the fetus. "Intelligibility" reflects the ability to distinguish complex sounds: music and voices are distinguishable from the basal noise by 8-12 dB. The male voice with a mean frequency of 125 Hz is better transmitted, but emerges in the range with high internal noise. Female voices with an average of 220 Hz receive greater attenuation, but emerge in a range with low internal noise.

Prenatal and postnatal auditory responsiveness

Studies of fetal response to sound have used a variety of stimuli, not always with rationale. Studies with airborne and vibroacoustic stimuli (VAS) providing vibration and airborne sound should be differentiated. The electronic artificial larynx used for VAS has a spectrum between 0.5 and 1 kHz and a sound pressure level averaging 135 dB. The first responsiveness to pure tones was detected at 23 weeks at 500 Hz, by 27 weeks to up to 500 Hz and only at 31 weeks responses were observed to 1000 - 3000

Hz. The sound pressure level required is 20-30 dB less at 35 than at 23 weeks. With the use of a habituation-dishabituation technique, a fetus aged 35 weeks was found to discriminate between frequencies of 250 and 500 Hz and different speech sounds; fetuses of 27 weeks were unable to make this differentiation.

Non-invasive recordings of auditory responses are based on compound potentials representing the activity of many cells; these recordings use the electroencephalographic (EEG) and magnetoencephalographic (MEG) methods. Human EEG responses to acoustic signals have been obtained after ruptured membranes. In MEG recordings neuromagnetic auditory brainstem responses are measured with intact membranes. Short stimuli are performed in a room guaranteeing electrical radio frequency shielding. Thus, we succeeded in recording stimulus-related auditory evoked neuromagnetic fields through the mothers' abdomen at 34 weeks (Figure 3). Latency shifts of brainstem components are proposed to reflect early brain maturation. Use of multi-channel magnetoencephalography specifically designed for the prenate might be expected to extend the window of observation.

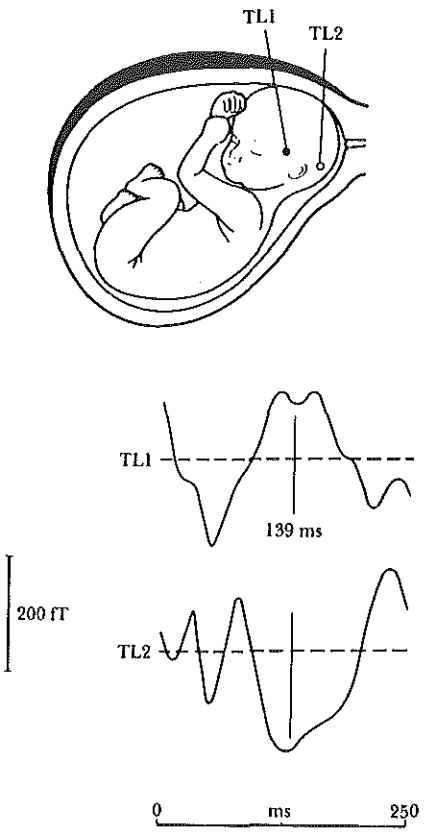

Figure 3 Waveforms obtained from brainstem recordings after click stimulation at 34 weeks using one-channel magnetoencephalography (17). TL = temporal lateral, ft=fentotesla.

Fetal auditory abilities can be examined by behavioural reactions. Problems arise when the fetus does *not* react, since we cannot say that the stimulus is not sensed. In general, reactions towards acoustic stimuli do not occur before 24 weeks. Fetal movement and FHR responses may be classified into immediate short (blinking, reflex movements) and long-term changes of activity and FHR patterns. With increasing gestational age, an increasing number of long reactions are observed after VAS. FHR baseline changes more dramatically than FHR variability. Fetal responses to speech were studied at 26-34 weeks' gestation, during periods of low FHR variability. A decrease in FHR was found, as the only demonstration of prenatal responses to speech stimuli. Female fetuses responded earlier than males. Twins are ideal models to differentiate the simultaneous influence of gestational age, state before stimulation, individual disposition (zygosity), position and sex of the prenates by analyzing inter-twin differences of FHR/fetal movement patterns towards vibroacoustic stimulation (VAS).

The detection of cortical potentials and auditory evoked potentials as early as 25 weeks before birth indicates functional maturity of the auditory pathway. Electrophysiological responses from the cochlea, the eighth nerve and the auditory brainstem are similar to those of the adult by 32-36 weeks. In neonates, broadband sounds (speech) are likely to elicit responses depending on states of hunger and sleep-wakefulness. Newborns differentiate band-width, duration, inter-stimulus interval, frequency and sound pressure: signals <4 kHz evoke responses more often than signals in the higher range. Lower frequencies generally evoke gross motor activity; high frequencies evoke freezing reactions.

Effects of prenatal hearing on postnatal development

While habituation reflects short-term memory, there is also proof of long-term memory from pre- to postnatal life, as observed by studies of behavioural modifications of neonates presented with stimuli with which they have been confronted prenatally. Newborns showed a preference for the voice of their mother and, with a baby's pacifier (dummy) connected to a tape recorder, they where able to distinguish between their mother speaking in her native or an unfamiliar language, and they preferred a lullaby that had been read twice a day by the mother during the last weeks of pregnancy to a new story. All this suggests the possibility of prenatal acquisitions and antenatal discrimination, however elementary it might be.

Settings of talking, music and meditation were performed during pregnancy. Postnatally, for talking only 58% of behavioural variables were identified as positive, 16% as ambiguous and 26% as negative (e.g. crying for obscure reasons, needing constant supervision). Music and meditation, however, correlated in 90-100% with positive attitudes. It is speculated that variations of timbre and hormonal changes associated with the content of speech may evoke associations in the infant.

Hearing has a close relation to the kinetic system: the "auditory-vocal-kinetic channel". Vocal expression can been heard in immature newborns and follow unpleasant maneuvers. Newborn "cryprints" are as unique as fingerprints. The fetus seems to store maternal speech features: even newborns born at 28 weeks had similar voice performance features to those of their mothers.

Prenatal acoustical responsiveness as a test for fetal well-being

The ability of VAS to elicit FHR accelerations has well been established. In this context, VAS was proposed to assess fetal well being and to discriminate between "non-reactive" non-stress tests (NST) due to hypoxia and a quiet state. FHR-reactions are reduced after VAS in fetuses with intrauterine growth restriction (IUGR). The conclusion, that nutritional deprivation is associated with delayed sensory maturation, is not necessarily true, since even when there is no reaction, the stimulus might well be received.

We compared the clinical value of NST, a ratio of cerebral versus umbilical blood flow, VAS and contraction stress test (CST) to predict poor outcome for IUGR and post-term pregnancies: Doppler velocimetry and the NST's were superior to VAS and the CST. In the only controlled trial, in which VAS was compared to NST, false-positive tests were slightly lowered and performance reduced from a mean of 27 to 23 min. The question remains of whether 4 min justifies frightening -or only wakening- an innocently sleeping fetus. For the time being, we recommend VAS only under controlled conditions, but not for routine use, as proposed by others.

Possible damage from environmental hazards during pregnancy

Fetal noise-induced hearing loss has been a matter of concern regarding working or living conditions of pregnant women supposing that noise can adversely change fetal hearing. From sheep experiments it was found that noise sources with low-frequency components and high intensity impulses had temporary effects, whereas long-term effects are still unknown. In summary, the Committee on Hearing, Bioacoustics and Biomechanics attempting to protect fetal hearing suggested that pregnant women should avoid noise exposures greater than 90 dB.

I.2.5 Olfaction and taste

Anatomy and physiology of nasal and oral flavor reception

About 1-2% of the human genome is allocated to receptors for the olfactory epithelium.[18] Olfactory receptors mediate the sense of flavor arising from volatile substances pumped into the nasal cavity during inhalation, swallowing or chewing. They also mediate neuroendocrine responses.[18,19]

The primary neuronal cells of the main olfactory system are embedded in the upper part of the nasal cavity. Their dendrites merge into the mucus and bear receptor-binding compounds. The receptor binding elicits in the neuron an electrical signal, which is transmitted along the axon penetrating the lamina cribrosa to meet in one of the paired olfactory bulbs, and via a neuronal network to the paleocortex including the hippocampus.[18] Impulses reaching the thalamic nuclei project to the frontal cortex where the conscious perception of smell takes place; pathways to the limbic system mediate affective and neuroendocrine responses. The vomeronasal organ with sensory cells on the nasal septum mediates endocrine responses activated by pheromones. More studies are required to determine the specific function in humans. By the third trimester all chemosensory receptors seem ready to be functional. The nostrils have become patent and amniotic fluid is swallowed and inhaled. Taste buds are found as

early as 12 weeks and displayed over the oral cavity, concentrating at birth on the tongue and on the anterior palate.[20]

Prenatal and postnatal olfactory responsiveness

Olfactory responses have been demonstrated in premature babies of 6 months. In the sheep, intranasal injections of odorant components induce FHR changes.[19] Intra-amniotic injection of a saccharine solution led to increased swallowing; injection of bitter or acid solutions reduces fetal swallowing, signifying awareness of different tastes during pregnancy even influencing fetal behaviour.[20]

The newborn may retain an olfactory memory trace: During the initial attempt to locate the mother's nipple, newborns preferred a breast with the areola moistened with amniotic fluid over an untreated breast.[18] The scent of amniotic fluid even has a calming effect shortly after birth, as measured by infants' rate of crying.[21] There is also evidence that babies recognize their mother by her scent, because 6-day-old infants turned preferentially in the direction of their own mother's odor pad rather than towards an alien breast odor. Neonates respond to scents by changes in respiration, facial expression and orientation. Even at less than 2 days old, they developed preferences (based on formation of new synapses) and oriented towards an odor that had been present in their nursery for the preceding 24 h. This phenomenon was still evident 2 weeks after the exposure was discontinued.[18]
Knowledge about olfaction and taste may not only have diagnostic and but also therapeutic implications, such as the initiation and stabilization of breast-feeding, newborn adaptation, attachment and social interaction as well as the reduction of apneic episodes.[21] We therefore try to provide the newborns admitted to our intensive care unit with some accessories of their mothers.

I.2.6 Vision

Anatomy and physiology of visual function

Different parts of the eye develop from different origins: the retina, iris and optic nerve are of neuroectodermal origin; the lens and cornea are of ectodermal origin; and the choroid, sclera, the ciliary body and ocular blood vessels are of mesodermal origin. Neuroblastic cells differentiate into photoreceptor cells, which later secrete interstitial retinol-binding protein, which plays a role in the defense against free radicals by binding vitamin E[22]. After 18 weeks, synapses appear on the eye rods. From 20 to 24 weeks, fetal eyelids may open. Macula and photoreceptors start development, which is not completed before birth and lasts for several postnatal months.[20] The retinal surface area doubles between 6 months and term.[23] Birnholz was the first to describe prenatal ocular structures as seen by ultrasound.[24] He described periods of active growth between 12 and 20 and between 28 and 32 weeks. At term, the eye is well developed, growing only three times compared to 20 times for the rest of the body to reach adult size. Myelination of the optic nerve starts between 6 and 8 months; myelination of the posterior visual pathway and superior colliculus start just before term.[23]

Prenatal and postnatal visual responsiveness

More than sound, light is attenuated by the maternal abdomen. Transmission of external light was detected to be around 2% at 550 nm and to reach 10% at around 650 nm[20]. In addition, the fetal position might prevent even a limited amount of light reaches the fetal retina. The restricted and irregular visual input impairs profound studies about behavioural responses to light *in utero*. Nevertheless, prenatal responses have been reported from 25 weeks onwards.[24] We have observed movements of the bulbs ("twinkling") by ultrasound as well as reactions of FHR and fetal movements towards stimulation with a flashlight from 28 weeks onwards, comparable to behavioural patterns towards VAS.[8] However, probably owing to the described problems, reactions of FHR/fetal movement patterns were recorded in only around 10%. Stimulations with light that was introduced during amnioscopy directly into the uterine cavity were more successful.[20]

After delivery, the newborn is suddenly exposed to light, and we do not know how this sudden impression is experienced. Recordings of visual responses based on compound potentials can be applied in preterm newborns such as the electroretinogram (ERG) or the visual evoked potential (VEP). The latter can be elicited even at 23 weeks; with increasing age the latency of the response signal decreases and its morphology gets more complex, signifying visual pathway maturation.[23] Behavioural tests have been introduced, such as the blink response and the registration of awareness and fixation. Dubowitz and Dubowitz used the ability of the preterm infant to focus, to follow and to track in order to draw conclusions about the developing visual system.[25] They also observed that newborns frequently do not open their eyes in the presence of strong lighting.[25]

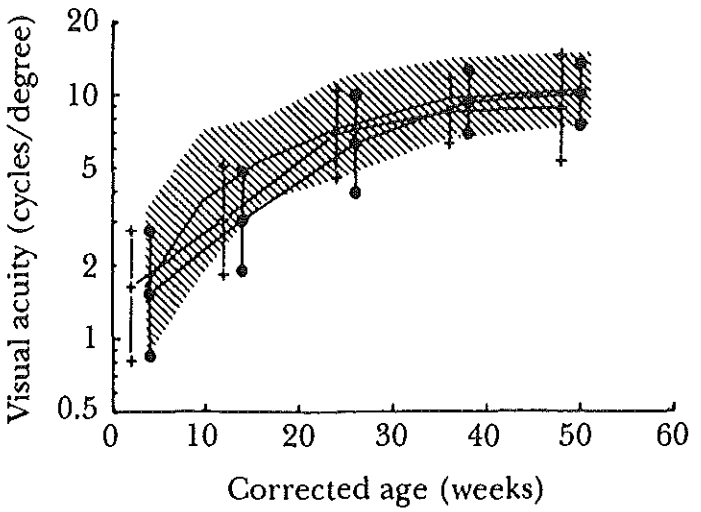

Figure 4 Development of binocular acuity in very low birthweight infants with neurological normal (circle) and abnormal (cross) development at the time of testing compared to mean low-risk preterm acuity. Vertical lines indicate 2 SD (26).

Further studies have all used "preferential looking"-based tests of visual acuity measurement. Although the visual development of preterm infants at different gestational ages lags behind that of term infants, when this is corrected for the degree of prematurity, both groups behave similarly. Preterms infants sometimes exhibit more rapid development than term infants.[23] All in all, around 50% of infants of very low birth weight show visual impairments across a range of functions.[26] The highest incidence was found at around 6 month; beyond this age fewer deficits were observed, suggesting delayed rather than permanently impaired visual development. Visual abnormality is frequently related to neurological impairment, suggesting that it is of cerebral rather than of ophthalmological origin (Figure 4). Still, in time these infants may show progress, thanks to neural plasticity.

Possible damage during and after pregnancy

The very small premature infant spends his/her first weeks in a constantly illuminated environment. Light is transmitted even through the eyelid at a rate of 1-10% predominantly at the red end of the spectrum.[23] Exposure to light has been demonstrated to cause retinal damage in animals even at intensities encountered in neonatal intensive care units. It is an intriguing question in how far this can be extrapolated to humans, and might require that light be reduced or adapted to the sleep-wakefulness rhythms of the neonates. Retinal photodamage mainly occurs by a raise of oxygen tension, occurring unnaturally for the very premature infants. At present, retinopathy is the most common ocular disease being characterized by abnormal proliferation of the immature retina, which can progress to retinal scarring and visual handicap up to blindness (for review, see reference 24).

I.2.7 Conclusions

An increasing amount of data demonstrate that learning abilities of the newborn organism reflect continuity from prenatal life. The long history of denial and the short history of limited research relating to prenatal perception and the implications for further life highlight our ignorance of the effects of early sensory development. The use of modern technology in order to understand pathophysiological processes and to create stimulative interventions, but even more important our own respect and awareness towards the unborn and newborn infant are prerequisite to discover that this topic represents a wealth for ongoing and future research. Early capabilities reflect complex neurological development and thus one of the most important objectives of perinatal care. It is our responsibility to avoid unnecessary stress of the fetus and newborn from overstimulation by unnatural exposure to light or sound and to treat pain as effectively as we do in adult patients. However, health is understood as the physical, mental and social well-being that goes much further than just the absence of suffering or illness. As important as it is to teach children abilities at certain times, it might favor the development or prevent sensory retardation to create integrated stimulations for the unborn or newborn. This should be a matter of concern of public health projects. Encouragement and avoidance of stimuli have to be considered and balanced in observational or interventional projects according to critical developmental phases. Whether we go so far as to found prenatal universities is of secondary importance, as long as we strive to understand physiology and pathophysiology of early sensory

development and to create designs suitable to induce a comprehensive expression of all our genetic potential.

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II

Paracetamol in Pain Management of the Newborn.

"Even if we have no facts, observations, or theories to suggest that infants feel pain, we have nothing to lose by assuming that they do." (Owens ME. A crying need. 1986)

II.1 Analgesic Therapeutic Range of Paracetamol and Correlation with Pain Assessment Instruments: Myth or Reality?

Based on the article:

Analgesic therapeutic range of paracetamol and correlation with pain assessment instruments: Myth or reality?
RA van Lingen, JT Deinum, R van Diëen, JN van den Anker, D Tibboel.
Submitted.

II.1.1 Abstract

Objective: To test the evidence that blood concentrations of paracetamol between 10 and 20 mg/l represent therapeutic analgesic values, and to determine the correlation between dose and/or concentrations and validated pain assessment scores.
Design: Meta-analysis of studies from the literature and an inquiry among pharmaceutical companies.
Results: Seventy-eight studies on paracetamol (43 on antipyretic, and 35 on analgesic effects) were found suitable to be included. Of the studies describing analgesic effects, 9 contained data on infants, 21 on children aged 1-17 years, and 7 on adults. Fifteen studies were dose-effect studies, 10 dose-concentration studies, and 10 dose-concentration-effect studies.
A wide range for mean maximum blood concentration was found; in infants and children doses of 7.5-40 mg/kg resulted in mean maximum blood concentrations ranging from 5.5 to 22.5 mg/l, while in adults single doses of 500-1500 mg resulted in mean maximum blood concentrations between 5.8 and 55.9 mg/l.
The pain scores taken into consideration rely on self-reporting (>3 years of age) or on objective validated pain measurements. In the different studies, concentrations of 5.9 - 29.8 mg/l in adults and of 7.5-22.5 mg/l in infants and children were reported to result in appropriate analgesia.
Conclusion: The recommended therapeutic range from 10-20 mg/l for the analgesic effect of paracetamol as reported in the literature is not supported by well-designed clinical studies providing level I evidence. Recent reports indicate that analgesic efficacy may be obtained when blood concentrations range from 5-25 mg/l, and that these concentrations are related to adequate concentrations in the cerebrospinal fluid.

II.1.2 Introduction

For over 100 years, paracetamol (acetaminophen, APAP) has been used as an analgesic. It is the most commonly used over-the-counter medication in the USA,[1] and the most frequently prescribed medication in the UK and the Netherlands.[2,3] Although its efficacy in antipyretic treatment has been shown in many studies,[4-10] its analgesic efficacy in children has never been studied by determining the plasma or serum concentration-effect relationship. In adults, two studies with intravenously administered paracetamol and two studies with oral paracetamol have been performed, using the visual analog scale (VAS) or the nociceptive flexion reflex threshold (RIII) as an effect parameter for acute pain.[11-14]
Paracetamol is a typical example of a pharmacologic agent that has been widely used for analgesia in the pediatric population based on data extrapolated from adults,[15] as often occurs.[16]
Internationally accepted dosages for paracetamol are 0.5-1 g for adults, with a maximum of 3 g/day orally or 4 g/day rectally, 90 mg/kg/day for children and infants from 3 months and older, and 60-90 mg/kg/day for infants from 1-3 months.[7,17] In neonates single doses of 20 mg/kg[18,19] and multiple doses up to 80 mg/kg/day have been shown to be safe.[20]

Prescribing habits in a children's hospital showed a trend in the use of a lower daily dose in the younger age group, while at the same time 17% of prescriptions were above 95 mg/kg/day. [21]
Recently, higher loading doses have been proposed for rectal administration in order to reach therapeutic concentrations for analgesia.[22-25]

Recent studies on the central analgesic effects in adults indicate that, after intravenous administration, concentrations from 6-24 mg/l result in appropriate analgesia, evaluated by VAS and R-III.[11,12]
Many studies already refer to a range of 10-20 mg/l as therapeutic.[9,26] Wilson et al [6] showed that plasma concentrations between 4 and 18 mg/l are associated with an optimal antipyretic effect in children.

To test the evidence that serum or plasma concentrations of paracetamol between 10 and 20 mg/l represent therapeutic analgesic values, both in children and adults, we conducted a systematic search for published reviews and reports of both randomised and non-randomised controlled or non-controlled trials on the use of paracetamol in adults, children and infants. In particular, we focussed on correlation between dose and/or concentrations in relation to validated pain scores.
For all therapeutic ranges given, we searched for the original research performed or for the original guidelines.

II.1.3 Methods

A number of different search strategies in MEDLINE (1966 through October 1999), the Cochrane Library (1999, issue 4) and the World Wide Web were used, without restrictions. Keywords were paracetamol, acetaminophen, therapeutic concentrations, drug dose-response relationship and values. Additional publications were found by manually reviewing reference lists of retrieved articles and reports. Original papers in English, French, German, Italian, and Dutch were included. Animal studies were excluded, as were studies on combination drugs, such as paracod.
Pharmacokinetic data including peak serum concentration (C_{max}), area under the concentration-time curve (AUC), time to reach C_{max} (T_{max}), and serum-half-life of the drug ($T_{½}$), therapeutic ranges, and measures for pain were extracted from the articles. In cases that only a figure was given data were calculated from the figures and when possible, the original author was consulted.
Oral, rectal, or intravenous routes of administration were included in the search.
The type of study was stratified into one of the following categories: Dose-Effect (DE), Dose-Concentration (DC), or Dose-Concentration-Effect (DCE), in relation to age.
Pharmaceutical companies producing one or more forms of paracetamol were asked for the primary source of their therapeutic ranges, as mentioned on their labeling instructions. When a therapeutic range was provided we evaluated the original reference.

author	n	Age	Dose	Route	Cmax	AUC	Tmax	t1/2	Study type	Range	Score
van Lingen	21	1d*	20mg/kg	r	12.5±2.9	95.1±28	3.9	11.0	DCE	4-20mg/l	facies
	7	1d*	,,	r	7.5±4.0	71.7±41.7	5.1	4.8	DCE	4-20mg/l	facies
Lin	5	1-4d*	20mg/kg	r	8.38±3.92		1.3±0.7		DC	10-20mg/l	
van Lingen	10	1 d	20mg/kgq4	r	10.79±6.39				DCE	4-20mg/l	facies
Howard	44	1 d	15mg/kgq4pre	o					DE		comfort
Shah	38	2 d	20mg/kg pre	o					DE		facial activity
Autret	5	< 10 d	7.5 mg/kg	iv	13.2±4			3.5	DC	4-18 mg/l	
	7	1-12 m	7.5 mg/kg	iv	8.9±2			2.1			
Hansen	17	2-160 d	25 mg/kg	r	10.9±5.1		1.7±0.59	4.1±1.9	DC	10-20 mg/l	
Bean	62	5m-8 y	15mg/kg pre	o					DE	10-20 mg/l	OPS
Rod	100	0.75-16 y	7.5mg/kg post	iv					DE		behav crit
Korpela	120	1-7 y	0-60 mg/kg i	r					DE	10-20 mg/l	VAS
Gaudreault	20	1-8 y	20mg/kg i	r	10.7±1.5		2.0		DCE	10-20 mg/l	pain eval
Houck	18	1-10 y	35mg/kg post	r	12.4-31.5		1.9		DC		
Ragg	45	1-12 y	20mg/kg pre	o					DE		pain faces
Anderson	20	12m-17 y	40mg/kg post	r	17.4±7.4	0.64±0.299	2.3±1.2	0.7±0.67	DC	10-20 mg/l	
Romej	14	2-8 y	15mg/kg pre	o					DE		FLACC
	14	2-8 y	20 mg/kg post	r					DE		FLACC
Schachtel	77	2-12 y	15mg/kg	o					DE		VAS
Rusy	25	2-15 y	35mg/kg pre	r	7.3				DCE	10-25 mg/l	OPS
Mather	24	3-12 y	20mg/kg pre	o					DE		vomit-/+
Anderson	50	3-15 y	40mg/kg i	o	22.5±3				DCE		VAS/OPS
	50	3-15 y	40mg/kg	o	7.5±4.5				DCE		VAS/OPS
Montgomery	10	3.4±0.5 y	45mg/kg i	r	13±6		3.3±1.2		DC	10-20mg/l	
Birmingham	9	3.4-13 y	10 mg/kg i	r	5.5±1.9		1.8±0.9		DC	10-20 mg/l	
	9	,,	20 mg/kg i	r	8.8±3.4		4.8±2.1				
	10	,,	30 mg/kg i	r	14.2±5.1		3.5±0.1				
Sanderson	7	3.9-9.8 y	20 mg/kgq4 pre	o	6.75-21				DC	10-20 mg/l	

Coulthard	10	5.8±1.0 y	25.1±2.8 mg/kg post	r	13.2±7.4	97.3±55.2	2.1±1.3	3.6	DC		
	10	5.8±1.7 y	24.5±3.5 mg/kg post	r	14.5±3.8	92.2±33.6	1.9±0.5	2.9	DC		
	8	not given	8-18mg/kg post	o/r	7.7/4.9		2.0/1.6	2.0	DC	10-20mg/l	
Bertin	78	6-12 y	10 mg/kgq3	o					DE		facies
Boelen	64	not given	240-500 pre	r					DE		Oucher
Granry	87	6-12 y	15mg/kg post	iv					DE		VAS
Anderson	20	8.1±3.6 y	40mg/kg pre	o			±0.56		DCE	10-20 mg/l	VAS
	100	8.1±3.6 y	40mg/kg i	r			±0.25		DCE	10-20 mg/l	VAS
Romsing	9	8.6±2.6 y	17.6-18.2q5 post	r					DE	10-20 mg/l	Poker chip
	11	8.0±2.4 y	7.6-19.5q? post	r:prn					DE		Poker chip
	11	11.3±2.9 y	12.7-13.1q5 post	o					DE		Poker chip
	9	11.0±3.9 y	8.4-10.0q? post	o:prn					DE		Poker chip
Piletta	10	20-34 y	1000 mg	iv	24.3				DCE	6-24 mg	R-III/VAS
Piguet	11	22-30 y	500 mg	iv	14.5±4.6	18.5		2.3±1.6	DCE		R-III
		„	1000 mg	iv	29.8±11	38.9		2.3±0.3	DCE		„
		„	2000 mg	iv	55.9±13	80.3		2.3±0.4	DCE		„
Garrec	50	42±16 y	1000 mg	iv					DE		VAS
Lifshitz	10	22-48 y	1500 mg	o	21.4±7.7				DC		
	10	22-48 y	1500 mg	o	16.3±7.2#				DC		
Arendt	12	22-49 y	1000 mg	o	± 14	44.5±10.4			DCE		PTD
Nielsen	10	25-50 y	500 mg	o	5.8±1.5				DCE		PTD
	10	„	1000 mg	o	11.3±3				DCE		PTD
Yuan	18	18-42 y	325-1000 mg	o					DE		cold-pressor

Table I *Summary of demographic, pharmacokinetic, and pharmacodynamic data of 35 trials on analgesic effect of paracetamol. *preterm infants, d; days, m; months, y; years, i; at induction of anaesthesia, pre; pre operatively, post; post operatively, C_{max}; peak serum/plasma concentration (mg/l), AUC; area under the serum/plasma concentration-time curve (mg.h/L), T_{max}; time to reach C_{max} (h), $T_{½}$; serum/plasma half-life of the drug (h), OPS; objective pain score, FLACC; faces, legs, activity, cry, consolability, VAS; visual analog scale, R-III; nociception flexion reflex , PTD; pain threshold difference. Pharmacokinetic data are given as mean, mean ± SD, or range.*

II.1.4 Results

Seventy-eight studies on paracetamol were included in our study, some studies reported data of more than one age group. Eight out of these studies contained data on neonates, 16 on infants, 37 on children (>1 year of age), and 33 on adults (Appendix 1) (Figure 1). The majority of the studies employed a single dose design, only 10 (2 in adults) investigated multiple dose administration (Appendix 1).
Of those 78 studies 43 studies (17 in children and 26 in adults) concerned trials in febrile patients or pharmacokinetic trials in volunteers.
Out of the 35 trials describing an analgesic effect of paracetamol, 15 trials were dose-effect (DE), 10 dose-concentration (DC), and 10 other dose-concentration-effect (DCE) studies (Table 1, see page 32-33).[11-14,18-20,22-49]

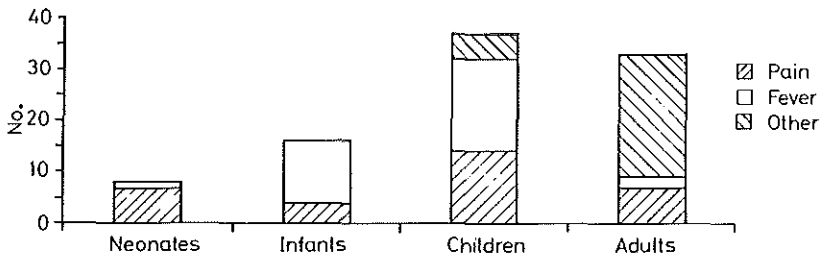

Figure 1 Paracetamol studies (n=78) in different age groups. Several studies contained data from more than one age group.

