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

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KEY WORDS tramadol; capillary electrophoresis; pharmacokinetics

ABSTRACT

AIM: To study the stereoselectivity in pharmacokinetics of the enantiomers of *trans*-tramadol (*trans*-T) and its active metabolite, *trans*-*O*-demethyltramadol (M1) in human subjects. **METHODS**: *Trans*-T hydrochloride sustained-release tablets were taken orally by 12 healthy male volunteers. After a multiple dosage schedule, the serum concentrations of (+)-*trans*-T, (−)-*trans*-T, (+)-M1, and (−)-M1 were determined in serum by high performance capillary electrophoresis (HPCE). **RESULTS**: (+)-*Trans*-T, (−)-*trans*-T, (+)-M1 and (−)-M1 in human serum were separated by HPCE. The linear range was 2.5 − 320 μg/L for the enantiomers of *trans*-T, and 2.5 − 50 μg/L for the enantiomers of M1. For the enantiomers of *trans*-T and M1, the intra-day and inter-day RSD were less than 15 % and 20 %, and the relative recoveries were 94.3 % − 106.2 % and 90.4 % − 107.8 %, respectively; the limit of quantitation was 1.25 μg/L. The serum concentrations of the enantiomers of *trans*-T reached a steady state in 12 subjects on d 4 after the initial administration. The steady state serum concentrations of (+)-*trans*-T were higher than that of (−)-*trans*-T at every sampling points in the subjects. The differences were significant in the main pharmacokinetic parameters between (+)-*trans*-T and (−)-*trans*-T except T_{max}. The serum concentrations of (−)-M1 were higher than that of (+)-M1 in most subjects and at most sampling time points. There were significant differences in C_{max} and C_{min} between the enantiomers of M1. **CONCLUSION**: The pharmacokinetics of *trans*-T and M1 was found to be stereoselective. (+)-*Trans*-T was shown to be absorbed completely, but eliminated more slowly. The pharmacokinetic stereoselectivity of M1 was different among human subjects.

INTRODUCTION

Tramadol (T) has two chiral centers in its chemical structure. The racemate of *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, whereas (−)-*trans*-T mainly inhibits norepinephrine reuptake. Preclinical studies suggest a complementary and synergistic antinociceptive interaction between the enantiomers of *trans*-T[2]. *Trans*-T is metabolized in the liver mainly to *trans*-*O*-demethyltramadol (M1), *trans*-*N*-demethyltramadol (M2), and *trans*-*N*-demethyl-*O*-demethyltramadol (M5). The metabolites may be further conjugated with glucuronic acid and sulfate before excretion into urine[3]. M1 is a pharmacologically active metabolite with a higher affinity to the opioid receptor than other enantiomers of *trans*-T. The monominergic component of *trans*-T analgesia is probably mediated by (+)- and (−)-*trans*-T and the opioid mechanism is probably due to M1. 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 no tolerance development after repeated administration[4].

To data, no information is available on the pharmacokinetics of the enantiomers of *trans*-T and M1. Recently, high performance capillary electrophoresis (HPCE) has been shown to be a technique with high resolution power and high efficiency for the determination of the enantiomers of *trans*-T and its metabolites[5,6]. In this paper, the simultaneous determination and pharmacokinetics of the enantiomers of *trans*-T and M1 was investigated in human subjects.

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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 was a gift from Department of Chemistry of Jinzhou Medical College (Jinzhong, China). Sulfobutylether-β-cyclodextrin was kindly provided by Lanzhou Institute of Chemical Physiology, Chinese Academy of Sciences (Lanzhou, China). 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 Twelve healthy male volunteers, aged 18 a − 32 a and weighing 55 kg − 75 kg, were involved in this study. All subjects were required to be free of cardiovascular, hepatic, renal, or gastrointestinal diseases, drug abuse or alcohol dependence, assessed by physical examination and review of medical history. In addition, the blood pressure, electrocardiogram, and results of chemical laboratory tests (blood chemistry, hematology, and urianalysis) were required to be within normal ranges. All subjects were required to abstain from the use of all other drugs for at least one week prior to and until after completion of the study. The subjects were also required to refrain from consuming alcohol or caffeine-containing beverages for 48 h prior to dosing and until the collection of the last blood sample. Each subject gave a written informed consent before participating in the study. The study protocol was approved by the Medical Ethics Committee of our hospital.

