Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Pharmaceutical and Biomedical Analysis 40 (2006) 397–403
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Kinetic disposition of lorazepam with focus on the glucuronidation capacity, transplacental transfer in parturients and racemization in biological samples

Olga Papini [a], Sergio Pereira da Cunha [b], Ângelo do Carmo da Silva Mathes [b], Carlo Bertucci [c,*], Elaine Christine Dantas Moisés [b], Luciana de Barros Duarte [b], Ricardo de Carvalho Cavalli [b], Vera Lucia Lanchote [a]

[a] *Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Avenida do Café s/n Campus da USP, 14040-903 Ribeirão Preto, SP, Brazil*
[b] *Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brazil*
[c] *Dipartimento di Scienze Farmaceutiche, Università di Bologna, Via Belmeloro 6, 40126 Bologna, Italy*

Received 9 June 2005; accepted 20 July 2005
Available online 6 September 2005

Abstract

The present study investigates the kinetic disposition with focus on the racemization, glucuronidation capacity and the transplacental transfer of lorazepam in term parturients during labor. The study was conducted on 10 healthy parturients aged 18–37 years with a gestational age of 36–40.1 weeks, treated with a single oral dose of 2 mg racemic lorazepam 2–9 h before delivery. Maternal venous blood and urine samples were obtained over a 0–48 h interval and the umbilical cord sample was obtained immediately after clamping. Lorazepam enantiomers were determined in plasma and urine samples by LC–MS/MS using a Chiralcel® OD-R column. In vitro racemization of lorazepam required the calculation of the pharmacokinetic parameters as isomeric mixtures. The data were fitted to two-compartment model and the pharmacokinetic parameters are reported as means (95% CI): $t_{1/2a}$ 3.2 h (2.6–3.7 h), K_a 0.23 h^{-1} (0.19–0.28 h^{-1}), $t_{1/2}$ 10.4 h (9.4–11.3 h), β 0.068 h^{-1} (0.061–0.075 h^{-1}), $AUC^{0-\infty}$ 175.3 (ng h)/ml (145.7–204.8 (ng h)/ml), Cl/F 2.6 ml/(min kg) (2.3–2.9 ml/(min kg)), Vd/F178.8 l (146.5–211.1 l), Fel 0.3% (0.1–0.5%), and Cl_R 0.010 ml/(min kg) (0.005–0.015 ml/(min kg)). Placental transfer of lorazepam evaluated as the ratio of vein umbilical/maternal vein plasma concentrations, obtained as an isomeric mixture, was 0.73 (0.52–0.94). Pregnancy changes the pharmacokinetics of lorazepam, with an increase in the apparent distribution volume, an increase in apparent oral clearance, and a reduction of elimination half-life. The increase in oral clearance may indicate an increase in glucuronidation capacity, with a possible reduction in the plasma concentrations of drugs depending on glucuronidation capacity as the major metabolic pathway.

Keywords: Lorazepam; Parturients; Pharmacokinetics; Glucuronidation; Racemization; Transplacental transfer

1. Introduction

Lorazepam, a potent benzodiazepine, has been shown to have marked anxiolytic and sedative properties. Lorazepam can be administered as a premedicant for elective caesarean section or to women during labor [1,2].

Lorazepam is marketed as a racemate of (+)-(*S*) and (−)-(*R*) enantiomers. The pharmacologically active (+)-(*S*) enantiomer has a 100- to 200-fold higher apparent affinity to the binding site of the receptor than (−)-(*R*)-lorazepam [3].

The pharmacokinetics of oral lorazepam has been well investigated in healthy male and female volunteers. Peak plasma concentrations of orally administered lorazepam are

DOI of original article:10.1016/j.jpba.2005.07.033.
* Corresponding author.
E-mail addresses: carlo.bertucci@unibo.it (C. Bertucci), lanchote@fcfrp.usp.br (V.L. Lanchote).

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.07.021

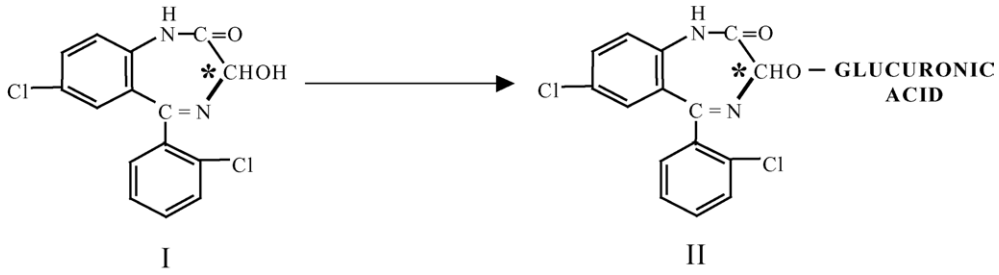

Fig. 1. Metabolism of lorazepam[13]; I-lorazepam- and II-lorazepam-glucuronide (asterisk (*) denotes the chiral center).

achieved within 2.5 h and mean values of absorption half-life generally are less than 30 min. The bioavailability of the 2 mg oral dose averages 89–93%. Lorazepam is less extensively bound to protein than are other benzodiazepines; its mean unbound fraction is 6.8%. The apparent volume of distribution of lorazepam (1.3–2.1 l/kg) indicates moderately extensive tissue uptake [4–10].

