Review:

Ontogeny of oral drug absorption processes

in children

Authors' list

Name	Academic qualification	Title
M.G. Mooij	MSc/MD	Junior research fellow
B.A.E. de Koning	MD, PhD	Pediatrician, fellow gastroenterology
M.L. Huijsman		Medical Student
S.N. de Wildt	MD, PhD	Pediatric intensivist, clinical pharmacologist

Correspondence

Mail: m.mooij@erasmusmc.nl	Telephone: 0031(0)1040736889

Keywords

Administration, oral; pharmaceutical preparations; absorption; child.

Abstract

Introduction: A large proportion of prescribed drugs to children is administered orally. Age-related change in factors affecting oral absorption can have consequences for drug dosing.

Areas covered: For each process affecting oral drug absorption a systematic search has been performed using Medline to identify relevant articles (from inception till February 2012) in humans. This review presents the findings on age related changes of the following processes affecting oral drug absorption: gastric pH, gastrointestinal motility, bile salts, pancreatic function, intestinal pH, intestinal drug metabolizing enzymes and transporter proteins.

Expert opinion: Clinicians should bear in mind the ontogeny of oral drug absorption processes when prescribing oral drugs to children. Our review shows large information gaps on almost all drug absorption processes. We present exciting future approaches aimed to reduce these gaps: 1. a drug dissolution/solubility intestinal model (TIM) to study differences in oral formulations simultaneous with age-related changes. 2. Generation of in vitro data on the intestinal ontogeny of drug metabolizing enzymes or transporter proteins. 3. Using these and existing data for population and physiology-based pharmacokinetic modeling. 4. (Labeled) microdosing studies to determine developmental changes in oral bioavailability in vivo.

Keywords

Administration, oral; pharmaceutical preparations; absorption; child.

Article highlights

- After a peak for the first few minutes postnatally, gastric pH remains around a value of 2-3 in children of all ages in the unfed state.
- Gastric emptying in infants appears to be faster than in adults until the age of 3 years.
- Proximal intestinal (duodenal) motor activity matures throughout the first weeks of life, whereas antral motor activity does not.
- Although there are clear clues of an age-related pattern for some drug metabolizing enzymes and transporter proteins in the intestine, the exact ontogeny still remains to be elucidated.
- In vitro studies (tissue drug metabolizing enzymes and transporters), and modeling and simulation using TIM, population pharmacokinetic (popPK) or physiology-based pharmacokinetic (PBPK) modeling are highly needed to increase our understanding on pediatric oral drug absorption.
- An important challenge is to conduct in vivo research, to validate these study findings, for example by using stable isotopes or microdosing to elucidate the ontogeny in oral drug absorption and to dose children adequately.

1. Introduction: orally administered drugs in children

A large proportion of drugs prescribed to children is administered orally [1]. Absorption of orally administered drugs may be affected by extrinsic factors (food and formulation) and intrinsic factors of a physiological nature. The latter includes volume of gastrointestinal fluids, the pH and buffer capacity of these fluids, contraction patterns, gastrointestinal transit, digestive enzymes, intestinal cellular transporters, drug metabolism enzymes, and intestinal bacterial flora [2]. Solubility and intestinal permeability of the individual drug will influence the impact of gastrointestinal (GI) processes on its absorption. A theoretical-based oral drug classification based on solubility and permeability characteristics of drugs, such as the biopharmaceutics classification system (BCS), may serve to predict which extrinsic or intrinsic variables will alter oral drug absorption [2-3].

As many of the GI processes change with age, oral drug absorption expectedly will change with age as well [4]. The current EU and US regulations aimed at stimulating the study of drugs across the pediatric age range, have given an impetus to promoting clinical trials in children [5-6]. Age-specific information on the processes governing drug disposition in children is needed for modelling and simulation approaches. Important progress has been made to elucidate age-related changes in drug hepatic drug metabolism and renal excretion [7-8]. In contrast, our knowledge on developmental changes in the GI processes involved in oral drug absorption is far less developed [4, 9-11].

The aim of this review is to present the available data on age-related variation in GI processes that govern oral drug absorption processes. We performed a systematic search in the literature using Medline. Reference lists of relevant retrieved papers were screened for additional relevant articles. We discuss current information gaps and provide suggestions for future research that may lead to develop evidence-based dosing guidelines for oral drugs in children.

2. Age-related changes in oral drug absorption processes

2.1. Gastric pH

Gastric pH is an important factor determining the stability of a drug passing through the stomach. Studies on gastric pH across the pediatric age range used pH measurement of gastric fluid aspirates and 24-hour intragastric pH monitoring [12-29]. Figure 1 displays the mean and median gastric pH values in healthy children in the first three months of life.

The mean gastric pH in newborns minutes after delivery is 7.05 and within a few hours it declines to a pH of 2.7 [13, 28]. A less acidic stomach environment in these newborns after delivery is most likely explained by swallowing of amnion fluids, which is supported by the decrease in pH within a few hours after birth [28]. More than seventy years ago Miller observed a decrease in acidity over the first 10 days of life [30]. Many more recent studies report that the gastric pH declines already within a few hours after birth [13, 28]. Miller titrated gastric juice with NaOH and then determined the amount of HCl as a measure of acidity. However, Miller did not provide information on the acidity of the primary gastric content; therefore we are unable to translate this outcome to pH. Many other studies subsequently showed that the gastric pH remains low at a pH around 2 and 3 in children of all ages [12, 14-27, 29].

More rarely, gastric pH can be described in terms of the proportion of time it peaks above 4 measured over 24 hours. In preterm infants, this proportion ranged from 46% to 70% over a 24-hour period [31-32]. In children up to two years of age it was around 51%; in older children it was 34% [33]. The higher proportion in younger children might be explained by the buffering effects of milk formula, older children are less frequently fed and receive more solid foods [23, 25, 33]. 24h-pH monitoring reflects the buffering effect as well; in preterm

infants the gastric pH first increased to 7 postprandially, but then immediately steadily declined to a pH of 2 [23]. Another study showed a similar pattern with a mean gastric pH returning to a value of 1.8 within 180 minutes postprandially [25]. Apparently, during the day younger children tend to have more often a basic gastric environment than older children, although the mean gastric pH remains around 2 or 3 in children of all ages.

Interestingly, this overview gives reason to contradict the widespread notion that absorption of gastric pH dependent drugs in both neonates and young infants is affected by high gastric pH [34-35]. Gastric pH may be high due to continuous enteral feeds, but is comparable to adult values when oral feedings are given at longer than 3 hour intervals. Children older than one week of age will typically receive such intermittent feeding, especially during the night. We have not been able to identify studies that compared effect of different feeding regimes (continuous and intermittent) on drug absorption in neonates.

The consequences of changes in gastric pH are relevant for acid labile drugs. These may be absorbed more efficiently in a higher gastric pH environment achieved by very frequent or continuous feeding regimens. Huang et al found that serum penicillin levels in premature and term newborns were higher than those in infants and children. These age-related changes were hypothesized to be either due to higher gastric pH in the first 10 days of life or to altered renal function [36].

