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Stereoselectivity in renal clearance of *trans*-tramadol and its active metabolite, *trans-O*-demethyltramadol

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KEY WORDS tramadol; kidney; perfusion; stereoisomerism; pharmacokinetics

ABSTRACT

AIM: To study the stereoselectivity in renal clearance of *trans*-tramadol and its active metabolite, *trans-O*-demethyltramadol. **METHODS**: The right kidneys were isolated from male SD rats and perfused with 100 mL of perfusate medium containing *trans*-tramadol 300 μg/L or *trans-O*-demethyltramadol 50 μg/L. After perfusion, the concentrations of the enantiomers of *trans*-tramadol and *trans-O*-demethyltramadol in the perfusate and urine were determined by high performance capillary electrophoresis. The enantiomeric ratios were calculated. **RESULTS**: After the kidneys being perfused with *trans*-tramadol hydrochloride, the concentration of (+)-*trans*-tramadol was higher than that of (−)-*trans*-tramadol, and the concentration of (+)-*trans-O*-demethyltramadol was lower than that of (−)-*trans-O*-demethyltramadol in the perfusate; meanwhile, (+)-*trans*-tramadol was more than (−)-*trans*-tramadol, and (+)-*trans-O*-demethyltramadol was less than (−)-*trans-O*-demethyltramadol in the urine. After the kidneys being perfused with *trans-O*-demethyltramadol, the concentration of (+)-*trans-O*-demethyltramadol was lower than that of (−)-*trans-O*-demethyltramadol in the perfusate, and (+)-*trans-O*-demethyltramadol was more than (−)-*trans-O*-demethyltramadol in the urine. **CONCLUSION**: The renal clearance of *trans*-tramadol was stereoselective. The *O*-demethylation of *trans*-tramadol was stereoselective in the kidneys, (−)-*trans*-tramadol being preferentially metabolized. The renal clearance of *trans-O*-demethyltramadol was also stereoselective, the (+)-enantiomer being preferentially cleared into the urine.

INTRODUCTION

Tramadol (T) has two chiral centers in its chemical structure. There are four isomers of T. The racemate of *trans*-T hydrochloride is used as a centrally acting analgesic[1]. (+)-*trans*-T and (−)-*trans*-T mainly inhibit the reuptake of serotonin and norepinephrine, respectively. (+)-*trans-O*-demethyltramadol [(+)-M1], the only one active metabolite of *trans*-T, has greater binding affinity to the μ-opioid receptor than do (−)-M1 and the enantiomers of *trans*-T. Thus *in vivo*, *trans*-T has a special dual model of antinoceptive action mechanism, which may contribute to its fewer adverse reactions and more desired efficiency[2,3].

In our previous paper, it was found that the serum concentration of (+)-*trans*-T was higher than that of (−)-*trans*-T in all tested subjects, and the serum concentration of (+)-M1 was lower than that of (−)-M1 in the most subjects after oral administration of *trans*-T hydrochloride[4]. While, Elsing *et al* reported that the urine concentration of (+)-*trans*-T was not higher than that of (−)-*trans*-T in two out of five subjects, and the urine concentration of (+)-M1 was higher than that of (−)-M1 in four out of five subjects after oral administration of *trans*-T hydrochloride[5]. In this paper, the renal clearance of the enantiomers of *trans*-T and M1 was investigated *in vitro*.

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, internal standard, was a gift from Chemical Department of Jinzhou Medicine College (China). Sulfobutylether-β-cyclodextrin, chiral selecter, was

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kindly provided by Lanzhou Institute of Chemical Physics, the Chinese Academy of Sciences. Other regents were of analytical or HPLC grade.