Lorazepam is mainly metabolized by glucuronidation (Fig. 1). At least 24 different UDP-glucuronosyltransferase (UGT) human genes have been identified and are classified in two families (UGT1 and UGT2) and three subfamilies (UTT1A, UGT2A and UGT2B) based on sequence homology. Lorazepam is not a substrate of UGT1A1, but appears to be a non-competitive inhibitor that may have a negative influence in patients with Gilberts's syndrome who already have an impaired glucuronidation of bilirubin [11]. It is not clear whether UGT2B7 metabolizes lorazepam and oxazepam although Patel et al. [12] suggested that 10% of Caucasians are poor glucuronidators of *S*-oxazepam. About 75% of an oral dose of lorazepam is excreted into human urine as lorazepam-glucuronide and 13.5% as oxidized metabolites and their glucuronides. Less than 1% is eliminated unchanged in urine [10,13]. Chaudhary et al. [14] reported a diurnal variation in lorazepam elimination consistent with a fast-induced increase in hepatic glucuronidation during the night. Oral lorazepam clearance in male or female healthy volunteers ranges from 5 to 7 l/h [7,9,15] and elimination half-life from 9 to 16 h [4–9].

UGT-catalyzed glucuronidation reactions are responsible for ∼35% of all drugs metabolized by phase II enzymes. Many of the individual UGT enzymes are expressed not only in liver but also in extrahepatic tissues, where the extension of glucuronidation can be substantial [16]. UGTs have not received substantial attention in the pharmacogenetic literature due to their overlapping activity and lack of selective probes [11]. Crom et al. [17] and Kearns et al. [18] assessed conjugation metabolism in healthy male volunteers or patients with cystic fibrosis using lorazepam administered by intravenous route. Herman et al. [10] reported that lorazepam cannot properly be used as a marker of conjugative metabolism due to the fact that it undergoes significant enterohepatic recirculation in humans. Herman et al. [19] associated neomycin and cholestyramine in an attempt to block the enterohepatic circulation of lorazepam and to permit an in vivo estimate of hepatic glucuronidation.

Pregnancy causes various physiologic changes that can lead to important variations in the pharmacokinetic process of absorption, distribution and elimination of drugs. Low concentrations of many drugs during pregnancy are consistent with increased hepatic blood flow, as well as increased volume of distribution and decreased binding to plasma proteins. Pregnant women are subject to an increased level of estrogens and progesterones resulting in changes in hepatic drug metabolism [20]. Previous studies in humans have demonstrated that pregnancy increases CYP2D6 and decreases CYP1A2 and *N*-acetyltransferase activities [21,22]. Luquita et al. [23] reported a decrease in liver UGTs (UDP-glucuronosyltransferases) activity in pregnant rats affecting family 1 isoforms and UGT2B1. In postpartum animals, protein level recovered (UGT1A5 and UGT2B1) or even increased (UGT1A1 and UGT1A6) with respect to control rats.

Lorazepam crosses the placenta and spreads through the tissues of the fetus. Mc Bride et al. [1] reported that fetal concentrations rarely exceeded that in the mother following administration of 2.5 mg i.v. Kanto et al. [2] found that the levels of lorazepam were equal in the maternal and umbilical circulation 0.8–7.7 h after administration of 2 mg i.m. or 11–15.5 h after administration of a 2.5 mg oral dose. The authors [2] reported a serum protein unbound fraction of $14.0 \pm 4.8\%$ in the maternal circulation and $20.8 \pm 3.1\%$ in the umbilical circulation. The metabolism of lorazepam in neonates differs considerably from that occurring in children and adults as a function of the low capacity of neonates to conjugate lorazepam with UDP-glucuronic acid. Cappielo et al. [24] reported that uridine 5′-diphosphoglucuronic acid, the endogenous substrate of UGTs, is present in the human fetal liver at a concentration five-fold lower than in the adult liver, indicating a potential limiting factor for glucuronidation in the human fetus.

