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Urinary excretion of the enantiomers of *trans* tramadol and its active metabolite, *trans O*-demethyltramadol, in human subjects

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Abstract **Aim** To investigate the urinary excretion of the enantiomers of *trans* tramadol (*trans* T) and its active metabolite, *trans O*-demethyltramadol (M1), in human subjects. **Methods** Twenty healthy volunteers (10 men, 10 women) were included in the study. After an overnight fast, a single 100-mg oral dose of *trans* T hydrochloride was ingested. Urine samples were collected for 24 h after dosing. The enantiomers of *trans* T and M1 were determined with a high performance capillary electrophoresis method. The enantiomers of M1 glucuronide conjugate ($M1_c$) was determined as the enantiomers of free M1 ($M1_f$) after enzymatic cleavage with β-glucronidase. **Results** In urine, (+)-*trans* T was more than (-)-*trans* T. There were no significant differences in the urine amount of (+)-*trans* T, (-)-*trans* T, or *trans* T between the male and female subjects. In urine, (+)-$M1_f$ was more than (-)-$M1_f$, (+)-$M1_c$ was less than (-)-$M1_c$, but (+)-M1 [(+)-$M1_f$ plus (+)-$M1_c$] was almost equal to (-)-M1 [(-)-$M1_f$ plus (-)-$M1_c$]. There were no significant differences in the urine amounts of $M1_f$, $M1_c$, M1 or their enantiomers between the male and female subjects. The glucuronide rate of (+)-M1 was lower than that of (-)-M1. There were no significant differences in the glucuronide rates of M1 or its enantiomers between the male and female subjects. **Conclusion** The urinary excretion of the enantiomers of *trans* T is different in human, (+)-*trans* T being excreted more. The urinary excretion of the enantiomers of M1 is stereoselective in human, (+)-M1 and (-)-M1 being excreted mainly as (+)-$M1_f$ and (-)-$M1_c$, respectively. There are no gender-related differences in the urinary excretion of the enantiomers of *trans* T or M1.

Key words tramadol; urinary excretion; stereoisomerism; pharmacokinetics

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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 display different binding properties for various receptors. (+)-*Trans* T preferentially inhibits serotonin reuptake and enhances basal serotonin release, whereas (-)-*trans* T mainly inhibits norepinephrine reuptake and enhances stimulation-evoked norepinephrine release. The preclinical studies suggest a complementary and synergistic antinociceptive interaction between the enantiomers of *trans* T[2]. *Trans* T is mainly metabolized in liver to *trans O*-demethyltramadol (M1), *trans N*-demethyltramadol (M2), and *trans N*, *O*-didemethyltramadol (M5). Some metabolites may be further conjugated with glucuronic acid and sulfuric acid before excretion into urine[3]. M1 is the only pharmacologically active metabolite, (+)-M1 having a higher affinity to the opioid receptor and (-)-M1 inhibiting monoamine reuptake [4]. The monoaminergic component in *trans* T analgesia is mediated by (+)-*trans* T, (-)-*trans* T, and (-)-M1; the opioid mechanism is due to (+)-M1. This dual model of action of *trans* T, opioid

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and nonopioid, may contribute to its high efficiency in certain pain, little or no respiratory depression and tolerance after repeated administration [5].

In our previous paper, the pharmacokinetics of *trans* T and M1 was found to be stereoselective in healthy male subjects; (+)-*trans* T was shown to be absorbed more completely but excreted more slowly; and the stereoselectivity in pharmacokinetics of M1 was much different among the subjects [6]. The aim of the present study was to provide further information on the urinary excretion of *trans* T, M1 and their enantiomers in healthy male and female subjects after oral administration of *trans* T hydrochloride.

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 Medicine College (China) and used as internal standard. Sulfobutylether-β-cyclodextrin was kindly provided by Lanzhou Institute of Chemical Physical，the Chinese Academy of Sciences. β-Glucuronidase (type HP-2) was purchased from Sigma (USA). 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.

Subjects

Twenty healthy Chinese volunteers (10 men and 10 women) met the entry requirements and completed the study. The age, height, and weight of the male volunteers ranged from 18 a to 26 a (20±3 a), 166 cm to 180 cm (173±4 cm) , 60 kg to 85 kg (69±8 kg), respectively. The age, height, and weight of the female volunteers ranged from 21 a to 33 a (25±4 a), 158 cm to 170 cm (163±4 cm), 50 kg to 60 kg (56±5 kg), respectively. All subjects were in good health on basis of the medical history, physical examination, electrocardiogram, and laboratory screening tests (routine hematology, blood chemistry, and urinalysis). Subjects did not have a history of hypertension, syncope, or significant diseases including cardiovascular and gastrointestinal diseases. If female subjects were of childbearing potential, they agreed to use a barrier method of contraception from 1 month prior to dosing until 1 month after completion of the study. All subjects were required to abstain from the use of all other drugs for at least two weeks prior to and until after completion of the study. The subjects were also required to refrain from consuming alcohol or caffeine-containing beverages from 48 h prior to dosing until after the collection of the last blood sample.

