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Stereoselective metabolism of metoprolol in isolated rat liver[1]

CHEN Xiao-Xiang, ZHANG Yin-Di[2], SHEN Jian-Ping
(*Institute of Clinical Pharmacology, Nanjing Medical University, Nanjing 210029, China*)

KEY WORDS metoprolol; liver; pharmacokinetics; stereoisomerism

AIM: To study the stereoselective pharmacokinetics of the enantiomers of metoprolol (Met). **METHODS:** The enantiomers of Met were metabolized in isolated rat liver with hemoglubin-free medium. The enantiomers of Met were analyzed with HPLC. **RESULTS:** The linear kinetics were found in 3 doses (0.16, 0.32, and 0.64 mg) of *R*-(+)- and *S*-(−)-Met. The time-concentration curve of Met was fitted in first order-kinetics compartment model. There were differences in the pharmacokinetic parameters (K, $T_{1/2}$, and Cl) between the enantiomers at the same dose ($P < 0.01$). The ratio of liver clearence of *S*-(−)-Met/*R*-(+)-Met was 0.14 − 0.17. **CONCLUSION:** Pharmacokinetics of enantiomers of Met in the isolated rat liver were stereoselective, with a preferential clearance of *R*-(+)-Met, which elimination was not a saturable process.

Metoprolol (Met) is a lipid-soluble β adrenoceptor blocker, mainly metabolized in the liver[1]. In rat, about 95 % of the oral dose was eliminated during the first pass through the liver[2]. Met is used as a racemic mixture, *R*-(+)- and *S*-(−)-Met. Most of β-blocking activity resides in the *S*-(−)-enantiomers[3]. The stereoselective metabolism of Met was found in human and rat liver[4,5], but the differences in the kinetics of the 2 isomers by studying on physiologic model have not been reported. In the present study, the pharmacokinetics of two enantiomers were studied to test the stereoselective metabolism of Met.

MATERIALS AND METHODS

Reagents *R*-(+)- and *S*-(−)-Met tartrate (powder, purity >99 %, mp *S*-(−)-Met 94 − 95 ℃, *R*-(+)-Met 93 − 94 ℃) were kindly provided by Prof M REIDENBERG of Cornell University of USA. Dextran was purchased from Shanghai Institute of Biochemistry. Tetrahydrofuran was purchased from Yixin Chemical Plants. Tetrahydrofuran was treated with KOH pellets overnight to remove water. The KOH was removed by filtration and the tetrahydrofuran was purified by distillation. Other reagents used were AR.

Liver perfusion Sprague-Dawley rats of either sex ($n = 36$, Jiangsu Animal Quility № was 950005) weighing 200 − 250 g were fasted for 12 h before liver perfusion. The livers were perfused in a recirculating system with an inlet catheter advanced into the portal vein and an outlet catheter into the superior vena cava[6]. The bile duct was cannulated. The perfusion medium consisted of K-H buffer 130 mL, including 0.2 % glucose, 1.5 % dextran and 0.005 % benzylpenicillin. The medium was oxygenated by perfusing it through a thin-wall tube that passed 95 % O_2 + 5 % CO_2 at a rate of 2 − 3 L·min^{-1}. The pH was maintained at 7.35 − 7.45. The liver was perfused 30 mL·min^{-1} at 37 ℃ using a recirculating system (Ambec model 1000, USA). After *R*-(+)- or *S*-(−)-Met was added into the reservior after 30 min perfusion, the reservoir was sampled 0.5 mL each at various times and 0.5 mL of medium was replenished.

Analysis of *R*-(+)-Met and *S*-(−)-Met Sample 0.5 mL was extracted with 2.0 mL acetic acid after alkalization with 0.05 mL NaOH 2 mmol·L^{-1}. The organic layer was evaporated at 60 − 70 ℃ in a stream of N_2. The residue was dissolved with 0.5 mL mobile phase and then assayed by Shimadzu LC-4A HPLC system with RF-530 fluorescence spectromonitor, λ_{em} 302 nm, λ_{ex} 284 nm[7]. The mobile phase comprised of 80 % water and 20 % tetrahydrofuran. Flow rate was 0.4 mL·min^{-1}. Absolute recoveries were 98 % + 3 %, the

[1] Project supported by the National Natural Science Foundation of China, № 38970853.
[2] Correspondence to Prof ZHANG Yin-Di. Phn 86-25-661-9141, ext 462. Fax 86-25-650-8960.
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coefficients of intra- and inter-assay were within 5 % and 6 %, respectively.

