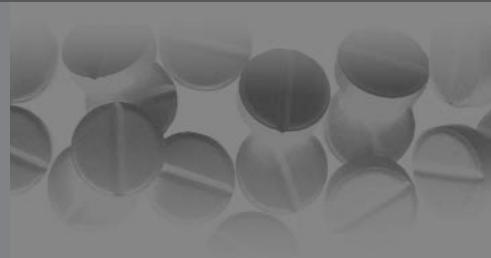


Midazolam pharmacokinetics in preterm infants: lack of an association with the CYP3A4*1B genetic polymorphism

Chapter 9



*S.N. de Wildt, R.H.N. van Schaik, G.L. Kearns, N.M. van Iperen, W.C.J. Hop,
D.J. Murry, J.N. van den Anker*

Submitted

Summary

Introduction The aim of this study was to investigate whether the CYP3A4*1B polymorphism is associated with altered midazolam clearance in preterm infants.

Methods The association between CYP3A4*1B genotype and midazolam pharmacokinetics was examined in preterm neonates (gestational age 26-34 weeks, postnatal age 3-13 days) who received a single intravenous (n=24) and/or oral (n=15) midazolam dose (0.1 mg/kg). All patients were genotyped for CYP3A4*1B with PCR-RFLP. Midazolam and 1-OH-midazolam concentrations were quantitated from plasma by GC-MS. Pharmacokinetic parameters were determined using standard noncompartmental techniques.

Results Median clearance of midazolam after intravenous midazolam did not differ significantly between patients with and without the CYP3A4*1B mutation [0.18 (range 0.06-0.40) vs 0.10 (range 0.04-0.26) L/kg/h, p=0.22]. Interestingly, median apparent oral clearance of midazolam was approximately 4-fold higher in the 3 preterm infants with the CYP3A4*1B mutation [0.43 (range 0.18-0.93 L/hr/kg)] as compared to 12 infants without the CYP3A4*1B mutation [0.12 (range 0.04-0.6) L/hr/kg, p=0.048]. However, multivariate analysis showed that after correction for indomethacin exposure, no difference in midazolam apparent oral clearance between infants with and without the CYP3A4*1B mutation could be detected.

Discussion The CYP3A4*1B mutation does not appear to alter midazolam disposition after intravenous and oral administration in preterm infants.

Introduction

Cytochrome P450 3A4 (CYP3A4) is abundantly expressed in human liver and intestine where it catalyzes the biotransformation of over 50 currently prescribed drugs in addition to several endogenous compounds and environmental pollutants. CYP3A4 expression shows large interindividual variability (1, 2). Co-variates known to affect CYP3A4 activity include age, medication, disease-state, nutrition, race and potentially, genotypic expression (3-6).

Recently, a CYP3A4 variant allele has been reported, CYP3A4*1B (7). This variant allele contains an A to G mutation in the nifedipine-specific element (NFSE) located -287bp from the transcription start site. The exact role of this NFSE element in the regulation of the CYP3A4 gene expression is currently unknown (8). Interestingly, the CYP3A4*1B mutation appears to be associated with a worse clinical presentation of prostate cancer and a lower risk for treatment-related leukemia in children (7, 9). In addition, Rebbeck and co-workers have shown that the CYP3A4*1B gene promoter variant may be associated with higher expression and activity of CYP3A4 *in vitro* as compared to the CYP3A4 wild-type promoter (10, 11). These observations could not, however, be confirmed by other investigators (12). A pharmacokinetic study in adults using the CYP3A4 probe midazolam showed a statistically significant decreased clearance of midazolam only after intravenous administration in individuals with the *1B mutation. In contrast, no effect of the CYP3A4*1B allele was detected using the erythromycin breath test, nifedipine clearance or oral midazolam clearance. (13, 14). The reduction of intravenous midazolam clearance in subjects with the *1B mutation was very modest and therefore was felt to be of minimal clinical importance with respect to its impact on CYP3A4 activity.

Genetically determined events that control the activity (i.e., affinity and capacity) of enzyme(s) responsible for the metabolism of several drugs appear to vary as a consequence of development (15). However, it is unknown whether specific polymorphisms in the CYP3A4 gene may be associated with the acquisition of functional activity (4). If the NFSE is associated with the expression of CYP3A4 activity after birth, genetic polymorphisms in this element may explain part of the large interindividual variation found in the pharmacokinetics of CYP3A4 substrates in newborn infants (16).

The aim of this study was to investigate if the plasma clearance of the CYP3A4 probe midazolam in newborn infants after intravenous or oral administration was associated with the CYP3A4*1B mutation.

