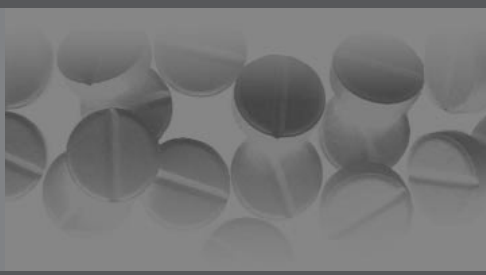
Introduction

Chapter 1



Drug therapy in children

From fetal life through adolescence, dramatic changes in pharmacokinetics and pharmacodynamics occur as a consequence of organ maturation and changes in body composition associated with normal development. Accordingly, effective and safe drug therapy in neonates, infants, children and adolescents requires a thorough understanding of human developmental biology and the ontogeny of the processes that govern the absorption, distribution, metabolism, excretion and action of drugs (1).

Pharmacokinetics

For those drug molecules that have the ability to be altered via biotransformation, metabolism is the major determinant of drug clearance. Although relative hepatic size and liver blood flow may affect the rate of drug metabolism during development, the maturation of drug-metabolizing enzymes is the predominant factor accounting for age-associated changes in nonrenal drug clearance (2).

In man, the quantitatively most important and well-studied group of drug metabolizing enzymes is the cytochrome P450 (CYP) superfamily. The CYP3A subfamily, the most abundant subfamily of the CYP isoforms in liver, kidney and intestine, consists of at least four isoforms: CYP3A4, CYP3A5, CYP3A7 and CYP3A43 (3). The individual CYP3A isoforms demonstrate a different ontogenic pattern of expression and activity. Accordingly, the pharmacokinetics of specific substrates for CYP3A isoforms may change as a function of developmental changes in enzyme activity (4).

In contrast to the cytochrome P450s, much less is known of phase II drug metabolizing enzymes [e.g., enzymes capable of catalyzing conjugation reactions such as sulfotransferases and uridine 5'-disphosphate (UDP)-glucuronosyltransferases (UGTs)] and how normal growth and development produces functional regulation of their activity. Collectively, phase II drug metabolizing enzymes play important roles in drug clearance by increasing the polarity of drug molecules and/or their metabolites produced by phase I drug metabolizing enzymes (e.g., cytochrome P450s). In humans, many drug conjugation reactions are catalyzed by the different isoforms of UGT (5) and hence, these particular phase II enzymes are quantitatively important as determinants of drug clearance. Failure to recognize the impact of ontogeny on UGT activity has produced therapeutic tragedies in pediatrics such as the 'grey-baby' syndrome associated with administration of chloramphenicol (an UGT substrate) to neonates and young infants (4).

Individual differences in drug metabolism throughout infancy, childhood and adolescence are only partially explained by the ontogeny of drug-metabolizing enzymes. Differences in diet, exposure to non-pharmacologic xenobiotics (e.g., environmental chemicals) and concomitant administration of other therapeutic drugs may contribute substantially to interindividual differences in drug metabolizing enzyme activity. It is now recognized that genetic variation directs the expression of many drug-metabolizing enzymes and therefore also contributes to interindividual differences in drug disposition and action (6).

In many instances, pharmacokinetic data (e.g., drug clearance, elimination half life) obtained in selected pediatric subpopulations for substrates of specific drug metabolizing enzymes have provided valuable insights as to developmental influences in their

activity and in some cases, the demonstration of clear, age-associated "break points". Despite this information, the impact of ontogeny on the regulation of CYP3A and the UGTs at the level of the gene (e.g., regulatory regions) is currently not known (6). However, in view of pharmacogenetic data now available for these and other drug metabolizing enzymes, it is reasonable to suspect that allelic variants of the CYP3A and UGT genes may contribute to the activity of these and other drug metabolizing enzymes.

Midazolam as probe of CYP3A activity.