Kanazawa et al. [25] reported the stereoselective analysis of lorazepam in plasma samples collected from a patient who was being treated with racemic lorazepam and found only concentrations of (+)-(*S*)-lorazepam. These data were not confirmed by independent studies. Actually Pham-Huy et al. [3] reported the spontaneous racemization of lorazepam in polar medium, and thus the impossibility of a quantitative

determination of the single enantiomers in plasma samples of rabbits.

Since lorazepam may be considered to be a marker of glucuronidation capacity and in view of the absence of clinical data about stereoselectivity and influence of pregnancy in the pharmacokinetics of lorazepam, the objective of the present study was to assess the pharmacokinetics of lorazepam and its conjugate as an isomeric mixture in parturients treated with a single dose of racemic lorazepam. LC–MS/MS analysis was performed on a chiral stationary phase in order to prove the in vitro racemization of lorazepam.

2. Patients and methods

2.1. Patients and clinical protocol

The study was conducted on 10 parturients ranging in age from 18 to 37 years and with a gestational age ranging from 36 to 40.1 weeks. Two of these parturients did not participate in the study of transplacental transfer (Table 1).

The research protocol was approved by the Research Ethics Committee of the University Hospital, Faculty of Medicine of Ribeirão Preto, University of São Paulo (No. HCRP 6259/99). The parturients were admitted to the Maternity of the Airport Complex (MATER), Ribeirão Preto, Brazil, and included in the study after giving written Free and Informed Consent. Only patients with normal hepatic and renal function were included in the study. The parturients received a 2 mg tablet of racemic lorazepam (Lorax®, Wyeth, São Paulo, Brazil) orally, 2–9.2 h before delivery by the vaginal ($n = 8$) or cesarian ($n = 2$) route. Maternal blood samples were obtained via a venous catheter at times 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 30, 36 and 48 h and urine was collected at 12 h intervals up to 48 h after lorazepam administration. Heparin was used as an anticoagulant (Liquemine® 25,000 IU, Roche) for blood collection tubes. Blood samples were also collected from the umbilical vein after clamping (volumes of approximately 5 ml). Plasma and urine samples were separated by centrifugation at 2000 × *g* for 10 min and stored at −20 °C until the time for chromatographic analysis.

2.2. Analysis of lorazepam and its metabolite lorazepam-glucuronide in plasma and urine by LC–MS/MS

The occurrence of racemization does not permit enantioselective analysis of lorazepam in plasma and urine samples. Analysis of lorazepam and lorazepam-glucuronide as an isomeric mixture (sum of the [(+)-(*S*) and (−)-(*R*)] concentrations) was performed by LC–MS/MS as previously reported by the group [26] using a Quattro Micro LC triple-stage quadruple mass spectrometer (Micromass, Manchester, UK) fitted with a Z-electrospray interface (ESI) operated in the positive ion mode.

Plasma or urine samples of 1.0 ml were spiked with 25 μl of nitrazepam solution (internal standard, 25 ng/ml) and with 1.0 ml 50 mM aqueous carbonate buffer. Lorazepam was extracted from plasma and urine samples with 5.0 ml of methyl *tert*-buthyl ether. The tubes were shaken horizontally for 30 min and then centrifuged for 5 min at 1800 × *g*. The organic phases were collected and evaporated to dryness in a centrifugal evaporator vacuum system (RCT90 and RC10.22 model) from Jouan AS (St. Herblain, France), set at 25 °C. The residues were reconstituted in 35 μl mobile phase (a mixture of acetonitrile–water–acetic acid (80:20:0.1%, v:v:v) and vortexed for 15 s and 20 μl were injected into the LC–MS/MS system. Lorazepam and the internal standard were separated using a Chiralpak® OD-R analytical column 0.46 cm × 25 cm, 10 μm particle size (Chiral Technologies, Inc., Exton, PA, USA). The effluent from the chromatographic column was split (0.2 ml/min) and attached to the inlet of a Quattro Micro LC triple-stage quadruple mass spectrometer. Quantitation was performed by MRM (dwell time

Table 1
Individual characteristics of the parturients investigated ($n = 10$)

