Gender-related differences in pharmacokinetics of enantiomers of *trans*-tramadol and its active metabolite, *trans-O*-demethyltramadol, in rats

LIU Hui-Chen, JIN Shu-Min, WANG Yong-Li[1]

Department of Pharmacology, Hebei Medical University, Shijiazhuang 050017, China

ABSTRACT

AIM: To compare the pharmacokinetics of the enantiomers of *trans*-tramadol (*trans*-T) and its active metabolite, *trans-O*-demethyltramadol (M1), in male and female rats. **METHODS:** Following a single oral dose of 10 mg/kg *trans*-T hydrochloride to rats, (+)-*trans*-T, (-)-*trans*-T, (+)-M1, and (-)-M1 in plasma were determined by a high performance capillary electrophoresis method. **RESULTS:** The females showed higher plasma concentrations of (+)-*trans*-T, (-)-*trans*-T, and (+)-M1 than the males. The enantiomers of *trans*-T were absorbed and eliminated more slowly in the females than in the males. (+)-M1 was eliminated more slowly in the females than in the males. All pharmacokinetic parameters but T_{max} of the two enantiomers of *trans*-T were significantly different in both sex rats. The (+)/(-)-enantiomeric ratios of the pharmacokinetic parameters for *trans*-T in the males were similar to those in the females. The values of C_{max}, $AUC_{0-\infty}$ of the two enantiomers of M1 were significantly different in both sex rats. The (+)/(-)-enantiomeric ratios of C_{max}, $AUC_{0-\infty}$ for M1 were lower than 1 in the males, larger than 1 in the females. **CONCLUSIONS:** Systemic exposure of (+)-*trans*-T, (-)-*trans*-T, and (+)-M1 was higher in female rats than in male rats. The stereoselectivity in pharmacokinetics of *trans*-T was similar, and that of M1 was different in male and female rats.

INTRODUCTION

Tramadol (T) has two chiral carbons and thus has four stereoisomers. *Trans*-T, a racemic mixture of 1*R*,2*R*-T [(+)-*trans*-T] and 1*S*,2*S*-T [(-)-*trans*-T], is used as a centrally acting analgesic[1]. The enantiomers of *trans*-T take as the action in different mechanisms. (+)-*Trans*-T preferentially inhibits serotonin reuptake and enhances basal sterotonin release, whereas (-)-*trans*-T preferentially inhibits norepinephrine reuptake and enhances stimulation-evoked norepinephrine release[2]. *Trans-O*-demethyltramadol (M1) is the only pharmaco-logically active metabolite. (+)-M1 has a high affinity to the μ-opioid receptor, whereas (-)-M1 inhibits monoamine reuptake[3]. This dual model of action of *trans*-T, opioid and nonopioid, may contribute to its efficiency in certain pain, little or no respiratory depression and tolerance after repeated administration[4].

In our previous papers, the pharmacokinetics of *trans*-T and M1 were found to be stereoselective in healthy human subjects[5]. It was also demonstrated that there was stereoselectivity in the distribution in central nervous system, renal clearance, and hepatic metabolism of *trans*-T and/or M1[6-8]. In this paper, we were interested in determining whether pharmacokinetics of the enantiomers of *trans*-T and M1 differed based on gender in rats.

[1] Correspondence to Prof WANG Yong-Li.
Phn & Fax 86-311-605-7291.   E-mail wangyl52@heinfo.net

MATERIALS AND METHODS

Chemicals and reagents *Trans*-T hydrochloride, (+)-*trans*-T hydrochloride, (-)-*trans*-T hydrochloride and M1 were kindly provided by Grünenthal Gmbh (Stolberg, Germany). *Cis*-T hydrochloride, a racemic mixture of 1*R*,2*S*-T and 1*S*,2*R*-T, was a gift from Chemical Department of Jinzhou Medical College (China) and used as internal standard. Sulfobutylether-β-cyclodextrin was kindly provided by Lanzhou Institute of Chemical Physical, the Chinese Academy of Sciences. Tris (hydroxymethyl) aminomethane (Tris), sodium hydroxide, phosphoric acid and ethyl acetate, from different commercial sources, were of analytical or HPLC grade. Double distilled water was used for the preparation of all solution.

