Paediatric and Perinatal Drug Therapy, 2007; 8 (1)

Problems and pitfalls performing pharmacokinetic studies in children

Brian J Anderson[1], Amanda L Potts[1], David W Herd[2]

[1]*Department of Anaesthesiology, University of Auckland, Auckland, New Zealand*

[2]*Department of Paediatrics, Auckland Children's Hospital, New Zealand*

Corresponding author

Associate Professor Brian Anderson, c/o PICU, Auckland Children's Hospital, Auckland, New Zealand
Email: briana@adhb.govt.nz

The apparent simplicity of the paediatric pharmacokinetic study is deceptive. The material reported in a paediatric pharmacokinetic publication does not include the trials and tribulations endured by investigators during the study; problems and pitfalls that are encountered again by others in further paediatric studies. Study design can not always be extrapolated from information based on adult experiences. Ethical considerations have been advanced to prevent harm to children. Recruitment, sampling for drug assay, adverse effect monitoring and data interpretation also present snags for consideration in a population that extends from premature neonates through to young adults. Consequently, paediatric studies differ from those conducted in adults and knowledge gained during the conduct of the one may not be applicable to the conduct of the other. The initiation and successful completion of a paediatric study by a novice is better undertaken with knowledge of these pitfalls and the guidance of others' experiences.

Paed Perinat Drug Ther 2007; 8: 4–17

Keywords: paediatric – pharmacokinetics

Introduction

Paediatric pharmacokinetic (PK) studies appear deceptively simple in design, execution and analysis. A drug is administered to children, samples from body fluid are taken to assay drug concentration at consecutive time points and those data analysed by reducing the sequence into a small number of characteristics. The reduction of a time-concentration profile into summary measures can be done without specific models (e.g. non-compartment models) or by estimating the parameters of mathematical models that describe that profile (e.g. compartment models).

The apparent simplicity of the paediatric PK study hides the pitfalls at each step of the conduct of such a study. Children were involved in all of the major therapeutic catastrophes that have shaped modern drug development[1-4]. Ethical considerations have been advanced to prevent harm to children[5]. Children are better studied in facilities that can cater to their needs and have the capacity to coordinate sampling with clinical activity. The drug assay may have to be made using body fluid samples much smaller than those used in adults. Analysis of data has advanced beyond interpretation of graphical representation and may require complex mathematical modelling. The impact of isomerism, active metabolites, chronobiology, drug interactions and disease processes requires

consideration. Good clinical research practice, regulatory requirements, drug assay costs, staffing and hospital overheads may impose considerable financial burden on any research project. The Food and Drug Administration (FDA) in the US has clear guidelines for PK studies in children and these guidelines cover many of the subjects in this current paper[6].

This review uses two recent PK studies investigating ketamine for procedural sedation[7] and analgesia in the Emergency Department (ED) and clonidine for postoperative analgesia and sedation in the Paediatric Intensive Care Unit (PICU) that were performed in our hospital in order to illustrate some of these problems and pitfalls (Table 1).

Ethical considerations

The two key considerations in medical ethics are respect for patient autonomy and beneficence on the part of the physician[8]. Respect for patient autonomy is part and parcel of the consent process. The patient should be aware of the pros and cons of treatment options and decide without coercion. Consent for children is usually sought from parents or care-givers. The assumption is that the consenting adult will act in the best interests of the child. Adults enter clinical trials for reasons that may not always apply in children, e.g. financial reward, increased attention from physicians, low perceived risk, future benefit to others with similar conditions, curiosity about scientific process, disgruntlement with existing therapies or simple altruism. Participation in a trial may improve outcome[9-11].

Children lack life's experiences that allow them to assimilate trial information and translate this information into realistic expectations. Children younger than 9 years of age often have poor understanding of the reason for a study or procedures involved. The ability of many children to volunteer may be compromised by their belief that failure to complete the study will displease others. Children often believe that it is not alright to withdraw from studies despite being explicitly informed that this is possible[12]. The concept of assent has been developed to give children input into their agreement to participate in a trial when fully informed consent is inappropriate. Age-appropriate explanation is the key to this process[13].

Beneficence implies that the physician holds the best interests of the patient utmost. This must be interpreted in terms of the family as well as the individual for children. The risk should be minimal and the benefit/risk ratio high. Minimal risk is defined as no greater than the risk encountered by the subjects in the aspects of their everyday lives[14]. However, minimal risk to an adult is not the same as minimal risk to a child. Blood sampling in a premature neonatal PK study, for example, may increase the propensity for anaemia. Venepuncture is a mild impediment in adults, but 85% of children 2.5–6 years (yr) undergoing venepuncture report high levels of distress[15]. This distress is magnified when phlebotomists lacking paediatric expertise are involved.

Both ketamine and clonidine are routinely used in the populations studied. The ED policy dictated that ketamine should not be used in children under three months of age, although children under one year rarely have procedures suitable for ketamine sedation and have increased airway risks. PK studies are more acceptable to ethical committees if the drug to be investigated is already prescribed by the patient's physician. The foundations for ethical approval vary in different parts of the world. In the USA, one is allowed to do non-therapeutic research in children if the risk is minimal. In Europe this is not allowed and a drug can only be studied as part of a therapeutic randomised controlled trial or when the drug is already prescribed by the physician. If a drug is not labelled for children even when prescribed for clinical reasons, discussion arises about whether

Table 1 Comparison of the two hospital based PK studies

	Ketamine study	Clonidine study
Location	Emergency Department	Paediatric Intensive Care Unit
Population	Minor trauma healthy (*n*=54)	After cardiac surgery (*n*=41) Possible hepatic/renal dysfunction
Age range (yr)	1–15	0–15
Consent	On admission	1–5 days preoperative
Dose	1 mg/kg IV	1–2 microg/kg IV
Blood sampling	3–4 blood samples over 1 h Venous cannula inserted using topical analgesia 2 ml sample + 3 ml dead space discard	3–4 blood samples over 12 h Arterial cannula inserted under anaesthesia 2 ml sample Dead space blood returned to patient
Assay	Preparation/transport of serum by local hospital laboratory, assay by independent laboratory	Preparation/transport of serum by investigators, assay by independent laboratory
Monitoring	Video recording	Use of nursing charts

the trial is a drug trial or not, which makes it a lot harder to get the study approved.

Time for informed consent may be short, situations stressful and the need for treatment emergent in the ED. Informed consent requires a multiple page consent document often with additional age-specific information for the child. This consent process is a major impediment to running PK studies in EDs. In the ketamine study the primary investigator was on call for all procedures requiring ketamine, was present for all consent discussions and was able to facilitate clinical treatment. Without this commitment, the project may not have succeeded. However, in some jurisdictions, the patient's physician can not be the investigator and the person who asks informed consent. A research assistant or nurse is a minimum requirement and adds considerable logistical and financial cost to studies.