Parturient	Fetal gestational age (weeks)	Age (years)	Weight before delivery (kg)	Height (cm)	Associated drugs
1	36.0	30	80	156	Diclofenac, lidocaine, oxytocin
2	40.1	18	50	150	–
3	39.0	37	72	163	Bupivacaine, dipyrone, fentanyl, lidocaine, methyl ergometrine, oxytocin
4	38.2	20	75	170	Bupivacaine, fentanyl, oxytocin
5	37.0	20	72	159	Bupivacaine, diclofenac, fentanyl, oxytocin
6	38.2	24	65	157	Cefazolin, diclofenac, lidocaine, oxytocin, thionembutal
7	38.6	24	82	171	Bupivacaine, diclofenac, dipyrone, oxytocin
8	39.2	21	93	166	Bupivacaine, cefazolin, diclofenac, oxytocin
9	39.6	21	86	163	Bupivacaine, lidocaine, nifedipine, oxytocin, sufentanyl
10	38.2	18	93	162	Oxytocin, sufentanyl
Mean CI 95%	38.41 37.54–39.28	23.30 19.02–27.58	76.80 67.40–86.20	161.70 157.08–166.32	

of 1 s) of the protonated molecular ion $[MH]^+$ and its corresponding product ion using two functions, 321 > 275 for lorazepam and 282 > 236 for the internal standard. The calibration curves were linear over the concentration range of 0.4–40.0 ng of unchanged lorazepam as isomeric mixture/ml plasma or urine. The quantitation limit was 0.2 ng for each enantiomer of unchanged lorazepam/ml plasma or urine. The coefficient of variations (precision) and percent relative error (accuracy) obtained for both within-day and between-days assays were lower than 15%.

The plasma and urine concentrations of the lorazepam-glucuronide were calculated from the differences between the concentrations of unchanged and total lorazepam (unchanged + conjugate). Urine samples (100 μl) were spiked with 25 μl of nitrazepam solution (internal standard, 25 ng/ml), 1.0 ml 0.25 M aqueous acetate buffer, pH 5.0, and 50 μl of β-glucuronidase type HP2: *Helix pomatia* (Sigma, St. Louis, MO). Plasma samples (500 μl) were spiked with 25 μl of nitrazepam solution (internal standard, 25 ng/ml), 0.5 ml 0.75 M aqueous acetate buffer, pH 5.0, and 25 μl of β-glucuronidase type HP2: *Helix pomatia* (Sigma, St. Louis, MO). After homogenization, all samples were shaken continuously for 16 h at 37 °C. Eighty microlitres of an aqueous 1 M sodium hydroxide (previously washed with methyl *tert*-buthyl ether) were added to the urine hydrolyzed samples and 20 μl of the same solution were added to the plasma hydrolyzed samples to give pH 10. After shaking in a mixer for 30 s, the hydrolysates were extracted with 5.0 ml of methyl *tert*-buthyl ether. Routine determination of total lorazepam as isomeric mixture in working ranges of 20.0–300.0 ng/ml urine and 2.0–60.0 ng/ml plasma with accuracy and precision less than 15% was possible. Quantitation limits were 1.0 ng of each isomer of total lorazepam/ml plasma and 10.0 ng of each isomer of total lorazepam/ml urine.

2.3. Pharmacokinetic analysis

The pharmacokinetics of the isomeric mixture of lorazepam and lorazepam-glucuronide following single oral dose of racemic drug was evaluated by fitting the data to two-compartment open model. Peak plasma concentration (C_{max}) and time to reach C_{max} were obtained from the raw data of the individual parturients. Areas under the plasma concentration-time curve ($AUC^{0-\infty}$) were calculated by the trapezoidal rule with infinite extrapolation by dividing the last plasma concentration by the elimination rate constant (β). Apparent total clearance (Cl/F) is reported as dose/$AUC^{0-\infty}$ and apparent volume of distribution (Vd/F) as Cl/β. Parameters such as absorption ($t_{1/2a}$) and elimination half-lives ($t_{1/2\beta}$) and absorption rate constant (K_a) and elimination rate constant (β) were determined as described previously. The data obtained with the determination of the isomeric mixture of lorazepam and lorazepam-glucuronide in urine were used to estimate the amount of excreted lorazepam (A_e), renal clearance (Cl_R) using the equation $Cl_R = A_e/AUC$, and elimination fraction (Fel) using the equation $Fel = A_e/dose \times 100$. The elimination half-life in urine was estimated by the curve of excretion rate ($\Delta A_e/\Delta t$) versus midpoint of the urine collection interval [4,6,8]. Pharmacokinetic parameters were calculated using the software WinNonlin, version 4.0 (Pharsight Corp., Mountain View, USA).

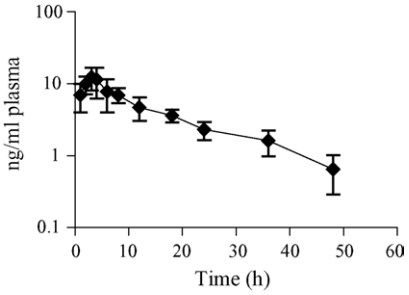

Fig. 2. Plasma concentration vs. time curve for the enantiomeric mixture of lorazepam. Data are reported as mean ± S.E.M. (n = 10 parturients).