Each subject was given an oral dose (100 mg) of *trans*-T hydrochloride sustained-release tablets with 200 mL of water at 6 am and 6 pm for 11 times. A standardized meal was provided 4 h after each dosing. The subjects remained under close medical supervision until 8 h after the last sample collection.

Blood collection After the initial administration, blood samples were collected just before the first dosing on d 4 − d 6. On d 6, the samples were collected at 0.75, 2, 3.5, 5, 6.5, 8, 10, 12, 16, 24, and 32 h after the last dose given in the morning. Serum was separated by centrifugation and stored at −24 ℃ until analysis.

Extraction of serum sample Sodium hydroxide 0.5 mol/L 500 μL, *cis*-T 1 mg/L 100 μL and 5 mL of ethyl acetate were added to 1 mL of human serum in a 10 mL tube. The sample was vortexed for 2 min and centrifuged at 2000 × *g* for 10 min. The organic layer was removed into another tube. After evaporation under a gentle stream of nitrogen, the residue was redissolved in 100 μL of water by vortexing for 2 min. Supernatant 30 μL was removed out for HPCE analysis.

HPCE Electrophoretic experiments were performed in a P/ACE 5000 automatic electrophoresis apparatus (Beckman, California, USA) equipped with UV detector set at 214 nm. 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 40 mmol/L (adjusted to pH 2.5 with phosphoric acid) and 0.8 mmol/L sulfobutylether-β-cyclodextrin, which was used as the chiral selector.

Pharmacokinetic parameters C_{max} and T_{max} were obtained directly from the observed concentrations. C_{min} was the average mean of the two concentrations before and 12 h after the last dosing. AUC_{0-t} and $AUC_{0-\tau}$ were calculated by the trapezoidal rule, where t is the last measurable time point and τ is the dosing interval. λ_z was estimated through linear regression of the nature logarithms of the observed concentrations during the terminal monoexponential phase versus time. $T_{1/2} = 0.693/\lambda_z$, $AUC_{t-\infty} = C_t/\lambda_z$, $AUC_{0-\infty} = AUC_{0-t} + AUC_{t-\infty}$, $C_{av} = AUC_{0-\tau}/\lambda_z$.

Data analysis Data were expressed as $x \pm s$. The serum concentrations and pharmacokinetic parameters between the two enantiomers of *trans*-T and M1 were compared through paired *t* test.

RESULTS

Under selected conditions, a complete baseline separation of the enantiomers of *trans*-T, M1, and *cis*-T was achieved without any interfering endogenous components at their retention time (Fig 1). The following retention time was determined: (+)-*trans*-T 9.47 min, (−)-*trans*-T 10.30 min, (+)-M1 8.12 min, (−)-M1 8.56 min. The *O*-demethylation of *trans*-T in human microsomer was found to be highly stereoselective, preferentially metabolizing the (−)-enantiomer. The order of elution of M1 was determined through analyzing each enantiomer of *trans*-T and its *O*-demethylated metabolite in microsomal fractions of human liver[7]. The order of elution of *cis*-T could not be determined as its single enantiomers were not available.