Materials and Methods

Patient population

This study was conducted using specimens from 30 preterm infants (gestational age: 26 to 33 weeks, postnatal age: 3 to 13 days) who participated in a pharmacokinetic study of midazolam. Infants were recruited from the Neonatal Intensive Care Unit of the Sophia Children's Hospital from June 1997 through May 2000. All children received midazolam prior to a stressful procedure (e.g. tracheal tube suction, elective nasopharyngeal intubation) and had an indwelling arterial catheter placed upon medical indication not

related to this study. No patients received drugs known to alter the activity of CYP3A4 and were excluded from participation if they received morphine, dobutamine or dopamine. Furthermore, patients were excluded from study participation if they had significant underlying renal, hepatic or neurological dysfunction.

This research protocol was approved by the Medical Ethical Committee of the Sophia Children's Hospital. Written, informed consent was obtained from parents or legal guardians of all infants prior to their enrollment in the study.

Drug administration and sample collection

Midazolam pharmacokinetics were determined after intravenous (IV, n=24) or after oral (PO, n=15) administration. Nine patients received both formulations in random order with a washout period of at least 72-hours between study periods. Midazolam (Dormicum® injection, Roche Laboratories, The Netherlands) was administered as a single intravenous (IV) dose, 0.1 mg/kg, diluted to a final concentration of 0.03 mg/ml in a 5% glucose solution. Midazolam (IV) was infused over 30 minutes through microbore tubing at a constant rate into a peripheral vein or into a central catheter. Midazolam (PO) at a concentration of 0.5 mg/ml was diluted with glucose 5% to a final volume of 0.5 ml and given as a single 0.1 mg/kg dose through the gastric tube, followed by 0.5 ml of glucose 5% to ensure that no midazolam remained in the tube. Arterial blood samples (0.2 ml each) were collected for the quantitation of midazolam and 1-OH-midazolam concentrations from plasma and were stored at -80°C until they were analyzed using a validated GC-MS method. Blood samples were obtained immediately prior to the infusion (IV) or bolus (PO) administration (i.e., at time = 0) and at 0.5, 1, 2, 4, 6, 12 and 24 h after dosing. The subjects were observed for the whole study period for adverse reactions, with vital signs checked prior to infusion and at the time of blood sampling.

Sampling for pharmacogenetic analyses

For all patients who had completed the companion pharmacokinetic study, a buccal brush was used to obtain epithelial cells for DNA isolation and subsequent analysis. From patients who enrolled in the pharmacokinetic study after development of CYP3A genotyping, we obtained 0.3 ml whole blood samples (EDTA anticoagulant) via an arterial catheter for DNA isolation and CYP3A*1B genotyping.

Drug assay methods

Plasma samples were analyzed for midazolam and 1-OH-midazolam by gas chromatography with mass spectrometric detection (Hewlett Packard 6890, Agilent Technologies Inc, Palo Alto, CA). The column used was a J&W Scientific DB-17 EVDX [0.2 micron, 25 meters (J&W Scientific, Folsom, CA)]. Diazepam (Elkins Sinn, Cherry Hill, NJ), 5µl of 500 ng/ml solution, was added to each sample as an internal standard and solid phase extraction was performed using a Varian Bond Elut Column (Varian Inc, Palo Alto, CA). The inter-day and intra-day coefficient of variation for the low standard (2 ng/ml) was consistently less than 10% for both midazolam and 1-OH midazolam. The lower limit of quantitation was 1 ng/ml for midazolam and 0.5 ng/ml for 1-OH-midazolam using 0.2 ml sample volume. All samples were analyzed in duplicate with the resultant mean concentration used in the pharmacokinetic analysis.

Genotyping

To detect individuals who carried the CYP3A4*1B mutation, genomic DNA was isolated from blood using the GenomicPrep Blood DNA Isolation Kit (Amersham Pharmacia Biotech, Buckinghamshire, UK). In a subset of patients DNA was isolated from buccal epithelium, using the MasterAmp Buccal Swab DNA Extraction Kit (BIOzym). DNA yields were estimated by measuring OD₂₆₀. Evidence for the CYP3A4*1B mutation was examined from each DNA sample using the PCR-RFLP method developed by van Schaik et al. (17).