3. Results

The curves for the plasma concentrations of the isomeric mixture of lorazepam and lorazepam-glucuronide versus time are presented in Figs. 2 and 3 as mean ± S.E.M. The pharmacokinetic parameters calculated for the isomeric mixture of lorazepam and lorazepam-glucuronide in maternal plasma are presented in Table 2.

The curves for the urinary excretion rate of the isomeric mixture of lorazepam and lorazepam-glucuronide versus midpoint of the urine collection interval are presented in Figs. 4 and 5 as mean ± S.E.M. The pharmacokinetic parameters calculated for the isomeric mixture of lorazepam and lorazepam-glucuronide in maternal urine are presented in Table 3.

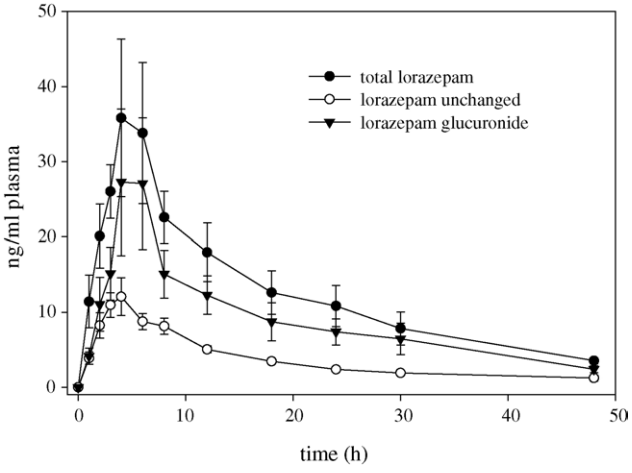

Fig. 3. Plasma concentration vs. time curves for the diastereomeric mixture of lorazepam-glucuronide. Data are reported as mean ± S.E.M. (n = 10 parturients).

Table 2
Kinetic disposition of lorazepam and its metabolite glucuronide in parturients treated with a single oral dose of 2 mg *rac*-lorazepam; mean (CI 95%)

	Lorazepam isomeric mixture	Lorazepam-glucuronide isomeric mixture
C_{max} (ng/ml)	12.96 (9.42–16.49)	35.55 (8.27–62.83)
t_{max} (h)	3.10 (2.57–3.63)	4.33 (2.90–5.77)
$t_{1/2a}$ (h)	3.16 (2.62–3.68)	1.37 (1.15–1.58)
K_a (h^{-1})	0.23 (0.19–0.28)	0.52 (0.44–0.59)
$t_{1/2}\beta$ (h)	10.35 (9.39–11.32)	18.17 (14.10–22.23)
β (h^{-1})	0.068 (0.061–0.075)	0.039 (0.032–0.047)
$AUC^{0-\infty}$ ((ng h)/ml)	175.25 (145.74–204.75)	481.19 (252.87–709.51)
Cl_T/F (ml/(min kg))	2.61 (2.34–2.88)	–
Vd/F (l)	178.78 (146.46–211.10)	–

–, Not determined.

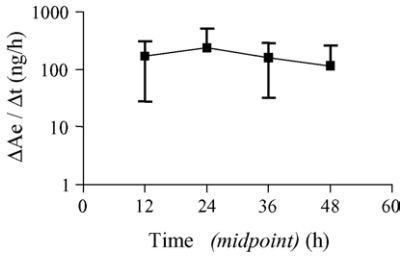

Fig. 4. Urinary excretion rate of an enantiomeric mixture of lorazepam vs. the midpoint of the collection interval. Data are reported as mean ± S.E.M. ($n = 8$ parturients).

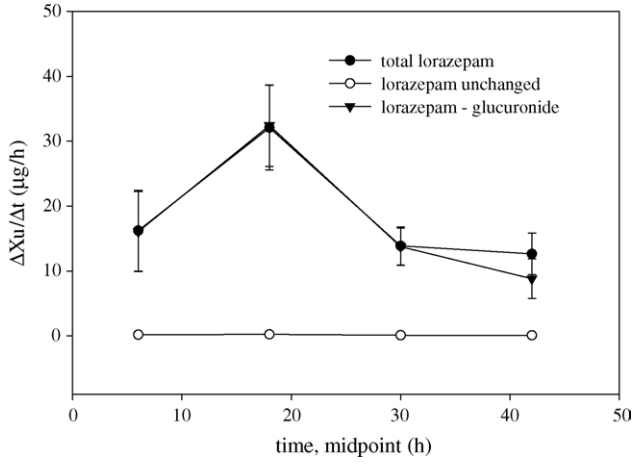

Fig. 5. Urinary excretion rate of a diastereomeric mixture of lorazepam-glucuronide vs. the midpoint of the collection interval. Data are reported as mean ± S.E.M. ($n = 8$ parturients).

