ELSEVIER

Drug metabolism in children and the elderly compared with that in the adult population. What is the impact of age-related changes in activities of phase I and phase II enzymes on the disposition of drugs?

Drug metabolism in the paediatric population and in the elderly

Margherita Strolin Benedetti[1], Rhys Whomsley[2] and Michael Canning[2]

[1] UCB Pharma, 21 rue de Neuilly, 92003 Nanterre Cedex, France
[2] UCB Pharma, Chemin du Foriest, 1420 Braine-l'Alleud, Belgium

This review focuses on one of the key factors accounting for differences in drug/metabolite exposure in paediatric and elderly subjects compared with that of the adult population, that is, differences in drug metabolism (both qualitative and quantitative) and in particular differences due to changes in the activity and/or concentration of drug metabolizing enzymes. Important differences have been found in the paediatric population compared with adults for both phase I (e.g. CYP3A7 versus CYP3A4 and CYP1A2, reductive and hydrolytic enzymes) and phase II (e.g. glucuronosyltransferases) enzymes. In the elderly, some phase I enzymes (e.g. esterases) appear to be impaired. From the information collected thus far, it would appear that phase II reactions, though sometimes decreased, are not extensively affected by old age.

Drug disposition is dependent on a number of factors that may each influence the pharmacokinetics of xenobiotics in man. The adult population is generally that in which the pharmacokinetic characteristics of drugs in development are evaluated; however, the disposition of drugs often differ in the elderly, who consume nearly 40% of all drugs, and in the paediatric population. Several practical problems and difficulties have discouraged the testing of drugs in paediatric and elderly populations for ethical, technical and logistical reasons. Both populations are generally viewed as vulnerable groups that may be put at risk when participating in studies on new drugs. Variations in drug disposition may arise through differences in absorption, distribution, metabolism or excretion; however metabolism is frequently the key determinant. This article will review what is presently known about differences in drug metabolism in the paediatric population and the elderly compared with adults, and in particular what is known about modifications in drug metabolizing enzyme activities and/or concentrations with age. The paediatric population analysed will be that ranging from preterm-newborn infants to adolescents and the old population will start from 65 years with no upper age cutoff. Information collected during the foetal life has generally not been taken into account. Whenever possible, studies documenting changes in drug metabolism in frail elderly people compared with fit elderly will be presented.

Corresponding author: Benedetti, M.S. (margherita.strolin@ucb-group.com)

MARGHERITA STROLIN BENEDETTI
Margherita Strolin Benedetti (PhD Chemistry, University of Bologna) is the author of 450 publications in international journals, most in the area of biochemical pharmacology, enzymology, pharmacokinetics, drug metabolism and drug–drug interactions. She is presently Associate Director for Scientific Affairs in the Department of Non Clinical Development at UCB. She previously directed research groups in other international pharmaceutical companies, contributing to the development of important drugs, and also worked as Research Assistant at the University of Geneva where she specialized in the study of drug metabolism with radiolabelled compounds.

RHYS WHOMSLEY
Rhys Whomsley (PhD Chemistry/Pharmacology, University of Wales) is the author of more than 30 publications in the area of drug metabolism and transport, pharmacokinetics and enzyme inhibition or induction. He has over 20 years experience in both scientific and regulatory aspects of drug development, leading teams in the pharma industry, contract research and at the University of Wales, where he focused on the development of steroidogenic enzyme inhibitors in the treatment of hormone-dependent cancers. He is currently Senior Principal Scientist Non-Clinical Development at UCB.

MICHAEL CANNING
Michael Canning (Veterinary Science, University of Bristol) is a veterinary pathologist by training and has over 17 years experience in non-clinical drug development and regulatory affairs with special interests in carcinogenicity and reproductive and juvenile toxicology. He is currently the Vice-president of Non-clinical Development at UCB having previously held positions in UK and continental Europe.

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When no specific mention of health status is given, it is assumed that the paediatric and the old populations discussed are healthy. Finally, when no mention is made concerning the paediatric population or the elderly for a family/subfamily or for a specific isozyme, this means that no corresponding information has been found from the literature search.

Drug metabolism

Metabolism renders drugs more water-soluble so that they can be easily excreted from the body after their desired effect has been exerted. This process occurs primarily in liver hepatocytes to generate metabolites that are inactive and relatively non-toxic; however, metabolites may occasionally be the source of toxic effects. For prodrugs, the parent drug is inactive but the metabolite is active. Drug metabolism mechanisms can be classified into phase I and phase II reactions, the former involving structural alteration of the drug molecule and the latter consisting of conjugation with another often more water-soluble moiety. Phase I reactions can be oxidation, reduction and hydrolysis. Oxidative reactions are frequently, though not necessarily, cytochrome P450 (CYP)-dependent. Involvement of enzymes other than CYPs in the oxidative metabolism of xenobiotics has been recently reviewed [1].

Both phase I and phase II metabolic pathways may be immature at birth and are subject to maturational changes. The different capacities to metabolize a drug in the paediatric or in the old population can produce lower or higher plasma concentrations of the active principle(s) than in adults depending on the enzyme system involved. There are examples of therapeutic agents that produce metabolites in children that are not normally present in adults. These metabolites may be responsible for some of the efficacy and/or toxicity observed with drug administration in children, an example is caffeine production in the neonate receiving theophylline [2]. Other therapeutic agents showing differences in metabolite production in children are valproic acid, paracetamol, chloramphenicol, cimetidine and salicylamide. However, in most cases, the differences between children and adults are in the ratios of the metabolites relative to the parent drug rather than in novel metabolites unique to the paediatric population as with a few exceptions, the paediatric population expresses the same complement of enzymes as the adult population, although the level of expression may differ. Concerning the elderly, to our knowledge, no example of the production of metabolites not normally present in adults has been reported to date.

The liver is quantitatively by far the most important organ for drug metabolism. It constitutes 5% of the bodyweight at birth but only 2% in the adult [3]. Advancing age is associated with a progressive reduction in liver volume and liver blood flow. As a consequence, the metabolic clearance is reduced primarily with drugs that display high hepatic extraction (hepatic metabolic clearance >70% of hepatic blood flow), whereas the metabolism of drugs with low hepatic extraction (hepatic metabolic clearance <30% of hepatic blood flow) usually is not or only slightly diminished. Other tissues such as the kidney can also be important for drug metabolism, and the developmental pattern of an enzyme can be tissue-dependent.