Pharmacokinetic analysis

Pharmacokinetic parameters were determined for midazolam following both intravenous and oral administration using standard non-compartmental methods. The area under the curve for both midazolam and 1-OH-midazolam were determined by the log-linear trapezoidal rule (AUC_{0t}) with extrapolation to infinity (AUC_∞) using the apparent terminal elimination rate constant (λz) determined via non-linear curve fit. The 1-OH-midazolam AUC_{0t} / midazolam AUC_{0t} ratio (i.e. metabolite:parent drug) was used as a "surrogate" measure of CYP3A4 activity. Only subjects with measurable concentrations of 1-OH-midazolam were used to construct this metabolic ratio. All pharmacokinetic analyses were conducted using models resident in the Kinetica® (version 2.0, Innaphase, Inc., Philadelphia, PA, USA) software package.

Statistical analysis

Data between subject groups with and without the CYP3A4*1B mutation were compared using the Mann-Whitney test. Multivariate linear regression analysis was performed to determine the predictive value of other co-variates, in addition to presence/absence of the CYP3A4*1B allele, on midazolam pharmacokinetics. For the multivariate analysis we used the logarithm of midazolam AUC_∞ and clearance to normalize the distribution of these parameters. These statistical analyses were conducted using the SPSS software package (version 9.0, Chicago, Illinois, USA). Data are expressed as mean \pm SD unless stated to the contrary. A significance limit of $\alpha = 0.05$ was accepted for all statistical analyses.

Results

Data from 30 patients were included in the analysis. Midazolam was administered intravenously in 15 patients and orally in six. In an additional 9 patients midazolam was administered both intravenously and orally. Patient demographic characteristics are summarized in Table 1. Out of the 30 patients studied, two were homozygous for the CYP3A4*1B mutation (one Black, one Hispanic) and four were heterozygous (three of Caucasian and one of unknown decent) (Table 2).

Median apparent oral clearance of midazolam was greater in the 3 preterm infants with the CYP3A4*1B mutation as compared to the 12 infants without the mutation [0.43 (range 0.18-0.93) vs. 0.12 (range 0.04-0.6) L/kg/h, $p=0.048$]. In addition, the apparent volume of distribution after oral administration was also larger in the patients with the mutation as compared to those without [3.7 (range 1.9 - 12.1) vs. 1.3 (range 0.3-9.2) L/kg, $p=0.048$]. However, elimination half-life nor metabolite:drug AUC ratio differed

Table 1 Demographic data of the patients

	Intravenous midazolam		Oral midazolam	
	N=24		N=15	
Parameters				
GA (weeks)	29.1 ± 2.3	(26.3-33.6)	28.0 ± 1.6	(26.0-30.7)
PNA (days)	5.8 ± 2.6	(3-11)	6.1 ± 2.7	(3-13)
Birth weight (g)	1092 ± 233	(745-1630)	1076 ± 240	(745-1630)
Study weight (g)	1105 ± 230	(770-1645)	1070 ± 232	(825-1660)
Apgar 1 min	6.1 ± 2.0	(2-9)	6.0 ± 2.0	(3-9)
Apgar 5 min	8.0 ± 1.2	(5-10)	7.9 ± 1.3	(5-10)

Data are expressed as means ± SD (range), GA = gestational age, PNA = postnatal age.

Table 2 Ethnic distribution of the CYP3A4*1B mutation

	Genotype			Total
	A/A	A/G	G/G	
Race				
Caucasian	21 (15*,10#)	3 (3,1)		24 (18,11)
Mediterranean	2 (2,2)			2 (2,2)
Black			1 (1,1)	1 (1,1)
Hispanic			1 (1,1)	1 (1,1)
Mixed-unknown	1 (1,0)	1 (1,0)		2 (2,0)
Total	24 (18,12)	4 (4,1)	2 (2,2)	30 (24,15)

* = number of patients who received intravenous midazolam, # = number patients who received oral midazolam.

AA = wild-type, A/G = heterozygous for CYP3A4*1B, GG = homozygous for CYP3A4*1B.

significantly between patients with and without the *1B mutation. Multivariate linear regression analysis was performed to test if, in addition to presence or absence of the CYP3A4*1B allele, any of the following co-variates was associated with altered midazolam AUC_{0-∞} or plasma clearance: gestational age, postnatal age, postconceptional age, birth weight, study weight, delivery mode, feeding, ventilation, prenatal exposure to betamethasone and/or indomethacin, postnatal exposure to indomethacin, caffeine or antibiotics. When the apparent oral midazolam clearance (i.e., CL/F; ml/min/kg) was

corrected for the effect of postnatal indomethacin treatment, the *1B mutation was no longer of significant predictive value for midazolam clearance. None of the other co-variates tested impacted the predictive value of the CYP3A4*1B mutation on the pharmacokinetics of oral midazolam.

