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Pharmacokinetics of atenolol enantiomers in 12 Chinese healthy men[1]

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KEY WORDS atenolol; stereoisomerism; pharmacokinetics; high pressure liquid chromatography; Mongoloid race

ABSTRACT

AIM: To study the pharmacokinetics of atenolol (Ate) stereoisomers in Chinese. **METHOD**: A single oral dose of 100 mg of racemic Ate tablets were given to 12 healthy volunteers of Han nationality. Plasma and urine concentrations were determined by the reversed phase HPLC method. **RESULTS**: The disposition of *d*-Ate and *l*-Ate was conformed to one-compartment model. Maximal plasma concentration (C_{max}): *l*-Ate (331 ± 79) $\mu g \cdot L^{-1}$, *d*-Ate (342 ± 78) $\mu g \cdot L^{-1}$. Area under blood concentration-time curve (AUC): *d*-Ate (2635 ± 610) $\mu g \cdot h \cdot L^{-1}$, *l*-Ate (2442 ± 588) $\mu g \cdot h \cdot L^{-1}$. Renal clearance ($Cl_r$): *l*-Ate (6.9 ± 1.2) $L \cdot h^{-1}$, *d*-Ate (6.5 ± 1.3) $L \cdot h^{-1}$. **CONCLUSION**: The disposition of Ate stereoisomers is of stereoselectivity.

INTRODUCTION

Atenolol (Ate) is a cardioselective β_1-adrenoceptor antagonist used for the treatment of hypertension, angina pectoris, and cardiac arrhythmias[1]. Ate is a raceme with 2 stereoisomers: *d*-Ate and *l*-Ate, the pharmacologic activity of the racemic Ate resides mainly in the *l*-enantiomer. The enantiomers of many drugs exhibited obviously differences in pharmacokinetics, pharmacodynamics, toxicology, and adverse reaction[2,3]. If a drug used clinically as a racemic mixture, its pharmacokinetics and pharmacodynamics must have been considered to be stereoselective. In this study we examined the pharmacokinetics of Ate enantiomers in Chinese.

MATERIALS AND METHODS

Drugs and reagents Racemic Ate tablet (50 mg/tablet), Henan Zhulin-Ante Pharmaceutical Co (lot № 940401). *l*-Ate standard ($[\alpha]^{25}$ − 16°), purity 99 % (lot № 33089-2), and *d*-Ate standard ($[\alpha]^{25}$ +16°), purity 99 % (lot № 33088-4), were purchased from Aldrich Chem Co. Internal standard (methoxamine HCl), purity > 99 %, was purchased from Sigma Co. Homochiral derivatizing reagent (*d*-1-phenylethylisocyanate, PEIC), purity > 99.5 %, was purchased from Fluka Co. Racemic Ate standard, purity 99 %, was provided by Tianjin Central Pharmaceutical Factory.

HPLC-grade methanol was purchased from Guangzhou Donghong Chemical Plants (AR, lot № 950701). All other chemical reagents were of AR. Water was distilled.

Chromatography The plasma and urine concentrations of Ate stereoisomers were determined as recently described[4]. In brief, the HPLC system (Hewlett Packard, USA) consisted of an HP ChemStation, an HP 1050 series pumping system, an HP 1046A fluorescence detector. The analytical column is an ODS Hypersil (250 mm × 4 mm, 5 μm). The mobile phase was composed of water-methanol-isopropanol-dichloromethane (vol = 53.2 : 41.6 : 4.8 : 0.4) at a flow rate of 1.0 $mL \cdot min^{-1}$. Fluorescent detector was set at λ_{ex} 235 nm and λ_{em} 300 nm.

Subjects In accordance with the declaration of Helsinki, 12 healthy male volunteers of Han nationality

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with free consent entered the study. They were aged ($29 \pm s$ 4) a, weighed ($65 \pm s$ 9) kg, and all the test results of their blood, urine, liver, kidneys, and electrocardiogram were normal. The volunteers abstained from any other drugs including alcohol, tobacco for 2 wk before the study and during the study days.

Study design The subjects, after a 12-h fasting, were given a single oral dose of racemic Ate tablets (100 mg) with 200 mL water. A uniform diet was supplied after 2 h and water 100 mL was asked to be drunk every 3 h.