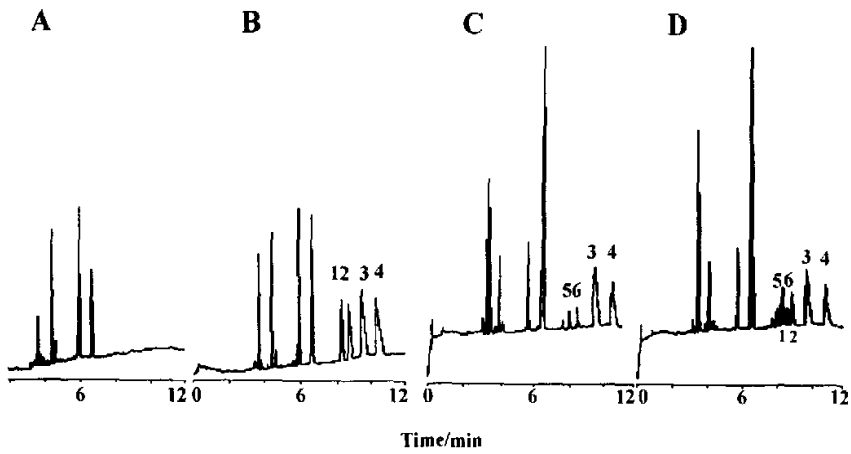

Fig 1. Typical electrophorograms. A: drug-free human serum; B: drug-free human serum spiked with *trans* tramadol and *cis*-tramadol (internal standard); C: serum sample from a volunteer who took *trans*-tramadol hydrochloride sustained-release tablets; D: the same volunteer's serum sample spiked with *cis*-tramadol. Enantiomers of *cis*-tramadol (1,2); (+)-*trans*-tramadol (3); (−)-*trans*-tramadol (4); (+)-*trans*-*O*-demethyltramadol[(+)-M1] (5); (−)-M1 (6).

The limit of quantitation (LOQ) was defined as the lowest concentration that could give a signal-to-noise ratio of 3 : 1. For the enantiomers of *trans*-T and M1, the LOQ was found to be 1.25 μg/L. The slowly eluted enantiomer of *cis*-T was used as the internal standard. The peak-area ratios of compounds were linearly related to the added amount of the enantiomers of *trans*-T and M1. For the enantiomers of *trans*-T, the linear range was 2.5 − 320 μg/L. For the enantiomers of *trans*-M1, the linear range was 2.5 − 50 μg/L. The linear regression equations were

(+)-*trans*-T: $Y = 0.03575 + 0.02066X$ ($n = 9$, $r = 0.9996$)

(−)-*trans*-T: $Y = 0.06300 + 0.02035X$ ($n = 9$, $r = 0.9994$)

(+)-M1: $Y = 0.04564 + 0.02062X$ ($n = 6$, $r = 0.9992$)

(−)-M1: $Y = 0.06420 + 0.02137X$ ($n = 6$, $r = 0.9994$)

Here, Y stands for peak-area ratio, and X represents concentration.

Intra-day variation, assessed by performing five replicate determinations of four spiked standards for the enantiomers of *trans* T and three spiked standards for the enantiomers of M1, gave accuracies ranging from 94.3 % to 106.2 % at all tested concentrations. The intra-day precision expressed as RSD was found to be less than 15 % for all compounds at all tested concentrations. Inter-day variation of the method was assessed by analyzing replicates of these standards in different days. Inter-day accuracies for the serum spiked samples were found to be ranging from 90.4 % to 107.8 % with RSD less than 20 % for all compounds tested at all concentrations (Tab 1).

The steady state serum concentrations of the enantiomers of *trans*-T already reached in 12 human subjects on d 4 after multiple oral doses of *trans*-T hydrochloride sustained-release tablets. At the steady state, the serum concentrations of (+)-*trans*-T were higher than that of (−)-*trans*-T at every sampling time point in 12 subjects. A time-dependent increase of (+)/(−)-*trans*-T ratio was observed during the sampling period. The serum concentrations of (−)-M1 were higher than that of (+)-M1 in most subjects and at most sampling time points. A time-dependent decrease in (−)/(+)-M1 ratio was observed during the sampling period (Tab 2). The concentrations of the enantiomers of M1 decreased fast after 16 h, and were below measurable levels in 9 subjects at 24 h, and in all subjects at 32 h. The average (+)/(−)-*trans*-T ratios at all sampling points were similar among 12 subjects. But the average (−)/(+)-M1 ratios at all sampling points were much different among 12 subjects. The lowest ratio was 0.89, and the highest was 1.90 (Tab 3).

After multiple oral doses of *trans*-T hydrochloride sustained-release tablets, there were differences in the main

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Tab 1. Mean intra-day and inter-day precision and recoveries of the enantiomers of *trans*-tramadol (*trans*-T) and *trans* *O*-demethyltramadol (M1). $n=5$. $\bar{x} \pm s$.