No relationship with the CYP3A4*1B mutation was apparent with respect to midazolam clearance or AUC_{0-∞} after intravenous midazolam administration. When a similar multi-variate analysis was performed for midazolam clearance after intravenous administration, no single association between midazolam clearance and CYP3A4*1B mutation was detected.

Discussion

Inheritance of the CYP3A4*1B mutation does not appear to alter midazolam disposition in preterm infants. The fact that there was no genotype/phenotype relationship with respect to the apparent oral clearance of midazolam in these infants is in agreement with data derived from healthy adults (19). However, the apparent absence of a genotype-phenotype correlation associated with the *1B mutation in our population of preterm infants who received intravenous midazolam is not in agreement with previous data from healthy adults that demonstrated a small but statistically significant reduction in midazolam plasma clearance (19). Examination of these data suggests, however, that this reduction in midazolam elimination is modest and given the normal, wide interindividual variability in CYP3A4/5 activity as reflected by midazolam clearance (19), may be of limited clinical significance.

In preterm infants other co-variates such as age and concurrent medication have also been identified as causes of variability in midazolam clearance (16,20). Even after correction for several co-variates, we could not detect an effect of the CYP3A4*1B mutation on midazolam clearance. Interestingly, all patients with the CYP3A4*1B allele had been exposed postnatally to indomethacin, while only half of the patients without the mutation had received indomethacin for closure of a patent ductus arteriosus. Therefore postnatal exposure to indomethacin was seen as a confounding treatment and as such, was considered during the final analysis. A limitation of our study, in addition to the uneven distribution of co-variates across the patient groups was the small sample size of our cohort. An effect or lack of effect of one or more CYP3A4*1B alleles may have been obscured due to a lack of statistical power. The normal intersubject variability in CYP3A activity and the effect of ontogeny on CYP3A activity necessitates a large sample of newborns to be included in such a pharmacogenetic study in order to enable discrimination of genotype-phenotype associations. A developmentally related surge in CYP3A activity during the first months of life as suggested by the data of Lacroix et al. (17), may completely obscure the role that specific mutations of the CYP3A4 gene might have on enzyme activity. As reflected by previous data on N-acetyl transferase 2 (NAT2) (15,23), developmental regulation of a gene product may have profound effect on the expression of both enzyme activity and phenotype.

Despite the reported allelic variants for CYP3A4, their significance with respect to the clearance of pharmacologic substrates for this enzyme remains inconclusive. In contrast, mutations in the CYP3A5 gene that appear to convey polymorphic activity of the enzyme were recently reported (18). One of the well described limitations of using pharmacologic “probe” compounds to assess the activity of CYP3A4 resides with the

potential for overlapping substrate specificities between CYP3A4 and CYP3A5. In the case of midazolam, both CYP3A4 and CYP3A5 are involved in its metabolism, with a differential capability of these isoforms on the formation of midazolam metabolites (19). The potential relevance of these findings to our data resides with the fact that we did not specifically assess CYP3A5 genotype or phenotype in our subjects but did examine the relative extent of midazolam conversion to 1-OH-midazolam as a potential "surrogate" marker for CYP3A4/5 activity. Thus, it is possible that the constitutive level of CYP3A5 activity in our subjects may have obscured our ability to discriminate a significant role of the *1B mutation as a determinant of midazolam disposition in preterm neonates.

Finally, data from Ozdemir et al. (6) suggest that approximately 60% of the individual variability in adult hepatic and intestinal CYP3A activity appears to be under genetic control. This is exemplified by the known polymorphisms for both CYP3A4 and CYP3A5, the latter being of functional significance with respect to the biotransformation of CYP3A5 substrates (18). Therefore, a continuous effort in elucidating the causes of the large variability in the pharmacokinetics of CYP3A substrates is necessary that takes into account the rapid discovery of new and potentially important genetic polymorphisms for the isoforms of CYP3A (8,24).

In conclusion, the results from our pilot study conducted in preterm neonates suggests that the CYP3A4*1B mutation does not appear to alter midazolam disposition after intravenous and oral administration of the drug. Before these results can be generalized and applied to the pediatric population, it will be necessary for much larger studies to be conducted in infants and children so that the impact of polymorphisms in both the CYP3A4 and CYP3A5 genes as a determinant of midazolam disposition in patients can be clearly elucidated.

Acknowledgements

We would like to thank Rebecca Brosens and Alberta Thiadens for their help with collection of the DNA samples.

