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Effect of dose of metoprolol on its elimination by isolated perfused rat liver *in vitro*[1]

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ABSTRACT The effects of dose of metoprolol (Met) on hepatic elimination was studied in isolated rat liver perfused at a flow of 25 ml·min^{-1}. The results showed that Met was eliminated by rat liver in accordance with one-compartment model. Linear kinetic eliminating processes (apparent first-order kinetics) were found in doses of Met 0.2, 0.5, 1.0, and 2.0 mg, $T_{\frac{1}{2}}$ were 8.3, 8.8, 9.6, and 10.6 min and the clearance rate were 11.7, 11.8, 9.6, and 8.6 ml·min^{-1}, respectively. Nonlinear eliminating processes were found in doses of Met 4, 8, and 12 mg. V_m and K_m were 0.98, 1.05, and 0.94 μg·min^{-1}·ml^{-1} and 15.6, 16.9, and 14.6 μg·ml^{-1}, respectively. It is concluded that hepatic Met elimination is independent on lower doses, but rested upon high doses.

KEY WORDS metoprolol; liver; perfusion; pharmacokinetics

Metoprolol (Met) is a selective β_1-adrenoceptor blocker, mainly metabolized in the liver[1]. Its pharmacokinetic properties have been studied extensively[2], but its model of elimination in isolated perfused liver was unknown. The present investigation dealt with the elimination and pharmacokinetic parameters of Met in a recirculating isolated perfused rat liver system.

MATERIALS AND METHODS

Reagents Met (Sigma), dextran and bovine serum albumin powder (Shanghai Institute of Biochemistry). Other reagents were all in AR grade.

Liver perfusion Sprague-Dawley rats ($n=38$) of either sex weighing 240±s 30 g were fasted for 12 h before liver perfusion. Anesthesia was induced by ip 20% urethane (1.0 g·kg^{-1}) and the abdomen was opened. The isolated liver system was set up according to the standard technique[3]. 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. The viability of the liver was monitored by bile flow. The perfusion was carried out at 37 ℃ in a thermostatical cabinet using a recirculating system. The inferior vena cava was ligated. The whole procedure was accomplished within 10 min. The perfusion medium consisted of 20 ml washed pig erythrocytes in 110 ml Krebs-Henseleit buffer with 1.5 g bovine serum albumin powder and 3.0 g dextron. A total volume of about 130 ml of the medium was oxygenated by perfusing it through a thin-walled tube that passed 95% O_2+5% CO_2 at a rate of 2—3 L·min^{-1}. The pH was monitored by a pH meter (Orion 720, USA) and maintained between 7.35 and 7.45 by slight adjustment in $NaHCO_3$, 375 mmol·L^{-1}. The maximal flow rate of the perfusion was kept at a level of more than 25 ml·min^{-1} for 15 min. Then the flow rate was adjusted to 25—30 ml·min^{-1} with an appropriate dose of Met added into the reservoir. The reservoir were sampled 0.5—1.5 ml each at 5, 10, 20, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, and 180 min and equal volume of drug medium was added. The flow rate was kept constant at 25 ml·min^{-1} during a period of 180 min.

Analysis of Met in blood Samples were centrifuged at 1500×g for 10 min. Plasma 0.3—1.0 ml was extracted with 3.0 ml acetacetate after alkalization with 0.1 ml NaOH 3 mol·L^{-1}(pH>12). The organic layer was evaporated at 60—70 ℃ under suction. The residue was dissolved with 0.5 to 1.0 ml mobile phase and then assayed by Shimadzu LC-4A HPLC with RF-530 fluorescence spectromonitor, EM 302 nm, EX 284 nm. The mobile phase comprised of water 80% and tetrahydrofuran 20%. Flow rate was 0.4 ml·min^{-1}. Absolute recoveries were about 99±4%. The coeffi-

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cients of variation of intra- and inter-assay were within 5% and 6%, respectively.

Pharmacokinetic analysis The first-order elimination rate constant (K) was obtained from the slope of the disappearance curve. The initial estimate of V_d was calculated from dose/C_0. Clearance was estimated from $K \times V_d$. Nonlinear processes of drug elimination were described by the Michaelis-Menten expression[4]:

$$-dC/dt = V_m C/(K_m + C)$$

Where V_m stood for the maximal elimination rate and K_m stood for the Michaelis half saturation concentration. V_m and K_m were estimated by the least square method[5]. The total clearance, varying with the concentration during Michaelis-Menten elimination, was calculated as dose/AUC, where AUC was the area under the plasma Met concentration *vs* time curve. Clearance during the first-order phase of elimination was estimated from $K \times V_d$.