Table 3
Urinary excretion of lorazepam and its metabolite glucuronide in parturients treated with a single oral dose of 2 mg rac-lorazepam; mean (CI 95%)

	Lorazepam isomeric mixture	Lorazepam-glucuronide isomeric mixture
A_e total (μg)	8.18 (2.67–13.70)	899.77 (534.58–1265.0)
Fel (%)	0.29 (0.12–0.45)	44.97 (26.65–63.29)
Cl_R (ml/(min kg))	0.0099 (0.0049–0.015)	1.12 (0.69–1.55)
$t_{1/2}$ (h)	12.75 (10.71–14.79)	11.5 (6.14–16.86)
Kel (h^{-1})	0.057 (0.048–0.065)	0.066 (0.040–0.093)

Placental transfer of the enantiomeric mixture of lorazepam, calculated as the ratio of umbilical vein/maternal vein plasma concentrations is presented in Table 4.

4. Discussion

The data concerning the metabolism of drugs in pregnant women are mainly related to the CYP-dependent oxidative capacity, with reports of CYP1A2 inhibition, increases in CYP2D6 activity, and little effect on the activities of CYP2C19 or CYP3A4 [21,22]. Heikkinen et al. [22] investigated the pharmacokinetics of fluoxetine and norfluoxetine in pregnancy and reported low concentrations of plasma fluoxetine explained at least in part by increased demethylation of fluoxetine by CYP2D6. It should be emphasized that there are no literature data regarding the capacity of CYP2D6 induction. Tsutsumi et al. [21] observed a reduction of about 13% in *N*-acetyltransferase 2 in early pregnancy. There are no data about the capacity of glucuronidation in parturients or about the pharmacokinetics of lorazepam as a separate isomer or as an isomeric mixture in parturients. There are no data regarding stereoselectivity in the kinetic disposition and metabolism of lorazepam in men and in non-pregnant women. Kanazawa et al. [25] only reported the enantiomeric concentrations in a plasma sample from a non-pregnant patient. The in vitro instability of the chiral center of lorazepam observed in various studies does not permit the application of the methods to biological fluids and consequently prevents the determination of the implications of enantioselectivity in pharmacokinetics. The present study reports for the first time the pharmacokinetics of lorazepam and its metabolite lorazepam-glucuronide as an isomeric mixture in parturients using LC–MS/MS with the description of racemization.

Comparison of the oral clearance of lorazepam as an isomeric mixture in the parturients investigated here (Table 2) with data reported in investigations of healthy volunteers treated with a single oral dose of racemic lorazepam permits us to speculate that pregnancy increases the oral clearance of lorazepam. The oral clearance values observed here in parturients ranged from 2.34 to 2.88 ml/min kg (95% CI), while other authors [6,8,9,15] investigating healthy

Table 4
Transplacental distribution of lorazepam as an enantiomeric mixture at delivery ($n = 8$)

Parturient	Cord blood (ng/ml)	Maternal blood (ng/ml)	Collection time[a] (min)	Cord blood/maternal blood
1	5.77	14.74	135	0.392
2	6.82	7.95	426	0.858
3	4.38	10.48	153	0.418
4	8.42	9.60	300	0.878
5	5.87	5.33	390	1.100
6	5.78	9.87	120	0.586
7	7.75	10.94	552	0.708
8	9.45	10.35	207	0.913
Mean CI 95%	6.78 (5.39–8.17)	9.91 (7.68–12.14)	293.4 (163.2–423)	0.73 (0.52–0.94)

Parturients were treated with a single oral dose of 2 mg rac-lorazepam; mean (CI 95%).
[a] Time between drug intake and blood collection from the umbilical cord and maternal vein.

volunteers (men and non-pregnant women) observed values of 0.84–1.80 ml/(min kg). Comparison of the present data with those reported in the literature is facilitated by the low interindividual variability observed in the pharmacokinetics of lorazepam.

The increase in apparent total clearance of lorazepam in parturients may be due to an increase in the capacity for conjugation with UGTs but also due to other factors such as a decrease in plasma protein binding and differences in the enterohepatic circulation. The increase in apparent oral clearance may indicate an increase in the capacity of glucuronidation, with the possible reduction in plasma concentrations of drugs depending on the capacity of glucuronidation as the main metabolic pathway. Miners et al. [27] reported higher paracetamol clearance in pregnant women due to increased activity of the glucuronidation and oxidative pathway metabolism and no changes in sulfation or renal clearance of the unchanged drug. Matar et al. [28] observed no effects of pregnancy on the pharmacokinetics of lamotrigine in dogs, indicating that pregnancy has little or no effect on glucuronidation, the principal pathway of lamotrigine elimination.