The ketamine study began without video surveillance; however it became obvious that video would be required to record accurate timing of events. Fortunately the ketamine study had initially been designed to include video and the ethical issues had been considered. Ethical consent was obtained quickly because of this. Video, apart from a number of technical challenges, highlights issues of privacy and consent. The ketamine study had four levels of consent; no video to be taken, video of the monitors for timing purposes only, video of the child for full psychological assessment by the primary investigator and a named clinical psychologist and video for public viewing. A separate consent for public display of the video was required to match institutional requirements. One parent asked for the video to be used only by the principal investigator and then destroyed. Ethics consent for video was facilitated by new technology that included digital video and encryption for easy storage and privacy. The ethics committee also suggested adding a request of the parents to allow the video to be used in future studies providing that full ethics approval is gained for those future studies. This ensures that valuable information gained may be re-used.

The ketamine study had a high enrolment rate (60/61) and this is because the study did not differ from standard clinical care apart from the drug sampling and observations. Parents were happy to consent because they felt the study may help future children. The promise of feedback (sending a summary of the results after trial completion) was also a positive factor in enrolment.

N-methyl-D-aspartate (NMDA) antagonists such as ketamine, are postulated to cause significant neuronal apoptosis during the periods of synaptogenesis in mammals. Neonatal rats exposed to ketamine have suffered widespread neuronal apoptosis and long-term memory deficits[16,17]. The applicability of extrapolating rodent data to the care of human neonates continues to be debated[18,19]. These concerns had some impact on the decision to study children 1–15 yr only. It is the physician's duty to provide information that a reasonable patient considers to be important. What is important to the individual will vary enormously.

Clonidine is not labelled for use in the paediatric population due to a lack of adequate well controlled studies. Accordingly, the PK and pharmacodynamic (PD) profile of this drug in children may not be well predicted by adult data and some adverse effects may not be predictable in paediatric patients because of these differences. The ethics of allowing physicians to study new compounds (e.g. the new alpha-2 agonist, dexmedetomidine) without allowing investigators access to pharmaceutical company preclinical data has recently been questioned[20]. Concerns about future harm to children from medication given at an early age dictate a long period of trial data storage and our National Ethics Committee advocated 26 years.

Practical considerations

Background reading

A literature review of the study drug is of value before embarking on the study. Safety issues, dose considerations and a clear understanding of intended data interpretation are paramount. Predicting dose in children, when only adult data are available can be difficult. Predicting dose in neonates, with their altered body composition, physiology, immature enzyme clearance pathways, effect responses and disease spectrum may be tackled by using very small empirical doses as pilot data. Estimation of dose based on ontogeny of clearance pathways may also be useful. Data on ontogeny of individual clearance pathways, derived from measurements of enzyme expression and activity in post-mortem livers[21,22] and from *in vivo* data from drugs that are cleared by similar pathways are useful. Models that incorporate this knowledge as well as additional covariates (protein binding, hepatic blood flow, liver size, renal maturation) are being developed to assist with first-time dosing in children[23-27]. Allometric scaling is applicable to children out of infancy[28].

One difficulty we encountered setting up the clonidine study was the lack of published PK paediatric clonidine studies. There was only one

published PK report concerning IV clonidine[29] and minimal information concerning clearance maturation. Best sampling times were based on adult studies and many of the adult studies used a one-compartment model. This approach using adult data alone was flawed. Adult data needs to be combined with developmental PK knowledge.

Clonidine clearance is dependant on the glomerular filtration rate (GFR) and CYP2D6. Maturation data of these pathways in neonates, infants and children are available to make an educated guess for age-related clearance changes, based on developmental milestones. Renal clearance is the main pathway and we might not expect CYP2D6 genetic polymorphism to play a major role, unless renal failure occurs (a possibility after paediatric cardiac surgery). Preliminary data from our study showed that a two-compartment disposition model was more appropriate. This required a change in the sampling times and the addition of an extra blood sample.

Coordination of study

Good clinical research studies can falter because of a lack of overall supervision. There may be a lack of understanding from novices about what is involved setting up a paper trail in accordance with good clinical research practice (GCP). Continued direct involvement from the principal investigator is necessary in a busy hospital practice. Research does not run on auto pilot. The imposition of additional workload associated with research is easily dropped by clinical staff with heavy routine duties. A simple measure such as payment for an occasional morning tea for these busy staff goes a long way to maintaining good will and subsequent trial compliance.

Recruitment

The difficulties of recruiting children to clinical trials need to be appreciated by all interested parties[30]. Parental consent and child assent are minimal prerequisites for recruitment of a child into a clinical trial. There are numerous challenges achieving this. Parents are generally reluctant to involve their child in a clinical study where the drug the child is receiving is not part of normal clinical care. Parents do not want their child to undergo any additional venepuncture, often an essential component of a PK study. In the clonidine study this was overcome by the use of pre-existing cannulae inserted during anaesthesia that allowed blood sampling with no additional venepuncture.

Although parents may be supplied with written information about the study prior to hospital admission, it can be hard to find an appropriate time to talk to the parents of a possible recruit. This occurs for a number or reasons; the parents may be talking to the surgeon, anaesthetist or nurse, the child could be having another procedure (ECG, cardiac ECHO, radiology), or simply that the parents are not always with the child. Often only one parent accompanies the child for their preoperative stay in the ward and that parent needs to discuss the study with their partner before making a decision. There may be limited time before the operation when consent can be obtained and parents can become overwhelmed with hospital processes and not make a decision. The main reason reported to us by parents for not wanting their child included in the study was that 'their child had been through enough already'. Adverse publicity concerning a monoclonal antibody (TGN1412) study at Northwick Park Hospital in London, March 2006, was cited as a reason for refusal of trial recruitment by a number of parents while media concerning that study remained topical.

The ketamine study recruited nearly half of all eligible participants and the main reason for not recruiting was that the primary investigator was unavailable. The high level of recruitment was aided by in-depth education of all staff about the beneficence of the study, the methods involved and how it impacts on future clinical management. All staff were free to feedback to the investigators.

Blood sampling and assays

Initial clonidine assays required at least 1 ml of plasma per sample. To achieve this volume of plasma, at least double the amount of blood was required from an indwelling arterial catheter. This is not a problem for children and older infants; however, in neonates this amount of blood, combined with the blood samples required for routine cares, may increase the risk of anaemia. Assays can often be performed with less than 1 ml of plasma but this may preclude additional assaying of the same sample if required, which may affect assay quality control. Three blood samples over an eight hour period were originally assayed for the clonidine PK analysis. However following preliminary analysis, a fourth blood sample was deemed necessary to improve accuracy of parameter estimation for a two compartment model. This was achievable because refinements in assay technique allowed smaller sample volumes.

The volume of blood required for ketamine assay was small (2 ml) although an additional 3 ml discard was required to ensure accurate sampling

from the cannula. This discard was not returned to the patient. Consent was given for taking blood from the existing cannula in the ketamine study. The cannula was placed using topical anaesthesia. If the drug under investigation cannot be sampled from the administration cannula (e.g. binding of drug to plastic cannula[31,32]) then a dedicated sampling cannula may be required. It is likely a second cannula would reduce patient enrolment.