Perfusate medium The perfusate medium (100 mL) consisted of Krebs-Henseleit (K-H) buffer (pH 7.4) containing bovine serum albumin (BAS) 65 g/L, glycine 2.3 mmol/L, *L*-glutamic acid 0.5 mmol/L, and *L*-cystine 0.5 mmol/L. The K-H buffer has following comqosition: NaCl 119.0, KCl 4.3, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25.5, $CaCl_2$ 2.5, and glucose 11.0 mmol/L. Before use, BAS was dissolved in K-H buffer (130 g/L) and purified by dialyzing against three exchanges of protein-free buffer over 3 d at 4 ℃. Dialyzed albumin solution was stored frozen at −20 ℃. On the day of a perfusion experiment, the concentration of BSA in the perfusate was adjusted to 65 g/L, and the three amino acids were added. Perfusate was then filtered successively through 0.45-μm and 1.2-μm filters[6]. *trans*-T hydrochloride or M1 was dissolved in the perfusate. The concentration of *trans*-T and M1 were 300 μg/L and 50 μg/L respectively.

Isolated kidneys Sixteen Sprague-Dawley rats (♂, 395 ± s 45 g) were supplied by the Animal Center of Hebei Medical University (Grade Ⅱ, Certificate No 04057). The rats were anesthetized with ip injection of 2 % pentobarbital sodium solution (60 mg/kg). Abdominal cavity was opened to expose the major abdominal blood vessels. The right ureter was catheterized with a polyethylene tube for urine sampling. The cannula was manipulated from superior mesenteric artery, across the abdominal aorta, and finally into the right renal artery[6]. Perfusion flow was initiated as soon as the cannula entered right renal artery without interruption of renal flow. After cannulation of proximal mesenteric and renal artery, the kidney was rapidly excised from the body, carefully trimmed of adhering tissue, and perfused on a pump at a constant pressure of 13.3 kPa. The rate was about 35 mL/min. The perfusate was kept at 37 ℃ and saturated with 95 % O_2 + 5 % CO_2. The kidney was perfused with drug-free perfusate for 5 min, then perfused in cycles with the perfusate containing *trans*-T or M1. There were 8 rats in both *trans*-T group and M1 group.

Assay of the enantiomers of *trans*-T and M1 Ten minutes after the kidney being perfused with each drug, the perfusate samples were collected at 10, 30, 50, and 70 min, and the urine samples were collected over 20-min intervals for 80 min. The samples were stored at −24 ℃ until assay. The concentrations of the enantiomers of *trans*-T and M1 in perfusate or urine were simultaneously determined by the HPCE method described before[4]. Briefly, the electrophoretic experiments were performed in a P/ACE System 5000 automatic electrophoresis apparatus (Beckman, California, USA) equipped with an UV detector set at 214 nm. The background electrolyte (BGE) contained Tris 40 mmol/L (pH 2.5) and sulfobutylether-β-cyclodextrin 0.8 mmol/L. The perfusate and urine samples were extracted with ethyl acetate. The organic layer was removed into another tube. After evaporation to dryness under a gentle stream of nitrogen, the dry residue was redissolved in 100 μL of water and electrokinetically injected into a capillary column (37 cm × 75 μm). The separation was carried with a voltage of 15 kV at 25 ℃.

Data analysis The results were expressed as $x \pm s$. To compare the contents of the enantiomers of *trans*-T or M1 in the perfusate or urine, paired t test was used.

RESULTS

There was no interference from the perfusate and urine. For the enantiomers of both *trans*-T and M1, the correlation coefficient for linear regression was more than 0.99; the relative recovery was from 96.4 % to 103.2 %; the intra-day relative standard deviation (RSD) was less than 12.3 %; the inter-day RSD was less than 16.8 %; and the limit of detection was 1.25 μg/L.

After the kidneys being perfused with *trans*-T, the concentration of (+)-*trans*-T was higher than that of (−)-*trans*-T, and the concentration of (+)-M1 was lower than that of (−)-M1 in the perfusate (Tab 1). Meanwhile, (+)-*trans*-T was more than (−)-*trans*-T, and (+)-M1 was less than (−)-M1 in the urine (Tab 2).