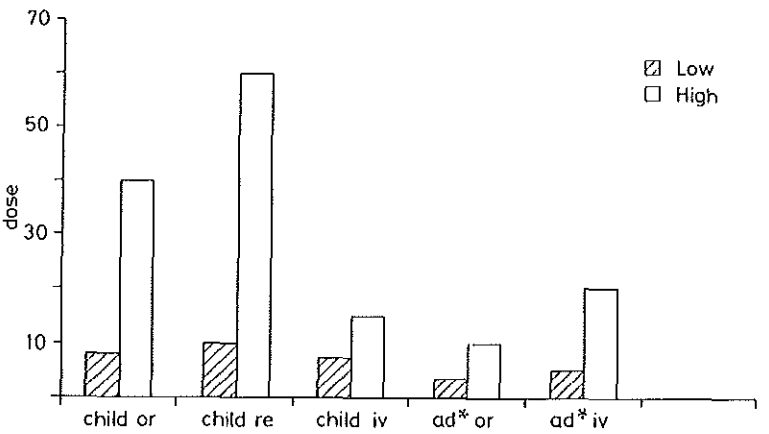

*Figure 2 Paracetamol dose range in 78 studies, in children aged 0-17 yrs (mg/kg), and in adults (*mg .10^2). or = oral, re = rectal, iv = intravenous administration.*

Paracetamol dose

In children, single oral dose administration ranged from 8-40 mg/kg and multiple dose administration was 15 mg/kg every 6 hours for 24 hours[27] or 17.6-18.0 mg/kg 5 times/day.[45] In adults, doses ranged from 0.33 to 1.5 g [11-14,47-49] (Figure 2).

For rectal administration in children, doses ranged from 10-45 mg/kg with either suppositories or a rectal solution.[18-20,23-26,30,33,35,36,38,41,43,46]

Propacetamol, a formulation for intravenous use, available only in Belgium, France, and Switzerland was used in 3 studies in infants and children in doses ranging from 15-30 mg/kg (equivalent to 7.5-15 mg of paracetamol)[29,32,44] and in 3 studies in adults with a dose of 1-4 g (0.5-2 g of paracetamol).[11,12,47]

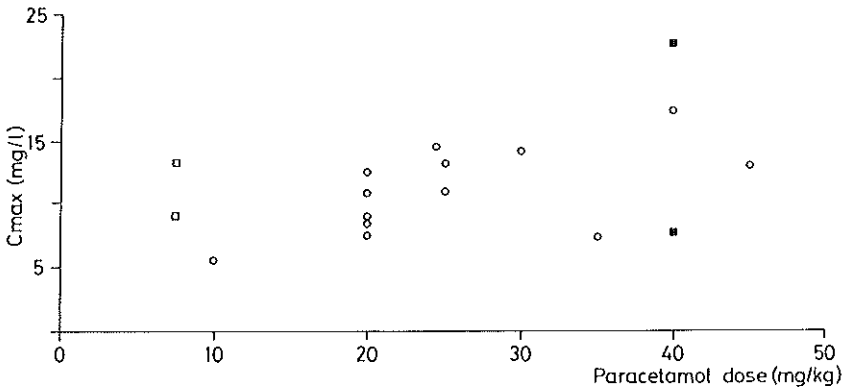

Figure 3 Mean C_{max} in children in relation to paracetamol dose.
Data from table 1. □ intravenous, ○ rectal, ■ oral administration.

Pharmacokinetics

Maximum serum or plasma concentrations (C_{max}) in children in relation to either oral, rectal or intravenous application are shown in figure 3.

Oral administration in children with 8-18 mg/kg led to a mean C_{max} of 7.7 mg/l,[41] 40 mg/kg to concentrations with a C_{max} between 7.5 and 22.5 mg/l.[22] Mean C_{max} was 5.8 ± 1.5 mg/l in adult volunteers after a single 0.5 g dose,[14] between 11.3 and 14 mg/l after 1 g,[13,14] and 21.4 ± 7.7 mg/l after 1.5 g of paracetamol.[48]

Rectal administration of 20 mg/kg in preterm infants <32 weeks,[18] and of 24.5-45 mg/kg in children >1 year of age,[23-25,30,35,41] usually resulted in serum or plasma concentrations with a mean C_{max} between 10.7 and 22.5 mg/l.

In preterm infants >32 weeks, a dose of 20 mg/kg resulted in a mean C_{max} between 7.5 and 8.4 mg/l,[18] while in infants and children doses from 10-25 mg/kg[19,20,25,26,41] and in one study a dose of 35 mg/kg[38] led to a mean C_{max} between 5.5 and 10.9 mg/l.

Intravenous administration of 7.5 mg/kg in infants resulted in a mean C_{max} ranging from 8.9 to 13.2 mg/l.[29] Doses of 0.5 to 1 g in adult volunteers gave mean C_{max} ranging from 14.5 to 55.9 mg/l.[11,12]

None of the studies reported values close to or higher than the toxic range of 120 mg/l.

AUC was determined in 4 studies in children after rectal administration and varied between 71.7 and 97.3 mg.h/l.[18,20,35,41] After oral and intravenous administration in adults, values were 44.5 ± 10.4[13] and between 18.5 and 80.3 mg.h/l[12] respectively (Table 1).

T_{max} in infants and children has been studied by 10 authors, mostly after rectal administration, with doses ranging from 10 to 45 mg/kg. In these studies, T_{max} between 1.3 and 5.1 h were found in neonates, and between 0.25 and 4.8 h in children.(Table 1)

Serum half-life of the drug ($T_{½}$) was high in neonates (3.5-11 h); in infants, older children and adults it varied from 0.7-4.1 h (Table 1).

Efficacy

In 15 DE and in 10 DCE studies 13 different pain scores were used, the results were favourable in 8 out of 15 DE studies and in 6 out of 8 DCE studies. As the design of these DE studies did not include serum concentrations, the paracetamol dose was related to 'therapeutic' antipyretic concentrations.

Pain was scored by means of a variety of measures; objective pain score (OPS), VAS, faces, legs, activity, cry, consolability (FLACC), Facies, Comfort, Poker Chip Tool, Oucher, RIII, pain threshold difference (PTD), and by behavioural criteria (Table 1). One study related the relief of postoperative pain to the presence or absence of vomiting (Table 1).

Only in the studies using the R III score a comparison was made to another pain score, i.e. the VAS.[11,12]

In the DE studies, the concentrations expected to be reached with a certain dose, were presumed to fall within the therapeutic range as stated beforehand, based on antipyretic data or on dose recommendations,[7,9] but an explanation was often lacking.

There was no positive or no measurable effect of paracetamol in the studies in infants with general painful procedures,[18,20] after circumcision[27] or during heel prick[28] and in children with tonsillitis,[37,42] regardless of dose, route or time of administration.

Postoperative analgesia after orthopaedic or visceral surgery was good after intravenous administration of 7.5-15 mg/kg postoperatively.[32,44] In the remaining 11 DE and DCE studies tonsillectomy or myringotomy was used as a pain model. The outcome was favourable if oral doses from 15-40 mg/kg were given preoperatively or at induction of anaesthesia,[22,31,34,36,45] and after a rectal dose of 40 mg/kg at induction.[45] Rectal doses <40 mg/kg or postoperative administration of paracetamol did not provide analgesia.[26,33,36,38,43,46]

Therapeutic concentrations

In 24 articles on both analgesia and antipyresis (Appendix 1), a therapeutic range was mentioned; in 21 cases the authors referred to a previous study. In two cases, the studies that were referred to did not even mention therapeutic values,[26,31] or referred to earlier published studies (5 times).

In only 6 studies (3 on antipyresis) original results were used to determine or propose therapeutic levels.[4,6,10,18,20,22] Of the 6 most cited publications, only the study by Wilson yielded an original therapeutic range,[6] the others proposed a range without providing supporting data,[7,53] or did not mention a range at all.[50-52]

All four DCE-studies in adults showed good analgesia after doses of 0.5-1 g resulting in plasma concentrations between 5.8 and 29.8 mg/l (Table 1). In children, good

analgesia was found with mean C_{max} concentrations between 7.5 and 22.5 mg/l, after single doses of 20-40 mg/kg.[22,26]
The proposed ranges are all close to the typically mentioned range of 10-20 mg/l, and vary from 4-25 mg/l.
Therapeutic ranges in relation to pain scores revealed that satisfactory analgesia was achieved in 50 and 75% of children undergoing tonsillectomy when blood concentrations were 10 mg/l or higher.[22,45] A cut-off value (i.e.3 on a scale of 1-10) for good analgesia was often not given.

All 5 pharmaceutical companies referred to the guidelines from the Dutch Council for Drug Administration (and the European Community), which are based on data from Temple, Rumack and Wilson.[6,7,53]

II.1.5 Discussion

Paracetamol has been known to be an effective antipyretic and analgesic drug for over 100 years.[54,55] It has been extensively used during the last 50 years, following renewed interest after the study of Brodie.[56] Our study shows that, up till now, only 4 studies took into account the ultimate aim of the drug: to reach a therapeutic concentration after an appropriate dose, evaluated by means of a validated pain score.

Several authors propose doses of 25-40 mg/kg followed by doses of 20 mg/kg every 4-8 hours in children,[23,25,40,41] and of 1000 mg q4 in adults to achieve blood concentrations between 10 and 25 mg/l.[11,14]
Differences between rectal and oral administration are due to differences in absorption. However, different suppositories can not be compared as they are non-equivalent products due to the different components leading to different bioavailability.[8,41,57] Age influences the absorption from the rectum, and resorption from the gastrointestinal tract is fast, but the rate-limiting factor is the time needed for gastric emptying,[58] which is slower in preterm infants.
Metabolism is slower in infants, but is adequate due to increased sulfation.[8,18,59]

Paracetamol is used extensively for both analgesia and antipyresis. However, only a few studies in infants and children have evaluated whether the administered doses result in the expected serum or plasma concentrations and the expected effect. This could lead to underdosing.[60] Especially when paracetamol is given for a period exceeding the 48 hours, and in the case of an underlying disease with decreased metabolism,[61] this might lead to overdosing and subsequent toxicity. Recently, Anderson proposed a faster determination of paracetamol levels in case of suspected overdose.[62] Although individual C_{max} varied widely, a toxic level was not reached in any of the reviewed studies. In multiple dose studies on analgesic effects,[20,40] and on antipyresis[50,63] no accumulation was found. However, none of these studies evaluated the effects of chronic use, i.e. for periods longer than 48-72 hours.
Although no toxic ranges were found in a multiple dose study in 21 children aged 0.5-6.4 years,[50] an increase in AUC suggested that paracetamol may accumulate substantially after repeated therapeutic doses over 2-3 days.

AUC was determined in a few studies only, which might be due to the fact that blood sampling took place during such an inadequate short time span, that blood concentrations were not yet decreasing.

$T_{½}$ is longer when impaired liver function is present, as in preterm or term infants where the liver function is not yet fully developed. Generally, $T_{½}$ increases with size, which means that, except for the neonatal period, it is shorter in children than in adults.[62] There is a lack of data regarding $T_{½}$ in children, which might be due in part to the fact that it is impossible to calculate $T_{½}$ when elimination of the drug is not yet apparent. $T_{½}$ seems to be independent of both dose and route of administration.

It is difficult to believe that for an analgesic that has been used in such large quantities and for such a long time, so few data on analgesic effect have been collected. This is partly due to its introduction at a time when drug trials did not yet exist. From the few studies in adults and children, that evaluated both concentration and effect, and from the majority of the dose concentration studies, it appears that oral doses of 15 mg/kg and rectal doses of 20 mg/kg 4 times a day after a loading dose of 30 mg/kg, are appropriate for analgesia, provided that blood concentrations are >10 mg/l. In preterm infants, a dosing interval of 8-12 hours should be maintained.[18]
In adults, the first few studies on the analgesic effect have been published.[11,12] However, in both adults and in children, prospective studies with validated pain scores as the R III/VAS combination (in adults) and the VAS, NIPS,[64] PIPP,[65] and Comfort are needed to assess the true analgesic value of paracetamol.

After a heel prick and during circumcision no effect was observed, since paracetamol is effective for moderate pain and not for severe pain. From the studies with tonsillectomy as a pain model it is evident that paracetamol should be administered preoperatively or at induction, preferably by oral route, or rectally after a loading dose of 30 mg/kg.
We agree with Anderson[66] that, as paracetamol has a central action for both antipyresis and analgesia, and maximum temperature decrease and peak analgesia are reported at 1-2 hours after peak plasma concentration, APAP should be given 1-2 h before any painful procedure.

The therapeutic range for paracetamol seems to be 10-25 mg/l, based on a few studies with propacetamol. Authors, pharmaceutical companies and governmental drug administration offices have been satisfied with data or references that are often not controlled, not true or non-existent.
In many studies, reference values were taken from the literature without checking the reference itself; some references quoted did not exist, did not mention the therapeutic concentrations,[50-52] or were review articles.[53,67-70]
The above-mentioned range applies to adults only, for children and infants it is not known; neonates may even have altered pharmacodynamics compared with older children.[71]

In newborns and even in infants, lower concentrations may be effective as paracetamol might pass the blood-brain-barrier more easily, but appropriate research with a

standard pain stimulus as RIII and studying levels in cerebrospinal fluid to confirm this hypothesis, is difficult to perform.
As most dosing recommendations are based on either single dose studies or on computer data, and since more reports are published with toxicity after apparently normal doses in children, but used for a longer time, more DCE studies after repeated therapeutic doses are needed.

We conclude that the therapeutic range from 10-20 mg/l for the analgetic effect of paracetamol as found in the literature is not supported by well-designed clinical studies, and in particular in children, does not correlate with pain assessment instruments. Recent reports indicate that analgesic efficacy might be obtained when blood concentrations are between 10 and 25 mg/l, in infants even from 5 to 25 mg/l, and that these concentrations are related to adequate concentrations in cerebrospinal fluid.
A loading dose of 30 mg/kg orally and 40 mg rectally followed by 15-20 mg/kg q6h in children, not exceeding 90 mg/kg/day and 4000 mg in adults is proposed.
Future dose-concentration-effect studies are needed to reach conclusive data on therapeutic analgesic ranges, especially in children.

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* multiple dose studies # therapeutic range included

II.2 Determination of Paracetamol and its Metabolites in Urine by High Performance Liquid Chromatography.

Based on the article:

Determination of Paracetamol and its Metabolites in Urine by High Performance Liquid Chromatography.
Hanneke Deinum, Aukelien Kuizenga, Richard van Lingen, Julius van Dam.
Submitted.

II.2.1 Summary

Paracetamol is a widely-used analgetic and antipyretic drug. Its use in the first weeks of life in preterm neonates is still a matter for debate, due to its unknown pharmacokinetics, pharmacodynamics and metabolism in this age group.
Analysis of plasma and urine of preterm neonates who have received a dose of paracetamol can provide more insight into the metabolism and possible formation of toxic metabolites of paracetamol in preterm neonates.
Although several methods for analyzing paracetamol in urine have been published, the majority of these are quite labour-intensive.
This paper describes a high-performance liquid chromatographic method for the determination of paracetamol and its metabolites in urine.
Retention times of paracetamol and its metabolites varied from 1.6 to 8.0 minutes, and calibration curves showed good linearity.

II.2.2 Introduction

Paracetamol (acetaminophen) is a widely-used analgesic which has few side effects in therapeutic doses. It is metabolised in the liver, with the metabolic biotransformation involving capacity-limited pathways of paracetamol-glucuronidation and paracetamol-sulphation. Taken together, these metabolites account for 82% and 68% of paracetamol excreted in urine for adults and children, respectively.[1]
In adults, a small percentage of a therapeutic dose is metabolised by cytochrome P450 to the potentially toxic intermediate N-acetyl-p-benzoquinoneimine. This reactive metabolite is subsequently conjugated with glutathione and excreted in the urine as the cysteine-conjugate or the mercapturic acid-conjugate. If the reactive intermediate is not immediately detoxified by conjugation (in the case of depletion of glutathione), it can bind covalently to essential hepatic cellular macromolecules and initiate cellular necrosis.[2-5]
It is known that the metabolism of paracetamol differs in adults, children and neonates; the percentage paracetamol excreted in urine as the glucuronide-conjugate increases with age.[6] Little is known about the metabolism of paracetamol in preterm infants.
Since we wanted to study the pharmacokinetics and metabolism of rectally administered paracetamol in preterm infants, an analytical method for the determination of paracetamol in serum and of paracetamol and its metabolites in urine was needed.
Many different methods have been published already. For a rapid and simple determination of paracetamol in serum, an High Performance Liquid Chromatography (HPLC) method has been described by Stevens and Gill.[7]
Several methods have been described for the determination of paracetamol and its metabolites in urine, but available assays are typically too labour-intensive, e.g. metabolites have to be hydrolyzed by enzymes before analysis,[8,9] or fail to identify or resolve critical paracetamol metabolites from endogenous peaks.[10]
We developed an HPLC method whereby paracetamol and its metabolites can be determined within 15 minutes.

II.2.3 Materials and Methods

Apparatus

The HPLC system consisted of a Waters 710 B autosampler (Waters, Etten-Leur, the Netherlands), a Thermo Separation Products SP 8810 pump (Thermo Separation Products, Eindhoven, the Netherlands), a Thermo Separation Products UV 2000 detector and a Thermo Separation Products Chromjet integrator.
The column used was a Licrospher RP C-18 (5 µm) 12.5 cm x 4 mm I.D. (Merck 50943).
The mobile phase consisted of formic acid: isopropanol: 0.1M potassium dihydrogen phosphate in water (0.1:1.7:98.2, v/v/v). The flow rate was 1.8 ml/min. UV detection was achieved at 240 nm.
The injection volume was 20 µl.

Reagents and chemicals

Isopropanol and methanol (HPLC grade), formic acid and potassium dihydrogen phosphate were obtained from Merck (Darmstadt, Germany), paracetamol (P) from Bufa (Uitgeest, the Netherlands), paracetamol-glucuronide (P-G), paracetamol-sulfate (P-S), paracetamol-cysteine (P-C) and paracetamol-mercapturate (P-M) were donated by Sterling Winthrop B.V. (Haarlem, the Netherlands).
De-ionized water was further purified for HPLC by passing it through six filters (Milli-Q system, Millipore, Etten-Leur, the Netherlands).
Standard solutions of paracetamol and its metabolites were in water.

Procedure

Calibration curves were prepared of P, P-S and P-G. In a pilot study, P-M and P-C were not detected in urine samples of preterm infants (collected in a plastic bag), therefore no calibration curves were prepared of P-C or P-M.
Calibration curves were prepared by adding the standard solutions of P, P-G and P-S to a pooled sample of "blank" urine of preterm infants, so that the final concentrations of paracetamol in urine were 5-10-20-40 µg/ml, P-G 5-50-100-200 µg/ml and P-S 10-100-500-1000 µg/ml, respectively.
Duplicate urine samples (100 µl) were diluted with 900 µl water and mixed, and 20 µl was injected on the column.
The precision of the method was determined by six replicate assays at two concentrations (of the calibration curve) for P, P-G and P-S.
The specificity of the method was determined by assay of six independent urine samples of patients who received other drugs (coffeine, gentamicine, carbamazepine, vancomycin, digoxin).
Detection limits (signal/noise ratio: 3) and determination limits (signal/noise ratio: 5) were determined.
Recovery of P, P-G and P-S in urine were measured in duplicate for four different concentrations and compared to a direct assay in water.

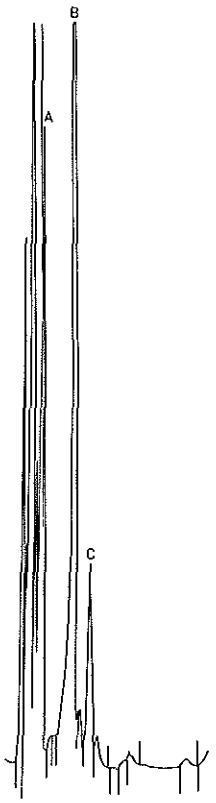

Figure 1 HPLC determination of paracetamol and its metabolites in urine of a preterm infant after a rectally administered single-dose paracetamol (20 mg/kg).
(A) paracetamol-glucuronide (t_R = 1.6 min.), (B) paracetamol-sulfate (t_R = 2.9 min.), (C) paracetamol (t_R = 3.7 min.)

II.2.4 Results

A chromatogram resulting from the analysis of urine from a preterm infant after a dose of 20 mg/kg paracetamol (rectally) is shown in figure 1.
HPLC determination of the metabolites of paracetamol in a pooled blank sample spiked with each of the metabolites (and an internal standard N-propionyl-p-aminophenol, which was not used in the final assay) was performed.
The retention times of P, P-G, P-S, P-C, and P-M were 3.7, 1.6, 2.9, 3.3 and 8.0 minutes, respectively.
Standard graphs of P, P-G, and P-S showed good linearity between peak heights and concentration, calculated by regression analysis (r^2 always > 0.99).
Validation results are given in the table.

	detection limit (mg/l)	determination limit (mg/l)	recovery (%)	precision (%)
Paracetamol	0.3	1.0	100	7
Paracetamol-glucuronide	2.3	3.2	95	3
Paracetamol-sulfate	6.3	10.1	95	3

II.2.5 Conclusion

The HPLC method described permits the rapid determination of paracetamol and its metabolites in urine.
The preparation of urine samples prior to chromatography is simple since only one diluting step is required. Both the sensitivity and precision of the method are good. Contrary to the methods described by others, which needed 1-5 ml urine,[8,9,10] we needed only 100 µl for analysis, making this method suitable for use in research studies including pharmacokinetics.

II.2.6 Acknowledgements

We gratefully acknowledge Kumar Jamdagni for his help in preparing the manuscript and Adri van Zoeren for preparing the figures.

II.2.7 References

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II.3 Pharmacokinetics and Metabolism of Rectally Administered Paracetamol in Preterm Neonates.

Based on the article:

Pharmacokinetics and metabolism of rectally administered paracetamol in preterm neonates.
RA van Lingen, JT Deinum, JME Quak, AJ Kuizenga, JG van Dam, KJS Anand, D Tibboel, A Okken.
Archives Dis Child Fet Neonatal Ed 1999;80:59-63.

II.3.1 Abstract

Aim: To investigate the pharmacokinetics, metabolism, and dose-response relation of a single rectal dose of paracetamol in preterm infants in two different age groups.
Methods: Preterm infants stratified by gestational age groups 28-32 weeks (group 1) and 32-36 weeks (group 2) undergoing painful procedures were included in this study. Pain was assessed using a modified facies pain score.
Results: Twenty-one infants in group 1 and seven in group 2 were given a single rectal dose of 20 mg/kg body weight. Therapeutic concentrations were reached in 16/21 and 1/7 infants in groups 1 and 2, respectively. Peak serum concentrations were significantly higher in group 1. Median time to reach peak concentrations was similar in the two groups. As serum concentration was still in the therapeutic range for some infants in group 1, elimination half-life ($T_{½}$) could not be determined in all infants: $T_{½}$ was 11.0 ± 5.7 in 11 infants in group 1 and 4.8 ± 1.2 hours in group 2. Urinary excretion was mainly as paracetamol sulphate. The glucuronide:sulphate ratio was 0.12 ± 0.09 (group 1) and 0.28 ± 0.35 (group 2). The pain score did not correlate with therapeutic concentrations.
Conclusions: A 20 mg/kg single dose of paracetamol can be safely given to preterm infants, in whom sulphation is the major pathway of excretion. Multiple doses in 28-32 week old neonates would require an interval of more than 8 hours to prevent progressively increasing serum concentrations.

II.3.2 Introduction

Even preterm neonates feel pain.[1] Soon after birth, many painful procedures may be required for routine neonatal intensive care, and there are few data on the use of analgesics in preterm infants.[2] In contrast to the use of paracetamol (acetaminophen, APAP) in term neonates,[3,4] the use of APAP in the first weeks of life in preterm neonates is controversial, because its pharmacokinetics, pharmacodynamics and metabolism in this age group remain unknown. Safety concerns may prevent its use in critically ill preterm neonates.

This study aimed to investigate pharmacokinetic variables after a single dose of rectally administered APAP in preterm infants, and to investigate whether there are any age dependent differences and any dose-response correlations between serum APAP concentrations and responses to pain during painful procedures.

II.3.3 Methods

Twenty-eight inborn neonates who were admitted to the neonatal intensive care unit were included into the study. Entry criteria were the need for insertion of arterial and venous catheters or other painful procedures, such as insertion of a chest drain. The study protocol was approved by the ethical review committee and informed parental consent was obtained. As pharmacokinetics and drug metabolism change during the last three months of gestation,[5,6] and pain sensitivity may be altered after 32 weeks,[7] the neonates were stratified into 2 gestational age groups: 28-32 weeks (group 1) and 32-36 weeks (group 2). The gestational age of the neonates was estimated from

maternal menstrual history, by routine ultrasound examination during pregnancy, and from postnatal physical characteristics (Farr score).[8] Patients were excluded if they had congenital anomalies, if the mother received tocolysis with indomethacin, or if she had been given analgesics (other than local analgesia) within 24 hours before delivery. None of the infants had sepsis or other congenital infections, or documented periods of shock.

The infants were studied on the first two days after birth. Nasal intubation, if necessary, was performed in the delivery room and the patient was transported to intensive care. In each patient heart rate, respiratory rate, arterial blood pressure and oxygen saturation were monitored continuously, together with rectal and peripheral temperatures. According to standard procedures in intensive care, umbilical venous and arterial access was obtained, and parenteral nutrition was started within 24 hours of birth.

All patients were given APAP rectally after arrival in intensive care, within one hour of birth, the dose used being as close to 20 mg/kg as the available strengths of suppository (20 mg for birthweights 750-1249 g, 30 mg for 1250-1749 g and 40 mg for 1750-2250 g) would allow. The nurses ensured that the suppository was retained. Suppositories contained 20, 30 or 40 mg APAP (particle size <45 μm), and hard fat (Witepsol H 15), a synthetic mixture of mono-, di-, and triglycerides of the saturated fatty acids C10-C18. The suppositories were prepared and analysed for APAP content and content uniformity by the quality assurance laboratory of the hospital pharmacy.

Before APAP was administered a 0.1 ml blood sample was taken from the arterial catheter. Subsequently samples were taken at 30, 60, 120 minutes, 4, 6, and 8 hours in the first 10 neonates, with an additional sample at 12 hours in 18 neonates. After collection serum was separated and frozen at -20 ^{0}C, until assayed. Urine was collected for 48 hours using a plastic bag, and frozen in separate (3 hours) aliquots until analysis.

The assay was performed within a month of sample collection, using a modified high performance liquid chromatography (HPLC) method.[9] Serum samples were extracted with perchloric acid and after centrifugation the supernatant was injected into the HPLC column. Urine samples were diluted with distilled water (1:9) before injection into the column. Standards for serum APAP, and urine APAP, APAP-glucuronide (APAP-G) and APAP-sulphate (APAP-S) were injected at the start and the end of each run. HPLC conditions, retention times, and ultraviolet detection were as described before.[9] Limit of detection for serum APAP was 0.2 mg/l, recovery was 96%, and precision 2%. Detection limits in urine were 0.3, 2.3, and 6.3 mg/l for APAP, APAP-G, and APAP-S respectively; recovery for APAP was 100%, for APAP-G and APAP-S 95%; and precision for APAP was 7%, for APAP-G and APAP-S 3%.

The values for each metabolite were converted to the equivalent weight of APAP, from which the metabolite was derived by correction for molecular weight. Standard metabolites were a generous gift of Sterling Health Company, Haarlem, The

Netherlands. The calibration curves were linear over the range 0.5-40 mg/l (APAP) and 5-1000 mg/l (metabolites).

Pharmacokinetic data and variables were calculated from standard equations using the KINFIT program (Mediware, Groningen, The Netherlands). Concentration time curves were constructed to determine peak serum concentration (C_{max}), time to reach C_{max}, time to reach therapeutic concentrations (adult values 10-20 mg/l),[4,10] and serum half-life of the drug ($T_{½}$). For $T_{½}$ a minimum of two time points in the elimination phase was used. Kinetic constants for APAP elimination were calculated according to the two compartment model as used by Miller.[11] For calculations a bio-availability of 90% was assumed.