Plasma and urine sampling Blood samples were collected into the heparinized glass tubes at 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, 24 h after the medication. The samples were centrifuged at $2000 \times g$ for 20 min to get plasma. The urine was collected at 0 – 3, 3 – 6, 6 – 9, 9 – 12, 12 – 24 h. Plasma samples (0.5 mL) and urine samples (0.2 mL) diluted 5 times with water were added with methoxamine HCl (100 mg·L^{-1}) 8 μL, then were extracted with ethyl acetate and isopropanol after alkalization with NaOH. The organic layer was evaporated to dryness under a nitrogen stream at 40 ℃, the residues were added 2 μL ammonia and 0.25 % PEIC chloroform solution 0.2 mL, stood at 4 ℃ for 15 min, then dried under a nitrogen stream at 25 ℃. Water 1 mL and chloroform 2 mL were added to the samples, and the remaining organic layer was evaporated under N_2 at 40 ℃. The residue was dissolved in 60 μL of the mobile phase. Aliquots of 20 μL of the supernatant were injected.

Pharmacokinetic analysis The plasma concentration-time curves were analyzed with MCPKP program (XIA Wen-Jiang *et al*, Institute of Traditional Chinese Veterinary Medicine) on a COMPAQ-486 personal computer to determine the compartment model and the phamacokinetic parameters. The maximal plasma concentration (C_{max}) and the time after dosing at which maximal plasma concentration was reached (T_{max}) were the observed actual values. The area under blood concentration-time curve ($AUC_{0-\infty}$) was determined by a statistical moment model. The urine pharmacokinetic parameters were calculated by:

K_e (elimination constant):

$$\lg \frac{\Delta X_u}{\Delta t} = -\frac{Kt_c}{2.303} + \lg \frac{K_e K_a F X_0}{K_a - K},$$

X_u^∞ (Total Excretion) $= X_u + (\Delta X_u/\Delta t) \cdot t/K$,

X_u^∞ % (Excretion Rate) $= (X_u^\infty / X_0) \times 100$ %,

Cl_r (Renal Clearance) $= X_u^\infty /$AUC.

All data were analyzed with paired t test.

RESULTS

Chromatography The baseline resolution of the diastereomers of Ate was obtained in plasma, with retention time of 9.3 min for *l*-Ate and 10.2 min for *d*-Ate. The internal standard was a racemic drug resolved into 2 peaks, and retention time were 13.0 and 13.7 min, respectively. The retention time of *l*-Ate, *d*-Ate, and methoxamine in urine were 9.7 and 10.6 min, 13.5 and 14.1 min, respectively. The peaks were sharp, well-seperated, and not interfered by the plasma or urine (Fig 1).

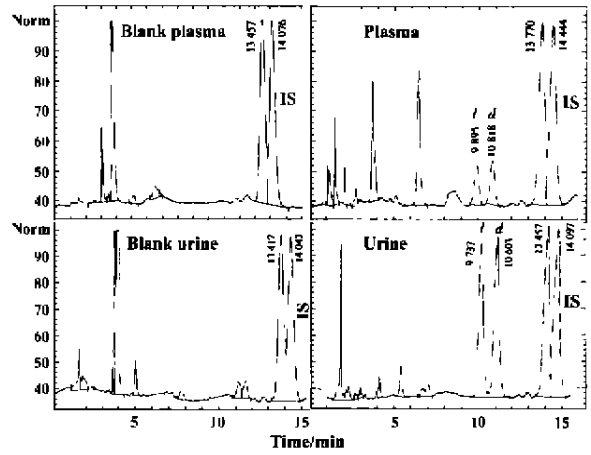

Fig 1. HPLC collected from a healthy man after *po* 100 mg of racemic Ate tablets.

Accuracy and reproducibility The standard curves for the enantiomers were linear over the range of 10 – 1000 μg·L^{-1} in plasma (*l*-Ate: $r = 0.9915$, *d*-Ate: $r = 0.9937$) and 25 – 1500 μg·L^{-1} in urine (*l*-Ate: $r = 0.9952$, *d*-Ate: $r = 0.9963$). The minimal absolute recoveries of *l*-Ate and *d*-Ate were 85.7 % and 86.3 %, respectively in plasma, and 84.8 % and 85.5 %, respectively in urine. The inter-day RSD of *l*-Ate and *d*-Ate were < 5.1 % and 4.7 %, respectively in plasma, and were < 2.4 % and 2.0 %, respectively in urine. The intra-day RSD of *l*-Ate and *d*-Ate were < 4.3 % and 3.8 %, respectively in plasma, and were < 2.0 % and 1.9 %, respectively in urine.

Pharmacokinetics The plasma Ate enantiomers concentration-time curves were fitted to a first

order absorption 1-compartment open model (Fig 2).