Developmental aspects of phase I reactions reviewed in this article are essentially a review of the enzymes involved in such reactions. The problem is more complex for phase II reactions as more than one enzyme can be involved in the mechanism of these reactions. The availability of the conjugating agent is also an important consideration.

Phase I reactions

Oxidative enzymes

A review paper on the ontogeny of human phase I oxidative enzymes has been recently written by Hines and McCarver [4].

CYP system

The CYP isoenzyme superfamily comprises over 50 proteins that catalyze the oxidative metabolism of many structurally diverse drugs and chemicals. These enzymes are located in the lipophilic membranes of the smooth endoplasmic reticulum of the liver and other tissues, which, when isolated, re-form into vesicles called microsomes. The CYP families 1–4 are mainly involved in xenobiotic metabolism, while other CYP families are mainly involved in the metabolism of endogenous substrates. CYPs are responsible for the phase I metabolism of the most clinically used drugs and are also responsible for the metabolic activation of many chemical carcinogens and toxins. For further information on age-related changes in drug-metabolizing CYP activities in man, please see references [5–7].

CYP1A

The CYP1A subfamily consists of two isoforms, CYP1A1 and 1A2. CYP1A1 is essentially extrahepatic. CYP1A2 is involved in the metabolism of aromatic amines, paracetamol, imipramine, phenacetin, warfarin, caffeine and theophylline. In the metabolism of caffeine and theophylline, CYP1A2 is involved in all demethylations (*N*-1, *N*-3 and *N*-7) as well as in ring hydroxylation (*C*-8) although other CYP isozymes (CYP2E1, 2A6 and 3A4/5) also contribute to these reactions [8,9] (see Figure 1).

Paediatric population: The maturational development of CYP1A2 has been described by Strolin Benedetti *et al.* [10] and references contained therein. CYP1A2 is barely detectable in early neonatal microsomes, becomes readily detectable in infants aged one to three months, is present at low levels (about 30% of the adult level) in infants <1 year and attains approximately 50% of the adult value at one year and 81% at two years. Levels of CYP1A2 comparable to those of adults have been found in children of three years or more. *N*-3-demethylation of caffeine depends on CYP1A2 not only in adults but also in neonates and infants. Maturation of caffeine metabolic pathways in infancy (premature newborns and older infants) has been extensively studied. Total demethylation, *N*-3- and *N*-7-demethylation increase exponentially with postnatal age; the percentage of methyl groups removed as a function of postnatal age increases up to 120 days, where it accounts for about 59, 91 and 80% of total, *N*-3-demethylation and *N*-7-demethylation, respectively. *N*-1-Demethylation shows no variation with postnatal age, and maturation of this pattern occurs after 19 months of age. *N*-3-Demethylation appears to be slightly more important in young infants than in adults. *C*-8-Hydroxylation is nearly mature at one month of age in some infants and may be higher in infants than in adults. The biotransformation of caffeine in human liver microsomes from neonates, infants and adults has been studied later by the same group [11]. The *N*-3-, *N*-7- and *N*-1-demethylation

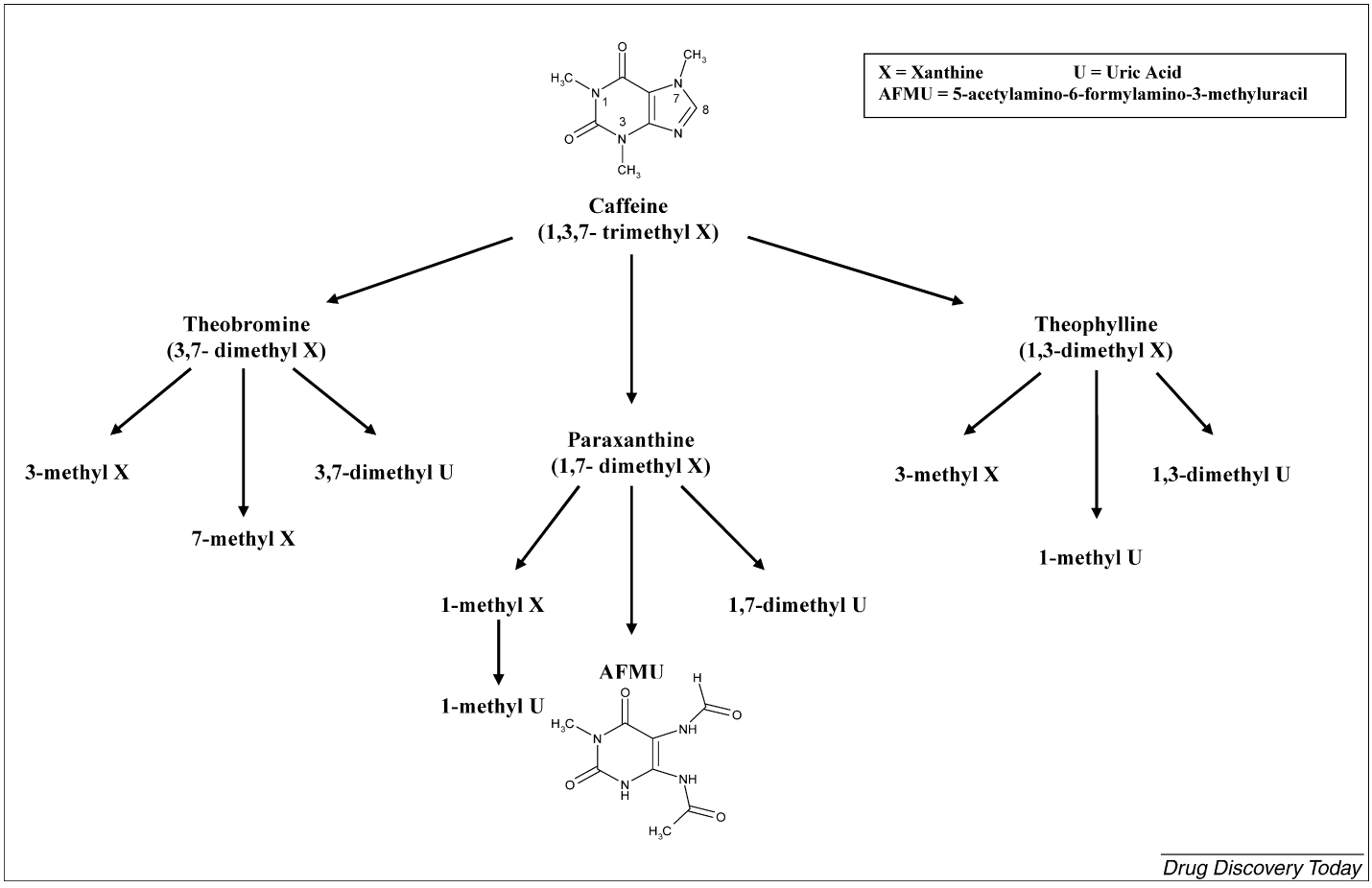

FIGURE 1

Major primary metabolites of caffeine and some further metabolic pathways.