The apparent distribution volume reported by Ellinwood et al. [8], Blin et al. [9] and Blin et al. [15] in the investigation of healthy volunteers of both sexes orally treated with racemic lorazepam ranged on average from 93 to116 l. The data obtained here in the investigation of parturients indicate an increase in the apparent volume of lorazepam distribution, with the observation of values of 146.46–211,10 l (95% CI) (Table 2). The increase in the apparent distribution volume of drugs is expected in pregnancy as a function of the increase in extracellular fluid, the reduced plasma albumin concentration and the existence of the fetus as another compartment [20,29].

The elimination half-life is a parameter that depends on the apparent distribution volume and on clearance, and it may be prolonged, unchanged or reduced in gestation depending on the proportion of alteration in the apparent distribution volume and clearance. The mean elimination half-life of the isomeric mixture of lorazepam was 10.35 h (95% CI 9.39–11.32) for the 10 parturients investigated (Table 2). Greeblatt et al. [4], Ellinwood et al. [8], Herman et al. [10] and Blin et al. [9] reported mean values of approximately 16 h in the investigation of men and non-pregnant women.

The mean lorazepam fraction eliminated in an unchanged form in urine was 0.29% (95% CI 0.12–0.45%) (Table 3). Greenblatt et al. [4] and Herman et al. [10] reported less than 0.5% recoveries of lorazepam from urine after oral administration of the benzodiazepine to healthy volunteers.

The transplacental transfer of the isomeric mixture of lorazepam, calculated as the ratio of umbilical vein/maternal vein plasma concentrations, was 0.52–0.94 (95% CI) 163–423 min (95% CI) after oral administration (Table 4). The ratio of umbilical vein/maternal vein plasma concentrations of lorazepam was higher than 1 for 1 parturient and higher than 0.5 for 6 of the 8 parturients investigated. Whitelaw et al. [29] obtained ratios of 0.6–1 after oral administration, and McBride et al. [1] reported values of 0.59–1.33 after intravascular administration of the benzodiazepine to parturients. Kanto et al. [2] obtained similar values, 0.88 on average, after intramuscular administration, and 1.25 after oral administration.

The data concerning the kinetic disposition of isomeric mixture of lorazepam-glucuronide are present in Table 2. The percentage of lorazepam conjugation, calculated as the ratio of the $AUC^{0-\infty}$ values for conjugated lorazepam and conjugated + unchanged lorazepam, was 73.30, indicanting almost complete conjugation for lorazepam.

Elliott [13] investigated healthy male volunteers treated with a single oral dose of lorazepam (2 mg) and reported that 74.5% was excreted in the urine as lorazepam-glucuronide. Greenblatt et al. [4] investigated healthy male and female volunteers and reported conjugation capacity of 71.9% following oral lorazepam dose. The data concerning the urinary excretion of lorazepam-glucuronide in the investigated parturients (Table 3) show that 45.0% of the lorazepam dose was excreted as lorazepam-glucuronide. The lower recovery of lorazepam-glucuronide in urine of the investigated parturients could be due to incomplete urine collection although all patients were orientated to collect all urine following 48 h after lorazepam administration.

5. Conclusion

The in vitro stereochemical instability of lorazepam does not permit the monitoring of enantioselectivity phenomena in the drug pharmacokinetics. However, the used method is suitable for pharmacokinetic studies of lorazepam, the limit of quantitation being 0.2 ng/ml plasma for both the enantiomers. Pregnancy alters the pharmacokinetics of lorazepam as an isomeric mixture, with the occurrence of an increase in the apparent distribution volume, an increase in apparent oral clearance and a reduction in the elimination half life. The concentration of lorazepam in the fetal circulation is 50–100% of the maternal plasma concentration.

Acknowledgements

Contact grant sponsors: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CNPq), Consiglio Nazionale delle Ricerche, and MIUR, Rome, Italy.