Animals Sixteen Sprague-Dawley rats (8 male and 8 female) were provided by Experimental Animal Center of Hebei Medical University (Grade II, Certificate N$\underline{o}$ 04057). The mean weight of the male and female rats was (232±17) g and (216±14) g with range from 208 to 268 g and from 204 to 241 g, respectively. The rats were given ig with 10 mg/kg *trans*-T hydrochloride.

Sample collection By puncturing postbulbar veins, blood samples were collected in heparinized glass tubes before (time 0), 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, and 24 h after administration. Plasma was harvested after separation from blood cells by centrifugation. The plasma samples (0.2 mL each) were stored at -24 ℃ until analysis.

Analytical method[5] After addition of *cis*-T, the enantiomers of *trans*-T and M1 in plasma were extracted with ethyl acetate. After centrifugation, the organic layer was removed out and evaporated to dryness under a gentle stream of nitrogen gas. The residue was redissolved in 100 μL of water, then an aliquot (30 μL) was removed out for high performance capillary electrophoresis (HPCE) analysis. Electrophoretic experiments were performed on a P/ACE 5000 automatic electrophoresis apparatus equipped with an UV detector (Beckman, California, USA). Data were collected with Gold software. The capillary was a fused silica one with a total length of 37 cm, an effective length of 30 cm, and an inner diameter of 75 μm. The background electrolyte (BGE) contained Tris buffer (pH 2.5) 40 mmol/L and sulfobutylether-β-cyclodextrin (chiral selector) 0.8 mmol/L. The sample was injected into the capillary by electrophoretic injection at the anode. The separation was performed at 25 ℃ with a positive voltage of 15 kV. The UV detector was set at 214 nm.

Pharmacokinetic calculation The pharmacokinetics of the enantiomers of *trans*-T and M1 were determined by method of noncompartmental analysis. The maximum plasma concentration (C_{max}) and its corresponding time (T_{max}) were recorded as observed. The elimination rate constant (λ_z) was estimated as the absolute value of the slope of a least-square linear regression of the terminal phase of the logarithmic plasma concentration-time curve. The plasma terminal half-life ($T_{1/2}$) was calculated as $0.693/\lambda_z$. The area under the plasma concentration-time curve (AUC_{0-t}) from time zero to the time of last quantifiable concentration (C_t) was calculated using the linear trapezoidal method. The area under the plasma concentration-time curve from time zero to the infinite time ($AUC_{0-\infty}$) was calculated as the sum of corresponding AUC_{0-t} and C_t/λ_z value. The plasma oral clearance (CL/F) was calculated as Dose/$AUC_{0-\infty}$. The apparent volume of distribution (V/F) was determined using the equation: $V/F= $ (Dose/$AUC_{0-\infty}$)/λ_z. As a measure for the rate of absorption, the $C_{max}/AUC_{0-\infty}$ ratio was also calculated[9].

Statistical analysis Data were expressed as mean±SD. Paired *t*-test was used to compare the pharmacokinetic parameters of the two enantiomers of *trans*-T or M1 in the male or female rats. To compare the pharmacokinetic parameters of each enantiomer of *trans*-T or M1 in the male and female rats, unpaired *t*-test was used to all parameters except T_{max}, to which nonparametric Wilcoxon two-sample test was used.

RESULTS

Electropherogram Under the analytical condition above, the enantiomers of *trans*-T, M1, and *cis*-T could be well-separated, and there was no interference from rat plasma (Fig 1). The calibration curves of the enantiomers of *trans*-T and M1, constructed by plotting the peak area ratios against the corresponding concentrations in standard plasma samples, were linear over the concentration range from 3.125 μg/L to 400 μg/L. The intra-assay and inter-assay RSDs were less than 10 % and 15 %, and relative recoveries were 95 %-103 % and 90 %-104 %, respectively. The limit of detection (LOD), defined as the lowest concentration that could give a signal-to-noise ratio of 3:1, was 1.25 μg/L.