2.2. Gastrointestinal motility

2.2.1. Gastric emptying

Next to intestinal motility, gastric emptying is a primary determinant of the rate at which drugs are presented to the small intestinal mucosa for absorption. Gastric emptying is usually measured by the following methods: gastric emptying breath test, scintigraphic procedure by

Technetium-99M liquid gastric emptying scan or the paracetamol absorption test. Gastric emptying time is reported in various ways: gastric emptying time, gastric half-emptying time or residual gastric activity at 1 hour.

We identified three studies using the gastric emptying breath tests. Hoekstra et al tested the effect of glucose and fructose on gastric emptying using an L-glycine-1-^{13}C breath test in four healthy children (mean age 14.3 years; age range 12.1 – 16.0 years). Gastric half emptying time after fructose intake was 45.5 minutes (SD 4.9); after glucose intake 64.3 minutes (SD 2.4). Gastric half emptying time was significantly longer when fructose and glucose were administered together, that is 85.3 minutes (SD 7.0) [37]. The authors considered all values to be in the normal ranges established with other methods. Using the 13C-octanoic acid breath test, Perri et al found a gastric half emptying time of mean 121 minutes (SD 42) in nine healthy control patients (mean age 9 years; age range 4-16 years). These children had eaten a standard test meal (bread, ham, juice, egg), however, which hardly compares to a smaller fructose and/or glucose administration [38]. Hoffman et al subjected 22 patients with gastroesophageal reflux symptoms (mean age 13.2 years) to the 13C-octanoic acid breath test and compared half emptying results between patients with or without pathologic acid exposure (84 (SD 24) vs. 86 (SD 26) minutes) and with or without duodenogastroesophageal reflux (105 (SD 47) vs. 76 (SD 24) minutes) were compared. Results were not statistically significantly different [39]. In conclusion, breath test measurements for gastric emptying rate are highly variable and, probably for practical reasons, that tests have only been performed in relatively older children.

Scintigraphic imaging makes it possible to measure the gastric emptying time or gastric half emptying time as well as the residual gastric activity at one hour. We identified seven useful scintigraphy studies. In ten preterm infants (median gestational age 28.9 weeks; range 26-33) the median gastric half-emptying time at a postnatal median age of 9 days (range 6-37) was

60 minutes (30-180 minutes) [40]. Patients were all hourly fed although not receiving a standard meal size. The residual gastric activity at one hour was 37.5% (range 19-100%) [40]. Di Lorenzo and colleagues conducted a study in 477 patients across a wide age span; 291 patients with and 186 patients without gastroesophageal reflux disease (GERD) (based on pH and/or scintigraphy investigations) [41]. In children without bolus or acidic gastroesophageal reflux, gastric residual activity at one hour was around 65% in those up to 3 years of age; it decreased to 51% in the age group 4-6 years; and to 45% in children over 6 years of age [41]. Seibert et al reported an opposite outcome in children being evaluated for gastroesophageal reflux (GER); the percentage emptied at one hour instead of the percentage residual activity [42]. The percentage emptied at one hour was 48% (SD 16) in 44 infants (mean age 5.7 months, range 1-23) and 51% (SD 7) in eight children (mean age 9.1 years, range 2-14.5 years). When converted to residual gastric activity, values are still comparable (respectively 52% and 49%). Note that in the study reported by Di Lorenzo et al a delay in gastric emptying was not related to GER symptoms until the age of 6 years [41]. Cucchiara et al studied a poorly described control group suffering from diarrhoea and failure to thrive not related to gastrointestinal symptoms. The gastric emptying activity at one hour was 38.1% (SD 6.5) [43]. Miele et al reported a 43.3% (SD 8.7) gastric emptying activity at one hour in a control group of 11 children without gastrointestinal or neurologic disorders (mean age 5.6 years; SD 3.9 years; range 2-12 years) [44]. Describing gastric emptying alternatively as a mean emptying half time, Yahav et al reported 87.8 minutes (SD 22.9) mean gastric emptying time in a control group with a mean age of 10.4 months for which no other details are reported [45]. Demirbilek et al found an average gastric emptying time of 51.6 minutes (SD 8.04) in a selected group of children with GERD (mean age 3.2 years; SD 1.1); the selection might have resulted in bias [46].

To relate these results to adults, reported healthy adult gastric emptying times range between 56 (32- 85) and 104 (49-126) minutes, for liquid and solid markers respectively [47-48].

Finally, the paracetamol absorption test was used in two small cohorts. In 15 critically ill patients (median age 5.3 years; interquartile range 1.2-6.5) who were food tolerant, it revealed a median 1.5 (interquartile range 0.7-2.2) ratio of time to reach paracetamol peak to the maximum paracetamol concentration (Tmax/Cmax) [49]. In 7 adolescents (mean age 16.4 years; SD 0.7 years; range 15.5-17.5) it revealed a paracetamol absorption ratio of 1.4 for high fat meals and 0.5 for low fat meals [50]. The evidence of these two studies is too limited to conclude on age-related changes.

However, population pharmacokinetic analysis applied in another study yielded a significantly lower oral paracetamol absorption rate in the first days of life before stabilizing after one week [51]. The lag time reflects the time to reach and permeate the absorbing surface of the intestine [2]. Considering that a lag time was observed after oral paracetamol administration only and not after rectal administration, it suggests that gastric emptying may be the primary determinant of a lag time for oral absorption of paracetamol.

2.2.2. Antroduodenal contractions

Antroduodenal motor activity plays a role in the gastric emptying next to fundic contraction, pyloric sphincter relaxation and intestinal motor activity. It can be determined by antroduodenal manometry which measures intraluminal pressures of the distal stomach and the proximal small bowel.

Fasting antral motor activity and antral motor activity in response to intraduodenal feeding did not significantly differ between term and preterm infants [52]. In contrast, the proportion of antral clusters temporally associated with duodenal activity in preterm infants was significantly lower than that in term infants. Moreover, the degree of association of antral and

duodenal activity increased significantly with gestational age [53]. In preterm infants 29 to 32 weeks of gestational age, the frequency of contractions, the number of contractions per burst, and the intraluminal peak pressure of duodenal motility during contractions all increased with postgestational age, resulting in a more efficient motility [54]. Similarly Bisset et al reported that both the magnitude and organization of motor activity increased with increasing gestational age [55]. Berseth et al reported shorter lasting individual duodenal cluster activity during fasting periods in preterm than in term infants, but duodenal motor activity in response to feeding increased similarly in both groups [52]. The timing of introducing food seems to influence the preterm neonates' (28-32 weeks of gestational age) duodenal motor activity; introducing formula early (day 3-5 postnatally) resulted in more mature motor complexes than introducing formula late (day 10-14 postnatally) [56]. Preterm infants showed more immature duodenal motor activity response to bolus feeding then did term infants [57].

In conclusion, proximal intestinal (duodenal) motor activity in contrast to antral motor activity matures throughout the first weeks of life, with increasing frequency, amplitude, and duration of propagating contractions. Regrettable there are no such studies in healthy children beyond the newborn period.