Clinicians will be reliant upon expert laboratory services. For many drugs this may require transport of samples to distant or even foreign facilities. Laboratories that can work on small samples are required for paediatric studies and are fortunately common these days. Methodology needs to be reported in detail as well as variability details (between samples, over time, over concentration range). This information may require some initial samples for calibration and the ketamine study used six participants for this purpose. Excellent communication between the investigator, the local laboratory and the analysing laboratory is essential. Only one ketamine sample from 189 was mishandled by the local laboratory. This sample was frozen by mistake and this issue was addressed by adding new instructions that accompanied each sample for assay. Unanticipated problems will periodically occur and rapid addressing of these is the key to reducing wastage of samples. Blood samples in the clonidine study were spun down to plasma, stored and sent for assay to the analysing laboratory by the investigators. This self-preparation and transport of samples was considerably cheaper (by a factor of 7) than commissioning the local laboratory to do this.

Assay methods may be expensive and contribute considerable financial burden on young investigators' budgets. The local laboratory or university may be unable to do the assay because additional equipment such as a mass spectrophotometer is required. Samples may be assayed in batches for quality assurance and financial reasons. This can cause frustrating delays if there are deadlines linked to study completion and submission. Interim analysis can aid population PK studies (e.g. best blood sampling time prediction) as opposed to controlled trials where such activity is considered poor practice.

Sampling blood

Clonidine blood samples were taken from arterial cannulae that had been inserted for monitoring during anaesthesia and PICU stay. This provided a convenient way of taking blood samples without causing the child any additional pain on sampling and proved to be an important factor when parents were considering if their child should take part in the study.

Sampling of blood should be remarkably straightforward in children's ED and PICU. Such procedures are common and the staff skilled. Successful sampling for PK was achieved in all children in the ketamine study with only one child requiring multiple attempts. Cannulae may block, tissue, or fail to relinquish blood due to position relative to the vessel wall. Topical anaesthesia was used for cannulae insertion on children involved with the ketamine study. Arterial rather than venous samples may influence PK estimates for some drugs metabolised in the lungs.

Repeated blood sampling is ethically and practically possible in children undergoing a procedure or nursed in intensive care. This is unlikely to be the case with the majority of PK studies in children. In that case, sparse sampling is likely to be the only approach.

Sampling coordinated with clinical activity

The timing of blood samples in the clonidine study was as a window (e.g. the first sample at 5–15 min following drug administration) and not an exact time. This allowed nursing staff to take the study samples at the same time as routine blood samples for monitoring of electrolyte or blood gas status. When the study drug was administered on the hour, the subsequent timing for blood samples occurred approximately 15-20 min after the hour; this allowed time for the nurse recording of routine hourly observations. Occasionally blood samples for assay were put into the unheparinised containers by staff that were unfamiliar with the study and this resulted in the loss of these blood samples.

Sample times for the ketamine study were estimated *a priori* from adult data and attempted to be done at times that would provide maximum utility. Early samples were limited by clinical activity (moving the child, undressing, removing plaster cast); late samples were limited by the child being discharged. Accuracy of recording time was improved by addition of video although in future studies a reference digital clock will be placed in the view of the camera rather than trying to adjust for time differences between the monitor, the camera and the clock in the room.

Alternatives to blood letting

In general, children detest needles and venepuncture remains unpopular, despite the common use of topical anaesthesia. The very sight of blood being withdrawn from an indwelling

cannula can be reason for withdrawal from a study for the occasional child. Saliva has been successfully used to study paracetamol PK in children[33] and there is hope that this technique could be extended to other drugs such as ketamine[34]. Urine remains a popular alternative to blood for drugs that are eliminated through the renal system. 'Dried blood spots' from finger or heel pricks have been used extensively for neonatal disease screening and hold potential for sparse sampling in population PK studies[35]. Alternative collection methods (e.g. saliva, urine, breath) require specific adjustment to be used in neonates. These sampling methods need to be validated before they can be used.

Adverse effect monitoring

Side effect monitoring must include observation for standard known adverse effects as well as vigilance for serious idiosyncratic responses. This included cardio-respiratory, sedation and oxygen saturation monitoring as well as two doctors and a nurse in a fully equipped resuscitation capable environment during ketamine administration. PICU and ED are expert in managing these situations but other departments may not be able to dedicate the same resources to adverse effects monitoring. Most paediatric studies, however, are not conducted in such an environment.

The establishment of a data monitoring committee should be given consideration for paediatric studies[36]. Monitoring adverse effects in young children, where there may be no signs or inadequate laboratory reference values can be difficult. Independent monitoring contributes to safety and assists with data interpretation through timely and meaningful interim results[36].

Data analysis

Consideration must be given to data analysis before initiating the study because this will influence sample numbers and timing, as well as the number of subjects that are likely to be required, to ensure PK parameter estimates are robust. The parameter estimates from the mathematical models used to analyse the data can then be used to predict the time-concentration profiles of other doses. Attempts to predict what will happen in a further subject often become unstuck because a factor accounting for variability between subjects is missing. If the variability between patients is modelled, then it is possible to predict the magnitude of the difference between predictions and the observations in the next subject. It may be possible to explain the variability on the basis of physiological differences; in which case predictions are improved because unexplained residual variation is reduced.

There are three common approaches to modelling data collected from a group of subjects (Tables 2 and 3). The FDA, on its website has guidelines concerning population PK[37].

Naïve pooled data approach

Time concentration data are pooled together as if all doses and all observations pertain to a single subject. Samples are taken at the same time in each individual. No information is available on individual subject profiles or parameters. This approach may be satisfactory if data are extensive for each subject and there is only minor inter-individual variability, but may result in misrepresentation if data are few. Problems also arise interpreting results when data are missing from some subjects. No information can be gathered about the magnitude of inter-individual variability and its causes.

Standard two-stage approach

Individual profiles are analysed and the individual structural parameters, e.g. volume of distribution (V), clearance (CL) are then treated as variables and combined to achieve summary measures. Sampling times have greater flexibility but must be complete for each individual. If the estimates are not based on a similar number of measurements for each individual, or if the response in one individual is much more variable than another, some form of weighting is required.

Table 2 Comparison of the classical (e.g. naïve pooled and standard two-stage methods) and population PK approach (e.g. mixed effects modelling) in paediatric drug research

	Classical approach	Population approach
Number of subjects	Few subjects (e.g. 6–12)	Large population (e.g. 50)
Number of samples	Rich data for time-concentration profiles	Few blood samples required from each subject
Data recording	Data recording usually easily controlled	Timing of drug dosing and blood sampling may also be less precisely noted when historical data are used
Estimation of best sampling times	Sampling times based on absorption and disposition half-lives	Optimal sampling strategies required to maximise information when sample or subject limitations exist
Data pooling	Data pooling tricky	Pooling of data from different studies may be possible
Computer program	Basic PK programs readily available and easy to use	Specialised software. Complex and computationally intensive methodology

Table 3 Comparison of the classical (e.g. naïve pooled and standard two-stage methods) and population PK approach (e.g. mixed effects modelling) in paediatric drug research

	Naïve pooled	Standard two-stage	Population approach
Sampling times	Exact same times for each individual	Sampling times in a window for each subject	Sampling may be coordinated with clinical activities. Rigid times unnecessary
Missing data	Each individual must have all data	Truncated data or missing data often excluded from analysis	Interpretation of truncated individual sets of data or missing data may still be used for analysis
Covariate analysis	Covariate analysis impossible	Covariates may be difficult to assess	Covariate investigation routine
Variability	No information about the magnitude of inter-individual variability and its causes	Cannot distinguish inter-individual from residual variability	Can distinguish inter-individual, intra-individual and residual variability

The inter-individual variability can be estimated from the standard deviation of the individual estimates, but it is an overestimate of the true variability because each estimate also has variability due to imprecision of the estimate. It may be possible to identify covariates to explain some of the variability but this does depend on having relatively good individual estimates of the parameters.