activities were significantly lower in neonates and infants than in adults, as shown previously *in vivo*. A recent study has demonstrated that an infant's dietary exposure plays an important role in the development of CYP1A2 activity. A more rapid metabolism of caffeine was observed in infants consuming formula than in those breast fed [12].

Elderly: Zeeh *et al.* [13] demonstrated a decrease in the clearance of a monoamine oxidase inhibitor (brofaromine) and the activity of CYP1A2 measured by caffeine clearance in elderly subjects. Other studies, however, found no age-related differences in the paraxanthine to caffeine plasma ratio in young and elderly subjects [14,15].

CYP2A

The CYP2A subfamily consists of three isoforms, CYP2A6, 2A7 and 2A13 [16]. CYP2A6 catalyzes coumarin 7-hydroxylation specifically [17].

Paediatric population: Not much is known about the developmental activation of the CYP2A subfamily. CYP2A6 is not expressed in foetal liver. Shimada *et al.* [18] found low but detectable levels of CYP2A6 immunoreactive protein and coumarin 7-hydroxylation activity in a single infant liver sample studied. It has been shown that the urinary excretion of 7-hydroxycoumarin is similar in adults and in children of 6–13 years of age [19].

Elderly: According to reviews [20,21], it appears that the rate of elimination is decreased or unchanged for substrates of CYP2A.

CYP2C

The isozymes of the CYP2C subfamily are involved not only in the metabolism of a number of therapeutic agents, such as anticonvulsants and non-steroidal anti-inflammatory drugs as well as warfarin, omeprazole, tolbutamide, diazepam and propranolol, but also probably in the metabolism of endogenous agents such as arachidonic acid. CYP2C9 and 2C19 have been extensively studied, and there is an increasing awareness of the important role of CYP2C8 in drug metabolism.

Paediatric population: The CYP2C isozymes develop early during the neonatal period. Barely detectable in newborns, they represent 1/3 of the adult value at one month and remain unchanged until one year [22,23]. The expression of two isozymes of the CYP2C subfamily (CYP2C9 and 2C19) during development has been recently investigated [24]. From birth to five months, a 35-fold interindividual variation in CYP2C9 protein values was observed with 51% of samples exhibiting values commensurate with mature levels. CYP2C9 protein and activity values were less variable between 5 months and 18 years. This is consistent with changes in the pharmacokinetics of phenytoin, an anticonvulsant and substrate of CYP2C9, throughout childhood. In preterm infants the apparent half-life of phenytoin is prolonged (ca. 75 h) relative to term infants <1 week after birth (ca. 20 h) or term infants aged >2 weeks (ca. 8 h) [25]. CYP2C19 expression increased linearly from birth over the first five postnatal months and varied 21-fold in individuals aged between five months and ten years. Samples

from individuals older than ten years showed CYP2C19 protein expression and activity values similar to those of adults. A study comparing CYP activities in paediatric versus adult liver found no age-related differences in the oxidation of paclitaxel, a substrate of CYP2C8 [26].

Elderly: The content of CYP2C remains unchanged with age in both genders [27,28]. It appears that the rate of elimination is decreased for substrates of CYP2C9 and 2C19 [20,21]. For example, the urinary recovery of the CYP2C19 substrate 4-hydroxymephenytoin decreased with age by 35% between the youngest (<35 years) and oldest (>50 years) groups [14]. Similarly, in a study on Japanese subjects using omeprazole as probe substrate, CYP2C19 activity was shown to be decreased with age [29]. The stereoselective metabolism of citalopram enantiomers in elderly patients differs from that in younger patients further supporting age-associated changes in CYP2C19 activity [30].

CYP2D

CYP2D6 contributes to the metabolism of several classes of drugs such as tricyclic and non-tricyclic antidepressants, β-blockers, anti-arrhythmic drugs, as well as codeine, cinnarizine, deprenyl, captopril and ondansetron [10].

Paediatric population: The expression of CYP2D during development has been reviewed by Strolin Benedetti *et al.* [10]. A clear increase in the CYP2D6 protein expression has been found during the first postnatal week. At four weeks, levels of activity are ca. 20% of those in adults. In infants and children up to five years of age, the level had reached about two-third of the average adult levels. A phenotyping and genotyping study was carried out in 52 male and female infants to characterize the ontogeny of CYP2D6 in the first year of life. CYP2D6 phenotype consistent with genotype was achieved on average by 15 days postnatal age and remained relatively constant over the first six months of life. The well-known CYP2D6 polymorphism is known to be present in children.

Elderly: According to reviews [20,21] and a recent study by Bebia *et al.* [14], it appears that the rate of elimination is unchanged for substrates of CYP2D6.

CYP2E

CYP2E1 is involved in the metabolism of rather small molecules, including ethanol, paracetamol, aniline and *N*-nitrosodimethylamine [10].

Paediatric population: This isozyme rises steadily after birth and reaches 40% of adult values throughout the first year and 100% of adult values between one and ten years [10].

Elderly: CYP2E1 was reported to diminish with age [27]. When investigated using the substrates chloroxazone and paracetamol, CYP2E1 is unchanged or reduced with age [20]. These findings are challenged by a recent study, which showed a significant increase in CYP2E1 activity with age that appeared to develop earlier in life in men than in women [14].

CYP3A

CYP3A isoforms are involved in the oxidation of the largest range of substrates and are the major CYP isoforms present in the liver and small intestine.