Pharmacokinetics of the enantiomers of *trans*-T Mean plasma concentration-time curves of the enantiomers of *trans*-T following a single oral dose of 10 mg/kg *trans*-T hydrochloride in the male and female rats were
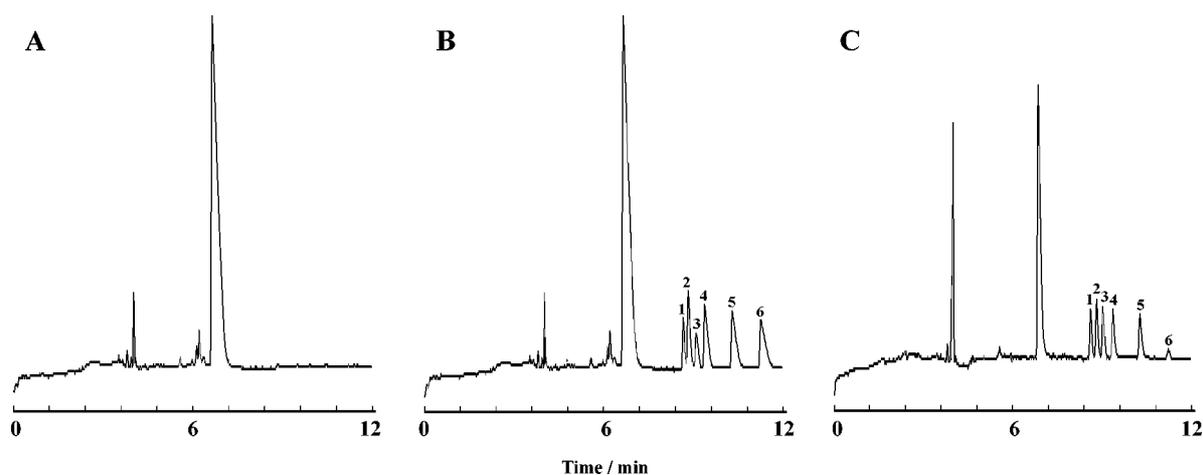
Fig 1. Typical electropherograms of the enantiomers of *trans*-tramadol (*trans*-T), *trans-O*-demethyltramadol (M1), and *cis*-tramadol (*cis*-T) in rat plasma. A) free of drugs; B) spiked with *trans*-T, M1, and *cis*-T; C) a plasma sample. 1: (+)-M1; 2: one enantiomer of *cis*-T; 3: (-)-M1; 4: another enantiomer of *cis*-T (internal standard); 5: (+)-*trans*-T; 6: (-)-*trans*-T.

depicted in Fig 2. The females showed higher concentrations of (+)-*trans*-T and (-)-*trans*-T than the males. The values of T_{max}, $AUC_{0-\infty}$, and $T_{1/2}$ for (+)-*trans*-T and (-)-*trans*-T, and the value of C_{max} for (+)-*trans*-T were significantly higher in the females than in the males.

Meanwhile, the values of CL/F, V/F, and $C_{max}/AUC_{0-\infty}$ for the enantiomers in the females were significantly lower than those in the males (Tab 1). It was indicated that the enantiomers of *trans*-T were absorbed and eliminated more slowly in female rats than in male rats.

Both in the males and in the females, all pharmacokinetic parameters but T_{max} of (+)-*trans*-T were significantly different from those of (-)-*trans*-T (Tab 1). It was indicated that the pharmacokinetics of *trans*-T was stereoselective in both sex rats. The (+)/(-)-enantiomeric ratios of the pharmacokinetic parameters for *trans*-T were shown in Fig 3. Since the (+)/(-)-enantiomeric ratios had no difference between the males and
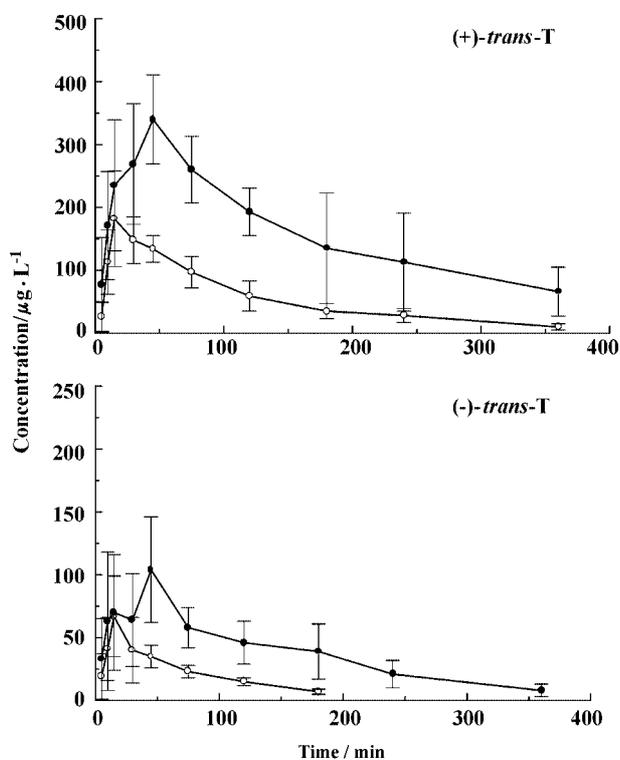