Mixed effects models

Mixed effects models provide a means to study variability in drug responses among individuals representative of those in whom the drug will be used clinically. The naïve and standard two stage approaches rely on "rich" data from a small group of subjects. In contrast, mixed effects models can be used to analyse "sparse" (2–3 samples) data from a large number of subjects. These models are “mixed” because they describe the data using a mixture of fixed and random effects. Fixed effects predict the average influence of a covariate such as weight as an explanation of part of the inter-individual variability in a parameter like clearance. Random effects describe the remaining variability between subjects that is not predictable from the fixed effect average. Explanatory covariates (e.g. age, size, renal function, sex, temperature) can be introduced that explain the predictable part of the inter-individual variability.

Interpretation of truncated individual sets of data or missing data is also possible with this type of analysis, rendering it useful for paediatric studies. Population modelling also allows pooling of data across studies to provide a single robust PK analysis[38-40] rather than comparing separate smaller studies that are complicated by different methods and analyses. Mixed effects modelling is a complex and computationally intensive methodology. There are a number of statistical programs available to undertake such analyses, but the most commonly used and versatile is that implemented in the Nonlinear Mixed Effects Model (NONMEM)[41-43]. Nonlinear regression is performed by an iterative process to find the curve of best fit by maximising the likelihood[44,45], an extension of line fitting through minimising the residuals (difference between observation and prediction).

Advantages to children of mixed effects population modelling

The first population PK studies were performed in adults using data from routine therapeutic drug monitoring (TDM)[46]. Interpretation of these routine clinical data was used to develop dosage guidelines that could be used for other patients. The benefits of using data without additional inconvenience in paediatric studies are obvious and paediatric TDM data is now used extensively for population studies[47-50]. A disadvantage of using historical TDM data may be that trough samples do not always adequately reflect area under a concentration time curve (AUC) and therefore are not optimal for PK analysis. Timing of drug dosing and blood sampling may also be less precisely noted when historical data are used, leading to more unexplained variability.

Sampling times are not crucial for population methods and can be fitted around clinical procedures or outpatient appointments. However, optimal sampling schedules can be determined through prior information and the Fisher Information Matrix[51-53]. Sampling time bands rather than exact times are equally effective[54] and allow flexibility in children. These approaches involve defining the PK model, inputting the parameter values and weighting scheme and specifying time ranges. Unfortunately sampling cannulae may block or tissue. Children or their parents may refuse repeat sampling and repeat venepuncture is frowned upon. Missing data, however, can still be used in a paediatric population analysis.

PK considerations

Assay sampling times

A literature review may establish the type of compartment model required (e.g. one- or two-compartment disposition model) and half-lives. This information assists estimation of approximate sampling times. In general, the number of samples required is equal to the number of structural parameters in the model (e.g. CL, V, absorption rate constant (Ka)); three samples will be required for a one-compartment first order absorption and elimination model while five are required for a two-compartment model (Ka, CL, V1, blood flow (Q), V2). Additional samples may be required if there are further covariate analyses. There may be limits on the quantity of blood for assay that can be taken from children. The quality of the analysis can be improved by dividing the study population into groups that have different sampling strategies[54].

It was possible to sample for ketamine assay in children presenting to the ED for a median time of only 28 min. Children were awake, willing to return home and displayed little enthusiasm for further sampling after this time. This duration was adequate to interpret ketamine PK, but inadequate for an analysis of its metabolite, norketamine. It would have been unjustified, logistically and ethically, to keep children waiting in the ED for further blood sampling. Time-concentration profiles for norketamine in both children and adults have been published in the literature. These published data were added to the observed truncated data from the current study in order to make metabolite parameter estimates.

Sample size

The appropriate number of patients for a population study is difficult to determine and will depend on the number of covariates under examination[55]. Data from a single-trough sampling design will require large numbers because data will be noisy. Multiple-trough studies also require large numbers to estimate inter-individual and residual variability with precision. Approximately 50 subjects are often used in a population study that has 3-6 samples per patient, but covariate investigation in a study investigating children ranging from neonates to adults may require larger numbers. Fewer subjects are typically used in a discrete population such as neonates but little can be learned about covariate relationships.

Pharmacogenetics

An understanding of pharmacogenomics has increasing relevance for PK drug studies in children and consideration should be given to investigating this covariate. Single nuclear polymorphisms (SNP) have considerable impact on adverse drug effects and CYP2D6, in particular, has major impact[56]. This enzyme's importance increases when it is responsible for greater than 50% of the clearance pathway, the drug has a steep dose-response curve and a narrow therapeutic window, or if an active metabolite is formed by enzyme. Clonidine is metabolised, in part, by CYP2D6 but SNP impact has not yet been assessed for this medicine.

Ketamine does have an active metabolite. Ketamine is metabolised by the CYP3A4, CYP2B6 and CYP2C9 enzyme systems[57,58] but the contribution pharmacogenomics add to ketamine inter-individual variability is unknown. The clearance formation enzyme responsible for ketamine N-demethylation to norketamine is CYP3A4[58]. Maturation of this enzyme occurs within the first year of life[27], but remains poorly quantified. Pharmacogenetic influences and concomitant drug therapy (e.g. induction of CYP3A4 with alcohol, inhibition with fluconazole, omeprazole, cimetidine) influence clearance. We might expect maturation of the metabolite clearance pathway to mirror renal maturation, which occurs also over the first year of life[59,60].

Binding

Both plasma albumin and alpha-1 acid glycoprotein concentrations increase over the first year of life and may effect PK parameter estimation[61]. However, although changes in plasma protein binding can have an important influence on individual PK parameters, acute changes in plasma protein binding will usually not influence the clinical exposure of a patient to a drug. Clearance has greater impact. As a consequence, no adjustments in dosing regimens are usually necessary for a drug with a high extraction ratio and narrow therapeutic index that is given parenterally (intravenous dosing of lidocaine is probably the predominant case for which protein binding changes are important) or, even rarer, a drug with a narrow therapeutic index that is given orally and has a very rapid PK-PD equilibration time[62].

Isomerism

Racaemic ketamine elimination is complicated by R(–)-ketamine inhibiting the elimination of S(+)-ketamine[63]. PK predictions based on

racaemic assays may overestimate the duration of pharmacological effect. This phenomenon has been described for ketorolac, which is supplied and administered as a racaemic mixture that contains a 1:1 ratio of the R(+) and S(–) stereoisomers. Pharmacological activity resides almost exclusively with the S(–) stereoisomer. Clearance of the S(–) enantiomer was four times that of the R(+) enantiomer in children 3-18 years[64] and the apparent volume of distribution of the S(–) enantiomer was greater than that of the R(+) form.