Paediatric population: The enzymes of the CYP3A subfamily are probably essential for the metabolism of steroid hormones of maternal, placental or foetal adrenal origin and thus develop at an early stage. The developmental expression of CYP3A has been reviewed by Strolin Benedetti *et al.* [10] and Stevens [31]. CYP3A4 is the major CYP expressed in adult liver, whereas CYP3A7 is the major CYP expressed in the foetal liver. CYP3A5 expression is highly variable and generally independent of age [31]. Although CYP3A4, 3A5 and 3A7 are structurally closely related, they differ in their capacity to carry out monooxygenase reactions. CYP3A7 is very active in the foetal liver; it reaches its maximal activity during the first week after birth before progressively declining to a very low level or being absent in adult liver. However, significant CYP3A7 expression has been recently demonstrated in a subset of the adult population. The expression is partly because of the CYP3A7*1C allele, where a mutation has caused a portion of the CYP3A4 promoter to be incorporated into the corresponding CYP3A7 promoter [31]. The activity of CYP3A4 is extremely weak or absent in the foetus and rises after birth to reach 30–40% of the adult activity after one month. The biotransformation of the serotonin 5-HT_4 agonist cisapride is mediated by CYP3A4 with only a minor contribution from CYP3A7. The metabolism of cisapride, negligible in less than seven-day-neonates, steadily increased after the first week of life to reach activities exceeding adult values. This explains why cardiac toxicity of cisapride has been reported in neonates that do not carry the risk factors known to affect children or adults. Similarly, the clearance of intravenous midazolam, a sedative and CYP3A4 substrate, is markedly lower in neonates than that in infants aged >3 months. The bioavailability of midazolam following oral administration has been reported to be increased in preterm infants compared with that of adults as a result of low CYP3A activity in the intestine [32]. Similar to CYP1A2, it appears that CYP3A4 development is also accelerated in formula-fed infants compared with that of breast-fed infants [12].

Elderly: Studies analyzing CYP3A expression and activity in the elderly have reached inconclusive results: according to some reports CYP3A remains unchanged [28,33]; others observed a decrease with age [27,34]. According to a recent review [35], it appears that age influences the clearance of certain CYP3A4 substrates with many studies showing that men are more susceptible to the age-related decrements in clearance. Erythromycin breath test data showed no age-related trends in very elderly and frail elderly persons [36]; however, erythromycin cannot be considered a pure CYP3A probe as it also undergoes P-glycoprotein transport. In addition, the study population received a mean of 11 pharmacologically active agents.

Flavin-containing monooxygenases

The flavin-containing monooxygenases (FMOs), encoded by a six-member gene family (FMO1-6), are important for the NADPH-dependent oxidative metabolism of a wide range of xenobiotics containing nucleophilic nitrogen-, sulfur-, selenium- and phosphorus-heteroatoms.

Paediatric population: The ontogeny of human hepatic FMOs has been reviewed by Strolin Benedetti *et al.* [10]. The highest level of FMO1 expression was observed at 8–15 weeks gestation. FMO1 expression subsequently declined and was completely suppressed within three postnatal days by a mechanism coupled with birth, not gestational age. FMO3 expression was observed at low levels between 8 and 15 weeks gestation and was generally undetectable

in the neonatal period, but was detectable by one to two years of age. Intermediate levels of FMO3 expression were observed until 11 years of age, at which time a gender-independent increase was observed from 11 to 18 years of age. In contrast to CYP3A isoforms, where the decline in CYP3A7 is accompanied by a simultaneous increase in CYP3A4/3A5 resulting in a relatively constant net CYP3A expression, the rapid postnatal suppression of FMO1 and the delayed onset of FMO3 expression result in a hepatic null FMO phenotype in the neonate.

Monoamine oxidases

Monoamine oxidases (MAOs), oxidative enzymes located in the mitochondria of liver, kidney, lung, gut, platelets and brain, are involved in the metabolism of endogenous as well as exogenous compounds [37]. Their high concentration in tissues other than liver is a demonstration that the effect of age should be also investigated for extrahepatic enzymes [38].

Paediatric population: A study analyzing the expression patterns of MAO A and MAO B during ontogeny in the frontal cortex of human brain found that MAO A activity was very high at birth, decreased rapidly during the first two years of life and remained constant thereafter [39]. By contrast, MAO B activity was low at birth, remained unchanged during early childhood and increased during advanced age. The development of this enzyme system in human peripheral tissues involved has not been investigated. As tyramine is metabolized by MAOs and a number of foods are rich in tyramine (e.g. cheese), it could be useful to investigate the developmental expression of MAOs in the gut.

Elderly: In humans, brain and platelet MAO activities have been measured with age. MAO B was found to increase significantly with age in most human brain areas, whereas MAO A activity either did not change or slightly decreased with ageing. Conflicting results, with no change or a slight increase, were reported for the activity of human platelet MAO in ageing [40].

Alcohol dehydrogenase

Alcohol dehydrogenases (ADH) are a family of mainly cytosolic isozymes that catalyse the reversible oxidation of alcohols to their corresponding aldehydes. The enzymes have been grouped into classes ADH1–6, of which five (ADH1–5) have been identified in man [1]. Endogenous compounds metabolized by ADH include steroids and retinol. With regard to the involvement of ADH in the metabolism of xenobiotic ethanol, ADH1 probably plays the major role in ethanol metabolism. Among xenobiotics, not only ethanol but also a number of drugs can be metabolized by ADH, for example the conversion of hydroxyzine to its major human metabolite cetirizine [41].

Paediatric population: The maturational development of alcohol dehydrogenases has been reviewed by Strolin Benedetti *et al.* [10]. Biokinetic data for ethanol in neonates clearly indicate reduced clearance of ethanol and its accumulation in plasma, findings that corroborate the previously reported developmental immaturity in ADH activity. It would appear that by 12–30 months of age, ADH activity equal to or greater than that observed in adults is reached. However, according to other studies, ADH activity reaches adult levels by five years of age.

Elderly: There are conflicting reports on the effect of advancing age on the activity of ADH. Most studies have focused on gastric ADH activity and have not differentiated between the different isoforms of ADH. The analysis of the data in both adult and elderly populations is complicated by the effects of atrophic gastritis, sex differences, alcohol consumption and helicobacter pylori infection on stomach ADH activity. Generally, no differences in ADH activities have been found between young and elderly subjects [42–44], although class IV ADH decreased with age in one study [42]. One study reported significantly higher circulating ethanol concentrations in elderly subjects [45]; however, another study observed lower circulating concentrations following oral administration in elderly subjects with normal gastric morphology that may be due to a deceleration in the speed of gastric emptying leading to an increased contact time with gastric ADH. In a study investigating the effect of age on hepatic ADH activity, no effect of age was observed in subjects aged 45–88 years [46]. Although ethanol concentrations have been reported to be higher in elderly subjects than in younger individuals after intravenous administration, this may be related to differences in distribution or a consequence of decreased ADH activity due to a lack of the cofactor NAD [47].