Because all infants were nursed in incubators with a relative ambient humidity of 60-70% and temperature settings that aimed at a neutral temperature between 36.5-37.2°C, the anti-pyretic effects could not be measured.
Pain was assessed by nurse pairs or nurse/doctor pairs with a modified five facies pain score, showing increasing levels of discomfort from 0 (no pain) to 4 (clearly/obviously in pain).[12,13]

Data were analysed with the Statistical Package for the Social Sciences, SSPS Inc., Chicago, Ill, USA.[14] Student's *t* tests were used for normally distributed data and Mann-Whitney U tests for non-parametric data to compare the two groups. Least squares regression was used to evaluate linear correlation between variables. P values ≤ 0.05 (two tailed) were considered significant.

Figure 1 (A) APAP serum concentrations following a single rectal dose of 20 mg/kg, after 30 minutes-8 hours in 10 infants (group I), (B) after 30 minutes-12 hours in 11 infants (group I), and (C) after 30 minutes-12 hours in 7 infants (group II).

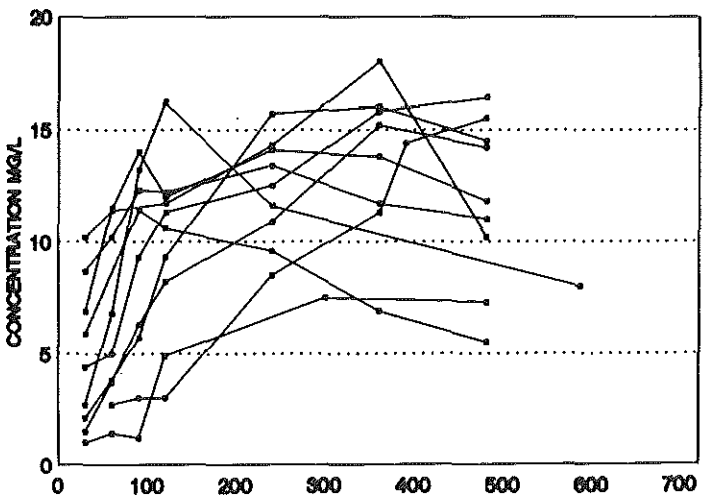

Figure 1A

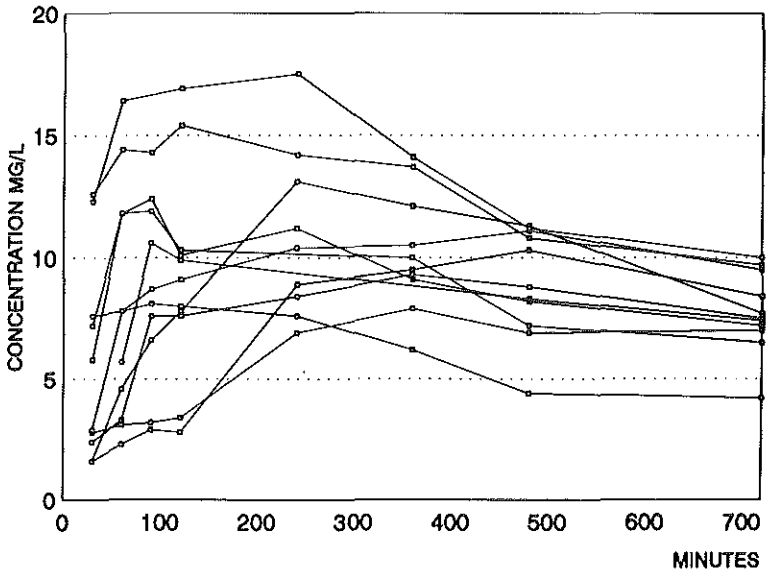

Figure 1B

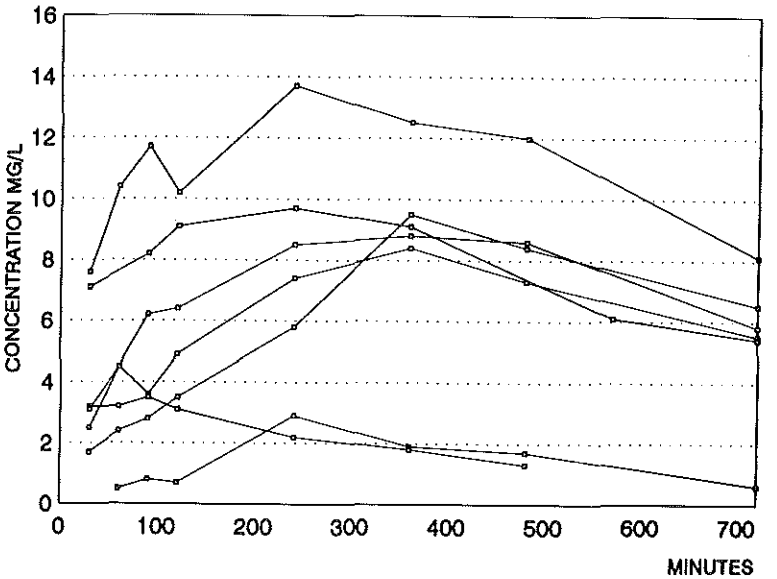

Figure 1C

II.3.4 Results

Of the 28 infants studied, 21 were in the 28-32 week group and 7 in the 32-36 week group. Demographic data are shown in table 1.
Serum APAP concentrations are shown in figures 1A-C.

	Group I 28-32 weeks (n=21)	Group II 32-36 weeks (n=7)
Maternal factors:		
HELLP/Preeclampsia	6	1
Solutio Placenta	1	-
Bloodloss Plac. previa	1	1
Sex:		
Male	13	2
Female	8	5
Method of delivery:		
Vaginal vertex	13	4
Vaginal breech	1	-
Ceasarean section	7	3
Diagnosis at admittance:		
RDS	7	1
SGA	5	-
Pneumothorax	-	1
Asphyxia	-	2
Preterm birth*	2	-
Artificial ventilation	10	5#
Surfactant	3	2
Birthweight (grams)**	1280 ± 284	1786 ± 323
Gestational age (weeks)**	30.21 ± 0.99	33.57 ± 0.77

Table 1. Demographic data of study participants.
*HELLP Haemolysis, elevated liver functions, low platelets, RDS Respiratory distress syndrome, SGA Small for gestational age. * as sole diagnosis, ** mean±SD, # one infant high frequency oscillation*

Analgesic therapeutic concentrations (adult values 10-20 mg/l)[4,10] were reached in 17 infants, in 16 out of 21 in group 1 (76.2%), and in one out of 7 (14.3%) in group 2. Therapeutic concentrations persisted for 8 hours in 13 infants (12 group 1 and one in group 2) and for 12 hours in one infant. C_{max} was mean 11.2 (SD 3.8) mg/l for all infants studied, data for groups 1 and 2 are shown in table 2; C_{max} was significantly higher in group 1. For individual values there was a significant negative correlation between C_{max} and gestational age (r -0.50, p=0.007). There was a significant inverse linear regression between APAP concentrations at T60 ($p<0.05$), T120 (p=0.008), and T240 ($p<0.05$) with gestational age (Figure 2) and no significant correlation between T_{max} and gestational age. As serum concentrations were still in the therapeutic range after 8 hours, $T_{½}$ could not be measured in 10 infants in group 1 because elimination of APAP was not yet apparent (Figure 1A). Therefore, $T_{½}$ could only be measured in 11 infants in group 1 but it was measured in all infants in group 2 (Table 2).

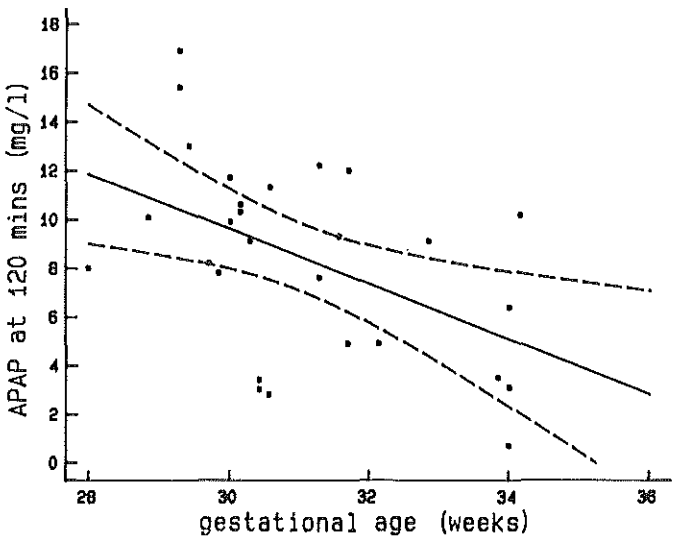

Figure 2 Linear regression analysis of gestational age on APAP levels at t 120 minutes. r-0.49, P 0.008. Dashed lines: 95 percent confidence limits.

In two infants only two pieces of data were available to calculate $T_{½}$; in the other 16 infants three or more time points were available. The area under the serum concentration time curve (AUC) was determined by the trapezoidal rule. The mean (SD) value in group 1 (95.1 (28.0) in 11 infants) was significantly higher than in group 2 (71.7 (41.7); p=0.046). Clearance increased with gestational age showing a significant positive correlation (r=0.52, p=0.008)(Table 2).

Parameter	GA 28-32 weeks (I)	GA 32-36 weeks (II)	p
C_{max} (mg/l)	12.5 ± 2.9	7.5 ± 4.0	0.001
range	7.5 – 18.0	1.5 - 13.6	
T_{max} (h)*	3.9	5.1	ns
range	0.8 – 10.5	1.0 - 9.5	
T½ (h)	11.0 ± 5.7	4.8 ± 1.2	0.011
range	3.5 - 25.2	3.6 - 6.8	
AUC (mg.h/l)	95.1 ± 28.0	71.7 ± 41.7	0.046
range	29.0 - 160.6	17.5 - 134.6	
Cl (l/h)	0.10 ± 0.04	0.56 ± 0.66	0.04
range	0.03 - 0.17	0.13 - 1.70	

Table 2. Pharmacokinetic parameters of paracetamol in preterm neonates after rectal single dose.
C_{max}, maximum concentration; T_{max}, time to reach maximum concentration; T½, elimination half-life; AUC, Area under the concentration-time curve; Cl, clearance.
Data are shown as mean ± SD or as median.*

Due to the available strengths of the suppositories, individual doses ranged from 16.6-26.6 mg/kg in group 1, and from 17.1-22.1 mg/kg in group 2; the relation between APAP dose and C_{max} is shown in figure 3. All doses of more than 18 mg/kg resulted in therapeutic concentrations in group 1, but not in group 2. There was a significant correlation between the rectal temperature and time to reach therapeutic concentrations (r -0.54, p<0.03), and no correlation between rectal temperature and C_{max}.

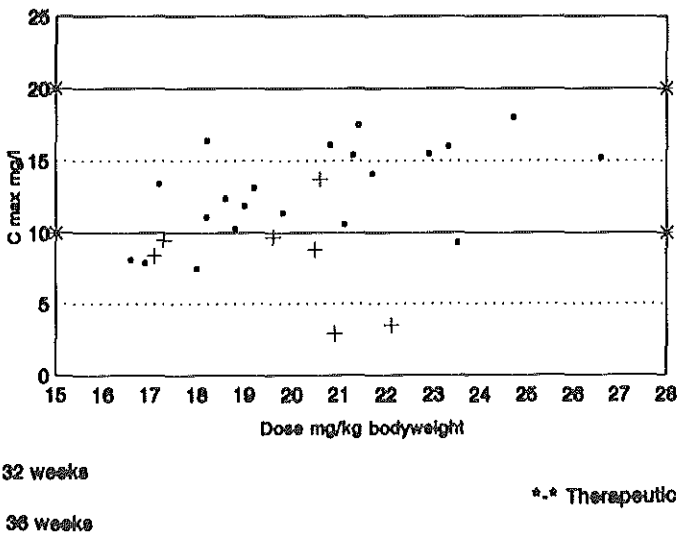

Figure 3 APAP peak serum concentration in preterm infants in relation to the dose, in two different age groups; group I 28-32 weeks (n=21), group II 32-36 weeks (n=7). Therapeutic levels represent adult values.

No significant losses occurred during urine collection. Of the rectal dose, 63.2 (24.4)% was recovered in the urine; 61.0 (24.4)% in group 1 and 74.2 (24.8)% in group 2 (NS; p=0.2). APAP was excreted mainly as APAP-S and to a lesser extent as APAP-G. Very small amounts of free APAP, and APAP-cysteine (APAP-C) were excreted and APAP-mercapturic acid (APAP-M) was not detected. The glucuronide:sulphate ratio (G:S ratio) was 0.12 (0.09) and 0.28 (0.35) for groups 1 and 2, respectively (Figure 4).

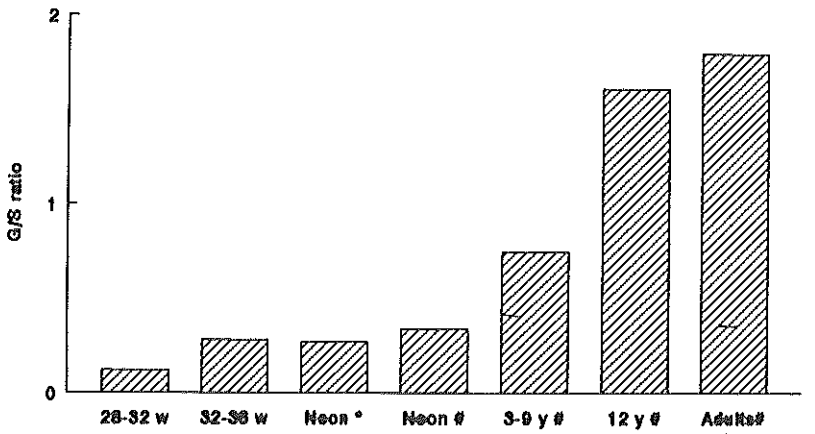

Figure 4 APAP metabolism, glucuronide to sulfate ratio in preterm infants in this study compared with term neonates (Levy et al 1975;55-6:818-25[25]), and term neonates, older children and adults (# Miller et al 1976;19-3:284-94[12]). (* reproduced by permission of Pediatrics and # reproduced by permission of Clinical Pharmacology and Therapeutics)*

Facies pain score reflecting the infants' responses was scored at the time of blood sampling. Pain scores from 0 to 2 were obtained in all infants and did not correlate with therapeutic concentrations. The reliability for the pain score was high; weighted Cohen's k=0.74.

II.3.5 Discussion

As far as we are aware this is the first study to provide data on the pharmacokinetics and metabolism of APAP in preterm infants. As the rectal mode of delivery results in less efficient absorption than the oral route, higher doses are needed in infants and in older children. After rectal doses of 16-26 mg/kg none of our infants reached concentrations above 20 mg/l, while toxic concentrations occur above 120 mg/l at four hours after ingestion.[15] In infants whose mothers ingested an overdose of APAP prenatally, high concentrations of APAP (75.5 and 260 mg/l) were documented in neonates with no apparent hepatic or renal toxicity.[16,17] The high therapeutic ratio in neonates may be related to reduced rates of metabolism by the cytochrome p 450 system in the neonatal liver and neonates' increased ability to synthesise gluthathione relative to adults.[18,19] Young mice have a fourfold greater gluthatione turnover and increased activity in the gluthatione peroxidase/reductase system than older mice.[18,19]

Renal toxicity is also prevented by the increased solubility of APAP-S as compared to APAP-G, the major metabolite in adults.
We speculate that multiple doses of APAP would also be safe, in the preterm infants studied.
Peak serum concentrations were reached later in preterm infants in our study than in term infants and adults,[4,20] but most 28-32 week old infants did achieve therapeutic concentrations. All the infants in our study were under 1 hour of age, and absorption may increase with gestational and postnatal age. Individual differences in rectal temperature (range 34.7°C-38.4°C) at time of administration of the suppository may alter the time needed for melting and absorption. A significant correlation occurred between rectal temperature and the time to reach therapeutic concentrations. Rectal temperature ranged from 34.8 to 36.8°C in 10 infants who did not achieve therapeutic concentrations (≥10 mg/l). Although no suppositories were expelled, differences in C_{max} and T_{max} in individual patients may be due to the variability of venous drainage from the rectum. Drugs administered into the proximal rectum will be subjected to the hepatic first pass effect, whereas drugs in the distal rectum will bypass the liver.[5] The inverse linear regression between APAP concentrations and gestational age may be due to the larger extracellular space and thus greater volume of distribution (Vd) with lower gestational age.[21] Although our sampling time was two to four times greater than the $T_{½}$ in adults and term neonates,[17,22-24] serum concentrations remained above therapeutic concentrations for more than 8 hours, which made it impossible to assess $T_{½}$ in 10 preterm infants. In infants where assessment was possible, the values indicated slow elimination, subsequent to a lower clearance and increased Vd.

Miller et al found shorter $T_{½}$ in three term infants and one preterm infant than in older children and adults after oral APAP.[11] Other studies found longer $T_{½}$ after rectal APAP in infants compared with oral doses in adults.[4,22,23] Our $T_{½}$ values were 11 hours in infants 28-32 weeks of gestation and 4.8 hours in infants 32-36 weeks of gestation and were inversely related to gestational age. This agrees with the published findings, with values of 2.7 to 4.9 hours in term infants in the first month of life and of 1.0 to 2.4 hours up to the age of 1 year.[10] Doses of 18 mg/kg or more produced therapeutic concentrations in the 28 to 32 week gestation group, and doses up to 26.6 mg/kg were given without any adverse effects. Because of the long $T_{½}$ we recommend that multiple doses should be given every 8 to 12 hours.

The pain scores we used[12,13] did not correlate with the concentrations of APAP; probably because most of the infants scored low on the pain score indicating minimal or no pain. It may be that this pain score is not particularly suitable for measuring pain in preterm infants or that it is only suited for the assessment of acute severe pain. In the original study this pain score was used for older infants,[12] and later modified for use in younger infants.[13] Manne et al showed that in older children simultaneous assessment of pain by patients, nurses and parents showed comparable results.[25] Postgestational age seems to be important when considering the pain response of infants less than 32 weeks of age.[26]
In several double blind cross over studies in healthy volunteers given normal therapeutic doses, analgesic effects occurred with concentrations <10 mg/l.[20]

If the same applies for the infants in our study, they were having suitable pain management and the pain score was appropriate.
Exaggerated concerns for overdosing and hepatotoxicity, or increased bilirubin concentrations have been the main reasons for withholding APAP from neonates. Neonates are capable of metabolising APAP, not by glucuronidation but also by sulphation,[11,22,27] and there is no correlation with plasma bilirubin concentration.[22]

Limited data show that even when APAP reaches toxic concentrations in preterm neonates, detoxification by the mixed function oxidase system is sufficient, without any adverse effects.[16,17] Even when glucuronide and sulphate conjugation (phase II reaction) have reached their maximum capacity, this normally minor pathway metabolises APAP as long as glutathione is not depleted. Part of the APAP is not metabolised and excreted unchanged in the urine.

Our findings that urinary excretion was primarily as APAP-S, resulting in a low glucuronide to sulfate ratio, agree with the results of earlier studies.[11,16,22] The values in our groups show a considerable decrease in G:S ratio with earlier gestational age. Figure 4 shows the comparison of G:S ratio in our study with two other studies in older infants, children and adults.[11,22] Our results are supported by several studies showing decreased fetal and neonatal UDPG-T activity,[28-30] and by the presence of a well developed sulphation pathway both in vivo[21] in term neonates and in vitro[31] in fetal liver at 19-22 weeks of age. Furthermore, two other studies showed even higher sulphation rates than glucuronidation in children aged 7-10 years, compared with adults.[11,32] Our results differ from those of a recent study in which 2 to 6 day old, term infants ingested paracetamol in their mothers' breast milk in amounts of 0.3-18.5 mg/kg, and excreted it mainly as APAP-G.[33] However, in this study urine sampling only took place for 3 hours; in other words with the longer $T_{½}$ in infants a substantial part was not yet excreted and maximal recovery of the ingested dose was 4.4%.[33]

We found only minute amounts of APAP-C and no APAP-M in the urine. As APAP-M was found in urine of a preterm infant after maternal overdose,[17] it might be that in preterm infants APAP-M is only formed after doses higher than our dose or after prolonged multiple doses, or that it is merely a reflection of their mothers' excretion pattern.

We conclude that rectally administered APAP is safe in preterm infants as single dose treatment. As prolonged rectal absorption from this dose is evident, a higher dose might be needed to shorten the interval to reach therapeutic concentrations. Prolonged rectal absorption and plasma clearance subsequent to developmental immaturity in the phase I and phase II pathways which govern APAP clearance were evident. Rectal APAP was apparently tolerated without any overt evidence of adverse effects.

Sulphation is the major metabolic pathway, while G:S ratio is positively related with post conceptional age. Furthermore, our study provides new ways of using APAP: in preterm infants the choice is no longer strong analgesics or no analgesic, but an intermediate analgesic for moderate pain.

II.3.6 Acknowledgements

We gratefully acknowledge the contribution by the nurses from intensive care to the collection of the data, and by Willem Grol for his statistical advice.

II.3.7 References

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II.4 Multiple-dose Pharmacokinetics of Rectally Administered Paracetamol in Term Infants.

Based on the article:

Multiple-dose pharmacokinetics of rectally administered acetaminophen in term infants.
RA van Lingen, JT Deinum, JME Quak, A Okken, D Tibboel.
Clin Pharm Ther 1999;66:509-15.

II.4.1 Abstract

Objective: To investigate pharmacokinetics and pharmacodynamics of rectally administered paracetamol in term neonates directly after birth.
Methods: In this prospective clinical trial, term neonates with painful conditions or who were undergoing painful procedures received multiple-dose paracetamol. Serum concentrations were determined serially with an HPLC method, and pharmacokinetic analysis was performed. Pain assessment was performed by means of a validated pain score.
Results: Ten consecutive term neonates received four rectal doses of paracetamol, 20 mg/kg body weight, every 6 hours. Mean peak serum concentrations (±SD) during multiple-dose administration were 10.79 ± 6.39 mg/l, 15.34 ± 5.21 mg/l, and 6.24 ± 3.64 mg/l for the entire group, boys, and girls, respectively. There was a significant difference between the boys and the girls (P=0.01). No serum concentrations associated with toxicity (>120 mg/l) were found. Median time to peak serum concentration was 1.5 hours after the first dose and 15 hours for multiple doses. Mean (± SD) half-life was 2.7 ± 1.4 hours in eight patients. There was no correlation between dose and serum concentration or between pain score and serum concentration. There was a significant inverse relationship between the preceding pain score and peak serum concentrations.
Conclusions In term neonates, multiple rectal doses of paracetamol, 20 mg/kg body weight, led to widely varying serum concentrations but did not result in therapeutic concentrations in all infants. Boys had higher peak concentrations. Because accumulation was not found, a dose of 30 mg/kg followed by doses of 20 mg/kg at 6- to 8- hour administration intervals are appropriate to reach therapeutic concentrations. A concentration-effect relationship could not be determined

II.4.2 Introduction

Although little is known about the pharmacokinetics and pharmacodynamics, paracetamol (INN, acetaminophen) is frequently used by both doctors and parents to treat minor and moderate pain in children. Fear of toxicity caused by accumulation in cases of multiple dosing has been the main reason that paracetamol has been refused to infants in the first days after birth.[1-3] Its effectiveness in treating neonates and both older and younger infants has not been clearly evaluated. In a recent study in preterm infants, we showed that a single rectal dose of paracetamol can be given safely.[4] Moreover, it has been proven that metabolism of paracetamol in newborn infants is comparable to that documented in older children and adults, not as a result of glucuronidation but because paracetamol is predominantly conjugated to sulphate.[4-6]

The appropriate analgesic dose for term infants is not known, but single doses between 10 mg/kg (oral) and 20 mg/kg body weight (rectal) that result in serum concentrations between 4 and 20 mg/l are reported to be sufficient to have an antipyretic effect.[7-9] By taking this range and by combining other data based on validated pain assessment instruments and oral dose, the therapeutic range for analgesia in adults and children (10-20 mg/l) has been extrapolated.[3,10,11] In older infants and children, the advised rectal dose is 20 mg/kg every 4 hours.[12,13] This study was therefore designed to

investigate the serum concentrations resulting from multiple rectal doses of 20 mg/kg paracetamol in term neonates and to determine the relationship between dose and effect and between concentration and effect.

II.4.3 Methods

Patients

The study included 11 consecutive neonates who were admitted to the neonatal intensive care unit. Entry criteria were traumatic delivery that resulted in hematoma, fractures, or scalp birth injuries or that resulted in the need for insertion of arterial and venous catheters or other potential painful procedures, such as insertion of a chest drain and subsequent drainage. The study protocol was approved by the ethical review committee of the Isala Clinics/Sophia Hospital (Zwolle, The Netherlands). Written informed parental consent was obtained for each patient before enrolment. The gestational age of the neonates was estimated from maternal menstrual history, by routine ultrasound examination during pregnancy, and by postnatal physical characteristics (Farr score).[14] Patients were excluded if they had major congenital anomalies, severe asphyxia (Apgar score ≤3 after 5 minutes), if their mother had received tocolysis with indomethacin (INN, indometacin), or if she had been given analgesics within 24 hours before delivery.

The infants were studied during the first 2 days after birth. Nasal intubation, if necessary, was performed in the delivery room in a standardized fashion without administration of medication, and the patient was transported to the neonatal intensive care unit. The heart rate, respiratory rate, arterial blood pressure, and oxygen saturation of each patient were monitored continuously, together with rectal and peripheral temperatures. Patients were nursed under a radiant heater. In each patient, umbilical venous and arterial access was obtained, and parenteral or enteral nutrition was started within 24 hours after birth according to standard procedures in the neonatal intensive care unit.

Within 1 hour of birth, patients were given paracetamol rectally. The dose used was as close to 20 mg/kg as the available strengths of suppository would allow (50 mg for birth weights 2500 to 2749 g, 60 mg for 2750 to 3249 g, 70 mg for 3250 to 3749 g, and 80 mg for ≥3750 grams. Subsequent doses were given after 6, 12 and 18 hours. Care was taken by the nurses to ensure that the suppository was retained. Suppositories contained 50, 60, 70, or 80 mg paracetamol (particle size <45 μm), and hard fat (Witepsol H 15), a synthetic mixture of monoglycerides, diglycerides, and triglycerides of the saturated fatty acids C10 to C18. The suppositories were prepared and analyzed for paracetamol content and content uniformity by the quality assurance laboratory of the hospital pharmacy.

Blood samples (0.2 ml) were taken at 30, 60, 90 minutes, and at 3, 9, 15, 21, 24 and 27 hours after the first dose had been administered. After blood collection, serum was separated and frozen at -20 ^{0}C, until analysis.

Analytical procedures
The assay was performed within a month of sample collection by a modified HPLC method, as described previously.[4,15] In short, serum samples were extracted with perchloric acid and, after centrifugation, the supernatant was injected into the HPLC column. Standards for serum paracetamol were injected at the start and end of each run. HPLC conditions, retention times, and ultraviolet detection have been described previously.[4,15] The limit of detection for serum paracetamol was 0.2 mg/l, the recovery rate was 96%, and the precision of the assay was 2%. The calibration curves were linear across the range of 0.5-40 mg/l.

Pharmacokinetics and pharmacodynamics
Pharmacokinetic data and parameters were calculated from standard equations using the Multifit program (J.H. Proost, University Centre for Pharmacy, Groningen, The Netherlands). Concentration-time curves were constructed to determine peak serum concentration (C_{max}), time to reach C_{max} (T_{max}), time to reach therapeutic levels (T_t), and serum half-life of the drug ($T_{½}$). The mean C_{max} was determined by averaging the maximum serum concentrations, and the mean T_{max} was determined by averaging the times of peak serum concentration. Area under the concentration-time curve (AUC) was calculated with the trapezoidal rule. For calculations of the quotient of clearance and bioavailability (Cl/F), a bioavailability of 90% was assumed.
Serum concentrations ranging from 10-20 mg/l were considered to be therapeutic.

Because all infants were nursed under radiant heaters with temperature settings between 36.8°C-37.2°C to achieve a neutral temperature, any antipyretic effects could not be measured. All infants had repeated painful procedures, including suctioning, skin puncture, dressing change or tape removal, and discontinuation of intravenous lines. Pain was assessed at standard times 1 hour after each dose was given by nurse pairs or nurse-doctor pairs, with a modified 5 facies pain score that showed increasing levels of discomfort from 0 (no pain) to 4 (clearly/obviously in pain), as described previously.[4,12] Observers were trained by video instruction for 3 hours, resulting in a high inter-rater reliability (Cohen's κ=0.74) as published previously.[4]

Statistical analysis
Data were analyzed with SPSS-5 (SPSS Inc, Chicago, III). Student's *t* tests were used for normally distributed data and Mann-Whitney *U* tests for nonparametric data to compare the two groups. Least-squares regression was used to evaluate linear correlation between variables, and *P* values $\leq$0.05 (two-tailed) were considered to be significant.