References

[1] R.J. McBride, J.W. Dundee, J. Moore, W. Toner, P.J. Howard, Br. J. Anaesth. 51 (1979) 971–978.
[2] J. Kanto, L. Aaltonen, P. Liukko, K. Mäenpää, Acta Pharmacol. Toxicol. 47 (1980) 130–134.
[3] C. Pham-Huy, G. Villain-Pautet, H. Hua, N. Chikhi-Chorfi, H. Galons, M. Thevenin, J.-R. Claude, J.-M. Warnet, J. Biochem. Biophys. Methods 54 (2002) 287–299.
[4] D.J. Greenblatt, R.I. Shader, K. Franke, D.S. MacLaughlin, J.S. Harmatz, M.D. Allen, A. Werner, E. Woo, J. Pharm. Sci. 68 (1979) 57–63.
[5] D.J. Greenblatt, Clin. Pharmacokinet. 6 (1981) 89–105.
[6] D.J. Greenblatt, J.S. Harmatz, C. Dorsey, R.I. Shader, Clin. Pharmacol. Ther. 44 (1988) 326–334.
[7] D.J. Greenblatt, L.L. von Moltke, B.L. Ehrenberg, J.S. Harmatz, K.E. Corbett, D.W. Wallace, R.I. Shader, Crit. Care Med. 28 (2000) 2750–2757.
[8] E.H. Ellinwood, D.G. Heatherly, A.M. Nikaido, T.D. Bjornsson, C. Kilts, Psychopharmacology 86 (1985) 392–399.
[9] O. Blin, A. Jacquet, S. Callamand, E. Jouve, M. Habib, D. Gayraud, A. Durand, B. Bruguerolle, P. Pisano, Br. J. Clin. Pharmacol. 48 (1999) 510–512.
[10] R.J. Herman, J. Duc Van Pham, C.B.N. Szakacs, Clin. Pharmacol. Ther. 46 (1989) 18–25.
[11] J. de Leon, Int. J. Neuropsychopharmacol. 6 (2003) 57–72.
[12] M. Patel, B.K. Tang, D.M. Grant, W. Kalow, Pharmacogenetics 5 (1995) 287–297.
[13] H.W. Elliott, Br. J. Anaesth. 48 (1976) 1017–1023.
[14] A. Chaudhary, R.A. Lane, D. Woo, R.J. Herman, J. Pharmacol. Exp. Ther. 267 (1993) 1034–1038.
[15] O. Blin, N. Simon, E. Jouve, M. Habib, D. Gayraud, A. Durand, B. Bruguerolle, P. Pisano, Clin. Neuropharmacol. 24 (2001) 71–81.
[16] T.K.L. Kiang, M.H.H. Ensom, T.K.H. Chang, Pharmacol. Ther. 106 (2005) 97–132.
[17] W.R. Crom, S.L. Webster, L. Bobo, M.E. Teresi, M.V. Relling, W.E. Evans, Clin. Pharmacol. Ther. 41 (1987) 645–650.
[18] G.L. Kearns, G.B. Mallory Jr., W.E. Evans, J. Pediatr. 117 (1990) 972–979.
[19] R.J. Herman, A. Chaudhary, C.B. Szakacs, D. Woo, R. Lane, M.A. Boctor, Eur. J. Clin. Pharmacol. 48 (1995) 253–258.
[20] R. Loebstein, A. Lalkin, G. Koren, Clin. Pharmacokinet. 33 (1997) 328–343.
[21] K. Tsutsumi, T. Kotegawa, S. Matsuki, Y. Tanaka, Y. Ishii, Y. Kodama, M. Kuranari, I. Miyakawa, S. Nakano, Clin. Pharmacol. Ther. 70 (2001) 121–125.
[22] T. Heikkinen, U. Ekblad, P. Palo, K. Laine, Clin. Pharmacol. Ther. 73 (2003) 330–337.
[23] M.G. Luquita, V.A. Catania, E.J. Sánchez Pozzi, L.M. Veggi, T. Hoffman, J.M. Pellegrino, S. Ikushiro, Y. Emi, T. Iyanagi, M. Vore, A.D. Mottino, Pharmacol. Exp. Ther. 298 (2001) 49–56.
[24] M. Cappiello, L. Giuliani, A. Rane, G.M. Pacifici, Eur. J. Drug Metab. Pharmacokinet. 25 (2000) 161–163.
[25] H. Kanazawa, Y. Kunito, Y. Matsushima, S. Okubo, F. Mashige, J. Chromatogr. A 871 (2000) 181–188.
[26] O. Papini, C. Bertucci, S.P. Cunha, N.A.G. Santos, V.L. Lanchote, J. Pharm. Biomed. Anal., doi:10.1016/j.jpba.2005.07.033.
[27] J.O. Miners, R.A. Robson, D.J. Birkett, Br. J. Clin. Pharmacol. 22 (1986) 359–362.
[28] K.M. Matar, P.J. Nicholls, A. Tekle, S.A. Bawazir, M.I. Al-Hassan, Epilepsia 40 (1999) 1353–1356.
[29] A.G.L. Whitelaw, A.J. Cummings, I.R. McFadyen, Br. Med. J. 282 (1981) 1106–1108.