After the kidneys being perfused with M1, the concentration of (+)-M1 was lower than that of (−)-M1 in the perfusate (Tab 3), and (+)-M1 was more than (−)-M1 in the urine (Tab 4).

DISCUSSION

The rat isolated perfused kidney is suitable for studying the renal handling of drugs, because tubular transport systems, in particular those involved in secretion, remain viable while making it possible to study

Tab 1. The concentrations of the enantiomers of *trans*-tramadol and *trans*-*O*-demethyltramadol (M1), and the (+)/(−)-enantiomeric ratios in the perfusate after the isolated rat kidneys being perfused with *trans*-T. $n = 8$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* (+)-enantiomer.

Time/min	(+)-*trans*-T/ $\mu g \cdot L^{-1}$	(−)-*trans*-T/ $\mu g \cdot L^{-1}$	(+)/(−)-*trans*-T ratio	(+)-M1/ $\mu g \cdot L^{-1}$	(−)-M1/ $\mu g \cdot L^{-1}$	(+)/(−)-M1 ratio
20	124 ± 26	106 ± 26[c]	1.19 ± 0.12	10 ± 2	54 ± 18[c]	0.20 ± 0.15
40	111 ± 20	93 ± 21[c]	1.23 ± 0.23	9 ± 5	32 ± 21[c]	0.29 ± 0.24
60	106 ± 22	84 ± 24[c]	1.38 ± 0.23	22 ± 21	51 ± 28[c]	0.35 ± 0.21
80	92 ± 21	72 ± 20[c]	1.32 ± 0.21	11 ± 4	37 ± 15[c]	0.26 ± 0.11

Tab 2. The amount of the enantiomers of *trans*-tramadol (*trans*-T) and *trans*-*O*-demethyltramadol (M1) and the (+)/(−)-enantiomeric ratios in the urine after the isolated rat kidneys being perfused with *trans*-T. $n = 8$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* (+)-enantiomer.

Time/min	(+)-*trans*-T/ ng	(−)-*trans*-T/ ng	(+)/(−)-*trans*-T ratio	(+)-M1/ ng	(−)-M1/ ng	(+)/(−)-M1 ratio
11 − 30	897 ± 301	797 ± 310[b]	1.17 ± 0.11	101 ± 52	246 ± 92[c]	0.50 ± 0.25
31 − 50	902 ± 280	728 ± 253[c]	1.22 ± 0.05	122 ± 82	238 ± 81[c]	0.53 ± 0.27
51 − 70	625 ± 163	452 ± 99[c]	1.37 ± 0.15	144 ± 73	252 ± 83[c]	0.47 ± 0.31
71 − 90	517 ± 101	357 ± 34[c]	1.34 ± 0.10	123 ± 68	234 ± 72[c]	0.52 ± 0.32

Tab 3. The concentrations of the enantiomers of *trans*-*O*-demethyltramadol (M1) and the (+)/(−)-enantiomeric ratios in the perfusate after the isolated rat kidneys being perfused with M1. $n = 8$. $\bar{x} \pm s$. $^bP < 0.05$ *vs* (+)-M1.

Time/ min	(+)-M1/ $\mu g \cdot L^{-1}$	(−)-M1/ $\mu g \cdot L^{-1}$	(+)/(−)-M1 ratio
20	21 ± 5	26 ± 4[b]	0.86 ± 0.15
40	20 ± 11	26 ± 10[b]	0.85 ± 0.14
60	19 ± 4	23 ± 8[b]	0.82 ± 0.14
80	16 ± 5	20 ± 4[b]	0.81 ± 0.17

Tab 4. The amount of the enantiomers of *trans*-*O*-demethyltramadol (M1) and the (+)/(−)-enantiomeric ratios in the urine after the isolated rat kidneys being perfused with M1. $n = 8$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* (+)-M1.

