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AGE-DEPENDENT PROPRANOLOL CLEARANCE IN PERFUSED RAT LIVER*

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Abstract—The effect of age on the hepatic clearance of propranolol was studied by perfusing the liver isolated from 3- to 104-week-old rats. Propranolol levels in the recirculating perfusate declined bi-exponentially with time in all age groups. When the liver isolated from 7-week-old rats was perfused with propranolol (1 μg/ml, 100 ml), hepatic clearance of this drug by the perfused liver (CL_{perf}) increased from 0.589 to 1.14 ml·min^{-1}·(g liver)$^{-1}$ with the increase of the perfusion flow rate from 1.0 to 2.0 ml·min^{-1}·(g liver)$^{-1}$, confirming evidence of "perfusion-limited" hepatic clearance for this drug. Furthermore, there was no initial concentration(dose)-dependence in CL_{perf} up to 2.5 μg/ml (i.e. 250 μg/organ). The effect of age on CL_{perf} was then investigated by perfusing the isolated liver with 1.0 μg/ml propranolol at 2.0 ml·min^{-1}·(g liver)$^{-1}$. Elimination of this drug from the perfusion medium was relatively rapid in 5- to 7-week-old rats, yielding the highest CL_{perf} in these relatively young rats [approximately 1.0 to 1.1 ml·min^{-1}·(g liver)$^{-1}$]. In contrast, CL_{perf} values in both immature and older rats were 0.5 ml·min^{-1}·(g liver)$^{-1}$ or less. The *in vitro* intrinsic hepatic clearance estimated in 5- and 7-week-old rats was about ten times as high as that in 104-week-old rats.

Among age-dependent changes, those that are directly sensitive to age include hepatic metabolic rate and/or activity and renal function. There have been few pharmacokinetic works on the age-dependent change in hepatic drug metabolic function, as compared with the numerous works leading to rational therapeutics (i.e. application of clinical pharmacokinetic consideration) in elderly patients with reduced renal function.

Propranolol is considered a good model drug to test the effects of aging on hepatic clearing of highly extracted drugs. Several reports have demonstrated that the total body clearance of this drug decreases with age from about 20 to 80 years in both normal subjects and patients [1–4]. Recently, we have also reported that, in rats, both systemic clearance and intrinsic hepatic clearance of this drug decrease with age from 7 to 24 weeks [5]. Under first-order conditions, the *in vivo* intrinsic clearance for several drugs including propranolol was approximated by the ratio of *in vitro* kinetic parameters (V_{max}/K_m) in 9- to 10-week-old male Sprague–Dawley rats [6]. However, there have been no reports clarifying mechanisms for this age-dependence in the *in vivo* intrinsic hepatic clearance of propranolol except our previous work showing a significant correlation between hepatic clearance of this drug and liver blood flow [5].

In vitro liver perfusion study or uptake and metabolism experiments using the hepatocytes may enable one to predict some probable mechanisms for the age-dependence found in the *in vivo* hepatic clearance of particular drugs. The present work was designed to characterize and compare elimination kinetics of propranolol when the liver isolated from male Wistar rats aged between 3 and 104 weeks were perfused. In the preliminary experiments, effects of perfusion rate and initial substrate concentration (i.e. dose) on the hepatic clearance were tested in 7-week-old rats. In addition, the present *in vitro* data were compared with our previous *in vivo* data to discuss the age-dependent hepatic elimination of this drug.

MATERIALS AND METHODS

Materials. Propranolol hydrochloride (*dl*-racemate, Sumitomo Chemical Co., Osaka, Japan) was donated by I.C.I.-Pharma Ltd. (Osaka, Japan). All other chemicals and reagents including *n*-heptane and isoamyl alcohol used for the extraction of unchanged propranolol from the perfusate were of analytical grade.

Perfusion of rat liver with propranolol. A Miller-type organ perfusion apparatus equipped with a peristaltic pump was used for the present *in vitro* liver perfusion experiments at 37°. Male Wistar rats that were 3 (60–85 g) to 104 (790–850 g) weeks old were used throughout the experiments (N = 4) after overnight fasting as described previously [5]. After anesthetizing each rat with urethane (800 mg/kg, i.p.), pre-perfusion through the liver with pH 7.4 buffer solution (121 mM NaCl, 6 mM KCl, 0.6 mM $MgSO_4$, 0.74 mM KH_2PO_4, 12 mM $NaHCO_3$, 5 mM glucose, oxygenated with 95% O_2–5% CO_2) via the portal vein was performed for 5 min at approximately 10–40 ml·min^{-1}·organ^{-1} for 3- to 104-week-old rats in the same way as reported previously [7–9] without