Statistical analysis If a single factor analysis of variance was significant ($P < 0.05$), the differences between individual pairs of means were evaluated by Q test.

RESULTS

The time course of elimination of Met, after bolus injection of different doses Met into the perfusion fluid reservoir was illustrated in Fig 1. To assess whether or not it possesses nonlinear kinetic properties at dosages from 0.2 to 12.0 mg, the principle of superposition was used[4]. Plots of concentration in Fig 1 were divided by its corresponding dose of Met. The results showed that plots of Met at doses of 0.2, 0.5, 1.0, and 2.0 mg were superposable, but not superposable at doses of 4.0, 8.0, and 12.0 mg.

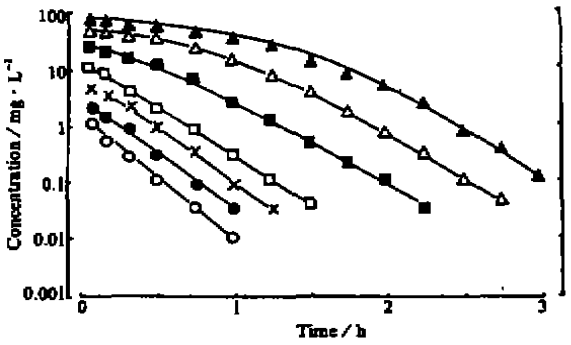

Fig 1. Metoprolol concentration in plasma after different doses in isolated perfused rat liver. 0.2 mg (○) $n=6$, 0.5 mg (●) $n=6$, 1.0 mg (×) $n=6$, 2.0 mg (□) $n=5$, 4.0 mg (■) $n=5$, 8.0 mg (△) $n=5$, 12.0 mg (▲) $n=5$.

The decrease in plasma concentration at doses of 0.2, 0.5, 1.0, and 2.0 mg was in linear kinetics fitted in first order-kinetics according to one-compartmental model. The regression coefficients were all > 0.99 ($P < 0.01$). Pharmacokinetic parameters were shown in Tab 1. $T_{\frac{1}{2}}$ and clearance values were found to be 8.3—10.6 min and 11.7—8.6 ml·min^{-1}, respectively ($P > 0.05$).

Tab 1. Linear pharmacokinetic parameters with metoprolol 0.2—2 mg in isolated perfused rat livers. $\bar{x} \pm s$. $^*P > 0.05$, $^\Delta P < 0.01$ *vs* 0.2 mg.

	0.2 mg ($n=6$)	0.5 mg ($n=6$)	1.0 mg ($n=6$)	2.0 mg ($n=5$)
V_d/ml	140±8	152±11*	132±10*	135±13*
$K \times 10^{-3}$/min^{-1}	84±11	78±10*	71±9*	65±18*
$T_{\frac{1}{2}}$/min	8.3±1.5	8.8±1.6*	9.6±1.3*	10.6±1.8*
Cl/ml·min^{-1}	11.7±2.5	11.8±2.6*	9.6±2.0*	8.6±2.5*
C_0/μg·ml^{-1}	1.4±0.2	3.3±0.4$^\Delta$	7.7±0.7$^\Delta$	15.0±1.3$^\Delta$
AUC/μg·min·ml^{-1}	17±4	43±8$^\Delta$	110±22$^\Delta$	228±45$^\Delta$

At doses of 4.0, 8.0, and 12.0 mg, a phase of apparent zero-order Met disappearance was followed by a phase of apparent first-order disappearance. Plots of Met concentration per unit dose *vs* time were not superposable, consistent with the saturable (Michaelis-Menten) elimination. The K_m and V_m values ranged from 14.6 to 16.9 $\mu g \cdot ml^{-1}$, and 0.94 to 1.05 $\mu g \cdot min^{-1} \cdot ml^{-1}$, respectively (Tab 2).

DISCUSSION

The results of superposition analysis in Fig 2 showed that linear kinetics were found with Met 0.2 to 2.0 mg, the initial concentration being 1.4 to 15.0 $\mu g \cdot ml^{-1}$. The initial plasma concentration of Met in the rat given iv 2 $mg \cdot kg^{-1}$ was less than 1.0 $\mu g \cdot ml^{-1}$ [6]. The different doses did not affect the elimination rate of Met at sub-therapeutic and therapeutic dose levels[2,6]. It was reported that oral bioavailability of Met was about 4%, $T_{\frac{1}{2}}$ for the elimination phase about 36 min and was independent of the dosage and route of administration[6]. During the first-order kinetic processes, it was observed that $T_{\frac{1}{2}}$ and the clearance of Met in this perfused rat liver were also independent of the dosage of perfusion.