2.2.3. Intestinal transit time

Overall, gastrointestinal motility can be expressed as orocecal transit time (OCTT). This can be measured by different techniques: hydrogen breath test, 13C Ureide breath test, radiotransmitting capsule, red carmine marker test or scintigraphy. Most common is the hydrogen breath test with lactulose as nonabsorbable carbohydrate substrate. This breath test has limited use in the general population, which may include hydrogen-non-responders. Also, lactulose may accelerate transit time by its osmotic laxative effect. Accordingly, Vajro et al reported in 11 control patients that the mean OCTT after a meal was significantly longer than

that after lactulose [58]. Although this method can be used to compare groups in standardized studies, it is merely an approach to the physiological situation of intestinal motility.

We identified four studies using the hydrogen breath test to measure OCTT in different pediatric age groups [58-62]. The populations were quite heterogeneous, but there does not appear to be an age-related difference in OCTT. In the whole age range from 1 to 17 years the mean OCTT was roughly between 60 and 110 minutes, as in adults [62]. The mean OCTT measured by the lactose-^{13}C-ureide breath test was 255 minutes (range 165-390) in children from 3 to 17 years of age [59]. This method cannot be used in infants below 6 months of age as they lack the intestinal bacterial enzymatic activity. In adults the latter test was validated in respect to scintigraphy [63]. The lactulose-H2 breath test yielded a significant shorter OCTT than did the labeled ureide test, which may be due to the effect of lactulose [64]. Fallingborg et al distinguished small intestinal and colonic transit times with the use of a radiotransmitting capsule in a small population of 12 healthy children (8 to 14 years) [65]. Small intestinal transit time was 7.5 hours and colonic transit time was 17.2 hours. Interestingly, from the number of observations in each segment they estimated that the capsule resided in the duodenum for 8% of the small intestinal passage; in the proximal part of the small intestines for 5%; in the mid part for 12% and in the distal part for 75%. The small intestinal transit time of 7.1 hours is considerably longer than that established by the breath tests. The fact that the capsule, which was larger than 2 mm, was located in the distal part of the terminal ileum for 75% of the small intestinal transit time suggests a longer ileo-cecal transit for large particles. By means of scintigraphy Bodé et al measured a mean OCTT of 3.1 hours (range 1.3-6.1 hours) in nine premature infants (mean gestational age 28.9 weeks) [40].

2.3. Bile acids

Bile is a complex secretory product produced by the liver. It eliminates waste products from the body and it promotes digestion and absorption of lipids by the intestines. In preterm neonates the concentration of the bile acids was found to be 4.55 mmol/l in the first few weeks postnatally [66]. In 65 healthy preterm newborns the total bile acid concentration was consistently higher in those fed with human milk in comparison with those fed with formula. Concentrations did not significantly increase over a 3 week follow-up period [67]. Concentration did not differ between small and appropriate for gestational age premature infants [66]. Challacombe et al compared three age groups, i.e. 2 days postnatal (n=12), 2 to 7 days (n=8), and 10 days to 7 months (n=14). Gestational ages were not documented. The total bile acid concentration in the oldest group was much higher than that in both other groups, and at a value comparable to those in adults [68].

Changes in biliary function can influence solubilisation and consequently absorption of lipophilic drugs [3].

2.4. Pancreatic function

The exocrine pancreas is a specialized secretory gland, which secretes juice rich in HCO_3^- and digestive enzymes that neutralizes the acidic gastric contents and help digest food. Functioning of the exocrine pancreas is typically measured by the fecal Elastase-1(E-1) concentration. The E-1 enzyme is highly specific for the pancreas and is not degraded during the intestinal passage. Age-related differences in E-1 concentrations were absent in a large cohort of healthy subjects (mean age 11.2 years (SD 0.5); age range 2 months to 52 years) [69]. Even as many as 96.8% of preterm and term infants up to the age of 12 months without known bowel or pancreatic disorders had adult E-1 values after 2 weeks of life, independent of gestational age [70]. However, up to 48 hours after birth none of the preterm infants had a fecal E-1 concentration of greater than 30 microgram/gram meconium, whereas 43% of the

term infants had normal adult values. This discrepancy may be due to either immaturity or insufficiency of the exocrine pancreatic function in premature neonates. However, the small sample size did not allow differentiating between these two possible causes. Deficient exocrine pancreas function as seen in cystic fibrosis patients was associated with lower oral bioavailability of mycophenolate mofetil [71]. This suggests an effect on oral drug absorption in neonates with immature pancreas function, but this has not been studied to date to our knowledge.

2.5. Intestinal pH

In comparison with gastric pH, remarkably little is known about the intestinal pH in children. Fallingborg et al measured gastrointestinal pH with a radiotransmitting pH-sensitive capsule in twelve healthy children aged 8-14 years. The mean value of pH rose from 1.5 in the stomach to 6.4 in the duodenum; in the distal part of the small intestine it reached an alkaline peak value of 7.4. The pH profile was almost identical to that in healthy adults. A broad conclusion on the development of the intestinal pH cannot be drawn as his small population consisted merely of older children. It would be worthwhile to repeat the experiment in other age groups [65].

2.6. Intestinal drug metabolism

Many developmental changes in hepatic drug metabolism and renal clearance have been well documented. Data on the ontogeny of intestinal metabolism remain scarce. What is known is that enzymes of the cytochrome P450, especially the 3A (CYP3A) subfamily are abundant in liver and gut and contribute to the first-pass metabolism of many orally administered drugs in adults [72]. Hepatic CYP3A forms present a developmental expression in fetal and pediatric

samples; CYP3A4 and CYP3A7 expression levels show to be age-dependent with respectively increasing and decreasing levels of total CYP3A expression levels [73].

CYP3A ontogeny can be reported as changes in mRNA, protein or activity level. We identified two in vitro studies on CYP3A ontogeny in the intestine. One studied fifty-nine histologically normal duodenal biopsies from children aged 1 month to 17 years for CYP3A mRNA by quantification and CYP3A proteins localization by immunohistochemistry [74]. The other studied duodenal biopsies and surgical sections from 104 children aged 2 weeks to 17 years and 11 foetuses for CYP3A protein expression by immunohistochemistry and activity by the formation of 6beta-hydroxytestosterone from testosterone [75]. CYP3A4 and CYP3A5 mRNA expression levels were to decrease with age. Showing expression levels were high in the first year of life and decreased thereafter [74]. This is in contrast with protein expression levels reported in the second study showing CYP3A protein expression significantly increased with age [75]. The discrepancy of decreasing mRNA expression and increasing protein levels with age might reflect a posttranscriptional regulatory mechanism that is not elucidated to date according to the authors [74]. Dissociation between protein and mRNA levels during the maturation process was already reported for CYP2D6 liver enzymes [76]. The location of the CYP3A protein in enterocytes assumes a maturation profile occurs. In the duodenal biopsies of children less than 6 months of age, CYP3A protein was detected in only half of the enterocytes, in the older children, however, CYP3A protein was expressed in all cells [74]. Moreover, the increase in CYP3A protein levels with age is mirrored with increasing CYP3A4 activity. It changes from undetectable in fetal samples, low in neonates and adult levels in children older than 5 years of age, as reflected by 6beta-hydroxytestosterone formation [75].