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recirculation. Size (o.d.) of the teflon catheter (portal cannula) was adjusted depending on the size (i.e. age) of the rats, usually ranging from 0.8 to 1.5 mm for 3 to 24 weeks and older. Approximate wet weight of the liver was measured immediately after isolation by keeping it in pre-perfusion solution. The pre-perfusion buffer was then replaced by the drug solution (1.0 μg/ml unless otherwise specified), which was prepared in the same buffer solution as above, with another pump. This replacement was completed in 1 min without recirculation. Another fresh drug solution (100 ml) was started perfusing through the liver in a recirculation system. Perfusion inflow rate was adjusted at 2.0 ml·min^{1}·(g liver)$^{-1}$ unless otherwise specified. Perfusion outflow was monitored by measuring the volume level in the graduated reservoir as described previously [9]. The outflow rate was not significantly different from the inflow rate for all age groups. For the drug assay, an aliquot (0.1 ml) of the perfusate was withdrawn from the reservoir periodically up to 30 min.

Preliminary experiments to examine the effects of perfusion rate and the initial perfusate level of propranolol on its elimination by the perfused liver were carried out in 7-week-old rats (N = 4). Perfusion flow was adjusted at three rates, 1.0, 2.0 and 3.0 ml·min^{-1}·(g liver)$^{-1}$, while the initial propranolol level in the perfusion buffer (100 ml) was changed at four different levels, 0.5, 1.0, 2.5 and 5.0 μg/ml [i.e. 50, 100, 250 and 500 μg per liver (9.27 to 9.68 g) respectively]. Perfusion outflow rate when the initial inflow rate was adjusted to 3.0 ml·min^{-1}·(g liver)$^{-1}$ tended to be reduced by approximately 15–30% of the inflow during the last half of the perfusion.

Assay of perfusate drug levels. Propranolol level in the perfusate was determined by slightly modifying the method of Vervloet *et al.* [10] as reported previously [11]. This analytical method including duplicate extraction procedures with *n*-heptane containing 1.5% isoamyl alcohol has been proved to be specific for unchanged propranolol.

Data analysis and statistics. Perfusate propranolol–time curves were analyzed according to least-squares regression analysis program MULTI [12] for bi-exponential decline expressed as $C = Ae^{-\alpha t} + Be^{-\beta t}$, where C is the perfusate drug level and A, B, α and β are hybrid pharmacokinetic parameters. The best fit of the data was achieved by weighting with the reciprocal of the concentration. Significant difference was quantified by Student's *t*-test.

RESULTS

Effect of perfusion flow rate on in vitro *hepatic elimination of propranolol.* Figure 1 shows perfusate propranolol level–time curves obtained in 7-week-old rats when the liver was perfused with 100 ml of 1.0 μg/ml drug at three different flow rates. The drug level when perfused at 1.0 ml·min^{-1}·(g liver)$^{-1}$ was always higher than those when perfused at 2.0 and 3.0 ml·min^{-1}·(g liver)$^{-1}$. All elimination curves in the liver perfusate declined bi-exponentially with time. The area under the curve (AUC) was then calculated from these data by the relationship, $AUC = A/\alpha + B/\beta$. Perfusion clearance (CL_{perf}) of propranolol in the present *in vitro* experiments was calculated according to the equation [9], CL_{perf} = (Initial load)/AUC, where the initial load was 100 μg (1.0 μg/ml multiplied by 100 ml). The smallest clearance value [0.589 ± 0.061 ml·min^{-1}·(g liver)$^{-1}$] was obtained at the lowest flow rate, but there was no difference between the values of 1.14 ± 0.13 and 1.08 ± 0.096 ml·min^{-1}·(g liver)$^{-1}$ when perfused at 2.0 and 3.0 ml·min^{-1}·(g liver)$^{-1}$ respectively.

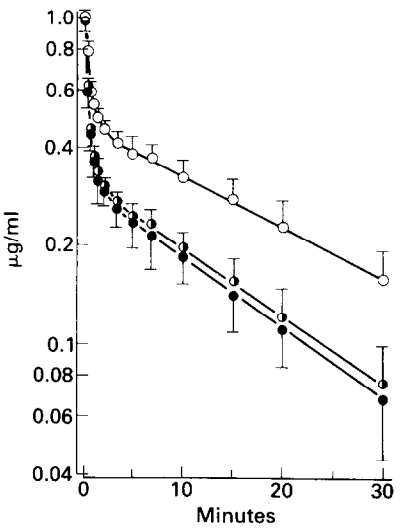

Fig. 1. Time course of propranolol remaining in the perfusate (pH 7.4, 37°) recirculating through liver isolated from 7-week-old rats. Initial substrate concentration was fixed at 1.0 μg/ml, whereas the perfusion flow rate was adjusted at 1.0 (○), 2.0 (●) or 3.0 (◑) ml·min^{-1}·(g liver)$^{-1}$. Each point is the mean ± S.D. of four rats. The solid line represents a computer-fitted bi-exponential curve analyzed according to the least squares regression program MULTI [12].