Pharmacokinetics analysis To assess whether it possessed linear kinetics properties at 0.16 – 0.64 mg, the principle of superposition was used[8]. The concentration-time courses were analyzed with CAPP program (LUO Jian-Ping *et al*, Department of Mathematics, Nanjing Medical Uuiversity). Statistic analysis was carried out using *t*-test.

RESULTS

Chromatograph of *R*-(+)-and *S*-(–)-Met The retention times of *R*-(+)- and *S*-(–)-Met were 9.16 and 9.15 min, respectively. Their metabolites were well-seperated with *R*-(+)- and *S*-(–)-Met (Fig 1).

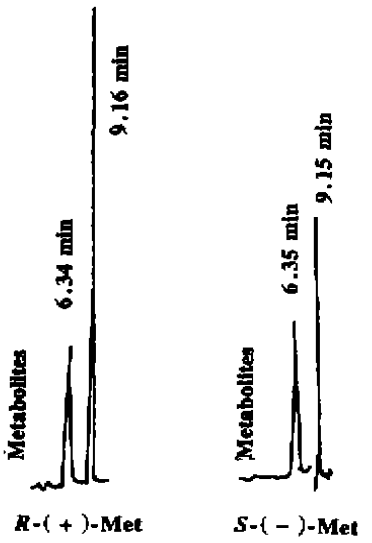

Fig 1. HPLC of *S*-(–)- and *R*-(+)-metoprolol (0.32 mg) after perfusion for 20 min.

Pharmacokinetics The time course of *R*-(+)-Met and *S*-(–)-Met concentration of 3 doses in the perfusing fluid reservoir was illustrated (Fig 2).

Linear kinetics were found with *R*-(+)- and *S*-(–)-Met 0.16, 0.32, and 0.64 mg (Fig 3).

The concentration was fitted in first order-kinetics according to one-compartment model. The regression coefficients were all >0.99 ($P<0.01$). For 0.16 mg *S*-(–)-Met and *R*-(–)-Met, $T_{1/2}$ were (24.4 ± 2.9) and (3.6 ± 1.4) min;

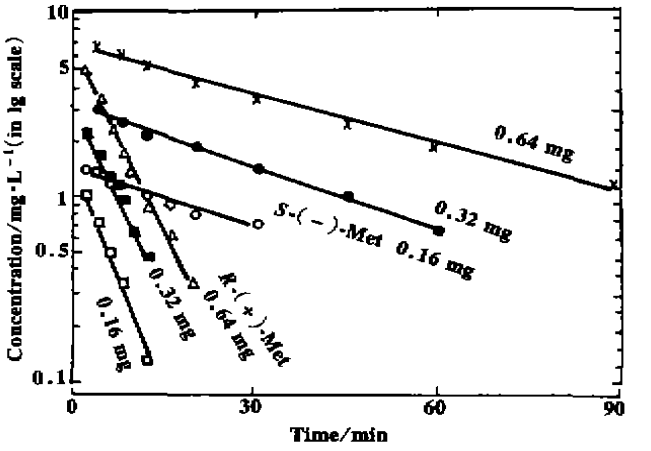

Fig 2. Metoprolol in perfusate in isolated rat liver.

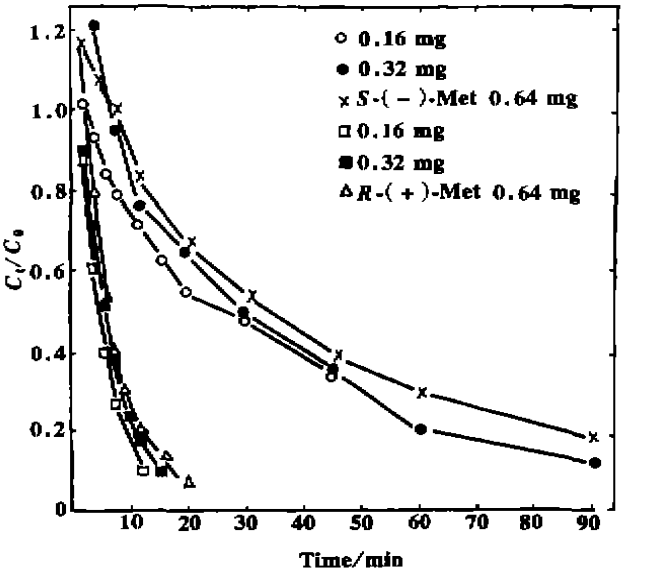

Fig 3. Metoprolol concentrations in isolated rat livers.