Intestinal CYP3A4, CYP3A5 mRNA levels have been established in pediatric liver recipients (age 0.1-15 years) at the time of transplant surgery [77]. Unfortunately the authors did not

study the effect of age within their cohort. Adult data show similar CYP3A4 and CYP3A5 expression levels [78]. This suggests that intestinal CYP3A expression does not change beyond childhood. However, because the range of levels reported in children was very wide, age-related changes from 0.1 year of age onwards can not be excluded [78]. Intestinal CYP3A5 mRNA levels were significantly higher in *CYP3A5*1* gene carriers (expressors) than *CYP3A5*3* homozygous patients (non-expressors) and observed in both the children and adult study. In *CYP3A5*1* carriers, CYP3A5 mRNA accounted for 20-30% of all CYP3A mRNA detected [77-78].

In vivo studies on oral bioavailability of CYP3A substrates in relation to age are scarce. Our own research showed that median midazolam oral bioavailability in preterm infants (28-32 weeks, <10 days of age) is significantly higher than in adults (50% vs. 30%) [79-81]. This likely reflects developmentally low intestinal and hepatic CYP3A activity, as midazolam is a validated probe drug for CYP3A4/5 activity.

Interestingly, the type of feeding (breast milk or formula) seems to impact the developmental pattern of combined intestinal and hepatic CYP3A in neonates. In children who received oral dextromethorphan six times between two weeks and 6 months of age, the urinary metabolite/dextromethorphan ratio as a measure of CYP3A4 activity clearly increased over this period. Moreover, this increase was faster for formula vs. breastmilk fed children [82]. This finding suggests a differential effect of components of these milk formulations on the induction of intestinal and hepatic CYP3A activity in the first months of life.

The ontogeny of other drug metabolizing enzymes in the intestine remains to be elucidated.

2.7. Intestinal drug transporter

Multidrug resistance protein 1 (MDR1/P-glycoprotein) is a plasma membrane glycoprotein acting as an efflux system. Based on in vitro studies it is currently considered the most

prominent gut transporter [83]. It is located in many tissues and specifically within the brush border in the small intestine. Its expression is genetically controlled by the *ABCB1* gene [84]. MDR1 action in the enterocyte reduces the bioavailability of orally administered drugs as these are expelled into the intestinal lumen. MDR1 protein can be localized by immunohistochemistry and mRNA quantification in intestinal tissue. In the earlier mentioned study evaluating 59 duodenal biopsies of children aged from 1 month to 17 year, MDR1 mRNA expression was highly variable and not related to age [74]. MDR1 protein was detected in all the enterocytes and was located on the apical surface. In the biopsies in children younger than 3 years, additional staining was located on a limited upper part of the lateral surface.

A possible age effect in relation to of the *ABCB1* genotype was found for oral bioavailability of the MDR1 substrate cyclosporine. 104 children with renal disease (age 0.36-16.3 years) were grouped by age and genotyped for *ABCB1* gene. The pre-hepatic extraction ratio of cyclosporine was *ABCB1* genotype dependent only in children older than 8 years, resulting in corresponding differences in oral bioavailability. No such association was found in younger patients, which suggests an interaction of age and genotype on MDR1 activity [85].

In the context of the previously mentioned study in pediatric liver recipients, MDR1 mRNA was determined as well and results were similar as for CYP3A, i.e. the median MDR1 mRNA expression did not differ between children and adults, but widely ranged in the pediatric population [77-78].

Interestingly, in noninflamed duodenal biopsies of children with Crohn's disease, MDR1 mRNA expression was significantly higher than that in normal biopsies. Expression of MDR1 was highly variable in both groups [86]. The effect of age was not examined in this study. The higher levels of MDR1 expression could have been induced by systemic inflammation present

in Crohn's disease, which is likely to lead to an elevated first pass metabolism of xenobiotics used in the treatment.

To our knowledge the intestinal ontogeny of other members of the ATP binding cassette transporters, such as multidrug resistance protein 2 (MRP2/ABCC2) or breast cancer resistance protein (BCRP/ABCG2), has not been studied to date [83, 87-89].

3. Conclusion

This literature review makes clear that GI processes that govern drug absorption change from the neonatal period up to adulthood. Consequently these changes could have an impact on drug absorption depending on the drug characteristics [3-4]. The review also brought to light important knowledge gaps regarding these processes and especially their impact on drug absorption.

Key findings in the research done so far are the following. Apart from a brief peak postnatally the gastric pH is about 2-3 in children of all ages. Postprandial its rise is due to the buffering effect of milk-based feeding. Especially in frequently fed neonates the pH may therefore be higher for a longer period during a 24 hour period, then in older children who eat less frequently. Gastric emptying time reported in the literature is highly variable. Standard gastric emptying tests do not reveal evident age-related changes. Population pharmacokinetic analysis shows a markedly paracetamol absorption decrease in the first few days of life, which suggests delayed gastric emptying. This delay could perhaps be explained by maturation of antroduodenal contractions.

There are no studies done examining antroduodenal contractions beyond the neonatal period. Intestinal transit time (in terms of mean OCTT) does not appear to be subject to age-related changes; it is roughly between 60 and 110 minutes in the age range of 1 to 17 years, as

measured by hydrogen breath test. The one study that used a capsule to measure OCTT showed a much longer transit time than any of the other studies using the lactulose breath test. This finding suggests that this latter test cannot be used to determine an absolute OCTT. It probably rather measures intra-individual changes in OCTT or differences between cohorts. A developmental change in biliary function appears to be present, with bile acids concentration reaching adult values around the age of four years. Pancreatic function appears to be sufficient in the large majority of healthy newborns, independent of gestational age. Intestinal pH has only been studied in a cohort of older children. Adult values were found in this cohort; therefore possible age-related changes remain to be elucidated. For a large proportion of drugs there seems to be a developmental pattern in CYP3A, which is the most important drug metabolizing enzyme in the intestines. CYP3A protein and activity levels were found to increase with age. These in vitro data are in line with higher oral bioavailability of midazolam in premature children compared to adults. Evidence on possible age-related effect on MDR-1 activity is contradictory and not elusive yet.

4. Expert Opinion

4.1 Information gaps

In summary, the main information gaps on the ontogeny of GI processes governing oral drug absorption have not yet been bridged. We need more knowledge on intestinal transit time, intestinal pH and the ontogeny of intestinal drug metabolizing enzymes and drug transporter proteins. The ultimate goal of research efforts in this field should be to predict more precisely the oral disposition of drugs in children across the pediatric age range. Below we describe some research approaches, both in vitro and in vivo, which are promising for future research to provide a better understanding of oral drug absorption in children.