II.4.4 Results

Eleven infants were included in the study; serum samples from one infant were lost. Of the remaining 10 infants, the lungs of nine were ventilated. Diagnosis at admittance was pneumonia in two infants and pneumothorax in two infants. The clinical details of these infants are shown in table I.
Paracetamol was detected in the serum of all infants studied. Individual serum concentration-time curves are shown in figure 1.

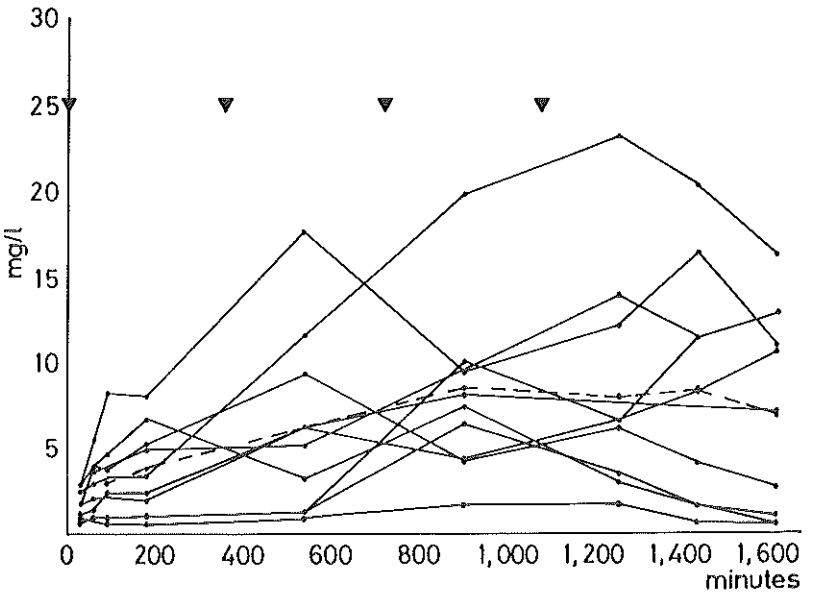

Figure 1 Paracetamol serum concentrations following multiple doses (at 0, 6, 12, and 18 hours) after 30 minutes - 27 hours (n=10).
Triangles: time of drug administration, ----- average concentration.

Sex	
Male	5
Female	5
Birth weight (grams)	3542 ± 777
Gestational age (weeks)	39.34 ± 2.35
Artificial ventilation	9
Mode of delivery	
- Vaginal	5
- Vaginal vacuum extraction	1
- Cesarian section	4
Diagnosis at admittance	
- Pneumonia	2
- Pneumothorax	2
- PPHN	1
- Wet lung (TTN)	5

Table I Clinical characteristics of study participants.

Absorption of paracetamol after the first dose was slow. C_{max} was reached between 30 and 180 minutes (median, 90). The C_{max} after the first dose reached a mean (± SD) value of 3.36 mg/l ± 2.11 mg/l. Even after multiple doses, it took a considerable time to reach a C_{max} within the therapeutic range (10-20 mg/l), with only the five boys reaching a C_{max} that exceeded 10 mg/l during the total study period.

There was a significant difference in the mean (± SD) C_{max} between the boys (n=5; 15.34 ± 5.21 mg/l) and the girls (n=5; 6.24 ± 3.64 mg/l; P = 0.01). The C_{max} for all infants throughout the entire study period was 10.79 ± 6.39 mg/l (range 1.7 to 23.2 mg/l; Figure 2). The coefficient of variation for all infants was 37.2 %. The median T_{max} values after the first dose and during multiple dosing was 1.5 hours and 15 hours, respectively.

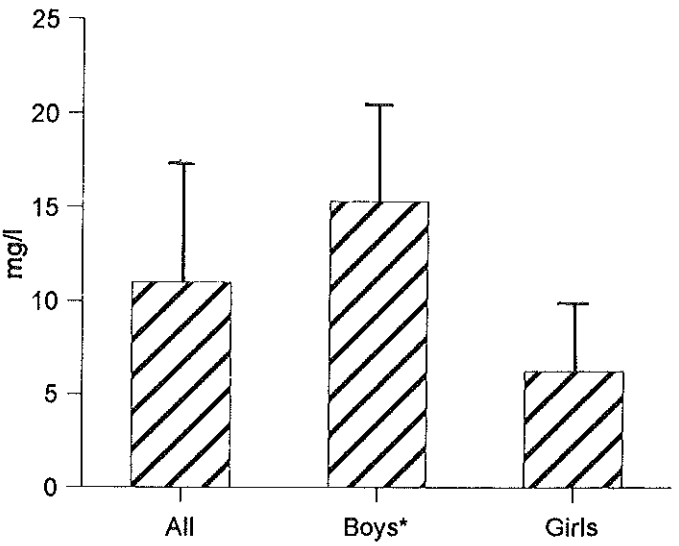

Figure 2 C_{max} (hatched bars) and SD (error bars) during the study period for all infants (n=10), boys (n=5), and girls (n=5).
** p = 0.01 boys vs girls.*

The serum $T_{½}$ after the first dose could be calculated for eight patients only (elimination of paracetamol was not yet apparent in two patients) and was 2.7 ± 1.4 hours.
The T_t for the five boys was between 9 and 27 hours; this was reached after two, three, or even four doses (n=2, 2 and 1). After the last dose, elimination seemed to be slower in four infants, with serum concentrations remaining in the therapeutic range even 9 hours after the last dose.

Because of the fixed strengths of the suppositories, the actual doses administered ranged from 16.6 to 21.2 mg/kg. A correlation between dose and C_{max} was not found. Almost all infants with a short T_{max} after the first dose had a low C_{max}, but this

correlation (r =0.57) did not reach a significant level. After multiple doses, no correlation between T_{max} and C_{max} was observed. The $T_{½}$, area under the serum concentration-time curve (AUC), clearance (Cl) for individual patients after all doses, and the quotient of clearance and bioavailability (Cl/F) are shown in table II.

Patient	$T_{½}$	AUC	Cl	Cl/F
G1	4.9	28.2	9.4	8.46
B1	8.6	461.8	0.8	0.72
G2	3.8	151.8	1.4	1.26
G3	1.2	115.2	3.9	3.51
G4	5.3	73.3	5.3	4.77
B2	-	397.2		
G5	52.4	164.5	0.3	0.27
B3	36	229.8	0.4	0.36
B4	-	118.6		
B5	11.8	160.4	1.2	1.08

Table II Pharmacokinetic data of study participants.
$T_{½}$, elimination half life (h); AUC, area under the concentration-time curve (mg.h/l); Cl, clearance (l/h); F, fraction of drug absorbed; G, girl; B, boy.

In all infants, pain assessment by means of the facies score, performed 1 hour after the dose was given before the infant's C_{max}, showed a significant inverse linear regression in relation to the C_{max} (r = -0.39, P = 0.023).
There was no correlation between the pain scores and the serum concentrations: pain scores from 0 to 1 were obtained in the infants when serum concentrations were >2.61 mg/l.

II.4.5 Discussion

As far as we are aware, this study is the first study to investigate both dose-concentration and concentration-effect relationships of paracetamol multiple-dose pharmacokinetics in term neonates. The pharmacokinetic effect of a single dose has been studied after rectal and intravenous administration in infants <14 days of age,[16,17] but there are still few data regarding multiple doses and analgesia.[18]
The antipyretic effect of paracetamol has been extensively studied in infants,[10,19,20] children,[7,10,19-21] and adults.[9,22] Plasma and serum values between 4 and 20 mg/l are considered to be therapeutic,[9] and by combining data from orally administered paracetamol that result in concentrations of 10 to 20 mg/l[23] with data from a dose-effect study,[10] a therapeutic range for analgesia has been proposed but has not been validated.[11] Using tonsillectomy as a pain model Anderson et al[24] found that

paracetamol (40 mg/kg orally) provided satisfactory analgesia for most children when plasma concentrations were within the range known to reduce fever. In the literature, data on the effect of paracetamol on fever or analgesia in children are either dose-concentration[16,20,25] or dose-effect studies.[26]

The values for C_{max} in our study are comparable with results from previous single-dose studies with a 20 mg/kg rectal dose of paracetamol in both preterm and term neonates,[4,16,27] and with high-dose rectal paracetamol (30-45mg/kg) in children aged 3 to 13 years.[25,28] In a recent study by Sanderson et al,[29] plasma values at 24 hours in children aged 3.9 to 9.8 years old ranged from 6.8 to 20.9 mg/l after a scheduled dosing regimen of 20 mg/kg orally with a 6-hour dosing interval. In comparison, we found values from 0.59 to 20.4 mg/l at 24 hours. Our data with a dosing schedule of 20 mg/kg four times a day do not support the estimates made by Autret et al[17] for infants under 10 days of age. With computer simulations of 15 mg/kg propacetamol four times a day (i.e. 30 mg/kg/day paracetamol) Autret et al[17] estimated that C_{max} would reach concentrations from 10.1 to 22.9 mg/l (mean ± SD, 17.46 ± 4.97 mg/l), whereas these values were reached in only half of our study infants with 80 mg/kg/day. We agree with Anderson et al[11] that with the large variability of results shown by the coefficient of variation of 40 %, which compares with our value of 37.2 %, we should be careful not to overstate the worth of mean values when considering individual patients, and that this coefficient of variation implies that some patients may not achieve a therapeutic C_{max}.

In all studies in infants, individual values vary widely, and when paracetamol is given rectally the T_{max} also varies widely and is seldom reached before 3 hours. This might be a result of the variability of venous drainage from the rectum, resulting in bypassing of the liver by drugs delivered distally, whereas drugs delivered in the proximal rectum will be subjected to the hepatic first-pass effect.[30]

Even after four doses, accumulation was not observed in our patients, as was the case in one study of children from 6 months to 6 years old to whom oral doses between 10 and 15 mg were administered.[23] In adults, orally administered paracetamol exhibits linear pharmacokinetics for doses of 18 mg/kg or less when given five times at 6-hours intervals.[31] In a computer model that simulated rectal dosing in children, a loading dose of 50 mg/kg followed by 30 mg/kg at 6-hour intervals achieved plasma concentrations of 9 to 18 mg/l, without accumulation.[32] However, the effect of paracetamol given for more than 24 hours in this age group is not known. Nahata et al[3] found accumulation after 2 to 3 days in an older age group (2 to 8 years) when given 22 to 27 mg/kg orally, whereas doses of 13.3 mg/kg or more may also accumulate after this period. The highest serum concentration recorded in our study was 23.2 mg/l, which is still well below the concentration associated with toxicity in adults (>120 mg/l).[1] However, elimination appeared to be slower after the last dose in our study.

In some studies with older children, a starting dose of 30 to 40 mg/kg has been proposed, but we refrained from following this schedule in this study because the metabolism in neonates is somewhat slower.[4,11,28,32] The $T_{½}$ varies widely between individuals, and in our study on the first day of life it ranged between 1.2 and 52.4

hours but decreased with postnatal age. In term infants it is reported to be between 2.7 to 4.9 hours in the first month of life and ranges from 1.0 to 2.4 hours up to the age of 1 year.[5,8]

On the basis of our study, a starting dose of 30 mg/kg is proposed, followed by 20 mg/kg rectally at dosing intervals that increase from 6 to 8 hours. In this way, the maximum daily dose of 90 mg/kg, is not exceeded.

Surprisingly, we found a significant difference in C_{max} between the girls and the boys; the mean value for girls was lower than for boys. Clearance was no higher in girls than in boys. Although we have tried to collect urine by means of an adhesive urinary bag in five infants, too many samples were lost to give a reliable impression of the excretion and recovery in the urine. Nothing is known about a gender-related difference in pharmacokinetics for any drug in this age group in humans. Data from our recent study in preterm infants do not show any difference.[4] Because our group is relatively small, this difference may become negligible when a larger sample group is taken.

Serum concentrations between 10 and 25 mg/l are suggested as the appropriate analgesic range.[32] However, no studies have been performed to compare serum or plasma concentration with effect in the age group under study. Except for the study by Anderson et al,[24] until now, therapeutic paracetamol concentration ranges have not been determined in infants or in older children and adults. Studies in adults on the central analgesic effect propose that the time course of paracetamol in cerebral spinal fluid may parallel that of analgesic effect because a time delay exists between serum concentrations and the analgesic effect of paracetamol measured by means of the R-III nociceptive flexion reflex. [33-35] Painful procedures such as those experienced by the infants in our study are reported to generate painful responses 75-100% of the time and therefore seem to be equivalent to each other.[36] A dose-effect study showed that 20 mg/kg oral paracetamol was ineffective for decreasing the pain from heel prick in term neonates.[37] Recently Anderson[38] stated that neonates may have altered pharmacodynamics compared with older children.

Although low serum concentrations were found, low scores of pain assessment were determined, suggesting appropriate analgesia in all infants. This may be the result of a greater permeability of the blood-brain barrier in neonates, leading to greater central analgesia. Because there was no correlation between the pain scores and the serum concentrations and because values that exceeded 2.6 mg/l were associated with facies pain scores from 0 to 1, we are not able to indicate a therapeutic paracetamol concentration range for this age group.

In conclusion, we found that paracetamol can be administered safely to neonates on the first day of life in rectal multiple doses of 20 mg/kg. A higher starting dose of 30 mg/kg, as recommended for older children, is proposed because (1) therapeutic concentrations were not reached in all infants, (2) the time to reach therapeutic concentrations was relatively long, and (3) accumulation was not found. Further

research with a longer sampling time is needed to determine whether paracetamol given to neonates for periods exceeding 24 hours is effective and safe.

II.4.6 Acknowledgements

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II.4.7 References

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II.5 Effect of Rectally Administered Paracetamol on Infants Delivered by Vacuum Extraction.

Based on the article:

Effects of rectally administered paracetamol on infants delivered by vacuum extraction.
Richard A van Lingen, Coby ME Quak, Hanneke T Deinum, Frans van de Logt, Jim van Eyck, Albert Okken, Dick Tibboel.
Eur J Obstetr Gynecol 2000; in press

II.5.1 Abstract

Objective: To evaluate whether paracetamol 20 mg/kg rectally relieves pain in infants delivered by vacuum extraction, and improves clinical condition.
Methods: Prospective, randomised, double blind, placebo-controlled study. Infants delivered by vacuum extraction were randomised either to the study group (n=61) and given paracetamol or to the control group (n=61) receiving placebo. Pain assessment was performed by a validated pain score and by scoring the clinical condition. Both scores and clinical symptoms in these groups were compared with symptoms in a reference group (n=66) with uncomplicated pregnancy and delivery in vertex position without vacuum extraction.
Results: Pain score did not differ between groups; clinical condition in the study group improved only after the first dose. There was a significant difference ($p<0.05$) in objective clinical symptoms in the vacuum extraction groups, compared to the reference group.
Conclusion: One dose of paracetamol given to neonates delivered by vacuum extraction improved significantly their clinical condition, but did not result in a significant change in objective pain scores. Subsequent doses of paracetamol did not show any effect on the clinical symptoms or appearance of the neonates studied.

II.5.2 Introduction

Vacuum extraction is frequently performed when there is insufficient progress in the second stage of labor, or when fetal distress is presumed.[1] The incidence of this procedure in the Isala Clinics, consisting of the Sophia Hospital, a hospital with a level III neonatal intensive care unit (NICU), and the Weezenlanden Hospital, is about 17%. This is higher than the incidence in the past few years in Dutch hospitals (5%-10%),[1] as vacuum assisted delivery is preferred over forceps delivery in our hospitals. During vacuum extraction, traction of 0.8 kg/cm^2 (550-600 mmHg) is used to reach sufficient force and this aggravates the formation of a caput succedaneum.
Several reports show that complications after vacuum extraction are mostly due to cup displacement and vary from moderate complications such as scalp injuries (redness, abrasions, lacerations, ecchymoses, ablation, blistering) in 12.6%, cephalhematomas (10%), and subgaleal hematoma, to more severe injuries as intracranial hemorrhage (0.4-0.8%), skull fractures (<0.1%) or rupture of the tentorium.[1,2,3] Extrapolated to the Netherlands with about 200,000 births and 5-10% vacuum extractions, this would lead to a maximum of 2500 cases with moderate, and 160 with severe complications. Retinal hemorrhage after vacuum extraction is frequently found (46.2%) and is thought to result from a rise in intradural pressure; however, the prognosis with regard to vision at a later age is good.[1]
Newborn infants are capable of experiencing pain, as substantiated by data published over the last decade,[4,5] but we still do not know under which condition neonates actually feel pain.[6]
We hypothesized that infants delivered by vacuum extraction experience pain due to the suction and the traction of the vacuum procedure, and that it may be relieved by the administration of paracetamol.

For this reason a prospective, randomized, double blind, placebo-controlled study was performed to evaluate whether paracetamol given in the recommended rectal dose of 20 mg/kg[7,8] relieves pain in newborn infants after delivery by vacuum extraction. Secondary endpoints were the changes in clinical symptoms, such as increased irritability, pain on handling, crying, vomiting, grunting or poor feeding and abdominal distension, and the changes in overall clinical condition, when compared with infants given placebo.
Moreover, both groups were compared with a (third) reference group, delivered in the vertex position without vacuum extraction and matched for gestational age and birthweight, to test the hypothesis that the above clinical symptoms are significantly more frequent in infants who required vacuum extraction than in those who did not.

II.5.3 Materials and methods

Patients
During a 15 month period infants born in vertex position after vacuum extraction in two level II hospitals were enrolled in our study. One of those hospitals also includes a level III NICU. Vacuum extraction was performed with either a hard cup (Malmström Bird Egnell, Lameris, Utrecht) or intermittently (4 times) with a soft cup (Silk Cup Egnell, Lameris, Utrecht). Infants were eligible for the study if they fulfilled the following entry criteria: birth weight >2500 grams, gestational age >36 weeks, Apgar score at 5 minutes $\geq$7, and the absence of congenital anomalies of the newborn. Entry criteria related to the infants' mothers were: uneventful pregnancy, no maternal analgesics <24 hours before delivery, and no maternal drug abuse. Infants admitted to the NICU for artificial ventilation because of respiratory insufficiency were excluded from the study. Furthermore infants were excluded during the evaluation phase of the study if data were incomplete, e.g. in case of early hospital discharge.
The same inclusion criteria and exclusion criteria, except for the delivery by vacuum extraction, applied to the infants born in vertex position in the reference group.

Informed parental consent was obtained prior to study entry. The study was approved by the hospital medical ethics committee.

Procedure
A computer-generated randomization procedure was carried out by one of our hospital pharmacists (JTD) who had no knowledge of the clinical status of the neonates. The NICU staff and the nurses, responsible for patient management and the nurses who performed scoring had no knowledge of the group designation. Randomization to paracetamol or placebo was stratified by birth weight.

In the delivery room separate numbered boxes were available for birth weight categories, each containing 4 suppositories with either paracetamol suspended in Witepsol H-15 as fatty suppository base or a placebo with only Witepsol H 15 base. The suppositories were prepared by the hospital pharmacy. Suppositories contained 50, 60, 70 or 80 mg paracetamol (Ph.Eur, particle size <45 μm). Hard fat Ph.Eur (Witepsol H 15), a synthetic mixture of mono-, di-, and triglycerides of the saturated fatty acids C10-C18 was used as a suppository base. The suppositories were prepared

in batches of 100; each batch was analyzed on content and content-uniformity by the quality assurance laboratory of the hospital pharmacy.
When an infant met all entry criteria the next numbered box for his or her birth weight category was used. Administration of the placebo or study drug was started immediately after inclusion. All infants were given the suppository rectally, in case of the study drug the dose of paracetamol used was as close to 20 mg/kg as the available strengths of suppository (50 mg for birth weight 2500-2749 g, 60 mg for birth weight 2750-3249 g, 70 mg for birth weight 3250-3749 g, and 80 mg for birth weight 3750 g) would allow. At 6, 12 and 18 hours hereafter they received another suppository from the same batch.

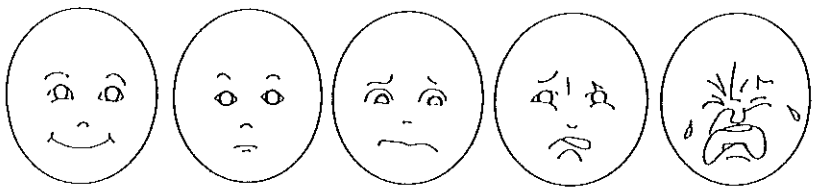

Figure 1 Facies scale, range 0 (no pain) - 4 (clearly in pain).[10] (reproduced by permission of the Dutch National Organization for Quality Improvement in Hospitals, CBO Utrecht).

Scoring
Pain was assessed with a modified 5 points facies scale[7] (Figure 1) at 1, 7, 13, and 19 hours after the first suppository had been given, assuming a maximum effect of paracetamol for term neonates between 30 and 60 minutes and a serum half life of 2-3 hours.[8] Scores ranged from 0 (no pain) to 4 (clearly in pain). Scoring was performed by nurses from the obstetric and pediatric wards, who were blinded for the group in which the infant was included. Nurses were trained by video instruction for three hours, which has been reported to result in a high inter-rater reliability (Cohen's κ=0.74).[9] In addition, nurses' perception of pain was noted at the same time and described as better, worse, or unchanged clinical condition, as compared to the clinical condition before administration, using the indices of pain as reported by Pigeon et al.[10]
Clinical symptoms in the first 24 hours were assessed before and after administration of paracetamol or placebo and were defined as shown in table I. In order to correct for symptoms due only to vacuum extraction, the same clinical symptoms were scored in a reference group of 66 term infants delivered after an uncomplicated pregnancy in vertex position without vacuum extracted head extraction. Two or more pain scores in the reference group were assessed at the same time points as in the other groups.
The following clinical data were recorded for all patients: birth weight, gestational age from last menstrual date or, preferably, from early (10 weeks) ultrasound, Apgar score at 5 minutes, heart rate, passage of meconium, and voiding. Labor related parameters such as fetal distress defined by CTG, and insufficient progress of labor in the second stage of labour, the indications for vacuum extraction, were recorded as well.

Symptom	Definition
Pain on handling	Noticing that the child dislikes to be handled, may cry with apprehension even when approached, but is quiet when left alone
Grunting	Grunting noise on expiration > 30 minutes after birth
Irritability	Hyperactive Moro reflex, increased muscle tone, restlessness, jittery even when undisturbed
Crying	Excessive crying longer than 1 minute, or incessantly for repetitive periods
Poor feeding	Listlessness or disinclination to feed
Bringing up	Returning small amounts of feeding
Vomiting	Returning the whole amount of one feeding
Abdominal distension	Increased abdominal circumference (by clinical impression from the nurse) in the time between 2 feedings

Table I Definition of clinical symptoms scored in the first 24 hours of life.

Data analysis

Data were analyzed using SPSS-5. In bivariate analysis, Student's t-tests were used when the data were normally distributed; otherwise, a nonparametric method (Mann-Whitney U test) was used to compare groups.

From our clinical practice, we assumed a complication rate of approximately 25% in the placebo group. To detect a significant difference (at the 5% level) to be 80% confident of detecting a difference in proportion of complications of 25% in the control group versus 5% in the study group, 58 patients were needed in each group. Ten more infants were added to compensate for possible dropouts. P values <0.05 (two-tailed) were considered significant.

II.5.4 Results

One hundred and forty infants entered the study, 122 of whom completed the study as designed. The first dose was administered within 1 hour after birth in all 140 infants. Eighteen infants were excluded since they failed to receive three or more doses of the study drug or placebo. Sixteen infants were discharged before all suppositories were given, and in two other infants the study was stopped on parental request. Sixty-one infants received paracetamol, 61 other infants entered the placebo group. The clinical data are presented in table II. There were no abnormalities in heart rate, passage of meconium, and voiding. No significant differences in group size, birth weight, gestational age, 5 minute Apgar scores or sex were observed. Indications for vacuum extraction were similar for the paracetamol and the placebo group. In both groups 2 infants were delivered by means of a soft cup. Labor related parameters were not different between these groups.

The reference group consisted of 66 infants delivered in vertex position without vacuum extraction after a normal pregnancy. The clinical data of these infants are also shown in table II. There were no significant differences with either vacuum extraction group.

	Paracetamol group (n=61)	Placebo group (n=61)	Reference group (n=66)
Sex			
Male	30	29	32
Female	31	32	34
Birth weight (g)	3575 ± 464	3556 ± 440	3486 ± 495
Gestational age (wks)	40.28 ± 1.20	40.16 ± 1.23	39.5 ± 1.5
Admission to neonatal ward	2	1	-

Table II Clinical data of study patients. Data are given in numbers or as mean ± SD. There were no significant differences between groups (Student's t-tests)

Pain assessment was performed in 66 infants in the reference group, in 59 infants in the paracetamol group and in 60 in the placebo group. In the reference group a facies score >1 was never observed. There were no significant differences in facies score between study and placebo group at any of the assessment times T=1, 7, 13 or 19 hours; scores in most infants were 0, 1, or 2; 40 in the paracetamol group and 43 in the placebo group. In 19 infants in the paracetamol group and in 17 infants in the placebo group one or two of the scores were 3 or 4 at any of the time points. There was no relation between facies score and analgesic effect.

There was, however, a significant difference in clinical condition between the paracetamol group and the placebo group as scored by subjective perception of nurses at T=1. In the paracetamol group the clinical condition improved in 33 infants, did not change in 5, and was worse in 21 as compared to that before administration. In the placebo group the scores were 21, 2, and 37, respectively ($P<0.01$).

In contrast, no difference in clinical condition between the paracetamol group and the placebo group at 7, 13 and 19 hours was observed. For individual patients the outcome at T=1 or any of the other time points was not predictive for the clinical condition at a later time point. Clinical condition was stable in all infants in the reference group.

Of the clinical symptoms, abdominal distension was scored only once in the placebo group. Excessive crying was noted in one case in the paracetamol group, and in 3 cases in the placebo group. Data for the other clinical symptoms are presented in Table III. There were no significant differences between the paracetamol and placebo group for any of the symptoms.

Multivariate analysis with one or more symptoms did not show any differences between the paracetamol and the placebo groups.

When compared with the infants in the reference group, however, there were significant differences between the numbers of episodes of vomiting and poor feeding in both the paracetamol and placebo groups (Table III). The same holds true for the number of infants that grunted or showed irritability or pain on handling (Table III).

	Paracetamol (n=61)	Placebo (n=61)	Reference group (n=66)
No. of times found			
- Bringing up	0.67 ± 1.00	0.90 ± 1.17	0.79 ± 1.12
- Vomiting	0.48 ± 1.27	0.29 ± 0.59	0.12 ± 0.45#
- Poor feeding	1.00 ± 1.61	0.86 ± 1.51	0.71 ± 1.05#
No. of patients			
- Grunting	9	8	2*
- Irritability	8	10	0#
- Pain on handling	8	11	0#

Table III Clinical symptoms
Number of times clinical symptoms were found within 24 hours of birth (mean±SD) and number of patients with clinical symptoms within 24 hours of birth.
* *P< 0.05, reference group vs paracetamol and placebo,* # *P< 0.01, reference group vs paracetamol and placebo (Mann-Whitney U test).*

II.5.5 Comment

We found that the administration of paracetamol directly after birth to infants born by vacuum extraction did not relief pain as assessed by the validated modified facies score.[7] The administration of one dose of 20 mg/kg paracetamol rectally directly after birth significantly improved the clinical condition as perceived by nurses but further doses did not make any difference.
Symptoms such as vomiting, poor feeding, grunting, irritability, and pain on handling were found significantly more after vacuum extraction than after vertex delivery without vacuum extraction, but did not diminish after administering paracetamol.

Although major sequelae, such as scalp injuries, cephalhematomas, intracranial hemorrhage and fractures have been reported in the literature,[1-3] minor complications or symptoms are not mentioned in pediatric, neonatal, or in obstetrics textbooks. Pain in relation to vacuum extraction has not been mentioned before in the literature (Medline search) and guidelines for preemptive analgesia directly after a traumatic delivery or after vacuum extraction in particular are not given either. In a recent study, Shah et al[11] assessed the efficacy of paracetamol in 75 term neonates undergoing heel prick. Facial action scores did not differ between groups, indicating that paracetamol fails to alleviate the acute sharp pain triggered by heel prick in term neonates.
In the present study we did not find differences in facies pain score between the paracetamol group and the placebo group. The results of our validation and the high interobserver rate (Cohen's κ=0.74) prove that the initial pain score was reliable.[9]

Labour related parameters did not differ between the paracetamol group and the placebo group. For 2 infants in the paracetamol group and one infant in the placebo group, who had been admitted to the neonatal ward because they 'were not doing well' after vacuum extraction, the vacuum extraction seemed to be the only cause for the symptoms.