Fig 2. Mean plasma concentration-time curves of the enantiomers of *trans*-tramadol (*trans*-T) in the male (○) and female (●) rats after a single oral dose of 10 mg/kg *trans*-T hydrochloride. *n*=8. Mean±SD.
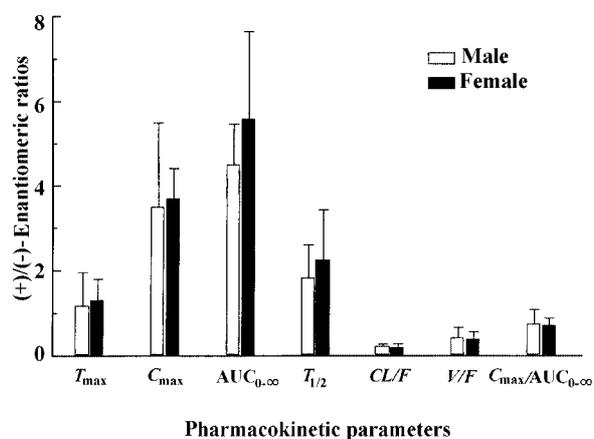

Fig 3. Enantiomeric ratios of the pharmacokinetic parameters for *trans*-tramadol (*trans*-T) in the male and female rats after a single oral dose of 10 mg/kg *trans*-T hydrochloride. *n*=8. Mean±SD.

Tab 1. Pharmacokinetic parameters of the enantiomers of *trans*-tramadol (*trans*-T) in the male and female rats after a single oral dose of 10 mg/kg *trans*-T hydrochloride. *n*=8. Mean±SD. [b]$P<0.05$, [c]$P<0.01$ *vs* male. [e]$P<0.05$, [f]$P<0.01$ *vs* (+)-enantiomer.

Parameters	(+)-*trans*-T		(-)-*trans*-T	
	Male	Female	Male	Female
T_{max} (min)	22±11	45±14[c]	22±8	38±14[b]
C_{max} (µg·L^{-1})	193±64	344±71[c]	74±30[f]	99±39[f]
AUC$_{0-\infty}$ (µg·L^{-1}·min)	21501±3564	79828±26604[c]	5257±703[f]	15196±5037[cf]
$T_{1/2}$ (min)	105±50	218±130[b]	66±29[e]	102±34[be]
CL/F (mL·kg^{-1}·min^{-1})	238±37	69±21[c]	1030±195[f]	361±117[cf]
V/F (L·kg^{-1})	36±19	20±9[b]	142±94[f]	54±26[bf]
C_{max} (AUC$_{0-\infty}$/min^{-1})	0.0091±0.0029	0.0047±0.0017[c]	0.0138±0.0046[f]	0.0066±0.0015[cf]

females, it was known that the stereoselectivity in pharmacokinetics of *trans*-T was similar in male and female rats.

Pharmacokinetics of the enantiomers of M1
Mean plasma concentration-time curves of the enantiomers of M1 following a single oral dose of 10 mg/kg *trans*-T hydrochloride in the male and female rats were depicted in Fig 4. Systemic exposure of (+)-M1, as measured by C_{max} and AUC$_{0-\infty}$, was significantly higher in the females than in the males. The value of $T_{1/2}$ for (+)-M1 was sig-

nificantly longer in the females than in the males (Tab 2).