4.1 In vitro drug dissolution/solubility model (TIM)

The Dutch Institute of Innovative Research has developed the TNO Gastro-Intestinal Tract model (TIM), a computer controlled dynamic system which mimics the physiological human conditions in stomach and intestines [90-91]. Parameters such as pH, temperature, peristaltic movements, transit time, secretion of digestion enzymes, bile and pancreatic juices can be adjusted. Intraluminal processing of drug dosage forms, including transit, release and dissolution can be simulated [92]. Removal of dissolved drug molecules from the intestinal compartments allows assessing the fraction of drug potentially available for small intestinal absorption [91]. This model has been extensively validated to simulate these processes in adults. It appears an interesting approach to test oral drug absorption in conditions with typical age-related physiological characteristics. Especially, the additional impact of existing oral formulations frequently given to children can be studied. It may also help to study the effect of drug manipulations to enhance drug ingestion by children (e.g. dissolving tablets in apple juice, apple sauce, ‘hiding’ in regular food, crushing, et cetera). Representative drugs of the different BCS classes can also be studied systematically for dissolution and solubility.

4.2 In vitro drug metabolism and transporter studies

The extensive studies on in vitro hepatic drug metabolism, for example by the group of Hines and colleagues could serve as an example [93]. Similarly, the ontogeny of drug metabolizing enzymes and transporters should be studied in intestinal samples from the different parts of the intestine and from children across the pediatric age span. New methods are quickly becoming available, not only to study drug transporter expression (mRNA) but also protein content, using sensitive LC-MS-MS methods.

4.3 Modeling and simulation: PB-PK models and population PK

The available data on age-related changes in relevant GI processes as well as possibly those from the TIM simulations can be incorporated in population based pharmacokinetics (PB-PK) software programs such as Simcyp®, PKsim® or GastroPlus®. These programs can then simulate fate of drugs given to children of different ages and provide guidance for age-appropriate dosing.

At this time, the usefulness of these programs is still hampered by the relative lack of physiological data across all age groups. Moreover, validation of the model is also still limited as we have little pharmacokinetic data to validate the model in especially the neonatal and infant age groups are scarce [94]. It is to be expected that increasing use of these programs will generate sufficient data to further validate the models.

4.4 Mechanism-based approach for in vivo studies

Another approach to learn more about the ontogeny of specific (intestinal) drug metabolizing enzyme and/or transporter pathways is a mechanism-based one [95]. The pharmacokinetics of drugs that represent a single pathway, studied in children of all ages, may provide valuable information on the ontogeny of that specific pathway. For example, determination of the plasma clearance of midazolam is a validated and widely used method to study interindividual variation in CYP3A activity in both adults and children [96].

To elucidate age-related changes in intestinal enzymes/transporters, independent of hepatic activity, we will need both oral and intravenous pharmacokinetic data, preferably from the same patients. At this time these data are scarce in children, even for CYP3A/midazolam. Full PK studies to determine bioavailability for a probe drug using a multi-day cross-over design are hardly feasible in children for ethical and practical reasons. As a major reason, children will not benefit from the drug but rather will experience the drug effect and risk adverse

events and have significant burden. Alternatively a stable-labeled isotope or a (very weak) radioactive-labeled microdose can be used [97-98]. In both a labeled probe drug is added to an intravenous therapeutic dose. Parent compound and metabolites can therefore be traced in serum and urine. This enables simultaneous determination of the pharmacokinetics of therapeutic IV and the labeled oral dose. It eliminates the risk of therapeutic effect/toxicity as the child already receives the drug for clinical reasons. A prerequisite for the use of microdosing in this context is that dose-linearity exists across the dosing range. For a number of drugs dose-linearity for microdosing has been established, whereas others clearly do not qualify [99-100].

Microdosing is a relatively novel technique used in adults. The microdose (one-hundredth of the predicted pharmacologic dose or 100 micrograms) contains a natural occurring radioactive carbon label (carbon 14, ^{14}C) which can be detected with highly sensitive methods as accelerator mass spectometry (AMS) [99]. Developmental changes in intestinal drug metabolizing enzymes can be delineated by investigating multiple age groups. Microdosing has been used once in preterm infants in a small pharmacokinetic study of ursodiol in the United States [101].

References

1. Schirm E, Tobi H, de Vries TW, Choonara I, De Jong-van den Berg LT. Lack of appropriate formulations of medicines for children in the community. Acta Paediatr 2003 Dec;92(12):1486-9.

2. Atkinson AJ, Abernethy DR, Daniels CE, Dedrick RL, Martkey SP. Drug Absorption and Bioavailability. *Principles of Clinical Pharmacology*. 2nd ed: Academic Press 2007:37-58.

3. Martinez MN, Amidon GL. A mechanistic approach to understanding the factors affecting drug absorption: a review of fundamentals. J Clin Pharmacol 2002 Jun;42(6):620-43.

4. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental pharmacology--drug disposition, action, and therapy in infants and children. N Engl J Med 2003 Sep 18;349(12):1157-67.

5. Jacqz-Aigrain E. Drug policy in Europe Research and funding in neonates: current challenges, future perspectives, new opportunities. Early Hum Dev 2011 Mar;87 Suppl 1:S27-30.

6. FDA. U.S. Food and Drug Administration. [cited; Available from: http://www.fda.gov/ScienceResearch/SpecialTopics/PediatricTherapeuticsResearch/default.htm

7. Hines RN, McCarver DG. The ontogeny of human drug-metabolizing enzymes: phase I oxidative enzymes. J Pharmacol Exp Ther 2002 Feb;300(2):355-60.

8. Smits A, Kulo A, de Hoon JN, Allegaert K. Pharmacokinetics of Drugs in Neonates: Pattern Recognition Beyond Compound Specific Observations. Curr Pharm Des 2012 Feb 27.

9. Strolin Benedetti M, Whomsley R, Baltes EL. Differences in absorption, distribution, metabolism and excretion of xenobiotics between the paediatric and adult populations. Expert Opin Drug Metab Toxicol 2005 Oct;1(3):447-71.

10. Yokoi T. Essentials for starting a pediatric clinical study (1): Pharmacokinetics in children. J Toxicol Sci 2009;34 Suppl 2:SP307-12.

11. Bowles A, Keane J, Ernest T, Clapham D, Tuleu C. Specific aspects of gastro-intestinal transit in children for drug delivery design. Int J Pharm 2010 Aug 16;395(1-2):37-43.

12. Cote CJ, Goudsouzian NG, Liu LM, Dedrick DF, Szyfelbein SK. Assessment of risk factors related to the acid aspiration syndrome in pediatric patients-gastric ph and residual volume. Anesthesiology 1982 Jan;56(1):70-2.

13. Datta S, Houle GL, Fox GS. Concentration of lidocaine hydrochloride in newborn gastric fluid after elective caesarean section and vaginal delivery with epidural analgesia. Can Anaesth Soc J 1975 Jan;22(1):79-83.

14. Goresky GV, Finley GA, Bissonnette B, Shaffer EA. Efficacy, duration, and absorption of a paediatric oral liquid preparation of ranitidine hydrochloride. Can J Anaesth 1992 Oct;39(8):791-8.