Nonlinear kinetics were observed with an increasing dosage of Met from 4.0 to 12.0 mg, the initial concentration being from 27.8 to 78.2 $\mu g \cdot ml^{-1}$. There was a progressive decrease in the total clearance from 4.8 to 2.7 $ml \cdot min^{-1}$. Dose-dependent elimination of Met by the isolated perfused rat liver was clearly indicated by the convex appearance of the plasma disappearance curves, as well as the lack of superposition of the logarithmic plasma concentration/dose *vs* time curves for doses from 4.0 to 12.0 mg. The clearance was greatest and constant at low plasma concentrations, and decreased as the concentration increased and the eliminating system became progressively more saturated. The reasons may be: 1) Higher doses of Met made the capacity of the drug-metabolizing enzyme saturated. 2) The hepatic clearance was reduced by β-adrenergic blocking activity of Met, as *dl*-propranolol also reduced its systemic clearance, but *d*-propranolol did not[7]. A linear correlation was found between the dose of Met and β-adrenergic blocking activity[8]. 3) The metabolites of Met were almost totally excreted by the kidney[2], but no kidney existed in the recirculating isolated perfused rat liver system. Thus, the high concentration of metabolites may inhibit the activity of the metabolizing enzyme.

It was concluded that the elimination of

Tab 2. Nonlinear pharmacokinetic parameter with metoprolol 4—12 mg in isolated perfused rat livers. $n=5$, $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* 4.0 mg.

	4.0 mg	8.0 mg	12.0 mg
V_d/ml	146±9	148±11^a	152±14^a
V_m/$\mu g \cdot min^{-1} \cdot ml^{-1}$	0.98±0.12	1.05±0.14^a	0.94±0.12^a
K_m/$\mu g \cdot ml^{-1}$	15.6±2.1	16.9±3.1^a	14.6±2.0^a
C_0/$\mu g \cdot ml^{-1}$	28±4	54±8^c	78±15^c
AUC/$mg \cdot min \cdot ml^{-1}$	0.84±0.14	2.3±0.4^c	4.4±0.8^c
Total clearance/$ml \cdot min^{-1}$	4.8±0.8	3.4±0.5^b	2.7±0.2^c
First-order clearance/$ml \cdot min^{-1}$	9.1±2.3	9.3±2.5^a	10.0±2.2^a

Met is independent of lower doses, but rested upon higher dosages.

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剂量对美托洛尔离体大鼠肝灌流消除的影响

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A **摘要** 用0.2，0.5，1.0和2.0 mg 美托洛尔进行离体大鼠肝灌流时，消除呈一级动力学. $T_{\frac{1}{2}}$分别为8.3，8.8，9.6和10.6 min. 用4.0，8.0和12.0 mg 美托洛尔灌流时，消除呈零级动力学. V_m 分别为0.98，1.05和0.94 $\mu g \cdot min^{-1} \cdot ml^{-1}$. K_m 分别为15.5，16.9和14.6 $\mu g \cdot ml^{-1}$. 提示低剂量美托洛尔肝消除无剂量依赖性，较高剂量呈剂量依赖性.

关键词 美托洛尔；肝脏；灌流；药物动力学 R96

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Antitumor activity and tumor necrosis factor production of *Phytolacca acinosa* polysaccharides I in mice[1]

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ABSTRACT The antitumor activities of *Phytolacca acinosa* polysaccharides I (PAP-I) and its effects on the induction of tumor necrosis factor (TNF) and immunological cytotoxicity of peritoneal macrophages were studied. PAP-I was given ip 5—20 mg·kg^{-1}·d^{-1}×7 d to ICR mice as priming agent with subsequent lipopolysaccharides (10 μg/mouse) iv for TNF production. TNF activity was measured by crystal violet staining assay using L929 cells. PAP-I showed priming activity for TNF production with hepto-splenic hyperplasia in a dose-dependent manner. The peritoneal macrophages treated with PAP-I 10 and 20 mg·kg^{-1} showed 67 and 74%, respectively, cytotoxicity (the control 34% cytotoxicity) against Meth A cells at effector:target = 40:1. PAP-I 10 and 20 mg·kg^{-1} prolonged the survival time of mice bearing ascites Meth A tumor from 21±4 to 32±10 and 38±8 d and inhibited the solid Meth A tumor growth with inhibition rate of 28.5 and 55.7%, respectively. These results sug-

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