After an overnight fast, urine was voided. Each subject was given two 50-mg *trans* T hydrochloride tablets, from Grünenthal Gmbh (Stolberg, Germany), with 200 mL of water. The subjects continued to fast for 4 h and allowed drinking water 2 h after administration. Standardized lunch and dinner were served 4 h and 8 h after dosing, respectively. The subjects remained under close medical supervision until 8 h after the urine collection.

Each subject gave a written informed consent before participating in the study. The study protocol was approved by the Medical Ethics Committee, Bethune International Peace Hospital.

Collection and extraction of urine samples

Urine was collected for 24 h after dosing. The volume of urine passed was recorded, and an aliquot from each sample was stored at –80℃ until analysis. After addition of 500 μL of 0.5 $mol \cdot L^{-1}$ sodium hydroxide and 100 μL of 2 $\mu g \cdot mL^{-1}$ *cis* T, the enantiomers of *trans* T and M1 in urine were extracted with 5 mL of ethyl acetate. After centrifugation at 2000 *g* for 10 min, the organic layer containing analyses was removed into another tube. After evaporation to dryness under a gentle stream of nitrogen, the residue was redissolved in 200 μL of water, and then an aliquot (30 μL) was removed out for analysis.

Analytical method

The enantiomers of *trans* T and M1 in urine were determined by a high performance capillary electrophoresis (HPCE) method as previously described [6]. Briefly, electrophoretic experiments were performed in a P/ACE 5000 automatic electrophoresis apparatus (Beckman, California, USA) equipped with an UV detector. 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, an inner

diameter of 75 μm. The background electrolyte (BGE) contained 40 mmol·L^{-1} Tris (adjusted to pH 2.5 with phosphoric acid) and 0.8 mmol·L^{-1} sulfobutylether-β-cyclodextrin, which were used as the chiral selector. The samples were 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. The enantiomers of M1 glucuronide conjugate ($M1_c$) were determined as the enantiomers of free M1 ($M1_f$) after enzymic cleavage with β-glucuronidase [7]

Data analysis

Paired *t* test was used to compare the pharmacokinetic parameters between the two enantiomers of *trans* T or M1. To compare the pharmacokinetic parameters between the male and female subjects, unpaired *t* test was used to all parameters except t_{max}, to which nonparametric Wilcoxon two-sample test was used.

Results

Under the analytical condition selected, the enantiomers of *trans* T, M1, and *cis* T could be well separated. There was no interference from urine (Fig 1). The calibration curves of the enantiomers of *trans* T and M1 were constructed by plotting the peak area ratios against the corresponding concentrations in urine samples. The calibration curves of the enantiomers of *trans* T were linear over the concentration range from 60 ng·mL^{-1} to 1000 ng·mL^{-1}. The calibration curves of the enantiomers of M1 were linear over the concentration range from 80 ng·mL^{-1} to 1280 ng·mL^{-1}. The intra-assay accuracy was acceptable if the mean concentration of standard replicates did not exceed ± 115 % of the normal concentration. The intra-assay precision, defined as the coefficient of variation (n=5) calculated in the determination of accuracy, was acceptable if the coefficient of variation was less than 15 %. The limit of detection (LOD), defined as the lowest concentration that could give a signal-to-noise ratio of 3:1, was 1.6 ng·mL^{-1} for the enantiomers of *trans* T and M1 in urine.

The amounts of *trans* T and its enantiomers excreted into urine were depicted in Table 1. In urine, *trans* T was about 22 % of the dose, and (+)-*trans* T was more than (-)-*trans* T. There were no significant differences in the amounts of (+)-*trans* T, (-)-*trans* T, or *trans* T between the male and female subjects. The (+)/(-)-ratios of *trans* T amounts were different among subjects. The (+)/(-)-ratios were almost equal to 1 (from 0.97 to 1.03) in four subjects, and over 1.03 in the other sixteen subjects.