15. Jahr JS, Burckart G, Smith SS, Shapiro J, Cook DR. Effects of famotidine on gastric pH and residual volume in pediatric surgery. Acta Anaesthesiol Scand 1991 Jul;35(5):457-60.

16. Kelly EJ, Chatfield SL, Brownlee KG, Ng PC, Newell SJ, Dear PR, et al. The effect of intravenous ranitidine on the intragastric pH of preterm infants receiving dexamethasone. Arch Dis Child 1993 Jul;69(1 Spec No):37-9.

17. Kelly EJ, Newell SJ, Brownlee KG, Primrose JN, Dear PR. Gastric acid secretion in preterm infants. Early Hum Dev 1993 Dec 31;35(3):215-20.

18. Kraus G, Krishna DR, Chmelarsch D, Schmid M, Klotz U. Famotidine. Pharmacokinetic properties and suppression of acid secretion in paediatric patients following cardiac surgery. Clin Pharmacokinet 1990 Jan;18(1):77-81.

19. Maekawa N, Mikawa K, Yaku H, Nishina K, Obara H. Effects of 2-, 4- and 12-hour fasting intervals on preoperative gastric fluid pH and volume, and plasma glucose and lipid homeostasis in children. Acta Anaesthesiol Scand 1993 Nov;37(8):783-7.

20. Miller BR, Tharp JA, Issacs WB. Gastric residual volume in infants and children following a 3-hour fast. J Clin Anesth 1990 Sep-Oct;2(5):301-5.

21. Nishina K, Mikawa K, Maekawa N, Tamada M, Obara H. Omeprazole reduces preoperative gastric fluid acidity and volume in children. Can J Anaesth 1994 Oct;41(10):925-9.

22. Oderda G, Rapa A, Chiorboli E, Ronchi B, Zavallone A, Strigini L. Measurement of postprandial changes in urine acid output to detect changes of gastric acid secretion after proton pump inhibitors in children. Dig Dis Sci 2002 Aug;47(8):1843-9.

23. Omari TI, Davidson GP. Multipoint measurement of intragastric pH in healthy preterm infants. Arch Dis Child Fetal Neonatal Ed 2003 Nov;88(6):F517-20.

24. Rogers IM, Drainer IK, Moore MR, Buchanan KD. Plasma gastrin in congenitial hypertrophic pyloric stenosis. A hypothesis disproved. Arch Dis Child 1975 Jun;50(6):467-71.

25. Smith LJ, Kaminsky S, D'Souza SW. Neonatal fat digestion and lingual lipase. Acta Paediatr Scand 1986 Nov;75(6):913-8.

26. Splinter WM, Schaefer JD. Unlimited clear fluid ingestion two hours before surgery in children does not affect volume or pH of stomach contents. Anaesth Intensive Care 1990 Nov;18(4):522-6.

27. Splinter WM, Stewart JA, Muir JG. Large volumes of apple juice preoperatively do not affect gastric pH and volume in children. Can J Anaesth 1990 Jan;37(1):36-9.

28. Whetstine LJ, Hulsey TC, Annibale DJ, Pittard WB. Supplemental oxygen and gastric pH in unfed preterm infants. South Med J 1995 Apr;88(4):458-61.

29. Yildiz F, Tryba M, Kuehn K, Hausdoerfer J. Reduction of gastric acid secretion. The efficacy of pre-anaesthetic oral cimetidine in children. Anaesthesia 1984 Apr;39(4):314-8.

30. Miller RA. Observations on the gastric acidity during the first month of life. Arch Dis Child 1941 Mar;16(85):22-30.

31. Lopez-Alonso M, Moya MJ, Cabo JA, Ribas J, del Carmen Macias M, Silny J, et al. Twenty-four-hour esophageal impedance-pH monitoring in healthy preterm neonates: rate and characteristics of acid, weakly acidic, and weakly alkaline gastroesophageal reflux. Pediatrics 2006 Aug;118(2):e299-308.

32. Omari TI, Haslam RR, Lundborg P, Davidson GP. Effect of omeprazole on acid gastroesophageal reflux and gastric acidity in preterm infants with pathological acid reflux. J Pediatr Gastroenterol Nutr 2007 Jan;44(1):41-4.

33. Demir H, Ozen H, Kocak N, Saltik-Temizel IN, Gurakan F. Does simultaneous gastric and esophageal pH monitoring increase the diagnosis of gastroesophageal reflux disease? Turk J Pediatr 2005 Jan-Mar;47(1):14-6.

34. Kleinman RE, Goulet OJ, Mieli-Vergani G, Sanderson IR, Sherman PM, Shneider BL. Walker's Pediatric Gastrointestinal Disease. Shelton, CT: People's Medical Publishing House, 2008.

35. Boyle JT. Acid secretion from birth to adulthood. J Pediatr Gastroenterol Nutr 2003 Nov-Dec;37 Suppl 1:S12-6.

36. Huang NN, High RH. Comparison of serum levels following the administration of oral and parenteral preparations of penicillin to infants and children of various age groups. J Pediatr 1953 Jun;42(6):657-8.

37. Hoekstra JH, van den Aker JH, Kneepkens CM, Stellaard F, Geypens B, Ghoos YF. Evaluation of 13CO2 breath tests for the detection of fructose malabsorption. J Lab Clin Med 1996 Mar;127(3):303-9.

38. Perri F, Pastore M, Zicolella A, Annese V, Quitadamo M, Andriulli A. Gastric emptying of solids is delayed in celiac disease and normalizes after gluten withdrawal. Acta Paediatr 2000 Aug;89(8):921-5.

39. Hoffman I, Tertychnyy A, Ectors N, De Greef T, Haesendonck N, Tack J. Duodenogastro-esophageal reflux in children with refractory gastro-esophageal reflux disease. J Pediatr 2007 Sep;151(3):307-11.

40. Bode S, Dreyer M, Greisen G. Gastric emptying and small intestinal transit time in preterm infants: a scintigraphic method. J Pediatr Gastroenterol Nutr 2004 Oct;39(4):378-82.

41. Di Lorenzo C, Piepsz A, Ham H, Cadranel S. Gastric emptying with gastro-oesophageal reflux. Arch Dis Child 1987 May;62(5):449-53.

42. Seibert JJ, Byrne WJ, Euler AR. Gastric emptying in children: unusual patterns detected by scintigraphy. AJR Am J Roentgenol 1983 Jul;141(1):49-51.

43. Cucchiara S, Bortolotti M, Colombo C, Boccieri A, De Stefano M, Vitiello G, et al. Abnormalities of gastrointestinal motility in children with nonulcer dyspepsia and in children with gastroesophageal reflux disease. Dig Dis Sci 1991 Aug;36(8):1066-73.

44. Miele E, Tozzi A, Staiano A, Toraldo C, Esposito C, Clouse RE. Persistence of abnormal gastrointestinal motility after operation for Hirschsprung's disease. Am J Gastroenterol 2000 May;95(5):1226-30.