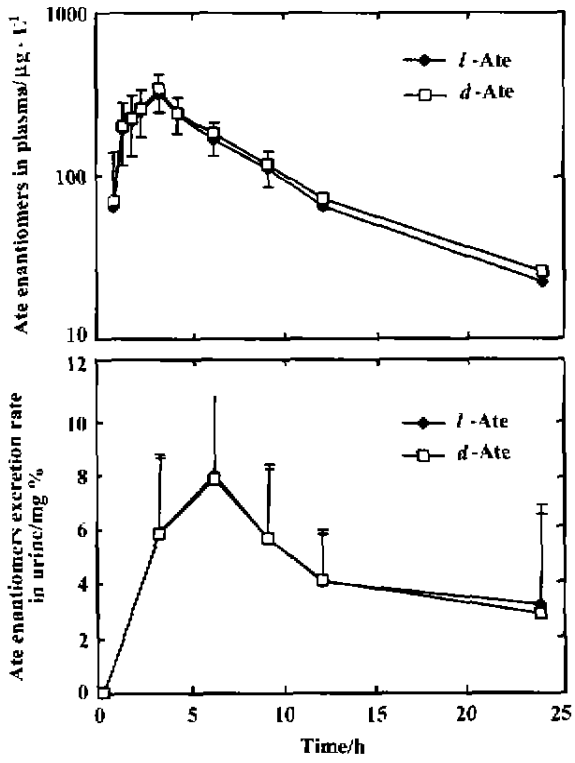

Fig 2. Atenolol enantiomers in plasma and urine after *po* 100 mg of racemic atenolol tablets in 12 Chinese healthy volunteers.

Fig 2 also showed the urine Ate enantiomers excretion rate-time relationship.

Tab 1 summarized all the pharmacokinetic data of the Ate tablets in plasma and in urine on 12 men. The plasma concentration of *d*-Ate was higher than that of *l*-Ate, the C_{max} of *d*-Ate was greater than that of *l*-Ate ($P < 0.05$), and the AUC of *d*-Ate was 1.08 times greater than that of *l*-Ate ($P < 0.05$). But the renal clearance (Cl_r) of *l*-Ate was 5.9 % higher than that of *d*-Ate ($P < 0.05$). The elimination rate constant (K and K_e) of Ate enantiomers in plasma and in urine showed no significant difference ($P > 0.05$).

Tab 1. Pharmacokinetic parameters of *l*-Ate and *d*-Ate in plasma and in urine after a single oral dose 100 mg racemic Ate. $n = 12$, $\bar{x} \pm s$.
$^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* *l*-Ate.

Parameters	*l*-Ate	*d*-Ate
In plasma		
K_a, h^{-1}	1.5 ± 0.8	1.5 ± 0.7[a]
T_{lag}, h	0.21 ± 0.20	0.24 ± 0.22[a]
K, h^{-1}	0.122 ± 0.014	0.116 ± 0.013[a]
$T_{\frac{1}{2}Ka}$, h	0.57 ± 0.27	0.59 ± 0.29[a]
$T_{\frac{1}{2}K}$, h	5.8 ± 0.7	6.0 ± 0.7[a]
T_{max}, h	2.0 ± 0.7	2.1 ± 0.7[a]
C_{max}, $\mu g \cdot L^{-1}$	331 ± 79	342 ± 78[b]
AUC, $\mu g \cdot h \cdot L^{-1}$	2 442 ± 588	2 635 ± 610[c]
V_d/F, $L \cdot kg^{-1}$	5.8 ± 2.0	5.4 ± 1.8[c]
Cl_{oral}, $L \cdot h^{-1}$	0.68 ± 0.14	0.63 ± 0.10[c]
AUC_{0-t}, $\mu g \cdot h \cdot L^{-1}$	2 255 ± 620	2 411 ± 625[c]
MRT, h	8.7 ± 0.7	8.7 ± 0.6[a]
MAT, h	0.9 ± 0.6	0.9 ± 0.6[a]
In urine		
K_e, h^{-1}	0.14 ± 0.07	0.14 ± 0.09[a]
$T_{\frac{1}{2}Ke}$, h	6.4 ± 3.0	7.0 ± 4.2[a]
Cl_r, $L \cdot h^{-1}$	6.9 ± 1.2	6.5 ± 1.3[b]
X_u^∞, mg	16 ± 6	18 ± 7[b]
X_u^∞, %	33 ± 11	35 ± 15[b]

DISCUSSION

β-Adrenoceptor blocking agents enantiomers show marked stereoselective pharmacokinetics and pharmacodynamics. The affinity of *d*-Ate is 10 times lower than that of *l*-Ate[5,6], it will be important clinical significance to study the difference in pharmacokinetics of Ate enantiomers.