Active metabolites

Norketamine has analgesic effectiveness one third that of its parent[65], but the role of this metabolite is rarely considered when studying ketamine effect. The onset and duration of metabolite effect remain poorly quantified. This lack of knowledge compromises the interpretation of PD data after ketamine administration. Ketamine is usually administered intravenously or intramuscularly. However, it can also be administered orally, where it undergoes significant first pass effect. For such drugs with significant first pass metabolism, the contribution of active metabolites may differ significantly depending on whether the drug is administered orally or intravenously.

Chronobiology

PK parameters may be influenced by physiological functions that show circadian variation[66]. Circadian changes in PK may result from time-of-day variations in absorption, distribution, metabolism or elimination. For example, hepatic metabolism of drugs is usually a function of hepatic blood flow and enzyme activity within the liver, both of which show circadian variation, and may result in the rate of hepatic metabolism of a drug changing as a function of time of day[67]. Similarly circadian changes in glomerular filtration, renal blood flow, urinary pH and tubular reabsorption have been documented. Changes in urinary pH, for example, results in the increased excretion of acidic drugs in the evening compared to the morning.

Circadian rhythms also influence PD end-points. Pain in children, for example, is often worse at bedtime. Cortisol concentration changes may need to be taken into account when developing PK-PD models.

Paediatric modelling

Growth and development are two major aspects of children not seen in adults. These aspects can be investigated using size and age as covariates. Problems exist because covariates can exhibit co-linearity. Clearance, for example, may increase with weight, height, age, body surface area, and creatinine clearance. All of these covariates may show a high degree of correlation and they are not mutually exclusive[68]. Any one covariate may or may not predict another.

Size adjustment

The range of body weights in children is far greater than that seen in adults and can vary 200 fold (e.g. 0.5–100 kg). Size is the primary covariate used in the investigation of co-linearity. Debate exists concerning the methodology used to adjust PK parameters to body size. Empirical approaches that focus on body weight or body surface area (BSA) have traditionally been used. However, the linear per kilogram and surface area models are inappropriate for scaling small children to adults[69]. The linear per kilogram under-predicts clearance while the surface area model over-predicts clearance in children. This error increases with decreasing weight[69,70]. Linear size models for young children have led to the idea that there is an enhanced capacity of children to metabolise drugs due to proportionally larger livers and kidneys than their adult counterparts[71]. This idea arises because clearance, expressed per kg of body weight, is larger in children than adults (Figure 1).

It now widely recognised that there is a nonlinear relationship between weight and drug elimination capacity[72]. It is possible to show that in almost all species including humans, the log of basal metabolic rate (BMR) plotted against the log of body weight produces a straight line with a slope of 0.75. West et al.[73,74] have used fractional geometry to mathematically explain this phenomenon. These allometric "¼ power" models can be applied to PK parameter estimates in children e.g. CL (0.75), half-life (0.25), V (1).

Effects of age

Once size is standardised, the effects of other covariates such as age[75], temperature[76], and renal function[77] can be investigated. Age is used to describe maturation of clearance or changes in body composition that influences distribution volumes. Albumin, globulin, lipoprotein and glycoprotein concentrations change over the first year affecting drug binding and the amount of free drug available for effect[78].

The quantitative models used to describe this maturation process vary depending on the span of the ages under investigation (Figure 2). A linear model is commonly used for a population

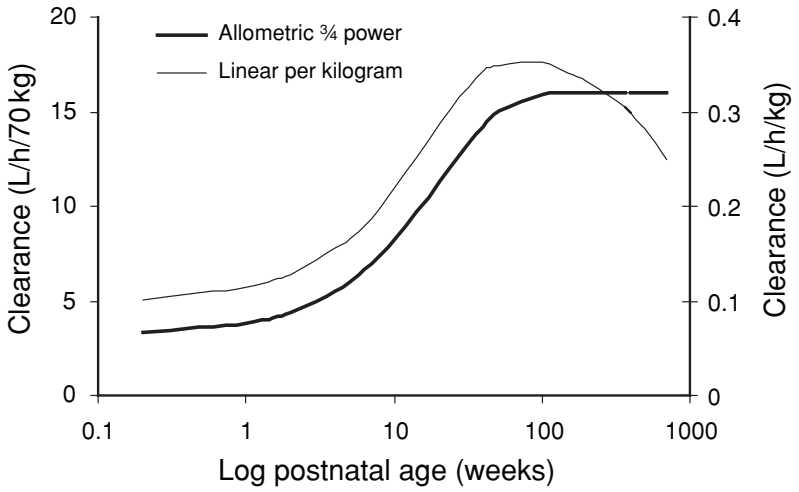

Figure 1 Age-related clearance changes for a hypothetical drug. Both models show an increase in clearance over the first year of life, attributable to clearance maturation. Clearance, expressed using the linear per kilogram model, decreases with age after 1 year to reach adult rates in adolescence. This course is not evident with the allometric ¾ power model.

sample limited to a small defined age band. An exponential model may describe the gradual increase of clearance in premature neonates[47], while a first order process has been used to describe clearance maturation and volume changes from birth to adolescence[75].

Maturation of clearance begins before birth, suggesting that postconception age would be a better predictor of drug elimination than postnatal age. A variable slope sigmoidal curve has recently been used to extrapolate adult vancomycin clearance values from neonatal data[79].

Renal function

Studies in drugs whose clearance is dependent on renal function require some measure of this function for interpretation. Estimates for amikacin clearance[47] mirror GFR estimates in premature neonates[80,81]. GFR matures during infancy and approaches an adult rate (6 l/h/70kg) by 6 months postnatal age[59,60]. Difficulties arise determining renal function in children although a number of formulae have been published that allow estimation of GFR from clinical characteristics[82-84].

Creatinine concentration decreases with age in the newborn. Consequently, vancomycin clearance estimates have been made using simply an inverse relationship to creatinine concentration in premature neonates[48]. Creatinine concentration in the first few days of life reflects maternal concentrations more than neonatal renal function and subsequent concentrations are influenced by tubular reabsorption[85].

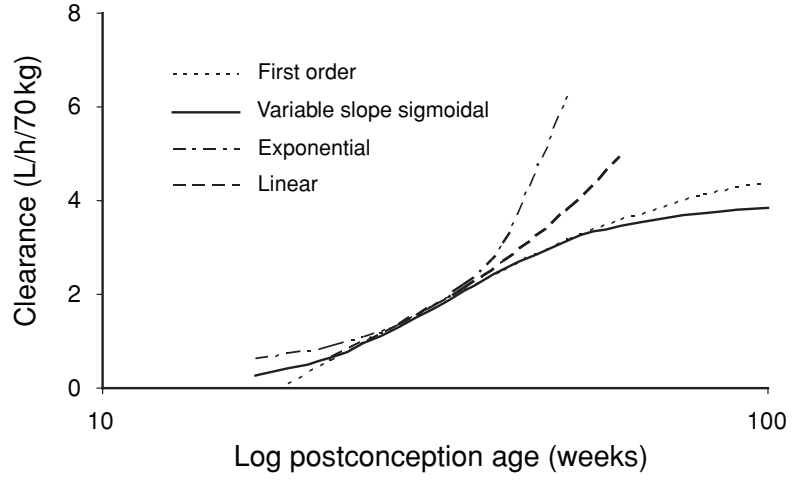

Figure 2 Clearance estimates are similar for all models (first order, variable slope sigmoidal, exponential, linear) over the narrow age band (24-36 weeks PCA). The first order model suffers because it assumes zero clearance at the x intercept. A variable slope sigmoidal model allows for gradual maturation of clearance in early life and a mature clearance to be achieved at a later age.