The facies score performed one hour after administration of paracetamol might have been too early to show pain effects. Previously we assumed that in term infants the maximal serum concentration is reached between 30 and 60 minutes, as shown by Hopkins et al[8], but recently we showed that gestational age must be taken into account: in preterm infants the maximal serum concentration is not reached until after 3.9 hours.[9]

In a recent other study we found that the maximum serum concentration in term infants of 3.36 ± 2.11 mg/l is reached between 30 and 180 minutes after a rectal dose of 20 mg/kg.[12]

In spite of the fact that appropriate analgesia has been reported,[12] and Anderson et al [13] recently suggested that neonates may have altered pharmacodynamics compared with older children, the given rectal dose of paracetamol might have been too small to show differences between the paracetamol and the placebo group. Higher starting doses of 25-40 mg/kg in older infants and children,[13-15] and one of 30 mg/kg in term infants on the first day of life have now been proposed.[12]

The above-mentioned symptoms might be expressions of pain caused by the vacuum extraction, considering they were fewer in the reference group, and no other causative factors have been identified so far.

Even though the facies pain scores did not significantly differ between the paracetamol group and the placebo group, the nurses' perception of pain (in terms of better, unchanged or worse clinical condition), suggested that infants in the paracetamol group had less pain than those from the placebo group after the first, but not after subsequent doses.

Although nurses' perceptions of pain are often based on subjective "feeling", signs and symptoms,[10,16,17] neonatal nurses have shown strong agreement (average 86%) on the indicators of neonatal pain.[10]

In a study on assessment of acute pediatric pain in older children, children's self-report and nurse ratings were significantly correlated, implicating that where observational coding is not possible, nurse ratings of acute pain may most closely approximate objective assessment of pain and distress behaviors.[18]

The difference after the first dose might have influenced our overall impression in scoring after the subsequent doses. The fact that no improvements were observed after the second and following doses might be due to rapid improvement of the clinical condition after a vacuum extraction during the first hours after birth, or to the fact that the acute pain was not present anymore and our scoring method was not appropriate for ongoing pain.

Although the endorphin system is completely functional in term infants,[19] we believe that the influence of high, endogenously produced, ß-endorphin levels in infants in umbilical blood,[20] is of little importance, as values in infants born after vacuum extraction and those born after delivery in vertex position did not differ significantly,[20] and because ß-endorphin has a short half life.[21]

We conclude that clinical symptoms occurred significantly more in babies delivered with vacuum extraction. Judging from the modified facies scale to assess neonatal pain, paracetamol did not significantly contribute in lowering the score in babies delivered by vacuum extraction
Judging from the subjective nurses' perception score of the clinical condition, the clinical condition improved following one dose of paracetamol.

Finally, we doubt that subjective feelings of nurses and the facies scale are suitable to assess pain in infants born after vacuum extraction, and suggest that multidimensional pain scores, which have recently become available, might be more appropriate.[22-24]
As the clinical condition of most infants was shown to improve after one dose of paracetamol, it seems justified to await the clinical course in individual patients, and to apply a multidimensional pain score to assess the possible need of more medication. In case overt pain is present following delivery a higher starting dose of 30 mg/kg might be more effective.

II.5.6 Acknowledgements

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III

Pharmacokinetics and Pharmacodynamics of Morphine in Ventilated Preterm Infants

"Then I prepared a draft of Red Shepenn for him and made it as strong as I dared, for I knew that pain could undo all my best efforts and destroy my patient as swiftly as a slip of my scalpel."(Wilbur Smith, River God, 1993)

III.1 Pain Assessment and Morphine Pharmacokinetics in Preterm Infants: The Usefulness of the Fluorescence Polarization Immuno Assay.

Based on the article:

Pain Assessment and Morphine Pharmacokinetics in Preterm Infants: The Usefulness of the Fluorescence Polarization Immuno Assay.
RA van Lingen, B Greijdanus, A Okken, DRA Uges.
Submitted.

III.1.1 Abstract:

Objective: To determine the concentration-effect relationship of continuous morphine in preterm infants in the first days after birth using a validated pain score, both at rest and during endotracheal suctioning, and to investigate whether a rapid method for the determination of morphine serum concentration, a modified fluorescence polarization immuno assay can be useful to adjust morphine dose.
Methods: In a prospective clinical trial, preterm ventilated neonates in three different age groups, who were subject to several painful procedures, received continuous morphine (10 µg/kg/h). Morphine serum concentrations were determined using a rapid Fluorescence Polarization Immuno Assay, and pharmacokinetic analysis was performed.
Pain scores before and after morphine, and during endotracheal suctioning, were assessed by a validated pain score.
Results: Of 24 infants included in the trial, 4 were aged <28 weeks, 14 between 28 and 32 weeks, and 6 >32 weeks. Morphine could be detected in 75 to 84 % of the samples during the first 10 days of life. Differences between the groups in mean morphine concentration were not significant, although there was a trend for lower concentrations with older age in the first 3 days. Mean morphine concentrations for all infants increased from 85.1 ± 33.4 on day 2 to 103.3 ± 70.8 µg/l on day 5. Except on day 2 morphine concentration was not correlated to gestational age. Mean estimated clearance was 2.3 ± 1.1 ml/min/kg for the total group, and increased with gestational age. The difference between the groups was not significant.
Pain scores were significantly higher before than after morphine (P <0.001) and also during than either before or after endotracheal suction (P <0.001), but were not related to morphine concentrations.
Conclusion: Morphine concentrations can be determined by this rapid method, but it can not be used for individual dosage adjustment. With the dose used in this study appropriate analgesia as assessed by a pain score was achieved during ventilation, but no concentration-effect relationship was found.

III.1.2 Introduction

Morphine is the most widely used analgesic in neonates.[1,2] It is used to alleviate and prevent pain and stress in case of invasive procedures in the neonatal intensive care unit (NICU), during and after interventions, and during assisted ventilation. During neonatal intensive care especially preterm infants are subject to many painful events, of which heelprick and endotracheal suctioning are the most common.[3] Furthermore, morphine is reported to increase synchronous ventilation,[4] and may reduce the incidence of poor neurologic outcome in ventilated preterm infants.[5]
An intravenous loading dose of 50 to 100 µg/kg followed by continuous infusion of 10 to 25 µg/kg/h is reported to provide good sedation and analgesia in term and preterm infants.[4,6,7-9] To prevent adverse effects it is recommended not to give doses in excess of 15 µg/kg/h.[6] However, a more recent study reported the administration of an intravenous loading dose of 50 µg/kg, followed by continuously given doses of 10-20 µg/kg/hour to neonates without any observed adverse effects.[10] The dose was even increased in case of inadequate clinical effect or when tolerance developed.[10] Several

authors do not report side effects with serum concentrations varying from 10 to 210 µg/l, at doses between 5 and 50 µg/kg/h.[11-14] Adequate sedation was reported in 50% of patients with a morphine serum concentration >125 µg/l.[14] In adults, morphine was consistently effective at plasma concentrations of 40 µg/l or greater.[15]

Morphine serum or plasma concentrations can be determined by various methods.[7,16] Concentrations vary widely and determination often takes days. Several groups investigated the pharmacokinetic effects of a single dose of morphine,[17-19] or of various continuous doses versus concentration or effect.[6,20] However, in contrast with studies in adults, the pharmacokinetics and pharmacodynamics in the newborn period were not studied.[15]
Pharmacokinetic-pharmacodynamic relationships of analgesia in preterm and full term infants have been examined only in studies using fentanyl,[21-23] and in one study using morphine as analgesic drug, and applying the Neonatal Facial Coding System as pain assessment instrument.[9] The effect of morphine is generally only measured by means of a sedation score.[14] This may be partly due to the absence of adequate methods to determine morphine concentrations in blood, and partly to the lack of validated pain scores for bedside use in this age group. Several validated pain measure instruments have been developped in the past ten years.[24]

If a correlation could be found between pain score and morphine concentration, the clinical benefit of a method to detect concentrations within hours would be obvious.
Thus the objectives of this study were twofold: 1. To determine a concentration-effect relationship in the first days after birth using the Neonatal Infant Pain Score (NIPS),[25] as a validated pain score for this age group, both at rest and during a painful procedure, i.e. endotracheal suctioning (ET), and 2. To investigate whether a rapid method for the determination of morphine serum concentration, a modified fluorescence polarization immuno assay (FPIA),[26] can be used to adjust morphine dose in preterm infants.

III.1.3 Methods

Patients
The study included 28 inborn preterm neonates who were admitted to the neonatal intensive care unit in the period from January, 1996 through April 1997.
Entry criteria were the need for artificial ventilation (IPPV), need for sedation or analgesia as assessed by a validated pain score, the NIPS, and postnatal age <48 hours.
The study protocol was approved by the ethical review committee of the Isala Clinics (Zwolle, The Netherlands). Written informed parental consent was obtained for each patient before enrolment.
The gestational age of the neonates was estimated from maternal menstrual history, by routine ultrasound examination during pregnancy, and by postnatal physical and neuromuscular characteristics (New Ballard score).[27] Patients were excluded if they had major congenital anomalies, severe asphyxia (APGAR score ≤3 after 5 minutes), if they needed muscle paralysis for adequate ventilation, if their mother had received indomethacin or had been given analgesics (other than local or epidural) within 24 hours before delivery.
All infants recieved appropriate fluid therapy and adequate nutrition.

According to standard procedures in the NICU, central venous catheters and umbilical or radial arterial catheters were inserted.

Infants were stratified into three different age groups: <28 weeks (group A), 28-31 weeks (group B), and 32-37 weeks (group C).
Morphine was administered as an intravenous loading dose (100 µg/kg) in 5 minutes on day 1, followed by 10 µg/kg of morphine continuously.[10] Extra morphine was given as the attending physician deemed it necessary. Total days on morphine therapy and time and amount of additional morphine were noted.

As the mean duration of artificial ventilation in our unit (level III NICU, no surgical patients) in general was between 7 and 8 days, we decided to study the pharmacokinetic and dynamic properties of morphine during a period of 10 days, with regular intervals during the time morphine was needed.
According to the study protocol morphine infusion was stopped by the attending neonatologist under the following circumstances: 1. After successful extubation, 2. If ventilator settings allowed extubation within several hours, 3. If the patient was judged to be pain free. If morphine had been given for more than 5 days, morphine administration was decreased by 50 % one day before discontinuation of the infusion, according to standard procedures to prevent possible symptoms of opioid withdrawal.[28]
Blood samples were taken at predetermined time points on days 2, 3, 5, 7, and 9 together with sampling for routine laboratory investigation, and at 12 and 24 hours after ending administration of the drug. After blood collection, serum was separated and frozen at -20^{0}C, until assayed.

Clinical observations included continuous monitoring of heart rate, respiratory rate, arterial blood pressure, oxygen saturation, and rectal temperature. Cranial ultrasound was performed routinely on days 1, 2, 3 and 5, and at the expected date of delivery.

Analytical procedures
For this study the assay was performed within a month of sample collection. A rapid (within one hour) and reproducible method was used for the quantification of morphine with the AxSym^R fluorescence polarization immuno assay (FPIA) analyser (Abbott Diagnostics Division, Amstelveen, The Netherlands) requiring 100 µl of serum.
In a polypropylene tube, 100 µl of serum was added to 50 µl solubilization reagent (Abbott 9797-30), and 150 µl whole blood precipitation reagent (Abbott 9797-33), mixed and centrifuged. The supernatant of the serum was transferred in a sample cup and analysed with the FPIA reagent for morphine in urine (Abbott 9673-60), conform the guidelines of the supplier, but using serum instead of urine.
Limit of quantification was 25 µg/l. Inter-day variation was 4.8 % (C = 103 µg/l; n = 100).
Cross reactivity for morphine-3-glucuronide (as given by the supplier) is 31-58 % depending on the concentration.

Pharmacokinetics

Pharmacokinetic data and variables were calculated from standard equations using the KINFIT program of MW/Pharm (Mediware, Groningen, The Netherlands).[29] An estimate of the individual serum clearance was obtained by dividing the morphine infusion rate by the apparent steady state mean serum concentration of morphine at day 2.[8]

Pain Assessment

Pain was assessed by means of the NIPS by experienced nurses, before and after starting morphine, twice during every 8 hour shift, and once daily before, during and 2 minutes after ET. Nurses were trained by means of a videotape to use the NIPS, until a good interrater reliability was reached (Cohen's kappa >0.70). A score of 0-2 was considered as good analgesia, 3-4 as moderate pain, and 5-7 as severe pain.
Inadequate analgesia (NIPS >3 at two consecutive controls or ≥5 once)[30] was managed by the attending physician by administering an extra bolus of morphine (50 µg/kg), or if this failed to improve analgesia, by increasing the morphine infusion rate by 5 µg/kg/h.
Severity of illness was assessed by means of the clinical risk index for babies (CRIB)[31,32] which includes birthweight, gestation, and clinical data up to 12 hours from birth, and predicts mortality and morbidity, and might predict the long-term clinical outcome.[33,34]

Statistical analysis

Data were analyzed with SPSS-8 (SPSS Inc, Chicago, Ill). Student's *t* tests were used for normally distributed data, Wilcoxon's Signed Rank tests for paired data, and Friedman's test or Spearman's rank correlation for nonparametric data. Mann-Whitney *U* tests were used for nonparametric data to compare the three groups. P values ≤0.05 (two-tailed) were considered to be significant, except for the pain scores, for which P values ≤0.01 (two-tailed) were considered to be significant.

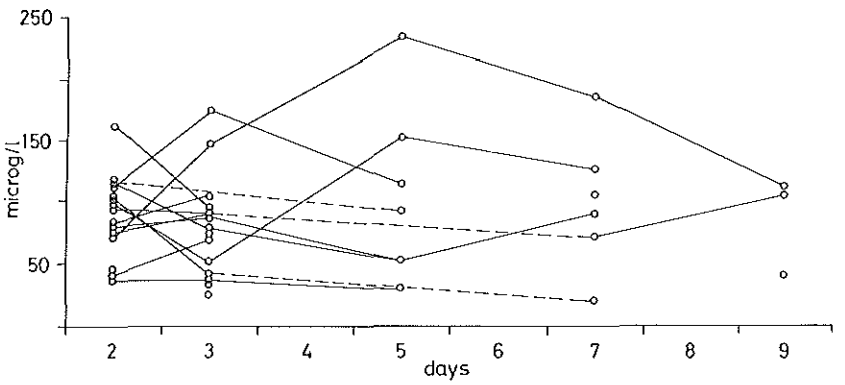

Figure 1. Morphine concentrations (µg/l) in individual patients (n=24) during the first 9 days of life.

III.1.4 Results

Parental consent was denied for 3 (all group A) of the 28 infants eligible for the study. Samples from one infant were lost. Clinical data of the remaining 24 infants are shown in table 1.

Morphine was detected in 16/21 (76 %) samples on day 2, 16/19 (84 %) on day 3, 7/9 (78 %) on day 5, 6/8 (75 %) on day 7, and 3/4 (75 %) on day 9. Twelve hours after discontinuation morphine was detected in 4/15 samples (range 38-57 µg/l), and in 5/16 samples (range 26-59 µg/l) after 24 hours. All values were considerably lower than the preceding values during morphine administration.

Morphine concentrations for individual patients are shown in figure 1.

Mean morphine concentration for the three groups could be determined on days 2 and 3 only, in groups A and B on day 5 as well (Table 2). There were no significant differences between groups.

In group B the mean morphine serum concentration on day 7 was 104.6 ± 60.0 µg/l (n = 5). In groups A and C insufficient data (0-1) were available after days 5 and 3 respectively, as morphine administration was stopped on clinical indication.

Mean morphine concentration did not differ significantly between groups on any day.

A significant negative correlation between gestational age and mean morphine serum concentration was observed on day 2 (Spearman Rank r=0.50, P <0.05); on all other days no correlation was found.

Estimated mean clearance (± SD) on day 2 was 2.3 ± 1.1 ml/min/kg (n=16) for the total group, and increased with gestational age, but the differences between the groups were not significant (Table 2).

NIPS score and morphine concentration did not correlate at any time point. Pain scores before and after morphine administration are depicted in figure 2A.; they significantly decreased after morphine administration (P <0.001).

Three infants needed an extra single morphine dose (50 µg/kg) after 3 days (one infant in group A, 2 in group B).

Except for day 9, pain scores during ET were significantly higher than before or after ET (P < 0.001 on day 2, P = 0.001 on days 3 and 5, P <0.05 on day 7). Pain scores (n = 19) during ET on day 2 are depicted in figure 2 B.

Pain scores before and after ET did not differ significantly, and almost always ranged between 0 and 2. In only 3 of 51 pain scores (5.9 %) before ET, and 4 of 51 pain scores (7.8 %) after ET a value of 3-4 was scored. Three of those 4 high pain scores after ET were in the same infants that had a high score before ET.

Severity of illness did not differ between the groups (Table 1). Two infants (one group A, one group B) died during the study period, for reasons not related to the study (both severe IRDS and persistent hypoxia). One other infant (group B) died at the age of 4 months due to septicaemia and paralytic ileus, caused by streptococcus A.

There were no significant differences in heart rate or mean arterial blood pressure between the groups after starting the morphine. In 14 infants hypovolemia and hypotension was already treated with volume expansion before morphine treatment was started, and in 7 infants (Table 1) inotropic support was given either before or after morphine administration.

Urinary retention as objectivated by ultrasound was found in 1 infant in group B.

Group	A (n = 4)	B (n = 14)	C (n = 6)	Total (n =24)
Birthweight (g)*	865 ± 91	1200 ± 385	2044 ± 316	1355 ± 535
Gestational age (weeks)*	26.5 ± 0.7	29.2 ± 0.9	33.8 ± 1.0	29.9 ±2.6
Sex				
Male	2	11	5	18
Female	2	3	1	6
Diagnosis at admittance				
RDS	4	12	2	18
Pneumonia/sepsis	-	1	2	3
Asphyxia	-	-	1	1
Pneumothorax	-	-	1	1
Hypoplastic lungs	-	1	-	1
Surfactant	3	11	2	16
Inotropic agents	2	4	1	7
PIVH I-II	-	1	-	1
PIVH III-IV/PVL	-	2	-	2
Died ≤ 28 days	1	1	-	2
Died >28 days	-	1	-	1
Artificial ventilation (days)*	22.0 ± 18.9	11.1 ± 7.7	7.0 ± 6.1	11.9 ± 10.6
Morphine (days)*	9.5 ± 6.5	6.2 ± 2.5	3.7 ± 2.2	6.1 ± 3.7
CRIB score*	6.0 ± 4.3	4.2 ± 2.5	2.7 ± 1.6	4.1 ± 2.8

*Table 1. Clinical data of study participants. Data given are number of infants, or * mean ± SD. No significant differences between groups except for gestational age group A vs B and C (P< 0.001), and B vs C (P<0.001), and birthweight A vs B(P<0.01) and C (P<0.001).*

	Cmax day 2	Cmax day 3	Cmax day 5	Clearance
Group A	116.0 ± 43.6 (n=3)	73.7 ± 31.8 (n=3)	60.5 ± 43.1 (n=2)	1.6 ± 0.7
Group B	87.7 ± 23.0 (n=9)	87.9 ± 44.6 (n=10)	120.4 ± 76.0 (n=5)	2.1 ± 0.8
Group C	56.3 ± 28.8 (n=4)	43.3 ± 23.0 (n=3)	-	3.4 ± 1.2
Total	85.1 ± 33.4 (n=16)	76.9 ± 41.3 (n=16)	103.3 ± 70.8 (n=7)	2.3 ± 1.1

Table 2. Mean morphine serum concentration (Cmax) ± SD (µg/l) and clearance (ml/kg/min) for all patients and for different age groups. All differences between groups not significant.

Figure 2A

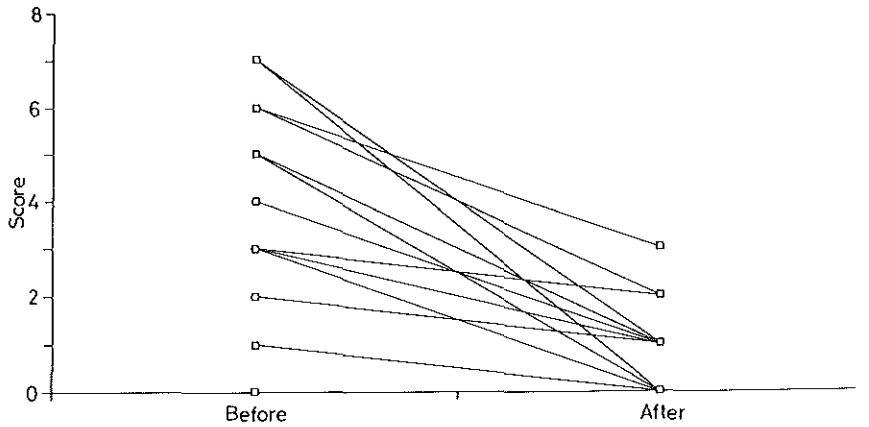

Figure 2B

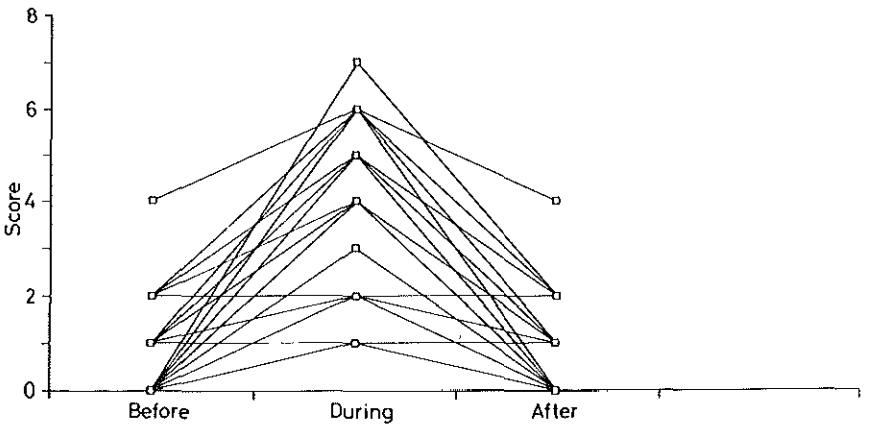

Figure 2. (A) NIPS scores before and after start of morphine administration (n=24) (P <0.001), and (B) before, during and after endotracheal suction on day 2 (n=19) (P <0.001 during vs before, and during vs after).

III.1.5 Discussion

The importance of analgesia in newborn infants in the NICU for their well-being, and improvement of short-term outcome is now widely known.[4,5,35] In this context, it is important to find dose and administration schedules which are effective and safe. In this study we investigated the pharmacokinetics and pharmacodynamics of ventilated newborn infants during the first week of life, in order to be able to adjust morphine

dosage following determination of the serum concentration and simultaneous pain assessment.
We have demonstrated that morphine concentrations can be detected by the FPIA method. With a relative low dose of 10 µg/kg/h morphine, serum concentrations between 25 and 233 µg/l were detected in the majority of infants.
A significant negative correlation between morphine serum concentrations and gestational age was detected on day 2, but on all other days correlations were not found.
Validated pain assessment scores showed good analgesia during continuous morphine administration, although without a correlation between morphine serum concentration and pain scores.
Differences between age groups were not significant. However, they could be determined only for days 2 and 3 postnatally, because numbers of patients in groups A and C on the other days, were too low.

In this study morphine was administered during the first 10 days of life, and pharmacokinetics and pharmacodynamics during this period have been determined. There are no other reports of a similar study of this length, which might be due to the difficulty of obtaining data, as not many infants need analgesia for a longer period. In a recent study by Scott et al,[9] 48 out of 496 eligible infants were studied during 3 days. It is obvious that only the most critically ill patients were studied.
In 14 infants in our study, 2 or more serum concentrations could be detected, with values ranging between 25 and 233 µg/l. These are in line with data found after morphine[6,7,9,13,14] and diamorphine.[36]
Comparable mean morphine concentrations were found in two studies using a continuous dose of 15 µg/kg/h[7] and 20µ/kg/h,[13] but our values were lower than those found by Scott,[9] with higher infusion rates (20-30 µg/kg/h), or until adequate sedation was reached with infusion rates of 7.5 to 30 µg/kg/h.[14] In a study comparing a 12.5 µg/kg/h continuous dose with a 50 µg/kg/h continuous dose no clinical advantage in using the higher dose was reported.[8]

The mean estimated clearance in our study on day 2 is in range with the values as reported by others in preterm infants,[8,14,37] and is considerably lower than in older children. This explains the higher mean morphine concentrations in our study as compared to those in older children.[16,38]
In children mean serum concentrations of 16 and 18 µ/l were found at 24-40 hours after a continuous infusion of 20 µg/kg/h.[16]
After discontinuation of the morphine infusion, morphine could still be detected in serum of 27 % and 31 % of the neonates after 12 and 24 hours, respectively. Whether this is still active morphine or either one of its metabolites is not clear, and will be subject of further study.
The FPIA enables us to determine morphine concentrations in a relatively short time, and theoretically it can be used the very same day to evaluate our therapy. However, in clinical practice it offers no benefit in that regard, as no correlation was found between morphine concentration and pain assessment score.
We found appropriate analgesia with morphine concentrations that were lower than those proposed by Hartley et al,[39] who aimed at a minimum target plasma

concentration in the region of 100 µg/l. Even higher concentrations were reported by Chay et al,[14] using individualised dosing, who found that a mean plasma concentration of 125 µg/l provided a desired comfort score in 50 % of babies.
The morphine concentrations of >40 µg/l, reported to correlate with appropriate analgesia in adults using patient controlled analgesia, are more in line with our values.[15]
In two other studies pain was scored in relation to morphine administration. Scott et al,[9] using the neonatal facial coding system (NFCS), found that facial activity discloses morphine analgesia, but is unrelated to morphine concentration, as in our study. Furthermore, the NFCS needs video taping and is in that respect not suitable for bedside use. Saarenmaa et al[23] compared morphine and fentanyl, and found similar changes in pain score for morphine and fentanyl administration, which compares to our results. However, as values were not reported it can not be concluded whether there was good analgesia or not.[23] Using our pain score this would mean that a decrease of 3 (moderate pain) to 0 (no pain) is equal to a decrease of 7 (severe pain) to 4 (moderate pain), but still in pain.

Severity of illness as measured by the CRIB score was not related to the analgesic effect of morphine as measured by the NIPS, duration of morphine administration as expected, was longer in the youngest age group, but was not related to severity of illness either.

We conclude that morphine concentrations can be determined by the FPIA method, but it can not be used for individual tailoring of dosage. With the dose used in this study appropriate analgesia as assessed by the NIPS was achieved during ventilation, but no concentration-effect relationship was found.

III.1.6 Acknowledgements

We gratefully acknowledge the contribution by the nurses from intensive care to the collection of data, Willem Grol for his statistical advice, Kumar Jamdagni and Ko Hagoort for their help in preparing the manuscript and Adri van Zoeren for skilful drawing of the figures.

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III.2 Pharmacokinetics and Pharmacodynamics of Morphine and its Metabolites: The Effect of two Different Dose Regimens in Ventilated Preterm Infants

Based on the article:

Pharmacokinetics and pharmacodynamics of morphine and its metabolites: The effect of two different dose regimes in ventilated preterm infants.
RA van Lingen, B Greijdanus, W Grol, A Okken, DRA Uges, D Tibboel MD.
Submitted.

III.2.1 Abstract

Objective: To investigate pharmacokinetics and pharmacodynamics of morphine and its main metabolites, morphine-3-glucuronide and morphine-6-glucuronide, in preterm ventilated neonates in the first ten days of life in relation to gestational age.
Design: Randomised controlled trial.
Setting: Level III neonatal intensive care unit.
Methods: In this prospective clinical trial, two groups preterm ventilated neonates stratified for gestational age, who were subject to several painful procedures, received continuous morphine in two different dose regimens of 10 (group A) or 15 μg/kg/h (group B). Serum concentrations of morphine and its metabolites were determined serially with a HPLC method, and pharmacokinetic analysis was performed. Pain assessment was performed by a validated pain score.
Results: Twenty-eight infants were randomised to either group A or B. Morphine could be detected in 80-100%, and morphine-3-glucuronide in 89-100% of the infants. The percentage of infants in whom morphine-6-glucuronide could be detected increased significantly from 41% to 90% over the first 9 days of life.
Mean morphine concentrations for all infants increased from 58.1 ± 37.6 μg/l on day 2 to 63.2 ± 29.1 μg/l on day 3 and decreased thereafter to 44.3 ± 16.3 μg/l on day 9. Morphine-3-glucuronide values increased significantly from 75.4 ± 41.0 μg/l to 130.8 ± 66.7 μg/l on day 9, and morphine-6-glucuronide concentrations increased from 25.1 ± 10.0 to 38.4 ± 14.0 μg/l on day 9. On all postnatal days except day 7, there was no relation between gestational age and morphine or its metabolites. Twelve and 24 hours after discontinuation of the drug, morphine-3-glucuronide was still detected in 12/14 (85.7%) and 13/17 (76.5%) infants in lower concentrations, but in the majority no morphine or morphine-6-glucuronide was found. Mean metabolite:morphine serum concentration ratios increased significantly during the first 7 days, the mean morphine-3-glucuronide:morphine-6-glucuronide ratio did not increase significantly.
Mean estimated serum clearance was 3.64 ml/min/kg for all patients, without a significant correlation with gestational age. There was a significantly higher clearance in group B than in group A. No influence of either inotropic drugs or severity of illness on clearance or morphine concentrations was present. Pain scores were significantly higher before than after start of morphine administration. Appropriate analgesia was present before and after endotracheal suction, but pain scores were significantly higher during suctioning (indicating moderate pain or distress). Pain scores did not correlate with morphine, morphine-3-glucuronide, or morphine-6-glucuronide concentrations at any time.
Conclusion: While in preterm infants morphine and morphine-3-glucuronide can be found after intravenous administration on all days, morphine-6-glucuronide can not be found in all infants in the first days after birth, though increasingly with higher postnatal age.
No correlation between morphine concentrations and pain scores was found. Therefore we suggest an initial dose of 10 μg/kg/h morphine. If tolerance develops the morphine infusion should be increased by 5 μg/kg/h, preceded by 50% of the loading dose.