CYP3A activity can be assessed *in vivo* with the use of pharmacological probes. According to the validation criteria for non-invasive probes for CYP3A activity, midazolam clearance is currently one of the best methods to assess CYP3A activity in adults (7). Midazolam, a short acting benzodiazepine routinely used in both pediatric and adult patients as a sedative and anticonvulsant agent, is metabolized by CYP3A4, CYP3A5 and, to a much lesser extent, by CYP3A7 to its main metabolite 1-OH-midazolam, a compound that also exhibits pharmacologic activity (8). This hydroxylated metabolite of midazolam is subsequently conjugated by one or more UGT isoforms to 1-OH-midazolam-glucuronide, a polar compound that is primarily excreted by renal mechanisms (9).

The careful study of midazolam disposition in neonates and infants, as reflected by its biotransformation and pharmacokinetics, will enable the study of this proven pharmacologic probe for CYP3A4. Specifically, a study of the impact of growth and development on the formation and elimination of 1-OH midazolam and its glucuronide metabolite provides a "window" through which to examine the ontogeny of both CYP3A4/5 and of the UGT isoforms responsible for the conjugation of 1-OH midazolam.

Pharmacodynamics

Despite known differences in drug-receptor interaction that are associated with development, clinicians rarely consider this information in the context of interpreting drug effect, be it therapeutic or toxic, in neonates and young infants. Extrapolation of adult pharmacodynamic data to neonates and infants must be done with care and with the knowledge that development may define the dose *vs*. concentration *vs*. effect relationship. The therapeutic plasma concentration range of drugs is not necessarily the same in children and adults and for most therapeutic drugs, has not been strictly defined for pediatric patients. Maturation processes of receptors and/or other cellular targets responsible for the determination of drug effects may alter the pharmacodynamic response in the developing human. As well, the large interindividual variability observed for pharmacokinetics of many drugs in neonates and infants renders the *a priori* definition of an age-appropriate dosage regimen from adult data difficult if not impossible. The only appropriate alternative is to have a thorough understanding of developmental differences in both pharmacokinetics and pharmacodynamics which is wrought only through the careful investigation of drugs in the pediatric subjects where they are intended for use and in turn, expected to improve health.

Midazolam for sedation in neonates, infants and children

Midazolam exerts its effects through potentiation of the effects of γ-amino butyric acid (GABA), an inhibitory neurotransmitter in the central nervous system. Through this effect, midazolam possesses sedative, anxiolytic, anticonvulsive, muscle relaxant and amnesic effects in both adults and children (10). While midazolam pharmacodynamics have been studied widely in adults, validated tools necessary to quantitate and characterize its sedative effects have scarcely been applied to pediatric intensive care patients (11). In the absence of accurate pharmacodynamic data for midazolam in neonates, current dosing recommendations of this drug for neonates and young infants are based upon prior therapeutic experience and the notion that these patients can be "titrated" to effect as is currently done for older children and adults. In the absence of specific pharmacodynamic data for midazolam in neonates, the therapeutic utility of this potentially useful agent may be compromised by the risks of either over- or underdosing.

In view of the profound age-related differences in pharmacokinetics and pharmacodynamics, physicians who prescribe drugs for neonates, infants and children must be aware of the interindividual differences that often result from developmental patterns of pharmacokinetic and pharmacodynamic processes. The research represented by this thesis provides an example of an integrated approach to critically examine the pharmacokinetics and pharmacodynamics of midazolam, an important and commonly used drug, in a population of neonatal and pediatric intensive care patients. The experimental approaches used were driven by an overall goal of translational science – namely, the discovery of new information that translates the discovery of new scientific information into that which can be used clinically.

The goals the research described in this thesis was to:

1. To review the current knowledge on the ontogeny and genetics of CYP3A and UGTs (Chapter 2 and 3)
2. To determine the pharmacokinetics of midazolam, in children from birth through to adolescence, as a surrogate measure of the ontogeny of CYP3A and UGT activity *in vivo* (chapter 4, 5 and 6)
3. To gain insight into the pharmacogenetics of CYP3A4 and its impact on the developmental expression of CYP3A4 *in vivo* (chapter 7, 8 and 9)
4. To evaluate the pharmacodynamics of midazolam in neonatal and pediatric intensive care patients (chapter 10 and 11)

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