Concentration μg/L	(+)-*Trans*-T RSD %	(+)-*Trans*-T Recovery %	(−)-*Trans*-T RSD %	(−)-*Trans*-T Recovery %	(+)-M1 RSD %	(+)-M1 Recovery %	(−)-M1 RSD %	(−)-M1 Recovery %
Intra-day								
5	14.8	106.2	10.4	94.3	12.8	98.4	13.3	98.1
20	6.0	106.0	8.5	104.7	4.1	100.9	4.7	101.3
40					8.6	100.9	8.7	100.8
80	6.7	98.4	2.5	97.4				
240	3.6	100.4	4.6	99.5				
Inter-day								
5	19.5	103.6	16.6	90.4	14.5	95.2	17.3	101.2
20	8.8	103.0	13.1	107.8	4.2	101.6	5.2	100.9
40					9.9	100.1	10.7	99.6
80	7.8	97.7	13.6	92.3				
240	3.6	100.0	8.5	102.5				

Tab 2. Mean serum concentrations and enantiomic ratios of *trans*-tramadol (*trans*-T) and *trans*-*O*-demethyltramadol (M1) in 12 healthy subjects at each sampling time after multiple doses of *trans*-T hydrochloride sustained-release tablets (100 mg/12 h, 11 times). $n=12$. $\bar{x} \pm s$. $^cP<0.01$ *vs* (+)-*trans* T; $^dP>0.05$, $^eP<0.05$, $^fP<0.01$ *vs* (+)-M1.

Time/h	*Trans* T (+)-/μg·L^{-1}	*Trans* T (−)-/μg·L^{-1}	*Trans* T (+)/(−) ratio	M1 (+)-/μg·L^{-1}	M1 (−)-/μg·L^{-1}	M1 (−)/(+) ratio
0	84 ± 18	68 ± 12^c	1.23 ± 0.12	10 ± 5	12 ± 4^d	1.2 ± 0.5
0.75	110 ± 34	93 ± 32^c	1.20 ± 0.11	13 ± 8	17 ± 7^f	1.4 ± 0.3
2	141 ± 34	122 ± 28^c	1.15 ± 0.06	15 ± 7	19 ± 6^f	1.36 ± 0.28
3.5	153 ± 28	133 ± 24^c	1.15 ± 0.05	17 ± 7	20 ± 5^f	1.37 ± 0.28
5	150 ± 41	124 ± 35^c	1.21 ± 0.11	16 ± 7	19 ± 6^f	1.30 ± 0.28
6.5	130 ± 31	109 ± 27^c	1.20 ± 0.07	15 ± 7	17 ± 6^f	1.3 ± 0.3
8	107 ± 32	89 ± 28^c	1.22 ± 0.07	12 ± 5	14 ± 5^f	1.26 ± 0.26
10	100 ± 25	81 ± 23^c	1.24 ± 0.08	11 ± 3	13 ± 3^f	1.24 ± 0.27
12	87 ± 25	70 ± 23^c	1.26 ± 0.07	10 ± 4	11 ± 4^e	1.21 ± 0.26
16	60 ± 19	45 ± 17^c	1.35 ± 0.12	7 ± 3	7.8 ± 2.8^d	1.15 ± 0.26
24	32 ± 11	22 ± 10^c	1.50 ± 0.27			
32	14 ± 6	7 ± 4^c	2.2 ± 0.6			

pharmacokinetic parameters between (+) and (−)-*trans*-T except for the T_{max}. There were significant differences in C_{max} and C_{min} between (+) and (−)-M1 (Tab 4).

DISCUSSION

The enantiomers of *trans*-T and some of its metabolites have been separated by HPLC with chiral stationary phases[8,9]. Recently, capillary electrophoresis was used for the stereoselective determination of *trans*-T and its metabolites in human urine[6]. The HPCE assay described here allowed the sensitive, precise, and rapid determination of the enantiomers of *trans*-T and M1 in human serum. In this paper, the pharmacokinetics of *trans*-T was found to be stereoselective. (+)-*Trans*-T was shown to be absorbed more completely, but eliminated more slowly in human body. Since the (−)/(+)-M1 ratios in serum were much different in different bodies, the pharmacokinetics of M1 was different among different human subjects. In addition, it has been reported that the (−)/(+)-M1 ratios in urine were less than 1 in 4 out of 5 human subjects[8]. It was found in this paper that the (−)/(+)-M1 ratios in serum were larger than 1 in 11 out of 12 human subjects. Therefore, it should be further studied whether or not the pharmacokinetic stereo-

Tab 3. The average enantiomic ratios of *trans*-tramadol (*trans*-T) and *trans*-*O*-demethyltramadol (M1) in each healthy subjects at all sampling points after multiple doses of *trans*-T hydrochloride sustained-release tablets (100 mg/12 h, 11 times). $\bar{x} \pm s$.