III.2.2 Introduction

For the last ten years morphine has increasingly been used as an analgesic in premature newborns. However, there are still insufficient data regarding its pharmacokinetic and pharmacodynamic properties in this age group.[1,2]
Because intubation and artificial ventilation are perceived to be sources of major discomfort and possible painful procedures, it is common practice to administer an analgesic drug like morphine during mechanical ventilation in adults and older children.[3] In the treatment of newborn infants one is reluctant to administer morphine, fearing adverse effects such as respiratory depression, constipation, tolerance, and withdrawal symptoms, and because of the difficulty of assessing pain in nonverbal patients.[4] Consequently there is a lack of data regarding the safety and effectiviness of morphine.[5]
In children and adults pain relief is considered the most important target in the administration of analgesia, and side effects are of secondary importance.[3] The same should apply to infants and newborns, and consequently efforts should be made to determine the dose-concentration-response relationship.
In the neonatal intensive care unit (NICU) babies are highly exposed to invasive procedures, such as heel prick and endotracheal suction, with 74% of the procedures performed on the 30% of infants born below 31 weeks gestation.[6]
Furthermore, pain sensitivity is higher in (premature) infants as a result of lower pain threshold,[7] repeated injuries,[8] and an initial lack of inhibitory processes.[9,10] In the most critically ill babies pain sensitivity might be higher due to allodynia.[11]
Pain and stress are suggested to play a role in poor neurological outcome in preterm infants when no analgesia is provided,[12] possibly due to fluctuations in arterial blood pressure.[13]
Finally, pain experienced during the neonatal period leads to different pain responses to subsequent painful events.[14,15]

The effects of morphine in preterm infants have been evaluated in several double blind randomised controlled trials,[12,16-19] pharmacokinetic studies,[20-26] and pharmacodynamic studies.[22,24,26]
Most of these studies have evaluated single dose effects,[20,21,25] short term (<72 h) effects of continuous morphine,[23,24,26] or different doses.[22] However, there is a lack of data for prolonged use of morphine. Moreover, a dose-concentration- response effect has been described considering only total morphine concentrations, with concentrations over a wide range suggested as being effective for analgesia.[22,27,28] A threshold value for the concentration necessary to obtain analgesia has not been set.
The morphine metabolite morphine-6-glucuronide (M6G) is considered to be responsible for most of the therapeutic benefit of morphine,[29] and the pharmacokinetics of morphine and its metabolites change rapidly in the first days of life. Consequently studies should include information about these metabolites morphine-3-glucuronide (M3G) and M6G as well. For this reason pharmacodynamic studies taking into account both M6G and M3G might give a better understanding of the mechanisms of pain relief.[30]

As severity of illness might influence the effect of morphine, and inotropic support has been reported to decrease the clearance of morphine,[31] these factors need to be considered.
Therefore, we conducted a study with the following objectives: 1. To determine pharmacokinetics and pharmacodynamics of morphine and its main metabolites M3G and M6G, following two different dose regimens in ventilated premature newborn infants in the first ten days of life; 2. To investigate their effects in relation to gestational age; and 3. To evaluate the effects of the use of inotropic agents, and severity of illness as evaluated by the Clinical Risk Index for Babies (CRIB).[32]

III.2.3 Methods

Patients
The study included 30 inborn preterm neonates admitted to the Isala Clinics neonatal intensive care unit in the period of May, 1997 through September, 1998. Entry criteria were the need for artificial ventilation (IPPV), need for sedation or analgesia as assessed by an objective pain score, the Neonatal Infant Pain Scale (NIPS),[33,34] and postnatal age <48 hours. The study protocol was approved by the ethical review committee of the Isala Clinics (Zwolle, The Netherlands). Written informed parental consent was obtained for each patient before enrolment.
The gestational age of the neonates was estimated from maternal menstrual history, by routine ultrasound examination during pregnancy, and by postnatal physical and neuromuscular characteristics (New Ballard score).[35] Patients were excluded if they had major congenital anomalies, severe asphyxia (Apgar score ≤3 after 5 minutes), if they needed muscle paralysis for adequate ventilation, if their mother had received indomethacin (INN, indometacin), or if she had been given analgesics (other than local or epidural) within 24 hours before delivery.

The infants were studied during the first 10 days with regular intervals during the time morphine was needed, and 12 and 24 hours after discontinuation of the drug. According to standard procedures in the NICU, central venous catheters and umbilical or radial arterial catheters were inserted, and parenteral nutrition was started within 24 hours of birth.
After a loading dose of 100 μg/kg morphine intravenously, patients were randomised to a group receiving 10 μg/kg/h (group A) or 15 μg/kg/h (group B). As pharmacokinetics and drug metabolism change during the last three months of gestation,[36,37] and pain sensitivity may be altered after 32 weeks,[38] the neonates were stratified into three gestational age groups: under 28 weeks, 28-31 weeks, and 32-36 weeks.

According to the study protocol morphine infusion, blinded for the dosage given, was stopped by the attending neonatologists, under the following circumstances:
1. after successful extubation;
2. if ventilator settings allowed extubation within several hours,
3. if they judged the patient to be pain free.

According to standard procedures, if morphine had been given for more than 5 days, morphine administration was decreased by 50% one day before discontinuation of the infusion, to prevent possible symptoms of opioid withdrawal.
Blood samples (0.4 ml) were taken from the indwelling arterial catheter at predetermined time points on days 2, 3, 5, 7, 9, and at 12 and 24 hours after ending the study medication. Total days on morphine therapy, possible additional morphine, total dosage, and total time of morphine administration were noted as well. After collection serum was separated and frozen at -20^{0}C, until assayed.

Inotropic drugs were started if the mean arterial blood pressure repeatedly (more than 2 times) fell under the standard value for gestational age and did not react to volume expansion by cristalloid solutions or saline, according to hospital routine.
Severity of illness was measured by CRIB,[32] which includes birthweight, gestation, and clinical data up to 12 hours from birth, and predicts mortality and morbidity, and might predict the long term clinical outcome.[40]
Clinical observations included continuous monitoring of heart rate, arterial blood pressure, and oxygen saturation of each infant. Cranial ultrasound was performed routinely on days 1, 2, 3 and 5, at the expected date of delivery, or more often whenever required by the clinical condition.

Analytical procedures
In a polypropylene tube 0.2 ml of serum and 0.4 ml of 0.01 M ammonium hydrogen carbonate (pH 9.3) were mixed and centrifuged. The supernatant was applied on an equilibrated C8 Bound-Elute (Merck, Darmstad, Germany) solid phase extraction column. After 5 minutes the column was washed with 0.01 M ammonium hydrogen carbonate, dried under vacuum, washed again with hexane and dried again. Morphine and its glucuronides were eluted with 0.5 ml of 0.05 M acetic acid in methanol/water (9/1 v/v) under vacuum for 20 s. The eluate was evaporated to dryness under nitrogen at room temperature. The residue was reconstituted in 150 µl of 0.05% (w/v) phosphoric acid. An aliquot of 75 µl was injected onto a HPLC column (Merck Lichrocart 250-4 fitted with a Merck Lichrocart 4-4 precolumn, both Lichrospher 60, RP select B 5µm). These analytical columns were eluted after 20 min. isocratic and then with a linear increasing gradient of 0 to 60% acetonitrile in 0.2 M potassium dihydrogen phosphate buffer (pH 3.0) in 8 min with a flow of 1.2 ml/min. Detection was with an extremely sensitive spectrofluorimetric detector L-7480 (Hitachi/Merck) with an 8µl flowcell, at excitation 210 nm, and emission 350 nm; response time 4 s. The limit of detection (signal to noise ratio >3) was for morphine, M3G, and M6G 8, 11, and 19 µg/l serum respectively and at 100 µg/l (n=5) the inter-day CV's were 1.1, 6.3, and 2.5%, and the bias -5.23, -1.4, and -0.019% respectively. The extracts were stable in the autosampler at 4°C during at least 22 hrs.

Pharmacokinetics
Pharmacokinetic data and variables were calculated from standard equations using the KINFIT program (Mediware, Groningen, The Netherlands). An estimate of the individual serum clearance was obtained by dividing the morphine infusion rate by the apparent steady state serum concentration of morphine at day 2.[23]

Pain assessment

Pain assessment was performed using a validated pain assessment instrument for this age group, the NIPS.[33] The NIPS was done before and after starting morphine, daily at predetermined intervals, being at the start and end of the nurses' shifts, and before, during and after endotracheal suctioning. A score of 0-2 was considered as good analgesia, 3-4 as moderate pain, and 5-7 as severe pain.

Inadequate analgesia (NIPS >3 at two consecutive assessments or >5 once)[34] was managed by the attending physician by administering an extra bolus of morphine (50μg/kg), or if this failed to improve analgesia, by increasing the morphine infusion rate by 5 μg/kg/h.

Statistical analysis

Data were analyzed with SPSS-8 (SPSS Inc, Chicago, Ill). To compare the two groups Student's *t* tests were used for normally distributed data, Wilcoxon Signed Rank tests for paired data, and Friedman test or Spearman's rank correlation for nonparametric data. P values ≤0.05 (two-tailed) were considered to be significant, except for the pain scores where P values ≤0.01 (two-tailed) were considered to be significant.

	Group A (n= 14)	Group B (n= 14)
Birthweight (g)*	1156 ± 387	1350 ± 783
Gestational age (wks)*	28.25 ± 1.7	29.46 ± 2.2
Sex		
Male	8	9
Female	6	5
Diagnosis at admittance		
RDS	13	13
Pneumonia	1	1
Surfactant	11	11
Inotropic agents	8	7
PIVH I-II	6	3
PIVH III-IV/PVL	4	2
Died ≤ 28 days	1	3
Died > 28 days	0	1
Artificial ventilation (days)*	14.9 ± 13.9	15.4 ± 10.5
Morphine (days)*	8.5 ± 6.5	8.1 ± 4.9
CRIB score*	4.7 ± 3.4	5.6 ± 3.0

*Table 1 Clinical data of study participants. Data given are number of infants, or * mean ± SD. Differences between groups for all data given are not significant.*

III.2.4 Results

Thirty-five infants were eligible for the study, but in five cases parental consent was denied. Thirty infants were included in the study; serum samples from one infant were lost and one infant was withdrawn from the study after initial parental consent. None of the infants died during the study period. Clinical data of the remaining 28 patients are shown in table 1 and revealed no significant differences for all the items evaluated. For all infants mean birth weight (± SD) was 1253 ± 614 g, and mean gestational age 28.85 ± 2.0 weeks.

Duration of morphine administration varied from 24 hours to 23 days (median 7 days). Blood samples were obtained in 27 infants on day 2 (1 missing sample), 22 infants on day 3 (2 missing samples, 4 morphine stopped), 19 infants on day 5 (3 missing samples, 6 morphine stopped), 17 on day 7 (1 missing sample, 10 morphine stopped), and 10 infants on day 9 (2 missing samples, 16 stopped). Morphine could be detected in 80-100%, and M3G in 89-100% of the infants (Figure 1). The percentage of infants in whom M6G could be detected increased significantly from 41% on day 2 to 90% on day 9 (P <0.01)(Figure 1).

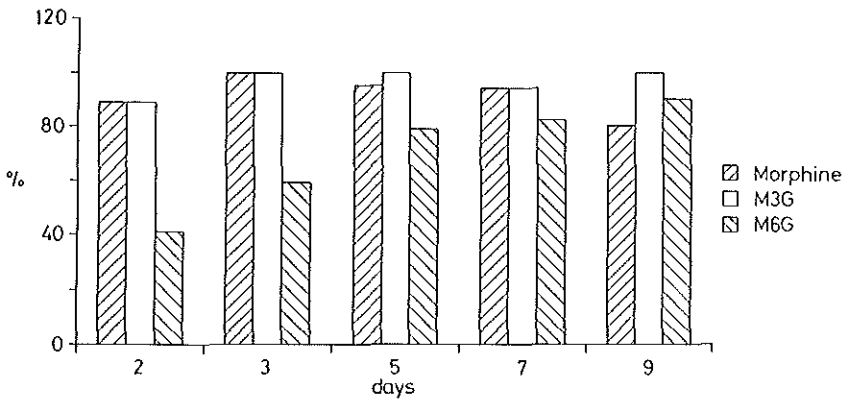

Figure 1 Percentage of infants in whom morphine and its metabolites could be detected during the study. M3G morphine-3-glucuronide, M6G morphine-6-glucuronide.

Mean serum concentrations of morphine, M3G, and M6G for the total group are shown in figure 2.
Mean morphine concentrations increased from 58.1 ± 37.6 µg/l on day 2 to 63.2 ± 29.1 µg/l on day 3 and decreased thereafter to 44.3 ±16.3 µg/l on day 9.
M3G values increased significantly from 75.4 ± 41.0 µg/l to 130.8 ± 66.7 µg/l on day 9 (P =0.016), and M6G concentrations increased from 25.1 ± 10.0 µg/l to 43.0 ± 11.7 µg/l, and 38.4 ± 14.0 µg/l on days 7 and 9, respectively (Figure 3A, B, C).
Differences between the groups A and B in mean serum concentrations for morphine, M3G, and M6G were not significant (Figure 3 A,B,C).

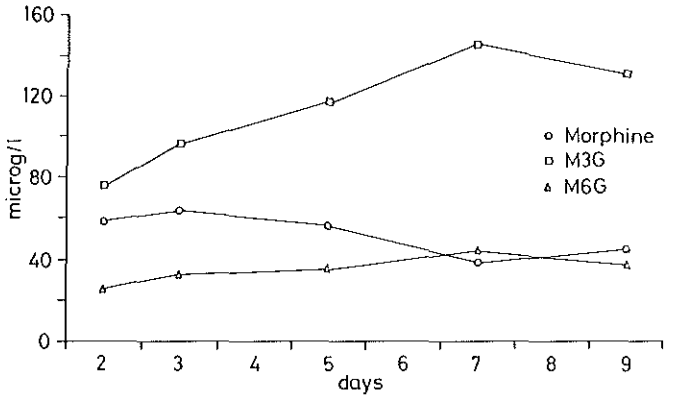

Figure 2 Mean morphine, morphine-3-glucuronide and morphine-6-glucuronide concentrations on consecutive days for the total group.

Because only 3 infants born after 32 weeks were included, differences between age groups were only determined for the group <28 weeks (n=10) and the group 28-32 weeks (n=15). Mean concentrations of M3G were significantly higher in the older age group on days 3 ($P<0.05$) and 9 ($P=0.007$), mean M6G concentration was significantly higher in the older age group on day 9 ($P=0.007$).

Figure 3A

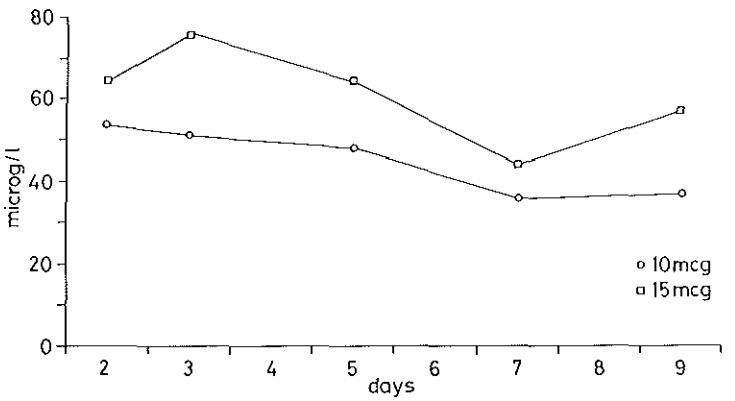

Figure 3B

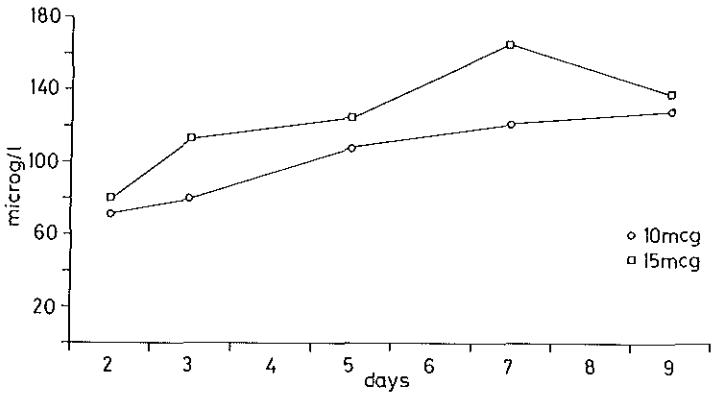

Figure 3C

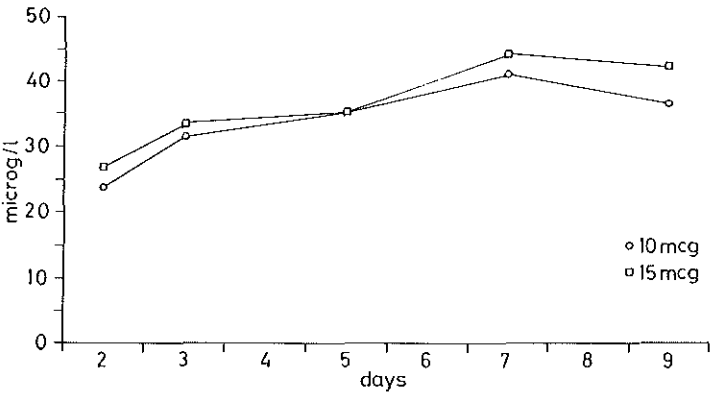

Figure 3. (A) Mean morphine concentrations, (B) mean morphine-3-glucuronide concentrations, and (C) mean morphine-6-glucuronide concentrations in groups A and B. Differences not significant.

Extra morphine was given in 7 infants; one infant (group B) needed only one bolus (day 2), in 3 infants (1 in group A on day 6, 2 in group B on days 3 and 7) the infusion rate was increased by 5 µg/kg/h, and in the remaining 3 infants (2 group A, 1 group B) an extra bolus was administered because of reintubation after accidental extubation.

After discontinuation of the drug, M3G was still found in 12/14 (85.7%) and 13/17 (76.5%) infants in concentrations of 41.0 ± 19.3 µg/l after 12 hours and 34.0 ± 27.4 µg/l after 24 hours respectively. There was no significant difference between groups A and B. In the majority of infants no morphine or M6G could be detected.
There was no difference between infants in whom the morphine dose was decreased by 50% before ending and those in whom it was stopped immediately.

Morphine concentrations on days 2 and 7 were significantly lower with higher gestational age (Spearman's rank correlation r=0.55, P<0.01, and r=0.64, P<0.01 respectively). On all other days there was no relation shown between gestational ages and morphine.
Mean M3:morphine, and M6:morphine serum concentration ratios increased significantly during the first 7 days (P <0.05), the mean M3G:M6G serum concentration ratio did not change significantly, although the ratio decreased from 4.1 on day 1 to 3.5 on day 9. There was no correlation of birthweight and gestational age with either ratios.

The estimated mean serum clearance on day 2 was 3.64 ml/min/kg for all patients, without significant correlation with gestational age. Mean serum clearance was significantly higher in group B (3.99 ml/min/kg) than in group A (3.37 ml/min/kg) (P =0.023). The use of inotropic agents was equal in both groups, and had no effect on the clearance in any dose compared to infants who did not receive inotropic agents.
Pain scores were significantly higher before than after start of morphine administration (P=0.001). There was good analgesia before and after endotracheal suctioning, but pain scores were significantly higher during suctioning (indicating moderate pain or distress), compared to those before and after suctioning (P<0.01).
Pain scores did not correlate with morphine, M3G, or M6G concentrations at any time.

Severity of illness, as measured by the CRIB, did not differ between groups. Neither a correlation between CRIB and morphine concentrations was found.
Incidence of PIVH grade III-IV or PVL was not significantly different between groups A and B.
Heart rate, and mean arterial blood pressure were comparable between groups; as over 50% of the infants had inotropic support, with no difference between groups A and B, the effects of morphine on heart rate and blood pressure could not be determined.
Decreased gastrointestinal motility was not found in our study. Urinary retention as objectified by ultrasound was found in 3 infants in group A, and in 1 infant in group B (ns).

III.2.5 Discussion

To our knowledge, this is the first study investigating both the pharmacokinetics and pharmacodynamics of morphine and its major metabolites M3G and M6G in preterm infants. We have demonstrated that a dose-dependent effect, for the doses used, was absent, and a correlation with gestational age could neither be determined.
In preterm newborn infants morphine and M3G can be found in serum after intravenous administration on all days; M6G was not detected on the first days after birth, but its appearance in serum increased with postnatal age. After discontinuation of the infusion, morphine and M6G disappear quickly, but M3G can be found in low concentrations after 12 and 24 hours. Although appropriate analgesia, as determined by a validated pain assessment instrument was present, no correlation between concentrations of either morphine or M6G, and pain scores was found. In only 4 infants possible adverse effects were found. Severity of illness as determined by the

CRIB score, did not influence the analgesic effect of morphine, and the use of inotropic drugs did not have an effect on morphine concentrations either.

The effects of continuous morphine taking into account both morphine and its main metabolites, their concentrations, and the relation to pain scores during a prolonged period, i.e. 7-14 days, in critically ill, ventilated preterm infants deserve great attention in determining objective guidelines for the use of morphine in this age group.
In the literature different doses of morphine and diamorphine have been reported for analgesia in preterm infants, with continuous doses ranging from 5 to 100 μg/kg/h.[12,23,27,42,43,44] Doses of 10 and 15 μg/kg/h are commonly used, which prompted us to investigate these dose regimens prior to a double blind placebo controlled multicenter trial. Initial higher doses offer no benefit, as no better analgesia is reported, and excessive doses might lead to adverse effects.

The mean serum concentrations of morphine (58.1 ± 37.6 μg/l) and M6G (25.1 ± 10.0 μg/l) we have found at 24 hours compare to the values reported in the literature; 24-96 μg/l,[23,24,27,42,43,45,46] and 19-48 μg/l,[23,42,43,45] respectively. Our values of M3G at 24 hours are higher than those reported after continuous morphine (33.3-62 μg/l),[23,45] but drastically lower than those found after administration of diamorphine.[42,43] Diamorphine is reported to have a more rapid onset of action and potency, probably due to its greater water solubility.[27]
Morphine concentrations between 58.1 and 63.2 μg/l were determined after 24-72 hours. Higher values of 64-118 μg/l were again found after diamorphine 15 μg/kg/h,[43] and values between 108.4 and 207.1 μg/l, after morphine in doses of 20 to 30 μg/kg/h,[26] which is twice the amount we used. The difference with the latter study might also be due to the determination of total morphine, where we measured unchanged morphine and its metabolites separately. After several days morphine concentrations will decrease, as more morphine is metabolized to M6G.
Morphine is metabolized to M6G and M3G, and to a lesser extent to morphine-3-sulphate, and morphine-6-sulphate. Although sulphation is the predominant pathway for acetaminophen in neonates and infants,[47] sulphation minimally contributes to the metabolism of morphine. [48]
The M3G:M6G concentration ratios show that the contribution of M6G to M3G remains the same, which is in agreement with the ratio found in urine,[49] and our values in preterm infants are comparable with those in full term neonates.[50]
Both the M3G:morphine concentration ratio and the M6G:morphine ratio significantly increase with the increasing glucuronidation capacity of the neonate in the first few days of life. M6G:morphine concentration ratios are consistent with reported ratios in newborns at 0.8,[42] and in infants at 1.9-2.1.[51]
Data from a recent review support the presence of a single glucuronidation enzyme after the neonatal period,[52] but with different isoenzymes for the 3- and 6-positions.[53] Assuming that this applies to neonates as well, this might explain why M3G glucuronidation develops very rapidly, i.e. within hours, and M6G glucuronidation increases in a few days, whereas the M3G:M6G concentration ratio remains constant. Contrary to Hartley et al,[45] who reported increased conversion to M3G and M6G with increasing birthweight, we did not find a correlation with birthweight or gestational age.

Contrary to adults and children, in both of whom concentrations in blood exceed those of unmetabolized morphine,[30,49] M6G, the most powerful metabolite is reported to be scarcely or not present at all in the first few days of life.[22,30,52] However, several other studies [23,45,50] have shown a rapidly increasing ability to glucuronidate morphine, apparently independent from gestational age, fitting with our results. During our long-term observations we found a correlation between concentrations and postnatal age. M6G concentrations did not exceed those of unmetabolized morphine, but equalled values on days 7 and 9, and it might be that after a longer period of morphine administration, or at an older age, M6G concentrations exceed those of morphine.

The high values of M3G from the first day onwards, might relieve the fear for respiratory depression, as this metabolite has a strong respiratory stimulating effect.[54] Moreover, it was observed that respiratory depression occurs only in the first few hours after the start of morphine administration,[2] and has the same risk for neonates as for infants and children.[55] A retrospective study of the use of opioids revealed that apnoea and respiratory depression occurred in 13% of all cases, but none were attributable to morphine,[56] although controversy still exists.[43,57] When morphine is used in ventilated infants, there is no reason to be afraid of respiratory depression, as ventilatory support can be increased temporarily, and several studies have failed to show respiratory depression.[22,46,58]
Afraid of respiratory depression or apnoea, many clinics are accustomed to stop morphine before extubation, but as can be extracted from our data, morphine and M6G disappear rapidly, whereas M3G is still detectable in most serum samples.

As morphine serum concentrations approach steady state values after 24 hours,[45] we used our values at day two to determine the apparent steady state clearance. Clearance in our groups is better than the estimated values of 2.2 ± 0.7 ml/min/kg,[1] but are in agreement with values of 2.16-4.6 ml/min/kg reported by several others, although these studies included term infants or preterm infants between 32-36 weeks.[22,23,27,42] Surprisingly, clearance was higher in group B infants.
Although many children received inotropic drugs, we found no effect on clearance. Our values were higher than those of Dagan, who reported lower values due to inotropic drugs.[31] However the groups are not comparable due to differences in gestational and postnatal age, underlying disease, and the reasons for admission, i.e. cardiac disease and post cardiosurgery.[31]

As M6G is found in relatively small amounts or not found at all during the first few days of life, this may account for a higher need of morphine for analgesia in early age.[22] Another explanation may be a diminished sensitivity of the μ receptors, which are responsible for the analgesic effect of morphine in the neonatal period. A difference in levels of the μ opiate receptor expression as a result of variation at the μOR gene locus in individuals, as recently proposed, might be another interesting possibility that needs further research.[59]
Recently steady state concentrations of 74.2-207.1 μg/l were shown to diminish pain as measured by facial expression, but no relation with morphine concentration was found.[26] We found good analgesia after morphine in preterm neonates judging from the NIPS, but without a correlation between analgesia and total morphine serum

concentration either.[47] In the present study we found good analgesia after the start of morphine administration, but again were not able to determine a correlation with morphine serum concentrations or with M6G serum concentrations.
As the pain assessment by means of the NIPS resulted in comparable values, we assume that during artificial ventilation for 1-9 days overall analgesia as assessed by the NIPS was adequate, with the exception of the moment of endotracheal suction, which is considered a strong pain and/or stress stimulus. Therefore, the recommended doses of 2 μg/kg/h, as calculated in a recent review,[2] are in our opinion to low to give adequate analgesia in the clinical setting.