Molybdenum hydroxylases (aldehyde oxidase and xanthine oxidase)

Aldehyde oxidase (AO) and xanthine oxidase (XO) are structurally similar enzymes involved in the biotransformation of exogenous and endogenous substrates. XO participates in a variety of biochemical reactions including the oxidative hydroxylation of hypoxanthine to xanthine and the subsequent hydroxylation of xanthine to uric acid, the final two steps of purine metabolism in mammals [1]. AO is involved in the metabolism of tamoxifen, famciclovir, zaleplon, zonisamide and ziprasidone.

Paediatric population: Plasma XO activity is higher in newborn infants than in adults [48]. AO activity is immature in neonates and infants until about one year after birth [49].

Elderly: XO activity, as measured by 1-methylurate/1-methylxanthine ratio in urine following caffeine administration, has been shown to be independent of age in subjects aged from 21 to 78 years [50]. A recent study also showed an absence of a significant age-related increase in XO expression in the vascular endothelium [51].

Reductive enzymes

Metabolism by reduction has generally been less appreciated in terms of its scope and importance. Nonetheless, biochemically important and chemically interesting examples of reductive metabolism abound. The enzymology of the reductive processes in mammalian tissues is reviewed in an article by Strolin Benedetti [52]. Little information is available on developmental changes in mammalian reductive reactions involved in drug metabolism. Information concerning the elderly is also limited.

NADPH-cytochrome P450 reductase

Cytochrome P450 reductase, containing flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), is the electron-donating partner to CYP enzymes. Reducing equivalents supplied by NADPH are transferred to the FAD of cytochrome P450 reductase and on to the CYP enzymes via the FMN of cytochrome P450 reductase.

Elderly: Schmucker *et al.* [28] reported that NADPH-cytochrome P450 reductase was not altered with age.

Aldo-ketoreductases

Aldo-ketoreductases are cytosolic enzymes carrying out the reduction of carbonyl groups and present in the liver as well as in other tissues such as erythrocytes. Hypolipidemic drugs such as fenofibrate and antitumor drugs (e.g. the anthracyclines) are metabolized by this enzyme system [53,54]. Ketoreductase activity is also involved in the metabolism of prostaglandin E_1 (PGE_1) used for the treatment of peripheral arterial occlusive diseases [55]. Reduction of the ketogroup may sometimes produce active metabolites as shown for idarubicin [56] and PGE_1 [55]. It is therefore very important to have information on the developmental changes of this enzyme system as well as on its changes in the elderly.

Paediatric population: Idarubicinol, the alcohol product of idarubicin ketoreductase biotransformation, was present in the cerebrospinal fluid of paediatric patients receiving idarubicin in a phase I leukemia study [57]. Anthracyclines, such as idarubicin, are not widely thought to penetrate the blood–brain barrier, which makes it difficult to determine if the presence of the reduced metabolite in the cerebrospinal fluid is due to higher peripheral and/or central ketoreductase activity or due to a different distribution of idarubicin and/or idarubicinol in paediatric patients. In the metabolism of PGE_1, a 13,14-dihydro-15-ketoprostaglandin E_1 is formed as an intermediary metabolite, which is reduced by a 15-ketoreductase to an active compound, the 13,14-dihydro-prostaglandin E_1. In a recent study [55], it has been found that enzymatic 15-ketoreduction of 13,14-dihydro-15-ketoprostaglandin E_1 in the liver from individuals aged ten years or below ($n = 15$) was significantly lower than that of those aged 13 years and above ($n = 22$) (there were no liver samples from individuals aged 11 or 12 years). The activity of such ketoreductase could be related to sexual maturity [58].

Hydrolytic enzymes

Esterases

Paediatric population: Esterase activity is reduced in the newborn compared with that in adults [59]. Plasma pseudocholinesterase and arylesterase activity rapidly increases from 28 weeks gestation to 1 year of age, after which no significant changes occur. By contrast, the erythrocyte acetylcholinesterase levels increase markedly between 40 weeks gestation and 1 year of age [60]. The sharp increase in erythrocyte acetylcholinesterase between term and one year is of interest as it suggests that the erythrocyte synthesized before birth is immature. Premature infant plasma degraded procaine hydrochloride and insecticide paraoxon more slowly than plasma from full-term infants, while the rates of hydrolysis for these two groups were significantly lower than those detected for children older than one year or adults. Drugs administered in the form of an ester, which require metabolism to the microbiologically active form *in vivo*, for example erythromycin estolate and chloramphenicol palmitate, may be incompletely hydrolysed in neonates [61], thus achieving only low serum concentrations. In infants and children, wide variations in the extent of chloramphenicol ester metabolism and excretion necessitate drug concentration monitoring to ensure that therapeutic serum concentrations are obtained [62]. Finally, a study on serum aspirin-esterase activity in epileptic patients [63], found important differences in the activity of this enzyme between children and adults: epileptic children showed significantly higher levels of hydrolytic activity than adult patients or control healthy subjects, although the group of epileptic patients younger than 10 years of age (6.6 ± 3 years) was admittedly too small to draw conclusion. Expression and activity of carboxylesterase (using *p*-nitrophenyl acetate as a substrate) have been studied in infant (2–24 months) and adult (20–36 years) liver. *In vitro* sensitivity to inhibition was also investigated using chlorpyrifos oxon [64]. No significant differences were observed during postnatal maturation.

Elderly: Studies analysing esterases in elderly people have shown that frailty is associated with a decline in metabolic activity of plasma aspirin esterase [64–67]. O'Mahoney *et al.* [68] found that some elderly patients experiencing injury or undergoing surgery have significantly impaired plasma aspirin esterase activity. This indicates that trauma and ill health can have substantial effects on enzymes of drug metabolism in older people. Studies of benzoyl, butyryl, and acetylcholinesterases have demonstrated no decline *in vivo* with age [69]. The transformation of articaine to its acid metabolite by tissue and plasma esterases is similar in young and elderly subjects [70].