Both in the males and in the females, C_{max} and AUC$_{0-\infty}$ of (+)-M1 were significantly different from those of (-)-M1 (Tab 2). It was indicated that the pharmacokinetics of M1 was also stereoselective in male and female rats. The (+)/(-)-enantiomeric ratios of the pharmacokinetic parameters for M1 were shown in Fig 5. The (+)/(-)-enantiomeric ratios of C_{max}, AUC$_{0-\infty}$ were lower than 1 in the males, and larger than 1 in the females. Therefore, the stereoselectivity in pharmacokinetics of M1 was different in male and female rats.
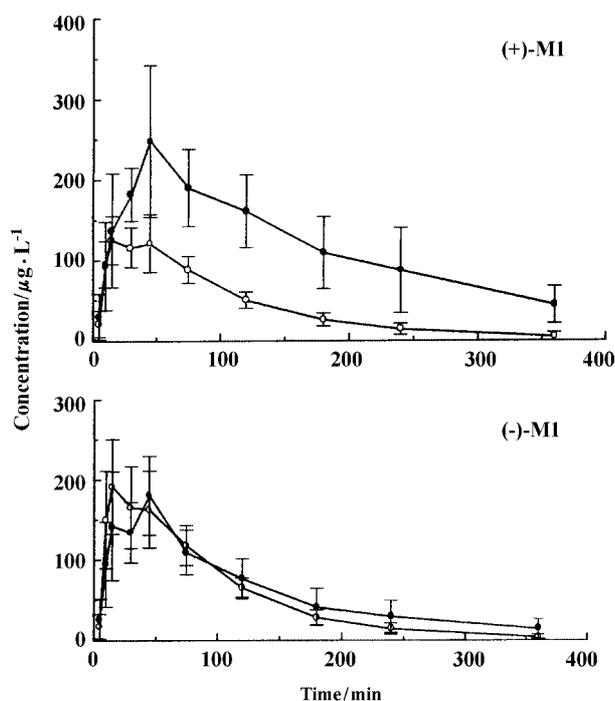

Fig 4. Mean plasma concentration-time curves of the enantiomers of *trans*-*O*-demethyltramadol (M1) in the male (○) and female (●) rats after a single oral dose of 10 mg/kg *trans*-T hydrochloride. *n*=8. Mean±SD.
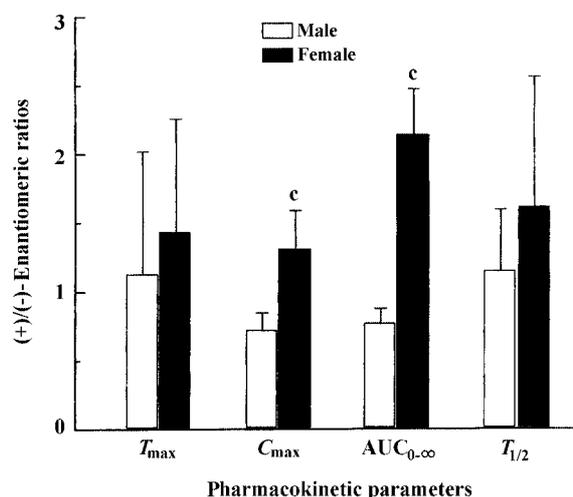

Fig 5. Enantiomeric ratios of the pharmacokinetic parameters for *trans*-*O*-demethyltramadol (M1) in the male and female rats after a single oral dose of 10 mg/kg *trans*-T hydrochloride. *n*=8. Mean±SD. [c]$P<0.01$ *vs* male.

DISCUSSION

In this paper, we observed that systemic exposure

Tab 2. Pharmacokinetic parameters of the enantiomers of *trans-O*-demethyltramadol (M1) in the male and female rats after a single oral dose of 10 mg/kg *trans*-tramadol hydrochloride. *n*=8. Mean±SD. [b]$P<0.05$, [c]$P<0.01$ *vs* male. [e]$P<0.05$, [f]$P<0.01$ *vs* (+)-enantiomer.

Parameters	(+)-M1		(-)-M1	
	Male	Female	Male	Female
T_{max}/min	34±21	49±11	41±24	39±11
C_{max}/µg·L^{-1}	136±32	250±93[b]	195±63[f]	190±50[e]
AUC$_{0-\infty}$/µg·L^{-1}·min	16242±2648	54083±17986[c]	21178±1987[f]	25418±8251[f]
$T_{1/2}$/min	78±28	163±98[b]	75±34	148±122

of (+)-*trans*-T, (-)-*trans*-T, and (+)-M1 was higher in female rats than in male rats after a single oral dose of *trans*-T hydrochloride. The monoaminergic component in *trans*-T analgesia is mainly mediated by (+)-*trans*-T and (-)-*trans*-T, and the opioid mechanism is due to (+)-M1[4]. Therefore, the gender-related differences in pharmacokinetics can result in differences in pharmacodynamic responses in male and female rats.