The results of our studies indicate that there were significant stereoselective differences in the C_{max} and AUC of Ate enantiomers. The reasons of the stereoselective differences in C_{max} and AUC are not clear[7]. The difference in C_{max} of the enantiomers could be explained by a difference in dose or bioavailability and by a difference in rate of elimination, rate of absorption, or volume of distribution. The difference in AUC can also be explained by differences in renal clearance, dose, or bioavailability of the enantiomers of Ate. In our study healthy volunteers were given a single oral dose of racemic Ate and the Ate enantiomers is equivalent. Ate is a hydrophilic agent, and only negligibly bound to plasma proteins, apparent volume of distribution (V_d) is low, therefore V_d may not be responsible for the differences of AT enantiomers. Analysing the pharmacokinetics of Ate, we consider that oral bioavailability and renal clearance of the Ate may be contributed to the stereoselective differences.

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The possible causes of stereoselective bioavailability include: 1) stereoselective first-pass hepatic metabolism, 2) stereoselective bacterial metabolism in the intestine, 3) stereoselective billiary secretion followed by reabsorption, 4) stereoselective active transport of Ate in the intestinal mucosal membrane. To analyze the characteristics of this drug, the suitable explanation for stereoselective bioavailability is that there may be a stereoselective active absorptive mechanism for Ate in the gastrointestinal tract. Because of the hydrophilic nature of Ate and its high pK_a (9.55), it would be expected to cross cellular membranes with difficulty. Therefore it is possible that Ate is absorbed through the intestinal mucosal membrane by a carrier-mediated process that is stereoselective.

The second reason is the renal clearance of Ate being of stereoselective. The route of renal excretion is glomerular filtration, active metabolism or transport. Glomerular filtration is passive, it is impossible that there is significant difference in glomerular filtration rate (GFR) of two Ate enantiomers, but stereoslective renal elimination may be possible if Ate is excreted by tubular secretion or tubular reabsorption. Our results demonstrate that the renal clearance of *l*-Ate is 5.9 % higher than that of *d*-Ate ($P < 0.05$), the C_{max} of *l*-Ate is lower than that of *d*-Ate. So the stereoselective differences in renal clearance of Ate enantiomers may share the reasons of lower plasma level of *l*-Ate.

REFERENCES

1 Frishman WH. Drug therapy: atenolol and timolol, two new systemic β-adrenoceptor antagonists. N Engl J Med 1982; 306: 1456-62.
2 Ariens EJ. Nonchiral, homochiral and composite chiral drugs. Trends Pharmacol Sci 1993; 14: 68-75.
3 Nation RL. Chirality in new drug development. Clinical pharmacokinetic considerations. Clin Pharmacokinet 1994; 27: 249-55.
4 Wang XM, Yu XY, Lin SG. Study on precolumn derivatization HPLC assay method of atenolol enantiomers in plasma and urine in man. Chin Pharm J 1997; 32 Suppl: 1-3.
5 Walle T, Webb JG, Bagwell EE, Walle UK, Daniell HB, Gaffney TE. Stereoselective delivery and actions of beta receptor antagonists. Biochem Pharmacol 1988; 37: 115-24.
6 Karim A. Enantioselective assays in comparative bioavailability studies of racemic drug formulations: nice to know or need to know? J Clin Pharmacol 1996; 36: 490-9.
7 Stoschitzky K, Egginger G, Zernig G. Stereoselective features of (R)- and (S)-atenolol: clinical pharmacological, pharmacokinetic, and radioligand binding studies. Chirality 1993; 5: 15-9.

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阿替洛尔对映体在12名中国健康人体内的药物动力学[1]

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关键词 阿替洛尔; 立体异构; 药物动力学; 高压液相色谱法; 蒙古人种

目的: 研究阿替洛尔对映体在中国人体内的药物动力学. **方法**: 12名男性汉族健康志愿者单剂量口服阿替洛尔片剂100 mg后, 用高效液相-荧光法测定血浆和尿液药物浓度. **结果**: 血药浓度-时间曲线拟合表明阿替洛尔对映体的体内过程符合口服一室开放模型, *l*-Ate和*d*-Ate的主要药代动力学参数: C_{max} (331 ± 79) and (342 ± 78) $\mu g \cdot L^{-1}$; AUC (2442 ± 588) and (2635 ± 610) $\mu g \cdot h \cdot L^{-1}$; Cl_r: (6.9 ± 1.2) and (6.5 ± 1.3) $L \cdot h^{-1}$. **结论**: 阿替洛尔对映体的药代动力学过程具有明显的立体选择性差异.

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