Phase II reactions

Acetylation

Many drugs such as sulfamethazine, hydralazine, isoniazid, *p*-aminosalicylic acid, *p*-aminobenzoic acid, phenelzine or procainamide, as well as toxic agents are conjugated with an acetyl group. *N*-Acetyltransferase (NAT), a cytosolic enzyme, is widely distributed in mammalian tissues.

Paediatric population: The maturational development of acetylation reactions has been reviewed by Strolin Benedetti *et al.* [10]. Acetylation of *p*-aminobenzoic acid (NAT1) appears to be present in premature and full-term newborns, slightly increased in infants and decreased in children, whereas the data in adults are not available. The maturation of *N*-acetyltransferase system (NAT2) involved in the metabolism of caffeine and isoniazid has been studied. Administering caffeine to premature newborn infants and 1–19-month-old infants demonstrates that acetylator status of this enzyme cannot reliably be determined before one year of age. Patients studied before one year of age may be either true slow acetylators or still immature fast acetylators. Maturation of isoniazid acetylation may occur even later than one year of age, as the percentage of fast acetylators increases with age and reaches a plateau at around four years.

Elderly: Comparison of rates of acetylation between age groups is complicated by polymorphic distribution, and studies to date do not agree on whether there is any change in acetylator phenotype distribution in relation to age [21].

Methylation

The transfer of methyl groups from *S*-adenosyl-*L*-methionine to methyl acceptor substrates is one of the most common reactions in nature. The methyl groups are transferred to a sulfur-nucleophile or nitrogen-nucleophile or oxygen-nucleophile. The conjugation reactions are carried out by *S*-methyltransferases, *N*-methyltransferases and *O*-methyltransferases, the latter including catechol-*O*-methyltransferases.

N-Methyltransferases

Paediatric population: In the newborn, *N*-7-methylation of theophylline to produce caffeine is well developed, whereas oxidative demethylation is deficient and develops over the ensuing months.

Thiopurine-S-methyltransferase

Thiopurine-*S*-methyltransferase (TPMT), a cytosolic enzyme present in many tissues including erythrocytes, catalyzes the methylation of thiopurines such as 6-MP, an antiblastic drug often used for the treatment of childhood acute lymphoblastic leukaemia, and is involved in the metabolism of other compounds such as azathiopurine, a prodrug converted to 6-MP [71].

Paediatric population: As TPMT is a polymorphic enzyme, not only ontogenic but also genetic specificities have to be taken into account in drug prescription. About 89–94% of the general population has a high TPMT activity, 6–11% an intermediate activity and approximately 0.3% a low activity. The efficacy of 6MP is significantly influenced by the inactivation by TPMT. Individuals with low activity experience severe or fatal toxicity with standard 6MP doses [72,73]. The maturational development of TPMT has been reviewed by Strolin Benedetti *et al.* [10]. When TPMT activity was measured in erythrocyte preparations from adults and newborns, a polymorphic distribution of TPMT activity was observed in both populations. The distribution of activity in neonates reflected the polymorphism characterised in adults. Peripheral erythrocyte TPMT activity appears to be 50% greater in neonates than in adults. Contradictory data exist in the literature on the activity of TPMT in the paediatric population compared with that of adults (both higher activity and slightly lower/similar activity reported in children than in adults).

Glucuronidation

Paediatric population: The maturational development of glucuronidation has been reviewed by Strolin Benedetti *et al.* [10]. As for CYPs, several UDP glucuronosyltransferase (UGT) isozymes have been identified; therefore caution should be taken in extrapolating information on developmental changes obtained with one isozyme to the others. Depending on the drug or endogenous compound investigated, glucuronidation does not approach adult values until three to six months of life, one year, three years or even later. Bilirubin glucuronidation, carried out by the isozyme UGT1A1, takes place at very low levels in the neonatal liver but increases to reach adult values at three months [74]. Chloramphenicol is an important example of a drug where lower glucuronidation capacity in the paediatric population can lead to toxicity. As glucuronidation is markedly deficient in most premature infants and some full-term babies, high concentrations of unchanged chloramphenicol may accumulate in a neonate receiving 'usual' doses on the basis of bodyweight. The serious toxicity (circulatory collapse or grey baby syndrome) associated with the administration of standard doses of chloramphenicol in neonates prompted numerous pharmacokinetic studies, which led to more precise dosage regimens based on weight, gestational and postnatal age. Although chloramphenicol is metabolized by UGT2B7, it is thought that other UGT isoforms may also participate in its elimination. Morphine is extensively metabolized by UGT2B7 with formation of both 3-glucuronides and 6-glucuronides (M3G and M6G). M6G has been shown to be a potent analgesic. M3G, in contrast, has no analgesic effect, but shows enhanced excitatory activity by a nonopioid mechanism. Studies on the metabolism of morphine in children and premature neonates show that morphine glucuronidation capacity is enhanced after the neonatal period. The elimination of epirubicin, which is used in the treatment of adult and childhood malignant diseases, is also largely dependent on glucuronidation by UGT2B7. An increase in epirubicin glucuronidation activity was observed with increasing age, with a positive correlation between UGT2B7 levels and postnatal age [75]. A relative deficiency in glucuronide conjugation of salicylamide and paracetamol is still observed in children of seven to ten years compared with adults [76]. The principal catalyst of paracetamol glucuronidation is UGT1A6, but UGT1A1 and 1A9 are also involved [77]. Strassburg *et al.* [78] studied the developmental aspects of 13 UGT genes. After six months of age UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B4, 2B7, 2B10 and 2B15 transcripts could be detected. Western blot analysis showed no differences in the expression of UGT1A1, 1A6 and 2B7 proteins. Lower mRNA expression in paediatric samples was observed for UGT1A9 and 2B4. However, even though the mRNA levels and protein expression were similar across the different age groups, hepatic glucuronidation activity in children aged 13–24 months was found to be lower than that in adults for ibuprofen, amitriptyline, 4-*tert*-butylphenol, estrone, and buprenorphine. The differences between the adult and paediatric enzymatic activities may be due to membrane factors including phospholipid content, long chain fatty acids and acyl co-enzyme A.