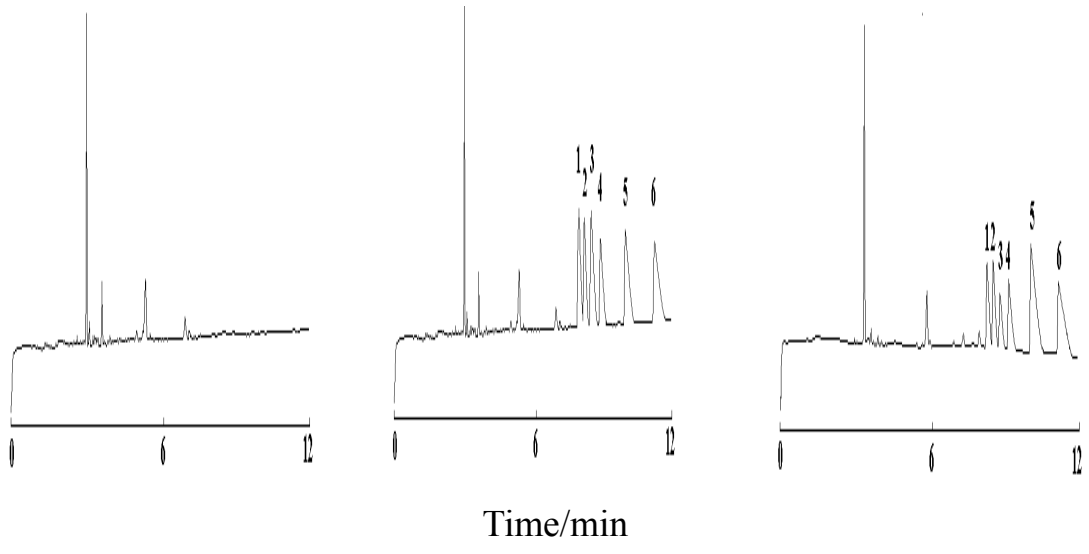

Fig 1 Typical electropherograms. A: drug-free urine; B: drug-free urine spiked with *trans* tramadol and *cis* tramadol (internal standard); C: a urine sample from a volunteer. 1: (+)-*trans O*-demethyltramadol ; 2: one enantiomer of *cis* tramadol; 3: (-)-*trans O*-demethyltramadol; 4: another enantiomer of *cis* tramadol; 5: (+)-*trans* tramadol; 6: (-)-*trans* tramadol.

The amounts of $M1_f$ and its enantiomers, $M1_c$ and its enantiomers, and M1 [$M1_f$ plus $M1_c$] and its enantiomers excreted into urine were depicted in Table 2. In urine, (+)-$M1_f$ was more than (-)-$M1_f$, (+)-$M1_c$ was less than (-)-$M1_c$, (+)-M1 was almost equal to (-)-M1. M1 was about 19 % of the dose. There was about one third of M1 excreted as $M1_c$. There were no significant differences in the amounts of (+)-$M1_f$, (-)-$M1_f$, $M1_f$, (+)-$M1_c$, (-)-$M1_c$, $M1_c$, (+)-M1, (-)-M1, and M1 between the male and female subjects. The (+)/(-)-ratios of M1 amounts were much different among subjects. The (+)/(-)-ratios were almost equal to 1 in four subjects, over 1.03 in six subjects, and lower than 0.97 in the other ten subjects.

The glucuronidation of the enantiomers of M1 in urine was depicted in Table 3. There were marked inter-individual variations in glucuronide rates of (+)-M1 than (-)-M1; and the mean glucuronide rate of (+)-M1 was lower than that of (-)-M1. There were no significant differences in the glucuronide rates of (+)-M1, (-)-M1, and M1 between the male and female subjects.

Table 1 Recovered amounts of *trans* tramadol (*trans* T) and its enantiomers in the urine of 20 volunteers after oral administration of two 50-mg *trans* T hydrochloride tablets.

Substance	Man	Woman
(+)-*trans* T/μmol	42±13	37±18
(-)-*trans* T/μmol	37±13**	33±17**
trans T/μmol	79±26	71±34

**P<0.01 compared with (+)-enantiomer with paired t test

Table 2 Recovered amount of free *trans O*-demethyltramadol ($M1_f$), M1 glucuronide conjugate ($M1_c$), M1 [$M1_f$ plus $M1_c$] and their enantiomers in the urine of 20 volunteers after oral administration of two 50-mg *trans* T hydrochloride tablets.

Substance	Man	Woman
(+)-$M1_f$/μmol	28±7	26±9
(-)-$M1_f$/μmol	16±4**	17±8**
$M1_f$/μmol	44±10	43±16
(+)-$M1_c$/μmol	5±5	3±2
(-)-$M1_c$/μmol	17±8**	14±7**
$M1_c$/μmol	22±12	17±8
(+)-M1/μmol	33±10	30±10
(-)-M1/μmol	34±8	31±7
M1/μmol	67±17	60±16

**P<0.01 compared with (+)-enantiomer with paired t test

Table 3 Glucuronidation of the enantiomers of *trans O*-demethyltramadol (M1) in the urine of 20 volunteers after oral administration of two 50-mg *trans* T hydrochloride tablets.