Due to the absence of differences between the two dose regimens, there is no need to use the higher dose initially. Higher doses leading to higher serum concentrations are reported to cause adverse effects such as bradycardia, arterial carbon dioxide retention, and urinary retention.[22] Contrary to that study, we found few adverse effects, which might be attributable to the fact that our serum concentrations are well under the value of 300 μg/l associated with adverse effects.[22]
Different doses of morphine for analgesia in preterm infants have been studied, with loading doses between 50 and 200 μg/kg, followed by 5 to 50 μg/kg/h continuously.[23,26,44] However, dose regimens higher than 12.5 μg/kg/h[23] offer no clinical advantage.
Recently, fentanyl was proposed to be superior to morphine during the first 2 days of life in newborn infants.[19] However, long term use was not evaluated and is associated with tachyphylaxis, rapid tolerance, chest rigidity, and greater likelihood of having an abstinence syndrome.[19,60,61]

As sicker infants, especially those with sepsis or meningitis, might react differently to pain stimuli due to hyperalgesia and allodynia, we evaluated the correlation between morphine concentration and severity of illness.
Severity of illness as measured by the CRIB score was not related to the analgesic effect of morphine as measured by the NIPS. Kahn et al[62] found that narcotics were used most frequently for the sickest patients, as measured by the score for neonatal acute physiology. This might be true for our NICU population as well, but in the present groups all infants were administered morphine, so no comparison could be made.

From our findings we conclude that an initial dose of 10 μg/kg/h is appropriate. If tolerance develops as shown by validated pain assessment instruments, the morphine infusion should be increased by 5 μg/kg/h, preceded by 50% of the loading dose.

III.2.6 Acknowledgements
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IV

General Discussion and Future Developments: To a Tailored Pain Management

De pijnboom groeit
hij heeft geen pijn
of soms
dat moet van het ontharsen zijn.

IV.1 General Discussion and Future Developments: To a Tailored Pain Management.

Appropriate pharmacological and non-pharmacological management of pain has been the subject of study throughout the ages. Following beliefs that certain types of pain could not be cured as they were a punishment of the Gods, a change took place in the 16th and 17th century. From that point on pain was seen as a result of both wounding (tissue damage) and/or as a signal of disease of certain organs, although the cause and mechanism was not always clear.[1]

Today in many circumstances the causes and mechanisms of pain are known, and consequently the choice of appropriate analgesia. However, in certain patient groups such as preterm infants, children less than 3 years of age, and severely mentally handicapped children in which selfreport is unreliable or not possible at all, there are still problems to be solved and myths to be unraveled. The age groups presented in this thesis represent one of the most difficult fields of pain assessment and management in the critically ill newborn infant.

Until about 20 years ago pain in neonates was believed to be non-existent, as pain pathways were not yet functional.[2,3] Following studies on the development of the pain pathways, indicating that neonates were very much capable of feeling pain, even when born prematurely,[4] new problems became clear. Nothing was known about both pain assessment and pain management during the first days and weeks of life.

As a result many clinicians were reluctant to provide analgesia. As a consequence, although they believed and knew that there was pain, clinicians did not even prescribe analgesics. This practice still exists in many clinics, and until now clinicians in the United States perform circumcision on newborn infants without analgesia or with insufficient analgesia.[5,6] In contrast, a good and easy method of analgesia, as the dorsal penile nerve block is used in Europe for a long time, and has been studied and recommended as early as 1983 in the United States as well.[7-9] Whether this attitude is due to ignorance or love of ease as it takes extra time, is not clear.

The clinical significance of pain and stress in the preterm neonate is recently summarized by Anand,[10] who stated that they have an increased sensitivity to pain, and that acute painful stimuli lead to prolonged periods of hyperalgesia. Repetitive painful experiences are associated with some of the neurobehavioral and developmental sequelae, observed after long term stay in neonatal intensive care units of babies.[11,12] This may lead to an altered development of the pain system associated with decreased pain thresholds during development in neonatal rat pups.[13] Postoperative mortality and morbidity in infants was decreased when potent anaesthetic agents were used.[14]

At the start of our studies four main problems were not solved.

1. In the first place a validated and reliable instrument to assess pain in the newborn infant was not available. Many tools were developed based on instruments in older children and adults and were mostly tested in circumstances of acute sharp pain, following minor surgery or procedures as the heel prick.[15-17] The so-called faces scales used in different settings were developed and tested in small studies without uniformity. This is demonstrated

in the different faces scales with for example even differences in the direction of the scale from no pain to worse pain in a Finnish scale.[18-20] The significance of unidimensional and multidimensional scales for pain assessment is clear as far as they can be related to self report. In contrast it is not clear in non-verbal subjects if it is related to effect only, and not to concentrations of analgesics.

2. The majority of drugs used in children and infants, is not officially tested and approved for this age group,[21,22] As drug metabolism (sulphation, glucuronidation) is different especially in the first period of life this subject needs thorough investigation in the near future.[23,24] Some studies on antimycotic and antibiotic drugs have already shown the difference in pharmacokinetics and metabolism in newborn infants,[25,26] but for analgesics only very few studies were performed.[27-30] Most studies describe either dose effect or dose concentration relationship. Most important however is the fact that analgesic therapeutic ranges become known by correlating concentration to effect as measured by validated pain assessment instruments, and controlling whether existing therapeutic ranges in adults apply to children as well. Another important source of information comes from self report and studies in volunteers using a pain stimulus as laser stimulation and the nociceptive flexion reflex threshold RIII.[31-33]

3. Following the results of laboratory experiments, involving repeated pain stimuli in newborn rats, changes in the brain due to hyperalgesia and plasticity,[34,35] the role of specific receptors and long term effects of pain in the neonatal period need to be studied in a clinical setting.

4. Doctors attitudes need to be changed: possibly due to changing views, studies reported in the literature, and more interest in pain in children, the attitudes are changing slowly, but still have not changed enough.[36-38] There was and apparently still is a need for practical guidelines that can be used in clinical practice.

This thesis focusses on the pharmacokinetics and pharmacodynamics of the two main analgesic drugs used in neonates: morphine and paracetamol, in correlation with validated pain assessment instruments, such as a modified facies scale and the Neonatal Infant Pain Scale (NIPS).[18,39,40]
The pharmacokinetic and pharmacodynamic effects of single doses of the above mentioned analgesic drugs were investigated whenever nothing was yet known in that particular age group. Whenever information was available about single doses, the pharmacokinetic and pharmacodynamic effects of multiple or continuous drug administration in relation to both gestational age and postnatal age was studied.

IV.2 Pain assessment

The faces scale proved to be a reliable instrument when validity was tested, but did not correlate with the concentrations of paracetamol in two studies (chapter II.3 and II.4)[41,42] for several reasons. Most of the infants scored low on the pain score

indicating minimal or no pain. It may be that this pain score is not particularly suitable to measure pain in preterm and term newborn infants or that it is only suited for the assessment of acute severe pain or moderate prolonged pain. Therefore, we were unable to pinpoint the therapeutic paracetamol concentration range for these age groups.

Although nurses' perceptions of pain are often based on subjective 'feeling', signs and symptoms,[43-45] neonatal nurses have shown strong agreement (average 86%) for the indicators of neonatal pain.[43]

In a study on assessment of acute pain in older children, children's' self-report and nurse ratings significantly correlated, implicating that in case observational coding is not possible, nurse ratings of acute pain may closely approximate objective assessment of pain and distress behaviours.[46]

This observation does not solve the problem of the absent correlation between pain scores and serum analgesic levels.

However, following vacuum extraction paracetamol did not significantly contribute in lowering the pain score, but judging from the subjective nurses'perception score of the clinical condition, the clinical condition improved following one dose of paracetamol (chapter II.5).

According to Pigeon et al,[43] there is less discrimination of the behaviours on the basis of intensity of pain. The utility of behavioural signs as crying, limb movement, agitated state, and tachycardia for the assessment of pain in "chronic" conditions in neonates, such as sepsis or necrotizing enterocolitis is questionable, as it is possible that such behavioural indicators may habituate to long-term pain.[43]

Multidimensional pain scores, which have recently become available, are probably more appropriate in this respect.[40,47,48]

Therefore, in two studies on the use of morphine (chapter III), a multidimensional validated pain score, the NIPS was used.[40]

Although appropriate analgesia was found, as shown by a significant decrease of the pain score after administration of morphine, a correlation between pain scores and morphine serum concentrations was not found. Our findings are in agreement with the results from other studies, with morphine infusions of 20-30 μg/kg/h.[28,49,50] This might be influenced by the cut off point used in the different studies to determine the presence of pain using the NIPS.[51]

Further development of the NIPS score by extending the number of items or the use of the premature infant pain profile (PIPP)[48] score or comparing these pain scores with the much more detailed, (but not usable for bed-side scoring), Neonatal Facial Coding System (NFCS),[17] is now under study evaluating the use of sucrose following heel prick pain, and might be of help in the future.

IV.3 Pharmacokinetics and pharmacodynamics of paracetamol and morphine.

Although paracetamol has been used as an analgesic for over 100 years,[52] its use in the paediatric population is based on data extrapolated from adults,[53] as occurs frequently.[22]

The therapeutic range of paracetamol of 10 -20 mg/l that we and others used in our studies was almost the same as that used to obtain an antipyretic effect.[54] A review of

the literature (chapter II.1) clearly shows that this recommended therapeutic range is not supported by randomized controlled trials providing level I evidence. However, recent reports indicate that analgesic efficacy may be obtained when blood concentrations range from 5-25 mg/l[31-33,41,42,55-58]

The commonly accepted loading doses of paracetamol of 10 mg/kg for oral administration and 15-20 mg/kg for rectally administration are not sufficient in alleviating pain and should be increased to 30 and 40 mg/kg for the oral and rectal route respectively.[55,59]

In neonates the metabolism of paracetamol is slower, but still adequate due to increased sulphation, provided that paracetamol is not used for periods exceeding 48 - 72 hours.[30,41,60]

In clinical studies using rectal paracetamol no toxic serum levels were reached after multiple doses and no accumulation was found.[42,61-63] Until further studies show otherwise, the daily dose should not exceed 90 mg/kg, and dose interval in neonates should be between 8 and 12 hours.

Morphine is the most widely used analgesic in neonates,[37,64] but consistency in prescribing is not yet achieved. Recently a 28.6-fold variation among NICUs in narcotic administration was shown.[38] Major differences in concentrations ranging from 10 to 422 μg/l after doses between 2 and 100 μg/kg/h, are presumed to result in adequate analgesia. Adverse effects after higher doses are commonly reported,[27,64] but it seems to occur after high doses (30-100 μg/kg/h) and probably result from altered pharmacokinetics and decreased clearance. If given for a longer period tolerance develops and higher doses should be given without an apparent increase in adverse effects. As part of the adverse effects occurs after discontinuation of morphine, when used for periods of 5 days or more, slow weaning of the patient over a prolonged period is required.[65,66]

As described in this thesis (chapters III.1 and III.2) and supported by other studies an initial dose of 10 μg/kg/h provides adequate analgesia,[67,68] however without a correlation between pain scores and concentration. This correlation is not achieved using higher doses either.[49,69]

Beneficial effects of morphine other than analgesia and stress reduction are increased synchronous ventilation,[70] stabilization of arterial blood pressure,[71] and a reduced incidence of intraventricular hemorrhage and periventricular leukomalacia.[72]

Morphine may also prevent abnormal behavioural and physiological responses to subsequent painful events.

The importance of simultaneous pain assessment and analgesia to evaluate whether starting or continuation of analgesia is indicated, is stressed by a recent study in newborn rats, where morphine given in the absence of pain resulted in a decreased sensitivity for morphine in adulthood.[73] Administration of opiates (and barbiturates and nitric oxide as well) during labour to mothers resulted in a higher proportion of infants that turned into opiate addicts as adults, as compared to their unmatched siblings.[74] In the only study available in which the outcome at 5-6 years of prematurely born children who received morphine as neonates was evaluated, McGregor et al did not find any adverse effects on intelligence, motor function, or behaviour.[75]

Now that the proper initial morphine dose (10 μg/kg/h) is established, it is time for double blind placebo controlled randomized trials, in order to evaluate and eventually develop new pain assessment instruments, together with the prolonged use of analgesics. This should lead to correlations between the most commonly used analgesics and pain scores. A multicentre study supported by the Dutch Research Council (NWO) is now in progress.
Moreover in the US the so-called NEOPAIN study evaluates the reduction of the incidence of poor neurologic outcome after preemptive morphine in preterm neonates, after encouraging results in a pilot study.[72]
The long term effects on infants, especially for neurodevelopmental outcome and pain response under standard conditions i.e. vaccination,[11,12] evaluating of the reaction to pain, are of paramount importance.

As more and more information is available on the central effects of morphine, but recently also of paracetamol,[32,76] the relationship between drug concentrations in cerebrospinal fluid and neurophysiological parameters as electrical activity and changes in blood stream velocity, need to be studied.

Pharmacodynamics of analgesics still pose an enormous problem, especially in case of (continuing) chronic pain. In the field of pharmacokinetics good progress has been made, and more studies on a wide range of drugs are forthcoming with the growing interest both in the Netherlands,[77] and internationally.[21]
For paracetamol the bioavailability after rectal and oral administration needs to studied in more detail, as it is hampered by erratic absorption due to the variability of venous dainage from the rectum[23] and by slow gastric emptying in (critically ill) newborn infants.[25,78] Studies with for instance propacetamol, an intravenous prodrug of paracetamol are warranted. A randomised trial comparing the pharmacokinetics and pharmacodynamics of oral versus rectal paracetamol after craniofacial surgery in a cohort of 40 children (mean age 8 months) is just finished.
Much research on the effects of paracetamol is performed by Anderson[57,58,79,80], and we agree that still a lot about paracetamol is not known and needs further study.[81] First of all a study is warranted to determine the objective therapeutic range.

IV.4 From laboratory to clinical research

Two recent studies have shed new light on the way morphine works. Matthes et al[82] showing that mice lacking the μ-opioid receptor do not show any of the normal responses to morphine, as both the analgesic and additional euphoric effect are completely absent. This also indicates that the δ- and κ-opioid receptors, are not involved in mediating the analgesic effect at the spinal level, as had been assumed previously.[83]
In this context, Uhl et al[84] postulated the existence of polymorphisms of the μ-opioid receptor, explaining the differences between human individuals and mouse strains in levels of μ-opioid receptor expression, and consequently the responses to painful stimuli, and to opiate drugs. Along the line of progress of the human genome project it is possible in the near future to "map" each individual, and as a consequence tailoring

of analgesia will become a possibility. A new study has started recently combining the analgesic effects of morphine and paracetamol to alleviate postoperative pain in infants, to evaluate the morphine sparing effect of paracetamol as suggested in adults.

Although this thesis focussed on pharmacological ways to manage pain, preventing pain and non-pharmacological pain management should not be forgotten.
Reducing stress by lowering noise, light and the huge number of potential painful procedures on the NICU, on average 14 daily,[85] and caregiving (by nurses) by means of individualized developmental care, may improve medical and neurodevelopmental outcome.[86] Non-nutrive sucking and administration of sucrose[87-92] are cheap and easy applicable methods in case of minor procedural pain, but might also be used as adjuvant therapy to well known analgesic agents.

IV.5 Attitude and education

However, none of the research published in this thesis, in journals or in textbooks, has any impact as the clinicians are not convinced that pain is present or will be expected in individual patients.
Educating the nurses and above all, educating the physicians by theoretical and practical instructions, providing them with validated tools for pain assessment, and guidelines for pain assessment and pain management, is the first condition to achieve better analgesia in newborn infants. In my opinion nobody can state that a neonate has no pain as long as no pain assessment has been performed. Pain assessment should be part of normal routine in the care for newborn infants, much in the same way as counting the heart rate, and measuring blood pressure.
Together with the proper use of analgesics, and personal "mapping" of drug metabolism, tailored pain management will be the future.

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V

Summary

V.1 Summary

Newborns admitted to the neonatal intensive care unit are subject to a large number of painful, invasive procedures. Pain can be present both in neonates where problems have occurred during delivery resulting in hematomas or fractures, and in neonates admitted to high care units or neonatal wards. In this thesis entitled "Pain management and analgesia in the newborn, an integrated approach" the pharmacokinetics and pharmacodynamics of two commonly used analgetic drugs - paracetamol and morphine - is presented.
To understand how analgetic drugs affect the neonate, a thorough understanding of pain and development of pain pathways in utero and during the transitional period from intrauterine to extrauterine life is needed. This is presented in chapter I.1. Furthermore, the goals of this thesis are specified: to investigate the pharmacokinetics, metabolism, and dose-response relation of single and multiple rectal doses of paracetamol and continuous intravenous doses of morphine in preterm and term infants.
The period before, during and shortly after birth is a period in which many changes occur in a relatively short period. As a result of preterm birth, several physiological and metabolic processes are not fully equipped to cope with extrauterine life, for example the metabolic processes of the liver. Other processes, such as the normal growth of lungs, are already extended to the extrauterine period, even after normal full-term birth.
Pain is one of the senses and is incorporated in a multidimensional process of development, together with touch, hearing, smell, taste and sight. As these are ongoing processes and fetal development can be influenced by intrauterine and extrauterine behavioural reactions as well as by physiological, hormonal and metabolic processes, the development of the senses is described in chapter I.2.

In chapter II, the pharmacokinetics and pharmacodynamics of paracetamol are investigated.
Firstly, the literature was reviewed to test the evidence that blood concentrations of paracetamol between 10 and 20 mg/l represent therapeutic analgesic values, and to determine the correlation between dose and/or concentrations and validated pain assessment scores (chapter II.1). This was done by means of a meta-analysis of studies from the literature and an inquiry among pharmaceutical companies.
Of 78 studies on both antipyretic and analgesic effect, 35 studies described analgesic effects. When stratified for age, 9 contained data on infants, 21 on children aged 1-17 years, and 7 on adults. The majority of these were dose-effect studies only, while in 10 studies both concentration and effect were studied.
The single doses used in children (7.5 to 40 mg/kg) by different routes of administration, were higher than those advised on the label instructions. They resulted in mean maximum blood concentrations from 5.5 to 22.5 mg/l, while concentrations between 5.8 and 55.9 mg/l in adults resulted from single doses of 500-1500 mg. Multiple doses were investigated scarcely.
Pain assessment was performed by several different pain measurement scores, either by objective validated pain measurements in preverbal children, or by self-report in adults and older children. Finally, appropriate analgesia was reported for

concentrations of 5.9-29.8 mg/l in adults and of 7.5-22.5 mg/l in infants and children, which is a wider range than the previous generally accepted range.
Therapeutic ranges reported in the literature are not based on randomised controlled trials. Our analysis showed that it is not the range of 10-20 mg/l that may indicate analgesic efficacy as previously reported, but a wider range from 5-25 mg/l.
For the purposes of this thesis, the range of 10-20 mg/l was used.
To determine paracetamol concentrations in our own patient group, a reliable and fast method was required. As earlier published methods were too labour-intensive, a modified high-performance liquid chromatography (HPLC) method was developed, enabling us to determine paracetamol and its metabolites in both serum and urine. With a retention time for the different metabolites varying from 1.6-8.0 minutes, and calibration curves showing good linearity, it proved to be an effective method (chapter II.2).
In chapter II.3, the results of a study investigating the effects of rectally administered paracetamol in preterm neonates are described. This drug, although in use for over 100 years, had not been studied in this manner in these age groups before.
Twenty-eight infants, stratified for gestational age of 28-32 weeks (n=21) and 32-36 weeks (n=7) received a single rectal dose of paracetamol of 20 mg/kg body weight (range 16-24 mg, due to available strengths). The mean maximal serum concentrations were between 1.5 and 18 mg/l. The mean maximal serum concentration in the 28-32 weeks group was 12.5 ± 2.9 mg/l and 7.5 ± 4.0 mg/l in the older age group. When doses were >18 mg/kg, most of the infants in the under 32 weeks age group had concentrations in the therapeutic range.
Metabolism in preterm infants appeared to be adequate, not by glucuronidation but mainly by sulphation as was seen from the very low Glucuronide:Sulphate ratio with earlier post conceptional age. During childhood, the significance of the contribution of glucuronidation to paracetamol metabolism increases, but it is not till after the age of 7-10 years, that it exceeds sulphation.
As excretion is slow, the administration of multiple doses in preterm infants would require an interval of >8 hours to prevent accumulation.
Extending our research of paracetamol to term infants, we investigated the pharmacokinetics and pharmacodynamics of rectally administered paracetamol in term neonates directly after birth (chapter II.4), and the effects of multiple dose paracetamol after a procedural painful event i.e. a vacuum extraction (chapter II.5) in two prospective clinical trials.
Ten consecutive term neonates suffering from painful conditions, or who were undergoing painful procedures, received multiple-dose paracetamol. Serum concentrations were determined serially using an HPLC method, and pharmacokinetic analysis was performed. Pain assessment was performed using a validated faces pain score.
Ten consecutive term neonates received 4 rectal doses of 20 mg/kg body weight every 6 hours. Mean peak serum concentrations (SD) during multiple dosing were 10.79 (6.39) mg/l, 15.34 (5.21) mg/l, and 6.24 (3.64) mg/l for the whole group, boys and girls respectively. There was a significant difference between the boys and the girls (P =0.01). No serum concentrations associated with toxicity (>120 mg/l) were found. The median time to reach peak serum concentration was 1.5 hours after the first dose and 15 hours for multiple doses. Mean $T_{½}$ (SD) was 2.7 (1.4) hours in 8 patients. There

was no correlation between dose and serum concentration, or between pain score and serum concentration. However, a significant inverse relationship between the preceding pain score and peak serum concentrations was found.
In term neonates, multiple rectal doses of paracetamol 20 mg/kg bodyweight resulted in widely varying serum concentrations, but did not result in therapeutic concentrations in all infants. As accumulation was not found, a dose of 30 mg/kg followed by doses of 20 mg/kg at 6-8 hour intervals of administration are appropriate to reach therapeutic concentrations. These new recommendations are in line with those recently published in the literature for older infants and children.
Pain after a procedural intervention was investigated in 122 infants delivered by vacuum extraction. The rationale for this study was the fact that in clinical practice paracetamol was often given or often not allowed, both without objective data to support the action taken. In a prospective, randomised, double-blind, placebo-controlled study paracetamol 20 mg/kg or placebo was given rectally to evaluate whether pain in infants delivered by vacuum extraction is relieved, and whether it improves clinical condition. Infants delivered by vacuum extraction were randomised either to the study group (n=61) and given a maximum of 4 doses of paracetamol or to the control group (n=61) receiving placebo. Pain assessment was performed by a validated pain score, a so-called faces score and by rating the clinical condition. In these groups, both scores and clinical symptoms such as vomiting, crying, and pain on handling were compared with symptoms in a reference group (n=66) with uncomplicated pregnancy and delivery in vertex position without vacuum extraction. It was found that pain scores did not differ between the vacuum extraction groups, while the clinical condition in the study group improved only after the first dose of paracetamol. There was a significant difference ($P<0.05$) in objective clinical symptoms in the vacuum extraction groups, compared to the reference group.
It was concluded that one dose of paracetamol given to neonates delivered by vacuum extraction improved their clinical condition significantly, but did not result in a significant change in objective pain scores. Subsequent doses of paracetamol did not show any effect on the clinical symptoms or appearance of the neonates studied. Consequently, the guidelines for paracetamol administration after vacuum extraction were changed to administration of one dose of paracetamol to all infants, and that depending on the pain score, subsequent doses should be administered.

In chapter III is described how the pharmacokinetics and pharmacodynamics of morphine was investigated in ventilated preterm infants.
Firstly, we investigated the effect of a continuous infusion of 10 μg/kg/h in different age groups, and the usefulness of a rapid method to determine serum concentrations to adjust the dosage (Chapter III.1). The objectives of the study were to determine the concentration-effect relationship of continuous morphine in preterm infants in the first days after birth using a validated pain score - the NIPS - both at rest and during endotracheal suctioning, and to investigate whether a rapid method for the determination of morphine serum concentration, a modified Fluorescence Polarization Immuno Assay (FPIA) can be useful when adjusting morphine dose.
In a prospective clinical trial, ventilated preterm neonates in three different age groups (<28 weeks, 28-32 weeks, and 32-37 weeks), who were subjected to several painful procedures such as insertion of intravenous catheters or cannula, endotracheal suction,

and elective reintubation, received continuous morphine (10 µg/kg/h). Morphine serum concentrations were determined using a rapid FPIA, and pharmacokinetic analysis was performed.
Pain scores before and after morphine, and during endotracheal suctioning, were assessed using the NIPS.
Of 24 infants included in the trial, 4 were <28 weeks, 14 between 28 and 32 weeks and 6 >32 weeks. Morphine was detected in 75 to 84 % of the samples during the first 10 days of life, and in some infants also at 12 and 24 hours. Differences between the groups in mean morphine concentration were not significant, although a trend was observed for lower concentrations with the older age groups in the first 3 days. Mean morphine concentrations for all infants increased from 85.1 ± 33.4 µg/l on day 2 to 103.3 ± 70.8 µg/l on day 5. No relation between morphine concentration and gestational age, except on day 2, was found. Mean estimated clearance was 2.3 ± 1.1 ml/min/kg for the total group, and increased with gestational age. No differences were found in clearance between the age groups.
Pain scores were significantly higher before morphine than after ($P<0.001$) and during endotracheal suction than either before or after ($P<0.001$), but were not related to morphine concentrations.
Morphine concentrations can be determined by this rapid method, but as there was no correlation with pain scores, it is not suitable for individual adjustment of dosage. With the dose used in this study appropriate analgesia as assessed by a validated pain score was achieved during mechanical ventilation, but no relationship between concentration and effect was found.
As studies on the effect of the metabolites of morphine increasingly indicated the importance of morphine-3-glucuronide as a stimulating substance for respiration and the importance of the analgesic properties of morphine-6-glucuronide, we studied the pharmacokinetics and pharmacodynamics of morphine and its metabolites in another prospective randomised controlled trial. The effect of the two most commonly used dose regimens were compared in ventilated preterm newborn infants. The aim of this study was to investigate pharmacokinetics and pharmacodynamics of morphine and its main metabolites morphine-3-glucuronide and morphine-6-glucuronide in the first ten days after birth. Furthermore, the effects of the use of inotropic agents, and the severity of illness were also studied.
Ventilated preterm neonates (n=28) who were subject to several painful procedures, received continuous morphine in two different dose regimens of 10 (group A) or 15 µg/kg/h (group B). Serum concentrations of morphine and its metabolites were determined serially with an HPLC method, and pharmacokinetic analysis was also performed. Pain assessment was performed by a validated pain score, the NIPS.
Morphine was detected in 80-100%, and morphine-3-glucuronide in 89-100% of the infants. The percentage of infants in whom morphine-6-glucuronide was detected increased significantly from 41% to 90% over the first 9 days after birth. Differences between the groups in mean serum concentrations of morphine and its metabolites were not significant.
Mean morphine concentrations for all infants increased from 58.1 ± 37.6 µg/l on day 2 to 63.2 ± 29.1 µg/l on day 3 and decreased thereafter to 44.3 ± 16.3 µg/l on day 9. Morphine-3-glucuronide values increased significantly from 75.4 ± 41.0 µg/l to 130.8 ± 66.7 µg/l on day 9, and morphine-6-glucuronide concentrations increased from 25.1

± 10.0 to 38.4 ± 14.0 µg/l on day 9. Morphine concentrations were significantly higher with higher gestational age on day 7; while on all other days, there was no relation between gestational ages and morphine, or its metabolites. Twelve and 24 hours after discontinuation of the drug, morphine-3-glucuronide was still detected in 12 of 14 infants (85.7%) and 13 of 17 infants (76.5%) in lower concentrations, but in the majority of infants, no morphine or morphine-6-glucuronide was found. Mean metabolite:morphine serum concentration ratios increased significantly during the first 7 days indicating a maturation of the metabolic pathways of morphine. The mean morphine-3-glucuronide:morphine-6-glucuronide ratio did not increase significantly.
Mean estimated serum clearance was 3.64 ml/min/kg for all patients, with no significant correlation with gestational age. There was a significantly higher clearance in group B than in group A. No influence of either inotropic drugs or severity of illness on clearance or morphine concentrations was found. There was a good analgesic effect of morphine as shown by the fact that pain scores were significantly higher before the start of morphine administration than after. Appropriate analgesia was observed before and after endotracheal suction, but pain scores were significantly higher during suctioning (indicating moderate pain or distress). Pain scores did not correlate with morphine, morphine-3-glucuronide, or morphine-6-glucuronide concentrations at any time.
In this study the dose-concentration-effect relationship of morphine in this age group during a period exceeding 72 hours was investigated for the first time. We concluded that in preterm infants, morphine and morphine-3-glucuronide can be found after intravenous administration on all days, and that morphine-6-glucuronide can not be found in all infants in the first days after birth, but this increases with higher postnatal age.
Although there was good analgesia, there is no correlation between concentration and pain scores. An initial dose of 10 µg/kg/h is appropriate. If tolerance develops the morphine infusion should be increased by 5 µg/kg/h, preceded by 50% of the loading dose, to maintain the analgesic effect. Apart from urinary retention in 3 infants, no adverse effects were found.
In chapter IV, the results of our studies are discussed in relationship to studies performed by others. The results contribute substantially to our understanding of the assessment and management of pain in newborn infants. Future studies are warranted to extend our knowledge of pharmacokinetics and pharmacodynamics of analgesic drugs in newborn infants. New studies to validate pain assessment scores are now in progress, and may, together with the above-mentioned studies, result into correlations between the most commonly used analgesics and pain scores. The reduction of the incidence of poor neurological outcome after preemptive morphine in preterm neonates, and the follow-up of infants for long-term effects, especially for neurodevelopmental outcome and under standard conditions (e.g. vaccination), evaluating of the reaction to pain, are of paramount importance.
The relationship between drug concentrations in cerebrospinal fluid and neurophysiological parameters, mechanisms of morphine (opioid) receptors and the existence of polymorphisms of the µ-opioid receptor are interesting in regard to our hope of personal "mapping" and, as a consequence, tailoring of analgesia.