Cl were (4.5 ± 0.5) and (30 ± 8) mL·L^{-1}, respectively, and no significant difference was found between the 3 doses ($P > 0.05$). Significant differences were found between the 2 enantiomers of the same dose, the *S*-(–)-/*R*-(+)-Met ratio of $T_{1/2}$ and *Cl* were 6.09 – 7.11, 0.14 – 0.17, respectively (Tab 1).

DISCUSSION

Metoprolol is a lipid-soluble β adrenoceptor antagonist. Single oral dose of metoprolol have been shown in one study in the human to undergo stereoselective oral clearence, producing the plasma concentration with a *S*-(–)-/*R*-(+)-

Tab 1. Pharmacokinetic parameters of *S*-(−)-Met and *R*-(+)-Met 0.16, 0.32, 0.64 mg in isolated rat liver. $n = 6$ rats, $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* *R*-(+)-Met.

Parameters	Dose/mg	*R*-(+)-Met	*S*-(−)-Met	*S*-(−)-/*R*-(+)-Met
V_d/mL	0.16	148 ± 30	156 ± 17[a]	1.05
	0.32	131 ± 34	140 ± 17[a]	1.07
	0.64	144 ± 13	129 ± 29[a]	0.90
$K \cdot 10^{-3}$/min^{-1}	0.16	209 ± 58	29 ± 4[c]	0.14
	0.32	186 ± 60	28 ± 7[c]	0.15
	0.64	156 ± 11	24 ± 10[c]	0.15
$T_{1/2}$/min	0.16	3.6 ± 1.4	24.4 ± 2.9[c]	6.78
	0.32	4.3 ± 2.1	26.2 ± 7.0[c]	6.09
	0.64	4.5 ± 0.4	32.0 ± 9.1[c]	7.11
Cl/mL·min^{-1}	0.16	30 ± 8	4.5 ± 0.5[c]	0.15
	0.32	23 ± 6	3.8 ± 0.9[c]	0.17
	0.64	22 ± 2.1	3.0 ± 1.0[c]	0.14

enantiomers ratio of 1.37[9]. This enantioselectivity have proposed to be due to preferential *O*-demethylation of the *R*-(+)-metoprolol, a major pathway, α-demethylation of the metoprolol, a minor pathway, is reported selectively for *S*-(−)-enantiomer, and should have the opposite effects on the clearence of the enatiomers of metoprolol[9]. In our study, linear kinetics were found of the dose of *S*-(−)-Met or *R*-(+)-Met 0.16, 0.32, and 0.64 mg with the principle of superposition, which was in good agreement with the previous report by using racemic Met[10]. The *S*-(−)-Met/*R*-(+)-Met ratio of the liver clearence between the three doses was 0.14 − 0.17. The isolated rat liver had the greater ability to eliminate *R*-(+)-Met than *S*-(−)-Met. The high stereoselectivity between the metabolism of the enantiomers implied a preferential elimination of *R*-(+)-Met. Further investigation with liver cytochrome P_{450} is needed to find the mechanism of the pharmacokinetic stereoselectivity between the two enantiomers.

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美托洛尔在离体大鼠肝上的立体选择性代谢[1]

陈小祥，张银娣[2]，沈建平
（南京医科大学临床药理研究所，南京 210029，中国）

关键词 美托洛尔；肝；药物动力学；光学异构

目的：研究美托洛尔光学异构体在离体大鼠肝灌流上的药物动力学的立体选择性差异。**方法**：美托洛尔光学异构体在离体大鼠肝灌流中作循环式灌流，用高效液相色谱法进行定量分析。**结果**：0.16，0.32 和 0.64 mg 三种不同剂量的美托洛尔光学异构体在离体大鼠肝脏中消除过程，经叠加原则认为不存在饱和机制，符合单室模型一级动力学过程，在三种剂量下，两种异构体间的动力学参数 $T_{1/2}$，K 和 Cl 均存在着显著性差异（$P < 0.01$），其 *S*-(−)-Met/*R*-(+)-Met 的 Cl 比值为 0.14 − 0.17。**结论**：离体大鼠肝脏对美托洛尔两种光学异构体的代谢具有立体选择性，其肝消除符合线性动力学过程。