Elderly: Temellini *et al.* [79] reported that UGT activity towards ethinylestradiol in microsomal preparations of human liver was not age-dependent. No influence of aging has been reported for the conjugation of propranolol [80]. According to Divoll *et al.* [81] and Miners *et al.* [82] no changes have been observed in paracetamol glucuroconjugation with age, whereas others reported frailty-associated reduction in the conjugation of paracetamol and suggested an impairment of glucuronidation in this population [83]. The glucuronidation of lamotrigine, mediated by UGT1A4 and 2B7 [84], is diminished in elderly subjects [85]. The clearance of oxazepam and retigabine, which are metabolized by UGTs, is also diminished in the elderly compared to young subjects [86,87].

Sulfation

The sulfotransferase (SULT) gene family encodes at least 11 distinct enzymes that catalyze the sulfate conjugation of a variety of endogenous and exogenous chemicals using 3′-phosphoadenosine-5′-phosphosulfate (PAPS) as a donor. Sulfotransferases and glucuronosyltransferases may have overlapping substrate specificities.

Paediatric population: The sulfate pathway is the dominant metabolic route for salicylamide and paracetamol in infancy and childhood as it is practically mature at birth [7,76]. The neonate can use sulfate conjugation to compensate for the less-developed glucuronidation, for example as an alternative route for morphine metabolism [7,88,89]. However, as there are several forms of sulfotransferases, the developmental pattern of the isoforms can be different [10].

Elderly: Temellini *et al.* [79] reported that sulfotransferase activity towards ethinylestradiol in cytosolic preparations of human liver was not age dependent. This observation was confirmed using paracetamol [90] or dehydroepiandosterone as substrate [91]. No

changes with age have been observed in the sulfoconjugation of paracetamol [81]. Wynne *et al.* [83] showed that the partial metabolic clearance to paracetamol sulfate was preserved per unit volume of liver with ageing and frailty; however, Miners *et al.* reported a lower partial metabolic clearance by sulfotransferases in elderly males than in young adult males whereas glucuronide and glutathione-derived conjugates were not significantly different between the two groups.

Conjugation with amino acids

Conjugation of xenobiotic carboxylic acids with endogenous amino acids has been shown to be an important pathway in the biotransformation of a number of compounds. In humans, the most frequently observed amino acid conjugates are those with glycine and glutamine; however, taurine conjugation can also occur to a minor extent.

Paediatric population: Glycine conjugation of benzoic acid to form hippuric acid is present but deficient in preterm neonates [92]. This is consistent with results reported by Pacifici *et al.* [93] that show over 40-fold higher activity in adult liver and kidney than in foetal samples. Conjugation with glycine instead of glucuronic acid is the dominant pathway of salicylate metabolism in neonates. Conjugation with glycine increases from newborns to infants and from infants to children [10]. The lack of readily available glycine seems to be only partly responsible for the decreased formation of glycine conjugate in newborns [10].

Elderly: In human liver homogenates, there is a weak, but significant, negative correlation between the rate of formation of hippuric acid and the liver donor's age [94].

Conjugation with glutathione

Glutathione conjugation is catalyzed by cytosolic glutathione *S*-transferases (GST). The GSTs can metabolize endogenous and exogenous toxins and carcinogens by catalysing the conjugation of diverse electrophiles with reduced glutathione. Thirteen different human members of the GST family have been identified belonging to five different classes.

Paediatric population: A review paper on the ontogeny of human phase II conjugation enzymes has been written recently by McCarver and Hines [95]. GSTA1 and GSTA2 expression levels increased 1.5–4-fold, respectively, to adult levels within the first one to two years of life. At birth, GSTM expression increased approximately fivefold to adult levels. By contrast, GSTP1 is present in the neonatal period but nondetectable in the adult liver.

Elderly: The concentrations of glutathione in human liver are unaffected by ageing [96,97]. Miners *et al.* [82] found no significant differences in the formation of glutathione-derived conjugates of paracetamol between elderly and young adult males. The expression of the components of the glutathione system in the colon was investigated with respect to age in macroscopically normal colon mucosa. In female subjects, there was a significant stepwise increase in GST activities and GSTP1 levels from the age of <50 years to >70 years [98].

Conclusion

In this review, the key factors accounting for differences in drug/metabolite exposure, that is differences in drug metabolism (both qualitative and quantitative) at the extremes of age have been discussed. Numerous studies have demonstrated age-related changes in the pharmacokinetics of various drugs. However, it is difficult to differentiate the effect of age because of variations in enzyme activity from effects caused by other factors such as altered liver mass, liver blood flow and changes in plasma drug binding. High interindividual variability may also mask age-dependent differences between the activities of specific drug metabolizing enzymes.

Nevertheless, important differences have been found in the paediatric population compared with that of adults both for phase I enzymes [oxidative (e.g. CYP3A7 versus CYP3A4 and CYP1A2), reductive and hydrolytic enzymes] (see Table 1) and phase II enzymes (e.g. *N*-methyltransferases and glucuronosyltransferases) (see Table 2). However, although determination of the metabolic fate of drugs is a vital part of the drug development process, drugs are rarely tested as substrates or inhibitors for CYP3A7, even though this is a major CYP isoform expressed in infants up to six months of age. Although CYP3A7 substrates are similar to those for CYP3A4, V_{max} values tend to be lower and K_m higher in most cases. Moreover, the potential for the generation of different metabolites from the same substrate by the two isozymes cannot be excluded. CYP3A7 is also less prone to inhibition than CYP3A4 [29]. Generally the major differences in enzyme activity in comparison with that of adults are observed in newborn infants, although for some enzymes (e.g. glucuronosyltransferases and other phase II enzymes) important differences still exist between infants and toddlers and adults. Each enzyme has a unique maturation profile. Changes in endogenous factors related to maturation such as hormone levels are likely to play a role in constitutive enzyme expression. Developmental changes in enzyme activities in human liver may also be attributable to the ontogeny of the transcription factors that regulate gene expression, including the xenobiotic receptors [99]. The availability of conjugating agent or organelle membrane composition may additionally contribute to differences in the paediatric and adult populations.