V.2 Samenvatting

Pasgeborenen die opgenomen zijn op de neonatale intensive care afdeling ondergaan een groot aantal pijnlijke invasieve handelingen en ingrepen. Pijn kan ook aanwezig zijn bij pasgeborenen, geboren na een moeizame gecompliceerde bevalling zoals bijvoorbeeld na een schouderdystocie, een vacuümextractie of een tangverlossing. Tenslotte kan pijn ook optreden bij pasgeborenen die opgenomen worden op de gewone zuigelingen afdeling, omdat de pasgeborenen ook daar, zij het in mindere mate pijnlijke handelingen, zoals een hielprik of infuus inbrengen, ondergaan.
In dit proefschrift, over pijnbeoordeling en behandeling bij de pasgeborene, wordt een geïntegreerde aanpak gepresenteerd van de manier waarop de twee meest gebruikte pijnstillende medicijnen paracetamol en morfine werken. Hierbij wordt onderzoek beschreven omtrent de wijze waarop deze medicijnen worden opgenomen, omgezet in een werkzame vorm en vervolgens worden uitgescheiden. Daarnaast wordt het effect op pijn bestudeerd.

Om te begrijpen hoe pijnstillers werken bij de pasgeborene is een globaal begrip van hoe pijn gevoeld wordt en de wijze waarop de pijnontwikkeling bij de ongeborene en gedurende de overgangsfase van intra-uterien naar extra-uterien leven verloopt, nodig (hoofdstuk I.1).

In de inleiding worden de doelstellingen van dit proefschrift geformuleerd. Deze zijn het bestuderen van de farmacokinetiek, het metabolisme en de dosis-effect relatie van enkelvoudige en meervoudige rectale doseringen van paracetamol en continue intraveneuze doseringen van morfine in zowel te vroeg geboren als op tijd geboren zuigelingen.
De periode voor, tijdens en kort na de geboorte is een periode waarin veel veranderingen optreden in een relatief kort tijdsbestek. Ten gevolge van de te vroege geboorte is een aantal ontwikkelingen van organen nog niet voltooid. Deze organen zijn niet in staat te functioneren, zoals bij op tijd geboren baby's, de rijping van de lever is bijvoorbeeld nog niet voltooid.
Andere processen, zoals de normale uitgroei van de longen, worden zelfs na een normale à terme geboorte gedurende de eerste levensjaren voltooid.
Pijn is één van de zintuigen en maakt deel uit van een multidimensioneel proces van ontwikkeling, samen met gevoel, gehoor, smaak, tastzin en gezichtsvermogen. De ontwikkeling van de zintuigen wordt beschreven in hoofdstuk I.2. De ontwikkeling van de ongeborene is tijdens het leven in de baarmoeder een continue proces en kan beïnvloed worden door gedragsmatige reacties en fysiologische, hormonale en metabole processen.

In hoofdstuk II wordt een aantal onderzoeken naar de farmacokinetiek en -dynamiek van paracetamol besproken. Er werd een litteratuuronderzoek verricht om aan te tonen dat serumconcentraties van paracetamol tussen 10 en 20 mg/l, de werkzame spiegel van paracetamol representeren en om de correlatie tussen de dosis en/of serumconcentraties en gevalideerde pijnscores te bepalen (hoofdstuk II.1).
Deze studie bestaat uit twee delen, een meta-analyse van in de litteratuur verschenen studies over paracetamol en een enquête gehouden onder de farmaceutische firma's die

paracetamol, of een combinatie van paracetamol met andere medicijnen, vervaardigen en/of distribueren.
Van 78 studies waarin het koortsverlagende (antipyretische) en/of het pijnstillende (analgetische effect) werd vermeld, werd in 35 gevallen gekeken naar het effect op pijn. Na indeling in leeftijdscategorieën, bleken 9 onderzoeken gegevens te bevatten over zuigelingen, 21 over kinderen tussen 1 en 17 jaar en 7 onderzoeken bevatten gegevens over volwassenen. Meestal waren dit dosis-effect studies, in 10 gevallen werden zowel de concentratie als het effect bestudeerd in relatie tot de dosis. De hoogte van de eenmalige dosering die gebruikt werd bij kinderen varieerde van 7,5 tot 40 mg/kg, waarbij paracetamol rectaal, oraal of intraveneus werd gegeven. Deze dosering was hoger dan de dosis geadviseerd in de bijsluiters. De doseringen gaven gemiddelde maximale serumconcentraties van 5,5 tot 22,5 mg/l, terwijl bij volwassenen, na een eenmalige dosering tussen 500 en 1500 mg, serumconcentraties tussen 5,8 en 55,9 mg/l werden gevonden. Het bepalen van de aanwezigheid van pijn ofwel het scoren van pijn werd gedaan met behulp van diverse pijnmeetinstrumenten. Bij kinderen die nog niet goed konden aangeven waar zij precies pijn hadden (onder 3 jaar) werd gebruik gemaakt van objectieve gevalideerde pijnmetingen, terwijl bij oudere kinderen en volwassenen meestal zelf-rapportage werd gebruikt. In die gevallen waarbij naar de serumconcentratie en het effect werd gekeken, bleek afdoende pijnbestrijding te bestaan bij concentraties van 7,5 tot 22,5 mg/l bij zuigelingen en kinderen en bij concentraties van 5,9 tot 29,8 mg/l bij volwassenen. In beide gevallen was de therapeutische breedte groter dan de tot nog toe geaccepteerde breedte, die zoals eerder vermeld tussen 10 en 20 mg/l gelegen is. Therapeutische spiegels die in de literatuur worden vermeld, zijn niet gebaseerd op gerandomiseerde gecontroleerde onderzoeken.
Uit de meta-analyse blijkt, dat niet de voorheen geaccepteerde therapeutische breedtes tussen 10 en 20 mg/l, maar een paracetamolserumspiegel tussen 5 en 25 mg/l de werkzame pijnstillende spiegel aangeeft.
Voor de studies in dit proefschrift (die zijn verricht voordat de meta-analyse was verricht) werd de therapeutische spiegel van 10 tot 20 mg/l aangehouden.

Om de paracetamolspiegels in onze eigen patiëntengroep te kunnen bepalen, was een betrouwbare en snelle methode nodig. Aangezien de tot nog toe gepubliceerde methoden te arbeidsintensief waren, werd een gemodificeerde High-Performance Liquid Chromatografie (HPLC) methode ontwikkeld. Op deze wijze konden paracetamol en de metabolieten in zowel serum als urine bepaald worden. Deze methode bleek goed te zijn, getuige de retentietijd van de verschillende metabolieten die 1,6 tot 8,0 minuten bedroeg en de verkregen kalibratiecurven, waarin een goede lineairiteit werd gevonden (hoofdstuk II.2).

In hoofdstuk II.3 worden de resultaten beschreven van het onderzoek naar de effecten van een paracetamolzetpil bij te vroeg geboren kinderen. Alhoewel paracetamol al meer dan 100 jaar gebruikt wordt, werd nog nooit op deze wijze en in deze leeftijdsgroep onderzoek verricht.
Achtentwintig kinderen werden, naar de zwangerschapsduur, ingedeeld in twee groepen; een groep van 28-32 weken (N=21) en een groep van 32 tot en met 36 weken (N=7). Zij kregen een eenmalige rectale dosis paracetamol van ongeveer 20 mg/kg

lichaamsgewicht met een spreiding van 16-24 mg/kg ten gevolge van de beschikbare sterktes van de zetpil. Een dosering van meer dan 18 mg/kg gaf bij het merendeel van de kinderen in de jongste leeftijdsgroep maximale serumconcentraties die "therapeutisch" waren.
De verwerking, omzetting en uitscheiding van paracetamol in deze groep te vroeg geboren kinderen bleek adequaat te zijn. Dit gebeurde voornamelijk door sulfatering en niet zoals bij volwassenen het geval is door glucuronidering, getuige de lagere G(lucuronide):S(ulfaat) ratio bij kortere zwangerschapsduur. Tijdens het verdere leven neemt het aandeel van de glucuronidering toe, maar pas na de leeftijd van 7-10 jaar is het aandeel van de glucuronidering in de omzetting van paracetamol hoger dan dat van de sulfatering.
Omdat blijkt dat de uitscheiding van paracetamol in de urine laag is, moet men bij eventuele toediening van meerdere doses paracetamol bij te vroeg geboren baby's een doseringsinterval van meer dan 8 uur aanhouden om eventuele ophoping in het bloed te voorkomen.

Uitbreiding van ons onderzoek van paracetamol naar kinderen die op tijd geboren werden, leidde tot onderzoek naar de farmacokinetiek en farmacodynamiek van meervoudige rectale toediening aan kinderen in deze groep direct na de geboorte (hoofdstuk II.4). Tevens werd het effect onderzocht van een meervoudige dosis paracetamol na een invasieve handeling tijdens de geboorte, namelijk de vacuümextractie (hoofdstuk II.5). Beide studies waren prospectieve klinische onderzoeken. Tien op tijd geboren baby's die opeenvolgend werden opgenomen op de afdeling en die pijn hadden of die pijnlijke handelingen of ingrepen ondergingen, kregen 4 x daags een dosering paracetamol. De serumconcentraties werden in serie bepaald door middel van eerder genoemde HPLC methode en farmacokinetische analyses werden uitgevoerd.
De pijnmeting werd verricht door middel van een gevalideerde pijnscore, een zogenaamde gezichtjesschaal, met gezichtjes die geen pijn uitdrukken variërend tot gezichtjes die erge pijn uitdrukken. De tien pasgeborenen kregen 4 zetpillen van 20 mg/kg lichaamsgewicht, elke 6 uur. De gemiddelde maximale serumconcentraties (standaarddeviatie) tijdens meervoudige toediening waren 10,79 (6,39) mg/l, 15,34 (5,21) mg/l en 6,24 (3,64) mg/l respectievelijk voor de hele groep, voor jongens en voor meisjes. Er was een significant verschil tussen de jongens en meisjes (P=0,01). Wij hebben geen serumconcentraties gevonden die geassocieerd zijn met vergiftiging (meer dan 120 mg/l). De gemiddelde tijd die nodig was om de maximale serumconcentratie te bereiken, was 1,5 uur na de eerste dosis en 15 uur voor alle doseringen. De gemiddelde halfwaardetijd (SD) was 2,7 (1,4 uur in 8 van de 10 patiënten). Er bleek geen correlatie te bestaan tussen de doses en de serumconcentraties en evenmin tussen de pijnscore en de serumconcentraties. Er was een significant omgekeerde verhouding tussen de voorafgaande pijnscore en de maximale serumconcentraties. Meervoudig rectale doseringen van paracetamol in een dosering van 20 mg/kg lichaamsgewicht leidden bij à terme geboren pasgeborenen tot wijd uiteenlopende serumconcentraties, maar leidden niet tot het bereiken van therapeutische spiegels bij alle kinderen. Aangezien er geen accumulatie werd aangetoond, is een dosis van 30 mg/kg gevolgd door doseringen van 20 mg/kg met een doseringsinterval van 6-8 uur, de optimale dosering om therapeutische waarden te

bereiken. Deze nieuwe aanbevelingen komen overeen met recent gepubliceerde aanbevelingen voor oudere zuigelingen en kinderen.
Pijn na een procedurele interventie, werd onderzocht in een groep van 122 kinderen die werden geboren na een vacuümextractie. In de klinische praktijk werd paracetamol in een aantal ziekenhuizen vaak gegeven, echter in andere ziekenhuizen niet toegestaan. In beide omstandigheden waren er geen duidelijk objectieve redenen aan te voeren voor het wel of niet geven van paracetamol. Daarom werd een prospectieve gerandomiseerde dubbelblinde placebo-gecontroleerde studie verricht, met paracetamol in een dosering van 20 mg/kg rectaal elke 6 uur, in vergelijking met een placebo. Hierbij werd geëvalueerd of paracetamol de pijn verlicht, dan wel bestrijdt, bij pasgeborenen die geboren zijn na een vacuümextractie. Daarnaast werd onderzocht of de klinische toestand verbeterde. Kinderen die geboren werden na een vacuümextractie, werden at random ingedeeld in de studiegroep (N=71) en kregen maximaal 4 doses paracetamol rectaal, óf in de controlegroep (N=71) waarbij zij een placebo kregen. De beoordeling van de pijn werd verricht door middel van een gevalideerde gezichtjesschaal én door de klinische conditie te scoren. Beide scores en klinische symptomen zoals spugen, huilen en pijn bij het verzorgen van deze kinderen werden vergeleken met symptomen bij de kinderen in een referentiegroep (N=66). Deze referentiegroep bestond uit kinderen die geboren waren na een ongecompliceerde zwangerschap en een normale bevalling (d.w.z. met het achterhoofd voor) zonder vacuümextractie. Het bleek dat de pijnscores niet verschilden tussen de twee vacuümextractiegroepen, De klinische conditie in de studiegroep verbeterde alleen na de eerste dosis paracetamol in vergelijking met de placebogroep. Er was een significant verschil ($p < 0,05$) in objectieve klinische symptomen in de beide vacuümextractiegroepen in vergelijking met de referentiegroep. Wij concludeerden dat een eenmalige dosis van paracetamol bij pasgeborenen, geboren na een vacuümextractie de klinische toestand duidelijk verbeterde. Dit resulteerde evenwel niet in een significante verandering in objectieve pijnscore. Het geven van herhaalde doses paracetamol had geen effect op de klinische symptomen of de klinische toestand van de pasgeborene. De in deze studie gebruikte dosis paracetamol en de methode die gebruikt werd voor de beoordeling van pijn, lieten geen enkel analgetisch effect van paracetamol bij pasgeborenen, geboren door middel van een vacuümextractie, zien. De paracetamol verbeterde hun klinische conditie echter wel. Naar aanleiding van de studie werden de richtlijnen voor toediening van paracetamol na een vacuümextractie veranderd in toediening van één dosis paracetamol aan alle kinderen vlak na de vacuümextractie; afhankelijk van de pijnscore kan eventuele aanvullende therapie worden gegeven.

In hoofdstuk III worden de onderzoeken naar farmacokinetiek en farmacodynamiek van morfine bij de premature pasgeborene beschreven.
In de eerste plaats werd het effect onderzocht, van een continue toediening van morfine in een dosering van 10 µg/kg/uur, bij kinderen uit verschillende leeftijdsgroepen en werd de bruikbaarheid van de snelle methode om serumconcentraties te bepalen onderzocht, met als doel de doseringen aan te kunnen passen (hoofdstuk III.1). De doelstellingen van de studie waren het bepalen van de concentratie-effect relatie van continue morfine in te vroeg geboren kinderen in de eerste dagen na de geboorte met gebruikmaking van een gevalideerde pijnscore, de

NIPS. De scores vonden plaats zowel gedurende een periode dat geen interventies plaatsvonden als gedurende endotracheaal (door de beademingsbuis heen) uitzuigen. Daarnaast werd onderzocht of een snelle, gemodificeerde Fluorescentie Polarisatie Imuno Assay gebruikt voor het bepalen van morfine serumconcentraties, van nut kan zijn om de morfinedoses aan te passen.
In een prospectief klinisch onderzoek kregen premature beademde neonaten continue morfine (10 µg/kg/uur). Deze pasgeborenen werden verdeeld in drie leeftijdsgroepen (<28 weken, tussen 28 en 32 weken, tussen 32 en 37 weken). Alle kinderen ondergingen verschillende pijnlijke procedures, zoals o.a. percutane intraveneuze catheters of infusen, endotracheaal uitzuigen, en electieve reïntubatie. De morfine serumconcentraties werden bepaald met gebruikmaking van een snelle Fluorescentie Polarisatie Immuno Assay en vervolgens werd farmacokinetische analyse verricht. Pijnscores vóór en ná het begin van de morfinetoediening en gedurende endotracheaal uitzuigen, werden bepaald met behulp van de NIPS.
Van de 24 kinderen die werden geïncludeerd in het onderzoek, waren er 4 jonger dan 28 weken, 14 tussen 28 en 32 weken en 6 ouder dan 32 weken postconceptionele leeftijd.
Morfine werd gevonden in 75-84% van de monsters gedurende de eerste tien levensdagen, en bij sommige kinderen ook nog 12 en 24 uur na staken van de morfine. Alhoewel er gedurende de eerste drie dagen een tendens was van lagere concentraties in de oudere groepen, waren de verschillen in de gemiddelde morfineconcentraties tussen de groepen niet significant.
De gemiddelde morfineconcentratie voor alle kinderen nam toe van 85,1 ± 33,4 µg/L op dag 2 naar 103,3 ± 70,8 µg/L op dag 5. Behalve op dag 2 werd geen relatie van de morfineconcentratie met de postconceptionele leeftijd gevonden. De gemiddelde geschatte klaring was 2,3 ± 1,1 ml/min/kg voor de totale groep en nam toe met de postconceptionele leeftijd. Er werden geen significante verschillen gevonden in klaring tussen de verschillende leeftijdsgroepen. De pijnscores waren significant hoger vóór, dan na morfine ($p < 0{,}001$), en eveneens significant hoger tijdens uitzuigen dan voor of 2 minuten na endotracheaal uitzuigen ($p < 0{,}001$). De pijnscores waren niet gerelateerd aan de morfineconcentraties.
Morfineconcentraties kunnen bepaald worden met behulp van deze snelle methode, maar aangezien er geen correlatie was met pijnscore is deze methode niet geschikt voor een individuele aanpassing van de dosering. Pijnmeting door middel van een pijnscore liet zien dat er in deze studie met de gebruikte doses goede analgesie werd verkregen gedurende de periode van beademing, maar dat er geen concentratie-effect relatie bestond.

Een toenemend aantal onderzoeken naar het effect van de metabolieten van morfine toonde aan, dat morfine-3-glucuronide en morfine-6-glucuronide respectievelijk van groot belang zijn als stimulerende stoffen voor de ademhaling en vanwege de pijnstillende werking. Daarom bestudeerden wij de farmacokinetiek en farmacodynamiek van morfine en zijn metabolieten in een prospectieve gerandomiseerde gecontroleerde studie. Het effect van de twee meest gebruikte doseringsvormen werd vergeleken in beademde premature pasgeborenen. Doel van het onderzoek was om farmacokinetiek en farmacodynamiek van morfine en zijn belangrijkste metabolieten morfine-3 en morfine-6-glucuronide te onderzoeken bij

kinderen in de eerste tien levensdagen. Daarnaast werden de effecten van het gebruik van cardiotonica (middelen die de werking van het hart ondersteunen en de bloeddruk verhogen), onderzocht, evenals het effect van de mate van 'ziek-zijn'. Achtentwintig te vroeg geboren, beademde kinderen die verscheidene pijnlijke handelingen of interventies ondergingen, kregen continue morfine toegediend in een dosering van 10 (groep A) of 15 (groep B) µg/kg/uur. Gedurende een aantal opeenvolgende dagen werden de serumconcentraties voor morfine en zijn metabolieten bepaald met behulp van een HPLC-methode en werden farmacokinetische analyses verricht. De mate van pijn werd bepaald door middel van een gevalideerde pijnscore, de NIPS. Morfine werd gevonden in 80-100% van de monsters en morfine-3-glucuronide in 89-100% van de serummonsters.

Het percentage kinderen, bij wie morfine-6-glucuronide werd gevonden in het serum, nam significant toe van 41% tot 90% gedurende de eerste 9 levensdagen. Verschillen tussen de groepen in gemiddelde serumconcentraties voor morfine en de metabolieten waren niet significant. De gemiddelde morfineconcentratie voor de totale groep nam toe van 58,1 ± 37,6 µg/L op dag 2 tot 63,2 ± 29,1 µg/L op dag 3 en nam hierna af tot 44,3 ± 16,3 µg/L op dag 9. De waarden van morfine-3-glucuronide namen significant toe van 75,4 ± 41,0 µg/L op dag 2, tot 130,8 ± 66,7 µg/L op dag 9 en morfine-6-glucuronide concentraties tenslotte namen toe van 25,1 ± 10,0 µg/L op dag 2 tot 38,4 ± 14,0 µg/L op dag 9. Op dag 7 waren de morfineconcentraties significant hoger bij een hogere postconceptionele leeftijd, op alle andere dagen was er geen relatie tussen de postconceptionele leeftijd en morfine of de postconceptionele leeftijd en de metabolieten van morfine.

Twaalf en vierentwintig uur na staken van de morfinetoediening werd in het merendeel van de kinderen geen morfine of morfine-6-glucuronide meer gevonden. Morfine-3-glucuronide daarentegen werd na 12 uur nog gevonden in 12 van 14 monsters (85,7%) en na 24 uur in 13 van 17 monsters (76,5%), echter in lagere concentraties dan de laatste waarden gedurende morfinetoediening. De gemiddelde metaboliet:morfine serumconcentratie ratio nam significant toe gedurende de eerste 7 dagen, hetgeen een aanwijzing is voor rijping van het morfinemetabolisme. De gemiddelde morfine-3-glucuronide:morfine-6-glucuronide ratio nam niet significant toe.

De gemiddelde geschatte serumklaring was 3,64 ml/min/kg voor de totale groep, zonder dat er een significante correlatie bestond met de postconceptionele leeftijd. Er was een significant hogere klaring in groep B dan in groep A. Zowel het toedienen van cardiotonica of de ernst van de ziekte waren niet van invloed op de morfineklaring of op de morfineconcentraties.

Er was een goed analgetisch effect van morfine. Pijnscores waren vóór het starten van de morfinetoediening significant hoger, dan na starten. Er was eveneens een goede pijnbestrijding vóór en na endotracheaal uitzuigen, maar tijdens uitzuigen waren de pijnscores significant hoger, hetgeen een aanwijzing kan zijn voor matige pijn of voor onwelbevinden. Pijnscores waren niet gecorreleerd met morfine, morfine-3-glucuronide of morfine-6-glucoronide concentraties op enig tijdstip.

Dit is het eerste onderzoek naar de dosis-concentratie-effect relatie van morfine bij kinderen in deze leeftijdsgroep gedurende een periode die langer is dan 72 uur. Wij kwamen tot de conclusie, dat na intraveneuze toediening aan premature pasgeborenen morfine en morfine-3-glucuronide gedurende alle dagen aangetoond kan worden in het

serum, maar dat morfine-6-glucuronide niet bij alle kinderen in de eerste levensdagen gevonden kan worden.
De mate waarin morfine-6-glucuronide kan worden aangetoond, neemt toe met hogere postnatale leeftijd.
Daarnaast concluderen wij, dat ondanks het feit dat er goede pijnbestrijding leek te bestaan, er geen correlatie was tussen de concentratie van morfine en de pijnscore. Een aanvangsdosis van 10 µg/kg/uur is voldoende. Indien tolerantie optreedt, moet om het pijnstillend effect te waarborgen de morfinetoediening worden verhoogd met een hoeveelheid van 5 µg/kg/uur, voorafgegaan door 50% van de oplaaddosis.
Behoudens het optreden van een blaasretentie bij drie kinderen, werden geen bijwerkingen gevonden.

In hoofdstuk IV worden de resultaten van dit proefschrift besproken in relatie tot andere in de litteratuur verschenen artikelen. De resultaten van dit proefschrift dragen in belangrijke mate bij aan ons begrip van pijnbeoordeling en pijnbestrijding bij pasgeborenen. Vervolgstudies zijn nodig om onze kennis over de farmacokinetiek en de farmacodynamiek van analgetica bij pasgeborenen te vergroten. Nieuwe studies met als doel de pijnscores te valideren, zijn reeds begonnen en zouden samen met de eerder genoemde onderzoeken kunnen leiden tot correlaties tussen de meest gebruikte analgetica en pijnscores. Van bijzonder belang is daarbij de preventie van een slechte neurologische uitkomst, door al vroeg morfine te geven aan te vroeg geborenen. Daarnaast is het goed de ontwikkeling van de kinderen te volgen, om eventuele lange termijn effecten op te sporen. Dit kan onderzocht worden tijdens een standaardsituatie, bijvoorbeeld tijdens vaccinatie, waarbij de reactie op pijn wordt bepaald.
De relatie tussen de geneesmiddelenconcentratie in de liquor cerebrospinalis (hersenvocht) en neurofysiologische parameters, alsmede de interaktie tussen het mechanisme van morfine receptoren en het bestaan van polymorfismen van de µ-opioid receptor, zijn belangrijke onderwerpen van huidig onderzoek. De huidige ontwikkelingen doen ons hopen, dat het in de toekomst mogelijk zal zijn om te komen tot het in kaart brengen van de pijngevoeligheid per individu en zo tot een aangepaste pijnbestrijding.

VI

Appendix

Abbreviations

APAP	Acetaminophen, paracetamol
AUC	Area under the serum concentration-time curve
C_{max}	Peak serum concentration
Cl	Clearance
CRIB	Clinical risk index for babies
CST	Contraction stress test
DC	Dose-Concentration
DCE	Dose-Concentration-Effect
DE	Dose-Effect
EEG	Electroencephalogram
ERG	Electroretinogram
ET	Endotracheal suctioning
F	Fraction of drug absorbed
FHR	Fetal heart rate
FLACC	Faces, legs, activity, cry, consolability
FPIA	Fluorescence polarization immuno assay
HELLP	Haemolysis, raised liver functions, low platelets
HPLC	High-performance liquid chromatography
IUGR	Intrauterine growth restriction
MEG	Magnetoelectroencephalogram
M3G	Morphine-3-glucuronide
M6G	Morphine-6-glucuronide
NFCS	Neonatal facial coding system
NICU	Neonatal intensive care unit
NIPS	Neonatal infant pain score
NST	Non-stress test
OPS	Objective pain score
PIPP	Premature infant pain profile
PTD	Pain threshold difference

RDS	Respiratory distress syndrome
SGA	Small for gestational age
T_{max}	Time to reach peak serum concentration
$T_{½}$	Serum half-life of the drug
T_t	Time to reach therapeutic levels
VAS	Visual analog scale
VAS	Vibroacoustic stimuli (I.1)
VE	Vacuum extraction
VEP	Visual evoked potential

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Promoveren, niet direct na je studie, maar wat later in het werkzame leven, geeft wat meer tijd om het een en ander te overdenken. Het nadeel is dan dat je alles uitgebreid en volmaakt zou willen doen, zodat het vaststellen van de datum net dat extra zetje was om alles af te ronden.
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Curriculum Vitae

De schrijver van dit proefschrift werd op 25 mei 1953 geboren te Leeuwarden.
In 1971 werd het eindexamen HBS-B (6-jarig) behaald aan Het Nieuwe Lyceum te Bilthoven.
Van september 1971 tot juli 1980 studeerde hij Medicijnen aan de Rijksuniversiteit te Groningen. Tijdens deze studie werkte hij van 1974 tot 1979 bij de taxi- en ambulancedienst ZTM te Groningen.
Van augustus tot november werkte hij als keuringsarts op de Bloedbank Groningen-Drente, waarna de militaire dienstplicht werd vervuld als officier-arts bij de Koninklijke Luchtmacht op de vliegbases Gilze-Rijen en Leeuwarden.

Vanaf januari 1982 tot april 1985 kreeg hij zijn opleiding tot kinderarts in het Zuiderziekenhuis te Rotterdam (Opleider: Prof. Dr. C.J. de Groot) en aansluitend in het Sophia Kinderziekenhuis/Academisch Ziekenhuis Rotterdam (Opleider: Prof. Dr. H.K.A. Visser).
Op 15 januari 1986 werd hij geregistreerd als kinderarts.
Hierna was hij tot januari 1989 als staflid verbonden aan de afdeling neonatologie van het Sophia Kinderziekenhuis (Hoofd: Prof. Dr. P.J.J. Sauer), waar de opleiding tot kinderarts-neonatoloog plaatsvond.
Van 1 januari 1989 tot 15 mei 1991 was hij als staflid verbonden aan de afdeling neonatologie (Hoofd: Prof. Dr. A. Okken) van de Kinderkliniek van het Academisch Ziekenhuis Groningen (Hoofd: Prof. Dr. H.S.A. Heymans).

Sinds 16 mei 1991 is hij werkzaam als kinderarts-neonatoloog binnen de maatschap van Zwolse Kinderartsen in het Sophia Ziekenhuis en later de Isala klinieken te Zwolle.