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

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KEY WORDS tramadol; pharmacokinetics; sex factors

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\textsubscript{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\textsubscript{max}, AUC\textsubscript{0-\infty} of the two enantiomers of M1 were significantly different in both sex rats. The (+)/(-)-enantiomeric ratios of C\textsubscript{max}, AUC\textsubscript{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 1R, 2R-[(+)-trans-T] and 1S,2S-[(-)-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 serotonin release, whereas (-)-trans-T preferentially inhibits norepinephrine reuptake and enhances stimulation-evoked norepinephrine release[2]. Trans-O-demethyltramadol (M1) is the only pharmacologically active metabolite. (+)-M1 has a high affinity to the \(\mu\)-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.
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 1R,2S-T and 1S,2R-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 No 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 ºC 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 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 ºC 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 (Cmax) and its corresponding time (Tmax) were recorded as observed. The elimination rate constant (λz) 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 (T1/2) was calculated as 0.693/λz. The area under the plasma concentration-time curve (AUC0-∞) from time zero to the time of last quantifiable concentration (Ci) was calculated using the linear trapezoidal method. The area under the plasma concentration-time curve from time zero to the infinite time (AUC0-∞) was calculated as the sum of corresponding AUC0-t and Ci/λz value. The plasma oral clearance (CL/F) was calculated as Dose/AUC0-∞. The apparent volume of distribution (V/F) was determined using the equation: V/F = (Dose/AUC0-∞)/λz. As a measure for the rate of absorption, the Cmax/AUC0-∞ 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 Tmax, to which non-parametric 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
depicted in Fig 2. The females showed higher concentrations of (+)-trans-T and (-)-trans-T than the males. The values of $T_{\text{max}}$, $\text{AUC}_{0-\infty}$, and $T_{1/2}$ for (+)-trans-T and (-)-trans-T, and the value of $C_{\text{max}}$ for (+)-trans-T were significantly higher in the females than in the males.

Meanwhile, the values of $CL/F$, $V/F$, and $C_{\text{max}}/\text{AUC}_{0-\infty}$ 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 $T_{\text{max}}$ 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

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**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.

**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.
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 $C_{\text{max}}$, AUC$_{0-\infty}$, $T_{1/2}$, and other pharmacokinetic parameters, was significantly higher in the females than in the males. The value of $T_{1/2}$ for (+)-M1 was significantly longer in the females than in the males (Tab 2).

Both in the males and in the females, $C_{\text{max}}$ 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 $C_{\text{max}}$, 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.

DISCUSSION
In this paper, we observed that systemic exposure

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**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. *P<0.05, **P<0.01 vs male. ***P<0.05, ****P<0.01 vs (+)-enantiomer.

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<tr>
<th>Parameters</th>
<th>(+)-trans-T</th>
<th>Female</th>
<th>(-)-trans-T</th>
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<tr>
<td>$T_{\text{max}}$ (min)</td>
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<td>45±14$^{c}$</td>
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<td>$C_{\text{max}}$ (µg·L$^{-1}$)</td>
<td>193±64</td>
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<td>AUC$_{0-\infty}$ (µg·L$^{-1}$·min)</td>
<td>21501±3564</td>
<td>79828±26604$^{d}$</td>
<td>5257±703$^{f}$</td>
<td>15196±5037$^{ef}$</td>
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<td>$T_{1/2}$ (min)</td>
<td>105±50</td>
<td>218±130$^{a}$</td>
<td>66±29$^{b}$</td>
<td>102±34$^{b}$</td>
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<tr>
<td>CL/F (mL·kg$^{-1}$·min$^{-1}$)</td>
<td>36±19</td>
<td>20±9$^{c}$</td>
<td>142±94$^{d}$</td>
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<td>$C_{\text{max}}$ (AUC$_{0-\infty}$/min$^{-1}$)</td>
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<td>0.0047±0.0017$^{c}$</td>
<td>0.0138±0.0046$^{f}$</td>
<td>0.0066±0.0015$^{ef}$</td>
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**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. **P<0.01 vs male.
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