45. Yahav J, Avigad S, Frand M, Shem-Tov A, Barzilay Z, Linn S, et al. Assessment of intestinal and cardiorespiratory function in children with congenital heart disease on high-caloric formulas. J Pediatr Gastroenterol Nutr 1985 Oct;4(5):778-85.

46. Demirbilek S, Karaman A, Gurunluoglu K, Akin M, Tas E, Aksoy RT, et al. Delayed gastric emptying in gastroesophageal reflux disease: the role of malrotation. Pediatr Surg Int 2005 Jun;21(6):423-7.

47. Graff J, Brinch K, Madsen JL. Simplified scintigraphic methods for measuring gastrointestinal transit times. Clin Physiol 2000 Jul;20(4):262-6.

48. Bennink R, Peeters M, Van den Maegdenbergh V, Geypens B, Rutgeerts P, De Roo M, et al. Evaluation of small-bowel transit for solid and liquid test meal in healthy men and women. Eur J Nucl Med 1999 Dec;26(12):1560-6.

49. Mayer AP, Durward A, Turner C, Skellett S, Dalton N, Tibby SM, et al. Amylin is associated with delayed gastric emptying in critically ill children. Intensive Care Med 2002 Mar;28(3):336-40.

50. Lodefalk M, Aman J, Bang P. Effects of fat supplementation on glycaemic response and gastric emptying in adolescents with Type 1 diabetes. Diabet Med 2008 Sep;25(9):1030-5.

51. Anderson BJ, van Lingen RA, Hansen TG, Lin YC, Holford NH. Acetaminophen developmental pharmacokinetics in premature neonates and infants: a pooled population analysis. Anesthesiology 2002 Jun;96(6):1336-45.

52. Berseth CL, Ittmann PI. Antral and duodenal motor responses to duodenal feeding in preterm and term infants. J Pediatr Gastroenterol Nutr 1992 Feb;14(2):182-6.

53. Ittmann PI, Amarnath R, Berseth CL. Maturation of antroduodenal motor activity in preterm and term infants. Dig Dis Sci 1992 Jan;37(1):14-9.

54. Morriss FH, Jr., Moore M, Weisbrodt NW, West MS. Ontogenic development of gastrointestinal motility: IV. Duodenal contractions in preterm infants. Pediatrics 1986 Dec;78(6):1106-13.

55. Bisset WM, Watt JB, Rivers RP, Milla PJ. Measurement of small-intestinal motor activity in the preterm infant. J Biomed Eng 1988 Apr;10(2):155-8.

56. Berseth CL, Nordyke CK, Valdes MG, Furlow BL, Go VL. Responses of gastrointestinal peptides and motor activity to milk and water feedings in preterm and term infants. Pediatr Res 1992 Jun;31(6):587-90.

57. al Tawil Y, Berseth CL. Gestational and postnatal maturation of duodenal motor responses to intragastric feeding. J Pediatr 1996 Sep;129(3):374-81.

58. Vajro P, Silano G, Longo D, Staiano A, Fontanella A. Orocoecal transit time in healthy and constipated children. Acta Paediatr Scand 1988 Jul;77(4):583-6.

59. Van Den Driessche M, Van Malderen N, Geypens B, Ghoos Y, Veereman-Wauters G. Lactose-[13C]ureide breath test: a new, noninvasive technique to determine orocecal transit time in children. J Pediatr Gastroenterol Nutr 2000 Oct;31(4):433-8.

60. Khin M, Bolin TD, Tin O, Thein Win N, Kyaw-Hla S, Thein Thein M. Investigation of small-intestinal transit time in normal and malnourished children. J Gastroenterol 1999 Dec;34(6):675-9.

61. Murphy MS, Nelson R, Eastham EJ. Measurement of small intestinal transit time in children. Acta Paediatr Scand 1988 Nov;77(6):802-6.

62. Vreugdenhil G, Sinaasappel M, Bouquet J. A comparative study of the mouth to caecum transit time in children and adults using a weight adapted lactulose dose. Acta Paediatr Scand 1986 May;75(3):483-8.

63. Geypens B, Bennink R, Peeters M, Evenepoel P, Mortelmans L, Maes B, et al. Validation of the lactose-[13C]ureide breath test for determination of orocecal transit time by scintigraphy. J Nucl Med 1999 Sep;40(9):1451-5.

64. Wutzke KD, Heine WE, Plath C, Leitzmann P, Radke M, Mohr C, et al. Evaluation of oro-coecal transit time: a comparison of the lactose-[13C, 15N]ureide 13CO2- and the lactulose H2-breath test in humans. Eur J Clin Nutr 1997 Jan;51(1):11-9.

65. Fallingborg J, Christensen LA, Ingeman-Nielsen M, Jacobsen BA, Abildgaard K, Rasmussen HH, et al. Measurement of gastrointestinal pH and regional transit times in normal children. J Pediatr Gastroenterol Nutr 1990 Aug;11(2):211-4.

66. Boehm G, Bierbach U, Senger H, Jakobsson I, Minoli I, Moro G, et al. Activities of lipase and trypsin in duodenal juice of infants small for gestational age. J Pediatr Gastroenterol Nutr 1991 Apr;12(3):324-7.

67. Jarvenpaa AL, Rassin DK, Kuitunen P, Gaull GE, Raiha NC. Feeding the low-birth-weight infant. III. Diet influences bile acid metabolism. Pediatrics 1983 Nov;72(5):677-83.

68. Challacombe DN, Edkins S, Brown GA. Duodenal bile acids in infancy. Arch Dis Child 1975 Nov;50(11):837-43.

69. Walkowiak J, Herzig KH, Strzykala K, Przyslawski J, Krawczynski M. Fecal elastase-1 is superior to fecal chymotrypsin in the assessment of pancreatic involvement in cystic fibrosis. Pediatrics 2002 Jul;110(1 Pt 1):e7.

70. Nissler K, Von Katte I, Huebner A, Henker J. Pancreatic elastase 1 in feces of preterm and term infants. J Pediatr Gastroenterol Nutr 2001 Jul;33(1):28-31.

71. de Winter BC, Monchaud C, Premaud A, Pison C, Kessler R, Reynaud-Gaubert M, et al. Bayesian estimation of mycophenolate mofetil in lung transplantation, using a population pharmacokinetic model developed in kidney and lung transplant recipients. Clin Pharmacokinet 2012 Jan 1;51(1):29-39.

72. Hines RN. The ontogeny of drug metabolism enzymes and implications for adverse drug events. Pharmacol Ther 2008 May;118(2):250-67.

73. Stevens JC, Hines RN, Gu C, Koukouritaki SB, Manro JR, Tandler PJ, et al. Developmental expression of the major human hepatic CYP3A enzymes. J Pharmacol Exp Ther 2003 Nov;307(2):573-82.

74. Fakhoury M, Litalien C, Medard Y, Cave H, Ezzahir N, Peuchmaur M, et al. Localization and mRNA expression of CYP3A and P-glycoprotein in human duodenum as a function of age. Drug Metab Dispos 2005 Nov;33(11):1603-7.