Subject	(+)/(−) *Trans*-T ($n = 12$)	(−)/(+) M1 ($n = 10$)
1	1.39 ± 0.34	1.41 ± 0.16
2	1.19 ± 0.15	1.52 ± 0.16
3	1.22 ± 0.11	1.43 ± 0.16
4	1.28 ± 0.18	0.89 ± 0.11
5	1.40 ± 0.61	1.15 ± 0.13
6	1.20 ± 0.25	1.05 ± 0.13
7	1.45 ± 0.54	1.18 ± 0.11
8	1.35 ± 0.36	1.45 ± 0.11
9	1.39 ± 0.19	1.90 ± 0.20
10	1.33 ± 0.23	1.09 ± 0.07
11	1.48 ± 0.48	1.43 ± 0.23
12	1.22 ± 0.24	1.19 ± 0.10

selectivity of the enantiomers of M1 is caused by different levels of protein binding and/or different rates of renal clearance of the enantiomers of M1, although the stereoselective *O*-demethylation of *trans*-T plays an important role. M1 is the main metabolite of *trans*-T in humans. It is interesting to note that the M1 production is carried out by the isoenzyme cytochrome CYP 2D6 and the polymorphism of this enzyme is the basis of a larger interindividual variability in the metabolism process of many drugs[7,10]. *Trans*-T appears to be a better analgesic in extensive metabolisers than in poor metabolisers who do not form the active *O*-demethylated metabolite, M1, due to the lack of P-450 2D6[10]. Since (+)-M1 binds to the μ-opioid receptor with much stronger affinity than (−)-M1, (+)-M1 might be a major source of the opioid component to the therapeutic efficacy of *trans*-T.

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Tab 4. Pharmacokinetic parameters of the enantiomers of *trans*-tramadol (*trans*-T) and *trans*-*O*-demethyltramadol (M1) in 12 healthy subjects after multiple doses of *trans*-T hydrochloride sustained-release tablets (100 mg/12 h, 11 times). $\bar{x} \pm s$. $^cP < 0.01$ *vs* (+) *trans*-T. $^fP < 0.01$ *vs* (+)-M1.

Parameters	(+)-*trans* T	(−)-*trans* T	(+)-M1	(−)-M1
T_{max}/h	4.2 ± 1.2	3.4 ± 1.0	3.8 ± 1.2	3.4 ± 1.0
C_{max}/μg·L^{-1}	164 ± 34	144 ± 28^c	18 ± 7	23 ± 6^f
C_{min}/μg·L^{-1}	86 ± 18	69 ± 16^c	10 ± 4	12 ± 4^f
$T_{1/2}$/h	7.8 ± 1.9	6.2 ± 1.6^c		
$AUC_{0-\tau}$/μg·L^{-1}·h			162 ± 65	179 ± 47
AUC_{0-t}/μg·L^{-1}·h	2306 ± 578	1839 ± 495^c		
$AUC_{0-\infty}$/μg·L^{-1}·h	2482 ± 642	1911 ± 534^c		
C_{av}/μg·L^{-1}	122 ± 28	102 ± 24^c	14 ± 5	15 ± 4

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人体内反式曲马朵及其活性代谢产物反式氧去甲基曲马朵对映体的药物动力学

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关键词 曲马朵；毛细管电泳；药物动力学

目的：研究反式曲马朵(*trans*-T)及其活性代谢产物反式氧去甲基曲马朵(M1)的人体药代动力学立体选择性. **方法**：12名健康男性受试者口服多剂量盐酸*trans*-T缓释片后，采用高效毛细管电泳(HPCE)法测定血清中 *trans*-T 及 M1 对映体的浓度. **结果**：血清中 *trans*-T 对映体浓度达稳态后，不同时间血清中(+)-*trans*-T 的浓度均高于(−)-*trans*-T 的浓度，两对映体除 T_{max} 以外的药代动力学参数的差异均有显著性. 在大多数受试者体内和大多数取血时间点，(−)-M1 的浓度高于(+)-M1 的浓度；在不同受试者体内，血清中 M1 对映体浓度的比值差别较大，两对映体的 C_{max} 和 C_{min} 差异有显著性. **结论**：*trans*-T 和 M1 具有药代动力学立体选择性. 人体对(+)-*trans*-T 比对(−)-*trans*-T 吸收完全，消除慢；在不同受试者体内，M1 的药代动力学立体选择性是不同的.

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