Renal considerations did not have an impact on the clearance of ketamine (the CYP3A4, CYP2B6 and CYP2C9) or even norketamine in healthy children with normal renal function. However, an assessment of renal function is required for postoperative cardiac children given clonidine. Clonidine is primarily cleared by renal mechanisms, with up to 60% of a dose excreted unchanged in the urine in adults[86,87]. Serum creatinine was used as a measure of renal function, but it is a crude measure in children immediately after cardiac surgery when they have undergone modified ultra-filtration while on cardiopulmonary bypass. An assessment of creatinine clearance would be better.

Integrating priors

Estimates from prior analyses of data (e.g. adult, paediatric or animal work) can be incorporated into paediatric studies when these values cannot be estimated from available current data. Paediatric studies investigating metabolite data can determine inter-compartment rate constants but knowledge of inter-compartment CL estimates may be of greater practical use. The amount of drug that is metabolised by different clearance pathways is often unknown. Observed data such as parent and metabolite concentrations only do not allow estimation of the fraction of drug metabolised to its metabolite. This may be calculated from a complete radio-labelled urine collection, but can be difficult to achieve. It was impossible to collect urine over the 24 h required to estimate this fraction for norketamine. Adult priors for metabolites have been used in paediatric studies[77,88], but none were available for norketamine. The V for norketamine was fixed the same as the central V of ketamine in order to model its clearance and gain insight into its disposition.

Effect measures

Wherever possible one should try and determine the PD response alongside the PK data. Relating the PK with PD is of paramount importance in determining optimal dosage regimens. The ability to relate drug concentration to clinical effect requires reliable, valid and sensitive assessment tools. The aim of this review is not to give a detailed description of the assessment and measurement of either pain or sedation. We would, however, like to bring to the attention of the reader some of the practical difficulties we experienced in relation to the two PK studies described.

The analgesic and sedative effects of clonidine were determined by recording the amount of morphine and diazepam administered in the treatment group. These data were compared to those from historical controls, a comparative technique fraught with problems. The use of a placebo group in paediatric studies generates considerable debate[89]. Standardised pain and sedation scales exist in the PICU that allow nursing staff to determine the need for analgesia and sedation. One problem encountered was that experienced nursing staff often preferred to administer sedatives and analgesics based on their clinical experience without reference to rating scales. Children recovering from cardiac surgery are often artificially ventilated overnight because inexperienced medical staff may not be competent at emergent re-intubation should it be required or because the later removal of chest drains is associated with pain that may require additional analgesic drugs with their risk of respiratory depression. Consequently there was a reluctance to reduce analgesic and sedative drugs at night. These idiosyncratic customs are often not apparent during initial study planning.

Vital signs are all recorded electronically and stored by the monitoring devices. This information can be downloaded and provides a much better data set than those manually recorded hourly on nursing observational charts. Electronic data allowed analysis of blood pressure changes that occurred over a 15-20 minute period following the administration of clonidine that are not seen when using hourly observation charts.

Multiple effects may be generated and require multiple assessment tools. Ketamine produces sedation at low doses and 'dissociative anaesthesia' at high doses as well as pain relief and amnesia. Undesirable effects include nausea, vomiting, tachycardia, hypertension and occasionally psychomimetic responses. Ability to detect clinical effect depends on how visible the effect is and the frequency of the effect. Ketamine is unique in that it produces 'dissociative anaesthesia' which manifests as a partially responsive state often with eyes open. Traditional sedation scales would therefore have difficulty measuring the effect but there is currently no reliable ketamine sedation scale. We used a 6-point Likert Scale[90] but a continuous measure could have been used. In the end the results appear to behave as a binary (dissociated or not-dissociated) and previous investigators have used an 'awakening point'. Analgesia can be measured with complicated tools but a simple Likert[90] or visual analogue scale can be sensitive enough to detect a clinically significant difference. Norketamine has a third the analgesic effect of ketamine and needs to be incorporated into any PD model.

Memory was recorded on a non-linear ordinal scale (amnesia, recognition and recall). The validity of this scale has not been explored. Nausea is a difficult measure in children. Vomiting is a more visible response and was recorded in the ED but not after discharge, when it may still be prevalent. Vomiting may be related to metabolites rather than ketamine concentration. Only one child had a psychomimetic response so drug concentrations during this event would have little predictive value.

Concomitant use of other medications will also alter the effect measures e.g. morphine given for pain or ondansetron given for nausea. In some circumstances there may be too many confounding factors to make an assessment of the effect due to ketamine.

Conclusions

The material reported in a paediatric PK publication invariably does not include the trials and tribulations endured by the authors during the study. These problems and pitfalls are subsequently repeated by others in further paediatric studies and are rarely reported. Paediatric studies differ from those conducted in adults and knowledge gained during the conduct of the one may not be applicable to the conduct of the other. The initiation and successful completion of a paediatric study by a novice is better undertaken with knowledge of these pitfalls and the guidance of others' experiences.