Substance	Man	Woman
(+)-M1/%	14±13	12±10
(-)-M1/%	50±13**	45±20**
M1/%	32±12	29±16

**P<0.01 compared with (+)-enantiomer with paired t test

Discussion

This study provided further characterization of the urinary excretion of the enantiomers of *trans* T, M1, and M1 glucuronide after oral administration of *trans* T hydrochloride in human subjects. The urinary excretion of the enantiomers of *trans* T is different in human, (+)-*trans* T being excreted more. The urinary excretion

of the enantiomers of M1 is stereoselective in human, (+)-M1 and (-)-M1 being excreted mainly as (+)-$M1_f$ and (-)-$M1_c$, respectively. There are no gender-related differences in the urinary excretion of the enantiomers of *trans* T or M1.

After oral administration of *trans* T hydrochloride, the mean (+)/(-)-ratios of $AUC_{0\text{-}24h}$ for *trans* T and M1 were 1.23 and 0.88 in human plasma, respectively[8]. Since the (+)/(-)-ratios of amount for *trans* T were about 1.13 in human urine, the more excretion of (+)-*trans* T may be due to its higher system exposure in plasma. The (+)/(-)-ratios of amount for M1 were about 0.96 in human urine. It is suggested that the urinary excretion of M1 is stereoselective, (+)-M1 being preferentially excreted into urine. In our previous research, (+)-M1 had been proved to be preferentially cleared into urine in rat isolated perfused kidney *in vitro* [9].

It had been found that there were 3 out of 5 subjects excreted more (+)-*trans* T than (-)-*trans* T, and 4 out of 5 subjects excreted more (+)-$M1_f$ than (-)-$M1_f$ into urine after oral administration of *trans* T hydrochloride[10]. In this study, inter-individual variations were also observed in the stereoselectivity in the urinary excretion of *trans* T and M1. Four subjects excreted racemate of *trans* T (the (+)/(-)-ratios were almost equal to 1); and the other sixteen subjects excreted more (+)-*trans* T (the (+)/(-)-ratios were over 1.03). Four subjects excreted racemate of M1, six subjects excreted more (+)-M1, and the other ten subjects excreted less (+)-M1 (the (+)/(-)-ratios were lower than 0.97). In addition, there were marked inter-individual variations in glucuronide rates of (+)-M1 than (-)-M1. Thus, further study should be carried out to know the reason for these inter-individual variations in stereoselectivity.

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Letter to the editor

With great interests, we have read the review entitled 'Bioequivalence and beyond: how to expand routine bioequivalence studies to generate new knowledge' by Yin OQP and Chow MSS[1]. In this context, authors summarized four methods to extract useful information from the bioequivalence data. We agree with the proposition they have raised and would like to add two more points on this topic.

1. Establishing limited sampling strategy (LSS) for therapeutic drug monitoring (TDM). Trough concentration, which correlates well with drug efficacy and toxicity, is widely used in the routine clinical TDM. However, sometimes, it can not reflect the pharmacology effects due to its large variations and poor relationships with the area under the curve (AUC) in certain drugs. LSS has been recommended in this situation. Firstly, a PK study is undertaken to estimate the value of AUC using a conventional sampling method, as the bioequivalence study we performed. The second step involves a multiple regression analysis in which a limited number of the sampling times (usually two or three) are determined that provide the best estimate of the AUC[2]. The information extracted from healthy volunteers may not be the same as the real patients, however, it is valuable to obtain an initial estimation for the further studies. We just finished a BE study on the cycloslin A and developed a LSS for its TDM in Chinese patients[3,4]. The results we got were consistent with the previous studies of other ethnic groups performed in the kidney transplant patients [5].

2. Developing models to link pharmacokinetics (PK), pharmacodynamics (PD, including therapeutic effects and unexpected drug effects.) and pharmagenomics (PG). It is well known that many factors can influence drug response, such as age, dosage, weight, food and genetics etc. To investigate all those variables is the one of the keys to explain the variability of drug response. We propose to gather data from all those three aspects to develop models which integrate the determinants as many as possible rather than only the information for PK-PD or PK-PG. It provides helpful knowledge for the individualized therapy. With the rapid development of computer science, more and more mathematical methods have been introduced into the pharmaceutical science. The nonlinear mixed effect model[6] and the neural network technique[7] are two of those techniques and provide powerful tools to explore BE data. As far as we know, there is no such study published. It may be attributed to the fact of lack of well-designed studies or the great difficulties to obtain all those data. However, it is obvious that combining PK, PD and PG would lead to better understanding of drug behavior.

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