The mechanistic processes underlying gender-related pharmacokinetics are complicated and can be divided into physiological and molecular factors. The physiological factors include bodyweight, organ size, glomerular filtration, and gastric motility. The molecular factors include drug transporters and drug-metabolizing enzymes. Recently, it is well known that gender-dependent metabolism in rats often results from differences in expression of hepatic enzymes[10]. In rats, *trans*-T is metabolized through *O*-demethylation and *N*-demethylation, M1 can be further metabolized to *N*,*O*-didemethyltramadol and conjugated with glucuronic acid and sulfuric acid[11]. Among the enzymes involved, CYP2D1 was proved to be responsible for the *O*-demethylation of *trans*-T and the formation of M1 in rats[8]. It is being under investigation that the roles of CYP2D1 and other factors in the gender-related differences in pharmacokinetics of *trans*-T and M1 in rats.

In addition, CYP2D6, known as the human counterpart of rat CYP2D1, is responsible for the *O*-demethylation of *trans*-T and formation of M1 in human[12]. It also was known that women have a higher CYP2D6 activity[13]. So, it is very possible that there are gender-related differences in pharmacokinetics of *trans*-T and M1 in human subjects.

REFERENCES

1   Guo WY, Zhan Q, Zhao Y, Wang L. Determination of *cis* and *trans* isomers of tramadol hydrochloride by capillary zone electrophoresis. Biomed Chromatogr 1998; 12: 13-4.

2   Raffa RB, Friderichs E, Reimann W, Shink RP, Codd EE, Vanght JL, *et al*. Complementary and synergistic antinociceptive interaction between the enantiomers of tramadol. J Pharmacol Exp Ther 1993; 267: 331-40.

3   Garrido MJ, Valle M, Campanero MA, Calvo R, Troconiz IF. Modeling of the *in vivo* antinociceptive interaction between an opioid agonist, (+)-*O*-demethyltramadol, and an monoamine reuptake inhibitor, (-)-demethyltramadol, in rats. J Pharmacol Exp Ther 2000; 295: 352-9.

4   Raffa RB. A novel approach to the pharmacology of analgesics. Am J Med 1996; 101: 40s-60s.

5   Liu HC, Liu TJ, Yang YY, Hou YN. Pharmacokinetics of enantiomers of *trans*-tramadol and its active metabolite, *trans-O*-demethyltramadol, in human subjects. Acta Pharmacol Sin 2001; 22: 91-6.

6   Liu HC, Wang N, Liu CS, Hu YQ, Liu JF, Hou YN. Distribution of the enantiomers of *trans*-tramadol and *trans-O*-demethyltramadol in the central nervous system of rats. Acta Pharmacol Sin 2001; 22: 871-5.

7   Liu HC, Zhang XJ, Yang YY, Wang N, Hou YN. The stereoselectivity in renal clearance of *trans*-tramadol and its active metabolite, *trans-O*-demethyltramadol. Acta Pharmacol Sin 2002; 23: 83-6.

8   Liu HC, Wang N, Yu Y, Hou YN. Stereoselectivity in *trans*-tramadol metabolism and *trans-O*-demethyltramadol formation in rat liver microsomes. Acta Pharmacol Sin 2003; 24: 85-90.

9   Fuder S, Stiegler S, Wetzelsberger N, Wieckhorst G, Lange R, Lucker PW. Effect of buffering on pharmacokinetics of ketoprofen enantiomers in man. Br J Pharmacol 1997; 44: 527-30.

10 Czerniak R. Gender-based differences in pharmacokinetics in laboratory animal models. Int J Toxicol 2001; 20: 161-3.

11 Lintz W, Erlacin S, Frankus E, Uragg H. Biotransformation of tramadol in man and animal. Arzneimittelforsch 1981; 31: 1932-43.

12 Paar WD, Poche S, Gerloff J, Dengler HJ. Polymorphic CYP2D6 mediates *O*-demethylation of the opioid analgesic tramadol. Eur J Clin Pharmacol 1997; 53: 235-9.

13 Meibohm B, Beierle I, Derendor H. How important are gender differences in pharmacokinetics. Clin Pharmacokinet 2002; 41: 329-42.