References

1. Berde C. Convulsions associated with pediatric regional anesthesia. Anesth Analg 1992;75:164-166.
2. Taussig HB. A study of the German outbreak of phocomelia: the thalidomide syndrome. JAMA 1962;180:1106-1114.
3. Burns LE, Hodgman JE. Fatal circulatory collapse in premature infants receiving chloramphenicol. N Engl J Med 1959;261:1318.
4. Kauffman RE. Fentanyl, fads, and folly: who will adopt the therapeutic orphans? J Pediatr 1991;119:588-589.
5. Morton NS. Ethical issues in research with children in the UK. Paed Perinat Drug Ther 2001;4:135-139.
6. FDA. Guidance for industry: general considerations for pediatric pharmacokinetic studies for drugs and biological products, 1998: http://www.fda.gov/Cder/guidance/1970dft.pdf.
7. Herd D, Anderson BJ. Ketamine disposition in children presenting for procedural sedation and analgesia in a children's emergency department. Pediatr Anesth 2007 (In press).
8. Simon R. Are placebo-controlled clinical trials ethical or needed when alternative treatment exists? Ann Intern Med 2000;33:474-475.
9. Peppercorn JM, Weeks JC, Cook EF et al. Comparison of outcomes in cancer patients treated within and outside clinical trials: conceptual framework and structured review. Lancet 2004;363:263-270.
10. Weijer C, Freedman B, Fuks A et al. What difference does it make to be treated in a clinical trial? A pilot study. Clin Invest Med 1996;19:179-183.
11. Schmidt B, Gillie P, Caco C et al. Do sick newborn infants benefit from participation in a randomized clinical trial? J Pediatr 1999;134:151-155.
12. Ondrusek N, Abramovitch R, Pencharz P et al. Empirical examination of the ability of children to consent to clinical research. J Med Ethics 1998;24:158-165.
13. Barfield RC, Church C. Informed consent in pediatric clinical trials. Curr Opin Pediatr 2005; 17: 20-24.
14. Koren G. Healthy children as subjects in pharmaceutical research. Theor Med Bioeth 2003;24:149-159.
15. Humphrey GB, Boon CM, van Linden van den Heuvell GF, van de Wiel HB. The occurrence of high levels of acute behavioral distress in children and adolescents undergoing routine venipunctures. Pediatrics 1992;90:87-91.
16. Fredriksson A, Archer T, Alm H, Gordh T, Eriksson P. Neurofunctional deficits and potentiated apoptosis by neonatal NMDA antagonist administration. Behav Brain Res 2004;153:367-376.
17. Wang C, Sadovova N, Fu X et al. The role of the N-methyl-D-aspartate receptor in ketamine-induced apoptosis in rat forebrain culture. Neuroscience 2005;132:967-977.
18. Soriano SG, Anand KJ, Rovnaghi CR, Hickey PR. Of mice and men: should we extrapolate rodent experimental data to the care of human neonates? Anesthesiology 2005;102:866-868.
19. Anand KJ, Soriano SG. Anesthetic agents and the immature brain: are these toxic or therapeutic? Anesthesiology 2004;101:527-530.
20. Tobin JR, Shafer SL, Davis PJ. Pediatric research and scholarship: another Gordian knot? Anesth Analg 2006;103:43-48.
21. Hines RN, McCarver DG. The ontogeny of human drug-metabolizing enzymes: phase I oxidative enzymes. J Pharmacol Exp Ther 2002;300:355-360.
22. McCarver DG, Hines RN. The ontogeny of human drug-metabolizing enzymes: phase II conjugation enzymes and regulatory mechanisms. J Pharmacol Exp Ther 2002;300:361-366.
23. 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;45:931-956.
24. Johnson TN. Modelling approaches to dose estimation in children. Br J Clin Pharmacol 2005;59:663-669.
25. Johnson TN. The development of drug metabolising enzymes and their influence on the susceptibility to adverse drug reactions in children. Toxicology 2003;192:37-48.
26. Edginton AN, Schmitt W, Willmann S. Development and evaluation of a generic physiologically based pharmacokinetic model for children. Clin Pharmacokinet 2006;45:1013-1034.
27. Edginton AN, Schmitt W, Voith B, Willmann S. A mechanistic approach for the scaling of clearance in children. Clin Pharmacokinet 2006;45:683-704.
28. Anderson BJ, Meakin GH. Scaling for size: some implications for paediatric anaesthesia dosing. Paediatr Anaesth 2002;12:205-219.
29. Lonnqvist PA, Bergendahl H. Pharmacokinetics and haemodynamic response after an intravenous bolus injection of clonidine in children. Paediatric Anaesthesia 1993;3:359-364.

30. Peden V, Choonara I, Gennery B et al. Recruiting children to a clinical trial. Paed Perinat Drug Ther 2000;4:75-78.
31. Martens HJ, De Goede PN, Van Loenen AC. Sorption of various drugs in polyvinyl chloride, glass, and polyethylene-lined infusion containers. Am J Hosp Pharm 1990;47:369-373.
32. Schneider JJ, Good P, Ravenscroft PJ. Effect of tubing on loss of clonazepam administered by continuous subcutaneous infusion. J Pain Symptom Manage 2006;31:563-567.
33. Hahn TW, Henneberg SW, Holm-Knudsen RJ, et al. Pharmacokinetics of rectal paracetamol after repeated dosing in children. Br J Anaesth 2000;85:512-519.
34. Gentili S, Torresi A, Marsili R, Chiarotti M, Macchia T. Simultaneous detection of amphetamine-like drugs with headspace solid-phase microextraction and gas chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2002;780:183-192.
35. Hoogtanders K, van der Heijden J, Christiaans M, et al. Therapeutic drug monitoring of tacrolimus with the dried blood spot method. J Pharm Biomed Anal 2006. Dec 19; [Epub ahead of print].
36. van den Ouweland FA, Brown E, Carr DJ. Data monitoring committees in paediatric research. Paed Perinat Drug Ther 2004;6:81-88.
37. FDA. Guidance for industry: population pharmacokinetics, 1999: http://www.fda.gov/CDER/guidance/1852fnl.pdf.
38. Anderson BJ, Hansen TG. Getting the best from pediatric pharmacokinetic data. Paediatr Anaesth 2004;14:713-715.
39. 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;96:1336-1345.
40. van der Marel CD, Anderson BJ, Pluim MA et al. Acetaminophen in cerebrospinal fluid in children. Eur J Clin Pharmacol 2003;59:297-302.
41. Sheiner LB, Beal SL. Evaluation of methods for estimating population pharmacokinetics parameters. I. Michaelis-Menten model: routine clinical pharmacokinetic data. J Pharmacokinet Biopharm 1980;8:553-571.
42. Sheiner LB, Beal SL. Evaluation of methods for estimating population pharmacokinetic parameters. II. Biexponential model and experimental pharmacokinetic data. J Pharmacokinet Biopharm 1981;9:635-651.
43. Sheiner LB, Beal SL. Evaluation of methods for estimating population pharmacokinetic parameters. III. Monoexponential model: routine clinical pharmacokinetic data. J Pharmacokinet Biopharm 1983;11:303-319.
44. Peck CC, Sheiner LB, Nichols AI. The problem of choosing weights in nonlinear regression analysis of pharmacokinetic data. Drug Metab Rev 1984;15:133-148.
45. Peck CC, Beal SL, Sheiner LB, Nichols AI. Extended least squares nonlinear regression: a possible solution to the "choice of weights" problem in analysis of individual pharmacokinetic parameters. J Pharmacokinet Biopharm 1984;12:545-57.
46. Sheiner LB, Rosenburg B, Marathe VV. Estimation of population characteristics of pharmacokinetic parameters from routine clinical data. J Pharmacokinet Biopharm 1977;5:445-479.
47. Allegaert K, Anderson BJ, Cossey V, Holford, NH. Limited predictability of amikacin clearance in extreme premature neonates at birth. Br J Clin Pharmacol 2006;61:39-48.
48. Grimsley C, Thomson AH. Pharmacokinetics and dose requirements of vancomycin in neonates. Arch Dis Child Fetal Neonatal Ed 1999;81:F221-F227.
49. Grasela TH, Sheiner LB, Rambeck B et al. Steady-state pharmacokinetics of phenytoin from routinely collected patient data. Clin Pharmacokinet 1983;8:355-364.
50. Grasela TH, Jr., Donn SM. Neonatal population pharmacokinetics of phenobarbital derived from routine clinical data. Dev Pharmacol Ther 1985;8:374-383.
51. Retout S, Mentre F. Optimization of individual and population designs using Splus. J Pharmacokinet Pharmacodyn 2003;30:417-443.
52. D'Argenio DZ. Incorporating prior parameter uncertainty in the design of sampling schedules for pharmacokinetic parameter estimation experiments. Math Biosci 1990;99:105-118.
53. D'Argenio D, Schumitzky A. ADAPT II User's Guide: Pharmacokinetic/Pharmacodynamic Systems Analysis Software. Los Angeles: Biomedical Simulations Resource, 1997.
54. Duffull S, Waterhouse T, Eccleston J. Some considerations on the design of population pharmacokinetic studies. J Pharmacokinet Pharmacodyn 2005;32:441-457.
55. Ribbing J, Jonsson EN. Power, selection bias and predictive performance of the Population Pharmacokinetic Covariate Model. J Pharmacokinet Pharmacodyn 2004;31:109-134.
56. Phillips KA, Veenstra DL, Oren E, Lee JK, Sadee W. Potential role of pharmacogenomics in reducing adverse drug reactions: a systematic review. JAMA 2001;286:2270-2279.
57. Yanagihara Y, Kariya S, Ohtani M et al. Involvement of CYP2B6 in n-demethylation of ketamine in human liver microsomes. Drug Metab Dispos 2001;29:887-890.
58. Hijazi Y, Boulieu R. Contribution of CYP3A4, CYP2B6, and CYP2C9 isoforms to N-demethylation of ketamine in human liver microsomes. Drug Metab Dispos 2002;30:853-858.
59. Arant BS, Jr. Developmental patterns of renal functional maturation compared in the human neonate. J Pediatr 1978;92:705-712.
60. Bergstein JM. Introduction to glomerular diseases. In: Jenson HB, ed. Nelson Textbook of Pediatrics. Philadelphia: W.B. Saunders Co, 2000:1574-1575.
61. McNamara PJ, Alcorn J. Protein binding predictions in infants. AAPS PharmSci 2002;4:E4
62. Benet LZ, Hoener BA. Changes in plasma protein binding have little clinical relevance. Clin Pharmacol Ther 2002;71:115-121.
63. Ihmsen H, Geisslinger G, Schuttler J. Stereoselective pharmacokinetics of ketamine: R(-)-ketamine inhibits the elimination of S(+)-ketamine. Clin Pharmacol Ther 2001;70:431-438.
64. Kauffman RE, Lieh-Lai MW, Uy HG, Aravind MK. Enantiomer-selective pharmacokinetics and metabolism of ketorolac in children. Clin Pharmacol Ther 1999;65:382-388.
65. Leung LY, Baillie TA. Comparative pharmacology in the rat of ketamine and its two principal metabolites, norketamine and (Z)-6-hydroxynorketamine. J Med Chem 1986;29:2396-2399.
66. Bruguerolle B. Chronopharmacokinetics. Current status. Clin Pharmacokinet 1998;35:83-94.
67. Reinberg A, Smolensky MH. Circadian changes of drug disposition in man. Clin Pharmacokinet 1982;7:401-420.