75. Johnson TN, Tanner MS, Taylor CJ, Tucker GT. Enterocytic CYP3A4 in a paediatric population: developmental changes and the effect of coeliac disease and cystic fibrosis. Br J Clin Pharmacol 2001 May;51(5):451-60.

76. Treluyer JM, Jacqz-Aigrain E, Alvarez F, Cresteil T. Expression of CYP2D6 in developing human liver. Eur J Biochem 1991 Dec 5;202(2):583-8.

77. Fukudo M, Yano I, Masuda S, Goto M, Uesugi M, Katsura T, et al. Population pharmacokinetic and pharmacogenomic analysis of tacrolimus in pediatric living-donor liver transplant recipients. Clin Pharmacol Ther 2006 Oct;80(4):331-45.

78. Fukudo M, Yano I, Yoshimura A, Masuda S, Uesugi M, Hosohata K, et al. Impact of MDR1 and CYP3A5 on the oral clearance of tacrolimus and tacrolimus-related renal dysfunction in adult living-donor liver transplant patients. Pharmacogenet Genomics 2008 May;18(5):413-23.

79. de Wildt SN, Kearns GL, Hop WC, Murry DJ, Abdel-Rahman SM, van den Anker JN. Pharmacokinetics and metabolism of oral midazolam in preterm infants. Br J Clin Pharmacol 2002 Apr;53(4):390-2.

80. Paine MF, Shen DD, Kunze KL, Perkins JD, Marsh CL, McVicar JP, et al. First-pass metabolism of midazolam by the human intestine. Clin Pharmacol Ther 1996 Jul;60(1):14-24.

81. Mandema JW, Tuk B, van Steveninck AL, Breimer DD, Cohen AF, Danhof M. Pharmacokinetic-pharmacodynamic modeling of the central nervous system effects of midazolam and its main metabolite alpha-hydroxymidazolam in healthy volunteers. Clin Pharmacol Ther 1992 Jun;51(6):715-28.

82. Blake MJ, Abdel-Rahman SM, Pearce RE, Leeder JS, Kearns GL. Effect of diet on the development of drug metabolism by cytochrome P-450 enzymes in healthy infants. Pediatr Res 2006 Dec;60(6):717-23.

83. Dietrich CG, Geier A, Oude Elferink RP. ABC of oral bioavailability: transporters as gatekeepers in the gut. Gut 2003 Dec;52(12):1788-95.

84. Cascorbi I. P-glycoprotein: tissue distribution, substrates, and functional consequences of genetic variations. Handb Exp Pharmacol 2011(201):261-83.

85. Fanta S, Niemi M, Jonsson S, Karlsson MO, Holmberg C, Neuvonen PJ, et al. Pharmacogenetics of cyclosporine in children suggests an age-dependent influence of ABCB1 polymorphisms. Pharmacogenet Genomics 2008 Feb;18(2):77-90.

86. Fakhoury M, Lecordier J, Medard Y, Peuchmaur M, Jacqz-Agrain E. Impact of inflammation on the duodenal mRNA expression of CYP3A and P-glycoprotein in children with Crohn's disease. Inflamm Bowel Dis 2006 Aug;12(8):745-9.

87. Zhao W, Fakhoury M, Deschenes G, Roussey G, Brochard K, Niaudet P, et al. Population pharmacokinetics and pharmacogenetics of mycophenolic acid following administration of mycophenolate mofetil in de novo pediatric renal-transplant patients. J Clin Pharmacol 2010 Nov;50(11):1280-91.

88. Ohmann EL, Burckart GJ, Brooks MM, Chen Y, Pravica V, Girnita DM, et al. Genetic polymorphisms influence mycophenolate mofetil-related adverse events in pediatric heart transplant patients. J Heart Lung Transplant 2010 May;29(5):509-16.

89. Zhao W, Elie V, Roussey G, Brochard K, Niaudet P, Leroy V, et al. Population pharmacokinetics and pharmacogenetics of tacrolimus in de novo pediatric kidney transplant recipients. Clin Pharmacol Ther 2009 Dec;86(6):609-18.

90. Blanquet S, Zeijdner E, Beyssac E, Meunier JP, Denis S, Havenaar R, et al. A dynamic artificial gastrointestinal system for studying the behavior of orally administered drug dosage forms under various physiological conditions. Pharm Res 2004 Apr;21(4):585-91.

91. Brouwers J, Anneveld B, Goudappel GJ, Duchateau G, Annaert P, Augustijns P, et al. Food-dependent disintegration of immediate release fosamprenavir tablets: in vitro evaluation using magnetic resonance imaging and a dynamic gastrointestinal system. Eur J Pharm Biopharm 2011 Feb;77(2):313-9.

92. Wilding IR, Kenyon CJ, Hooper G. Gastrointestinal spread of oral prolonged-release mesalazine microgranules (Pentasa) dosed as either tablets or sachet. Aliment Pharmacol Ther 2000 Feb;14(2):163-9.

93. Hines RN. Ontogeny of human hepatic cytochromes P450. J Biochem Mol Toxicol 2007;21(4):169-75.

94. Johnson TN, Rostami-Hodjegan A, Tucker GT. Prediction of the clearance of eleven drugs and associated variability in neonates, infants and children. Clin Pharmacokinet 2006;45(9):931-56.

95. Ince I, de Wildt SN, Tibboel D, Danhof M, Knibbe CA. Tailor-made drug treatment for children: creation of an infrastructure for data-sharing and population PK-PD modeling. Drug Discov Today 2009 Mar;14(5-6):316-20.

96. de Wildt SN, Ito S, Koren G. Challenges for drug studies in children: CYP3A phenotyping as example. Drug Discov Today 2009 Jan;14(1-2):6-15.

97. Gorski JC, Jones DR, Haehner-Daniels BD, Hamman MA, O'Mara EM, Jr., Hall SD. The contribution of intestinal and hepatic CYP3A to the interaction between midazolam and clarithromycin. Clin Pharmacol Ther 1998 Aug;64(2):133-43.

98. Lappin G, Shishikura Y, Jochemsen R, Weaver RJ, Gesson C, Brian Houston J, et al. Comparative pharmacokinetics between a microdose and therapeutic dose for clarithromycin, sumatriptan, propafenone, paracetamol (acetaminophen), and phenobarbital in human volunteers. Eur J Pharm Sci 2011 Jun 14;43(3):141-50.

99. Lappin G, Garner RC. The utility of microdosing over the past 5 years. Expert Opin Drug Metab Toxicol 2008 Dec;4(12):1499-506.

100. Lappin G, Kuhnz W, Jochemsen R, Kneer J, Chaudhary A, Oosterhuis B, et al. Use of microdosing to predict pharmacokinetics at the therapeutic dose: experience with 5 drugs. Clin Pharmacol Ther 2006 Sep;80(3):203-15.

101. Baillie R, Bosley J, Blood A, Vasquez H, Vuong L. Compartmental analysis of ursodiol PK neonates using AMS. Clin Pharmacol Ther;Feb 89.

Figure 1: Gastric pH measured in neonates by 24-h monitoring or gastric aspirates. References correspond with references in the text.