Period/ min	(+)-M1/ ng	(−)-M1/ ng	(+)/(−)-M1 ratio
11 − 30	337 ± 27	235 ± 44[c]	1.47 ± 0.22
31 − 50	239 ± 34	181 ± 47[c]	1.36 ± 0.22
51 − 70	163 ± 35	118 ± 23[c]	1.28 ± 0.20
71 − 90	106 ± 8	83 ± 14[c]	1.32 ± 0.25

renal disposition in the absence of confounding factors such as extrarenal metabolism[6]. In this experiment, the functional viability and performance of kidneys were assessed by measurement of perfusion pressure, perfusate pH, urine pH, and content of glucose, sodium, and *N*-acetyl-β-*D*-glucosaminidase (NAG) in the urine.

After the kidneys being perfused with *trans*-T, the concentration of (+)-*trans*-T was higher than that of (−)-*trans*-T in the perfusate. It indicated that the renal clearance of *trans*-T was stereoselective, and (−)-*trans*-T could be preferentially eliminated. *O*-demethylation of *trans*-T was catalyzed by the cytochrome CYP2D6 in kidney, which could preferentially metabolize (−)-*trans*-T and cause larger production of (−)-M1[7,8]. Since the (+)/(−)-enantiomeric ratio of *trans*-T in the perfusate was much similar to that in the urine, there was no significant difference in the urinary clearance between the enantiomers of *trans*-T. Therefore, the preferential *O*-demethylation of (−)-*trans*-T, which causing higher concentration of (−)-M1 in the perfusate, might play an important role in the stereoselective renal clearance of *trans*-T.

After the kidneys being perfused with M1, the concentration of (+)-M1 was lower than that of (−)-M1 in the perfusate. It indicated that the renal clearance

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of M1 was stereoselective, and (+)-M1 could be preferentially cleared. Since the (+)/(−)-enantiomeric ratio of M1 in the perfusate was contrary to that in the urine, the stereoselectivity in renal clearance of M1 might be mainly caused by the preferential urinary clearance of (+)-M1. This might be the reason that (+)- M1 was usually more than (−)-M1 in the urine after administration of *trans*-T. It was needed to study whether the renal clearance mechanism of the enantiomers of M1 was due to active secretion, active reabsorption, or carrier-mediated reabsorption.

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反式曲马朵及其活性代谢产物反式氧去甲基曲马朵肾脏清除的立体选择性

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关键词 曲马朵; 肾; 灌流; 立体异构; 药物动力学

目的: 研究反式曲马朵及其活性代谢物反式氧去甲基曲马朵肾脏清除的立体选择性. **方法**: 取雄性SD大鼠的右肾, 分别以含反式曲马朵(300 μg/L)或反式氧去甲基曲马朵(50 μg/L)的灌流液(100 mL)进行灌流; 利用高效毛细管电泳法测定灌流后灌流液、尿液中反式曲马朵及反式氧去甲基曲马朵对映体的浓度, 并计算对映体浓度比值. **结果**: 以反式曲马朵进行离体肾灌流后, 在灌流液中, (+)-反式曲马朵的浓度高于(−)-反式曲马朵的浓度, (+)-反式氧去甲基曲马朵的浓度低于(−)-反式氧去甲基曲马朵的浓度; 在尿液中, (+)-反式曲马朵较(−)-反式曲马朵多, (+)-反式氧去甲基曲马朵较(−)-反式氧去甲基曲马朵少. 以反式氧去甲基曲马朵灌流后, 在灌流液中(+)-反式氧去甲基曲马朵的浓度低于(−)-反式氧去甲基曲马朵的浓度; 在尿液中(+)-反式氧去甲基曲马朵较(−)-反式氧去甲基曲马朵高. **结论**: 反式曲马朵及反式氧去甲基曲马朵的肾脏清除具有立体选择性. 在肾脏中反式曲马朵的氧去甲基代谢具有立体选择性, 以(−)-反式曲马朵优先代谢成(−)-反式氧去甲基曲马朵. 反式氧去甲基曲马朵的尿排泄具有立体选择性, 以(+)-反式氧去甲基曲马朵占优.

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