In the elderly, among phase I enzymes, some esterases, in particular, appear to be impaired (see Table 1). Concerning phase II reactions, not many studies have examined the effect of old age on conjugation. From the information collected thus far, it would appear that phase II reactions, although sometimes decreased, are not extensively affected by age (see Table 2). The loss of some enzyme activities may be due to a loss of the smooth endoplasmic reticulum with age [28]. It has also been observed that elderly individuals possess fewer, larger hepatocytes than younger individuals possibly as a result of nuclear polyploidization [100]. Other enzyme activities (e.g. GSTs) may be influenced by levels of steroid hormones or reactive oxygen species.

For some enzymes (e.g. reductive enzymes, TMPT [101,102], epoxide hydrolase), little, none or contradictory information is available comparing activities in the paediatric, geriatric and adult populations. Epoxide hydrolase, classified as phase I or phase II enzyme according to the different authors, is an important example of enzymes for which some information is available in human foetal and adult tissues [10,95], but little information is available in the paediatric population and in the elderly. This enzyme system is responsible for the detoxification of many reactive metabolites generated by the microsomal monooxygenase system. If age-related changes in monooxygenase, which causes the production of a

TABLE 1

Summary of activities/concentrations of phase I drug metabolizing enzymes or metabolic reactions in the paediatric population and in the elderly compared with those of adults

Enzyme family/subfamily/isoenzyme or metabolic reaction	Paediatric population	Elderly	References/substrates
Oxidative enzymes			
CYP1A2	↘ until two years	↘ or ∼	[10,11,13–15]
CYP2A		↘ or ∼	[20,21]
CYP2A6	↘ until 6–13 years		[19]
CYP2C		∼	[27,28]
CYP2C8	∼		[26]
CYP2C9	↘ until one to two years	↘	[20,21,24]
CYP2C10		↘	[20,21]
CYP2C18		↘	[20,21]
CYP2C19	↘ until ten years	↘	[14,20,21,24,29,30]
CYP2D6	↘ until 12 years	∼	[10,14,20,21]
CYP2E1	↘ until two years	Contradictory data	[10,14,20,27]
CYP3A		↘ or ∼	[27,28,33,34]
CYP3A4	↘ until two years		[31]
CYP3A5	∼		[10,31]
CYP3A7	↗ until three to six months (very low or undetectable in adults)		[10,31]
FM01	Suppressed within three postnatal days		[10]
FM03	↘ until 12–18 years		[10]
MAO A	↗ until two years	∼	[37,39]
MAO B	∼	↗ or ∼	[37,39,40]
ADH	↘ until two to five years	↘ or ∼	[10,42–47]
AO	↘ until one year		[49]
XO	↗ (plasma) in newborns	∼	[48–51]
Reductive enzymes			
NADPH-cytochrome P450 reductase		∼	[28]
Aldo-ketoreductase(s) involved in PGE_1 metabolism	↘ until ten years		[58]
Hydrolytic enzymes			
Pseudocholinesterase	↘ until one year		[60]
Arylesterase	↘ until one year		[60]
Acetylcholinesterase	↘ until one year	∼	[60,69] acetylthiocholine iodide
Benzoylcholinesterase		∼	[69] benzoylcholine iodide
Butyrylcholinesterase		∼	[69] *S*-butyrylthiocholine iodide
Esterase	↘ until 2–11 years		[61,62] chloramphenicol esters
Esterase	↗ until 2–11 years (limited data)	↘ or ∼	[63,65–69] aspirin
Carboxylesterase	∼		[64] *p*-nitrophenylacetate

∼: similar to that of adults; ↘: lower than that in adults; ↗: higher than that in adults.

reactive epoxide, are not accompanied by corresponding changes in detoxifying epoxide hydrolase, unexpected toxicity may occur.

There is a growing awareness of the biological basis of age-related ADME and increasing interest in the development of in silico models to simulate drug disposition in virtual patient populations. These models integrate *in vitro* data on human drug metabolism and transport with demographic, genetic, physiological and pathological information to predict population distributions of drug clearance and metabolic drug–drug interactions [103]. These algorithms have been extended to include information on age-related development of drug metabolizing enzymes in the paediatric population and the high interindividual variability in microsomal protein levels in the elderly that might play a role in the extreme values of drug clearance observed in certain individuals. The prediction of variability in drug clearance is important to identify patient populations who may require dosage adjust-

TABLE 2

Summary of activities/concentrations of phase II drug metabolizing enzymes or metabolic reactions in the paediatric population and in the elderly compared with those of adults

Enzyme family/subfamily/isoenzyme or metabolic reaction	Paediatric population	Elderly	References/substrates
Acetyltransferases			
NAT1	Insufficient information		[10]
NAT2	↘ until one to four years		[10]
Methyltransferases			
N-Methyltransferases	∼		[10]
TPMT	↗ in neonates, contradictory data in children		[10,102,103]
Glucuronosyltransferases			
UGT1A1	↘ until seven to ten years	↘ or ∼	[10,74,79]
UGT1A4		↘	[84,85]
UGT1A6	↘ until seven to ten years	Contradictory data	[76–78,82,83]
UGT1A9	↘ until seven to ten years	Contradictory data	[76–78,82,83]
UGT2B7	↘ until 11 years	↘	[75,84,85]
UGT		↘	Oxazepam [86,87] [a]
UGT		↘	Retigabine [86,87] [a]
UGT		∼	Propranolol [80] [a]
Sulfotransferases			
SULT1A1	↘ until 12 years	↘ or ∼	[10,82,83,90]
SULT1A3	∼		[10]
SULT2A1	∼		[10]
SULT1E1		∼	[79]
Sulfotransferase		∼	Dehydroepiandosterone [91] [a]
Conjugation with amino acids			
Glycine	↘ until two years	↘	[10,94]
Glutathione transferases			
GSTA1	↘ until one to two years		[95]
GSTA2	↘ until one to two years		[95]
GSTM	∼		[95]
GSTP1	↗ in the neonatal period (undetectable in adults)	↗ (females)	[95,98]
GST		∼	Paracetamol [82] [a]

∼: similar to that of adults; ↘: lower than that in adults; ↗: higher than that in adults.
[a] Non-specific substrate or specificity unknown.

ment in order to avoid adverse reactions (e.g. chloramphenicol in the paediatric population) or a lack of efficacy due to differences in drug exposure. These predictions depend on the availability of high quality data across a wide range of enzyme systems. Further studies will be necessary to provide rigorously derived kinetic parameters that will enable a better understanding and prediction of pharmacokinetic behaviour of drugs in development in the paediatric and geriatric populations.

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