68. Bonate PL. The effect of collinearity on parameter estimates in nonlinear mixed effect models. Pharmaceutical Research 1999;16:709-717.
69. Holford NHG. A size standard for pharmacokinetics. Clin Pharmacokinet 1996;30: 29-332.
70. Mitchell D, Strydom NB, Van Graan CH, van der Walt W H. Human surface area: comparison of the Du Bois formula with direct photometric measurement. Pflugers Arch 1971;325:188-190.
71. Tenenbein M. Why young children are resistant to acetaminophen poisoning. J Pediatr 2000; 137:891-892.
72. Dawson WT. Relations between age and weight and dosages of drugs. Ann Intern Med 1940;13: 1594-1613.
73. West GB, Brown JH, Enquist BJ. A general model for the origin of allometric scaling laws in biology. Science 1997;276:22-126.
74. West GB, Brown JH, Enquist BJ. The fourth dimension of life: fractal geometry and allometric scaling of organisms. Science 1999;284:1677-1679.
75. Anderson BJ, Woollard GA, Holford NH. A model for size and age changes in the pharmacokinetics of paracetamol in neonates, infants and children. Br J Clin Pharmacol 2000;50:125-134.
76. Anderson BJ. Phenytoin elimination in a child during hypothermia for traumatic brain injury. Paed Perinat Drug Ther 2005;6:133-138.
77. Bouwmeester NJ, Anderson BJ, Tibboel D, Holford NH. Developmental pharmacokinetics of morphine and its metabolites in neonates, infants and young children. Br J Anaesth 2004;92:208-217.
78. Booker PD, Taylor C, Saba G. Perioperative changes in alpha 1-acid glycoprotein concentrations in infants undergoing major surgery. Br J Anaesth 1996;76:365-368.
79. Anderson BJ, Allegaert K, van den Anker JN et al. Vancomycin pharmacokinetics in preterm neonates and the prediction of adult clearance. Br J Clin Pharmacol 2007;63:75-84
80. Sonntag J, Prankel B, Waltz S. Serum creatinine concentration, urinary creatinine excretion and creatinine clearance during the first 9 weeks in preterm infants with a birth weight below 1500 g. Eur J Pediatr 1996;155:815-819.
81. van der Heijden AJ, Grose WF, Ambagtsheer JJ et al. Glomerular filtration rate in the preterm infant: the relation to gestational and postnatal age. Eur J Pediatr 1988;148:24-28.
82. Paap CM, Nahata MC. Prospective evaluation of ten methods for estimating creatinine clearance in children with varying degrees of renal dysfunction. J Clin Pharm Ther 1995;20:67-73.
83. Schwartz GJ, Feld LG, Langford DJ. A simple estimate of glomerular filtration rate in full-term infants during the first year of life. J Pediatr 1984;104:849-854.
84. Schwartz GJ, Gauthier B. A simple estimate of glomerular filtration rate in adolescent boys. J Pediatr 1985;106:522-526.
85. Guignard JP, Drukker A. Why do newborn infants have a high plasma creatinine? Pediatrics 1999;103: e49.
86. Lowenthal DT, Matzek KM, MacGregor TR. Clinical pharmacokinetics of clonidine. Clin Pharmacokinet 1988;14:287-310.
87. Davies DS, Wing LMH, Reid JL et al. Pharmacokinetics and concentration-effect relationships of intravenous and oral clonidine. Clin Pharmacol Ther 1977;12:593-601.
88. van der Marel CD, Anderson BJ, Romsing J, Jacqz-Aigrain E, Tibboel D. Diclofenac and metabolite pharmacokinetics in children. Paediatr Anaesth 2004;14:443-451.
89. Anderson B, Cranswick N. The placebo (I shall please)–is it so pleasing in children? Paediatr Anaesth 2005;15:809-813.
90. Likert R. A technique for the measurement of attitudes. Archives of Psychology 1932;140:55.

CrossRefs are available in the online published version of this paper:
http://www.librapharm.com
Paper PPDT-0177_3, *Accepted for publication:* 17 January 2007
Published Online: 15 February 2007
doi:10.1185/146300907X167781