References

- 1 Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: Studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp Ther* 1994;270(1):414-423.
- 2 Guengerich FP. Cytochrome P-450 3A4: regulation and role in drug metabolism. *Annu Rev Pharmacol Toxicol* 1999;39:1-17.
- 3 Tanaka E. Gender-related differences in pharmacokinetics and their clinical significance. *J Clin Pharmacol Ther* 1999;24(5):339-46.
- 4 de Wildt SN, Kearns GL, Leeder JS, van den Anker JN. Cytochrome P450 3A: ontogeny and drug disposition. *Clin Pharmacokinet* 1999;37(6):485-505.
- 5 Michalets EL. Update: clinically significant cytochrome P-450 drug interactions. *Pharmacotherapy* 1998;18(1):84-112.
- 6 Ozdemir V, Kalowa W, Tang BK, Paterson AD, Walker SE, Endrenyi L, et al. Evaluation of the genetic component of variability in CYP3A4 activity: a repeated drug administration method. *Pharmacogenetics* 2000;10(5):373-88.
- 7 Rebbeck TR, Jaffe JM, Walker AH, Wein AJ, Malkowicz SB. Modification of clinical presentation of prostate tumors by a novel genetic variant in CYP3A4. *J Natl Cancer Inst* 1998;90(16):1225-9.
- 8 Sata F, Sapone A, Elizondo G, Stocker P, Miller VP, Zheng W, et al. CYP3A4 allelic variants with amino acid substitutions in exons 7 and 12: evidence for an allelic variant with altered catalytic activity. *Clin Pharmacol Ther* 2000;67(1):48-56.
- 9 Felix CA, Walker AH, Lange BJ, Williams TM, Winick NJ, Cheung NK, et al. Association of CYP3A4 genotype with treatment-related leukemia. *Proc Natl Acad Sci U S A* 1998;95(22):13176-81.
- 10 Amirimani B, Walker AH, Weber BL, Rebbeck TR. RESPONSE: re: modification of clinical presentation of prostate tumors by a novel genetic variant in CYP3A4. *J Natl Cancer Inst* 1999;91(18):1588-90.
- 11 Rebbeck TR. More about: modification of clinical presentation of prostate tumors by a novel genetic variant in CYP3A4. *J Natl Cancer Inst* 2000;92(1):76.
- 12 Westlind A, Löfberg L, Tindberg N, Andersson TB, Ingelman-Sundberg M. Interindividual differences in hepatic expression of CYP3A4: relationship to genetic polymorphism in the 5'-upstream regulatory region. *Biochemical And Biophysical Research Communications* 1999;259(1):201-5.
- 13 Ball SE, Scatina J, Kao J, Ferron GM, Fruncillo R, Mayer P, et al. Population distribution and effects on drug metabolism of a genetic variant in the 5' promoter region of CYP3A4. *Clin Pharmacol Ther* 1999;66(3):288-94.
- 14 Walker AH, Jaffe JM, Gunasegaram S, Cummings SA, Huang C-S, Chern H-D, et al. Characterization of an allelic variant in the nifedipine-specific element of CYP3A4: Ethnic distribution and implications for prostate cancer risk. *Hum Mutat* 1998;Mutation in brief(Online):#191.
- 15 Leeder JS, Kearns GL. Pharmacogenetics in pediatrics. *Ped Clin North Am* 1997;44(1):55-77.
- 16 Lee TC, Charles BG, Harte GJ, Gray PH, Steer PA, Flenady VJ. Population pharmacokinetic modeling in very premature infants receiving midazolam during mechanical ventilation: midazolam neonatal pharmacokinetics. *Anesthesiology* 1999;90(2):451-7.
- 17 Lacroix D, Sonnier M, Monion A, Cheron G, Cresteil T. Expression of CYP3A in the liver. Evidence that the shift between CYP3A7 and CYP3A4 occurs immediately after birth. *Eur J Biochem* 1997;247:625-634.
- 18 Paulussen A, Lavrijsen K, Bohets H, Hendrickx J, Verhasselt P, Luyten W, et al. Two linked mutations in transcriptional regulatory elements of the CYP3A5 gene constitute the major genetic determinant of polymorphic activity in humans. *Pharmacogenetics* 2000;10(5):415-24.
- 19 Gorski JC, Hall SD, Jones DR, Vandenberghe M, Wrighton SA. Regioselective biotransformation of midazolam by members of the human cytochrome P450 3A (CYP3A) subfamily. *Biochem Pharmacol* 1994;47(9):1643-1653.