Effect of initial propranolol concentration on its in vitro *hepatic clearance.* This was also tested in 7-week-old rats but the perfusion flow rate was fixed at 2.0 ml·min^{-1}·(g liver)$^{-1}$. There was no effect of the initial propranolol concentration on the present *in vitro* hepatic clearance of this drug up to 2.5 μg/ml (i.e. 250 μg/liver), as shown in Fig. 2. However, a further rise in the initial drug concentration to

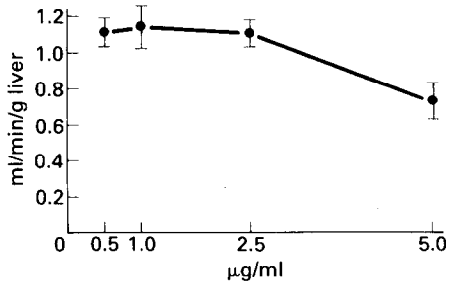

Fig. 2. Effect of initial propranolol concentration in the recirculating perfusate (pH 7.4, 37°) on its *in vitro* hepatic clearance (CL_{perf}) when liver isolated from 7-week-old rats was perfused. Flow rate was fixed at 2.0 ml·min^{-1}·(g liver)$^{-1}$. Each point is the mean ± S.D. of four rats.

5.0 μg/ml (i.e. 500 μg/liver) reduced CL_{perf} by about 35%.

Effect of age on in vitro *hepatic clearance of propranolol by the perfused liver.* Initial propranolol concentration and perfusion flow rate were fixed at 1.0 μg/ml and 2.0 ml·min^{-1}·(g liver)$^{-1}$ respectively. In any age group other than 7 weeks, the perfusate drug level–time curve also declined bi-exponentially with time. Pharmacokinetic analysis of each curve indicated that the value, which is principally a function of the hepatic elimination rate constant of propranolol, tended to increase in relatively young rats between weeks 5 and 11. AUC exhibited the smallest value in 7-week-old rats but was relatively large in 3- or 24- to 104-week-old rats.

Effect of age on CL_{perf} estimated in the same manner as described above is shown in Fig. 3. Relatively high clearance values, 1.01 and 1.14 ml·min^{-1}·(g liver)$^{-1}$, were obtained in 5- and 7-week-old rats respectively. These values decreased to approximately 0.5 ml·min^{-1}·(g liver)$^{-1}$ in 15-week-old rats and gradually with age thereafter. The perfusion clearance was also relatively low in 3-week-old rats. A similar age-dependent profile was obtained when this *in vitro* hepatic clearance was represented as per body weight (ml·min^{-1}·kg^{-1}), as shown with the broken line in Fig. 3.

DISCUSSION

In our previous report, it was suggested that the systemic clearance of propranolol is highly sensitive to the aging of rats and also correlates with the liver blood flow [5]. Similar age-dependence has been observed earlier in the systemic clearance of this drug in man [1–4]. However, there has been no direct evidence explaining a possible mechanism for the reduced metabolic rate or intrinsic clearance of propranolol in the aged liver.

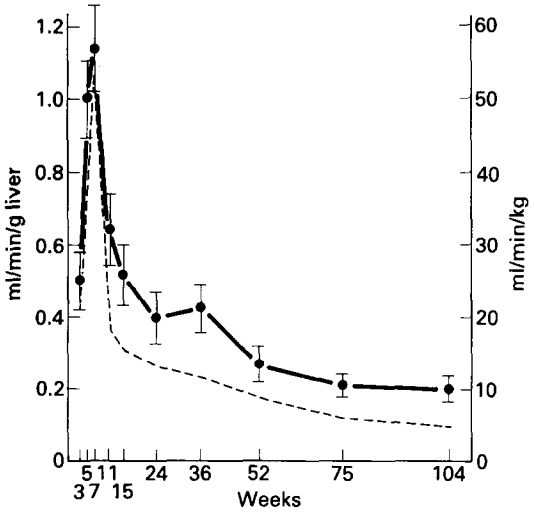

Fig. 3. Effect of age on the hepatic clearance of propranolol when the isolated rat liver was perfused with this drug at pH 7.4 and 37°. Initial substrate concentration was fixed at 1.0 μg/ml and the flow rate at 2.0 ml·min^{-1}·(g liver)$^{-1}$. Each point is the mean ± S.D. of four rats. The broken line represents the *in vitro* hepatic clearance expressed as ml·min^{-1}·kg^{-1} (right ordinate).

The present *in vitro* perfusion technique for characterizing hepatic elimination (metabolic) kinetics enabled us to study the effect of aging on the hepatic clearance of propranolol in rats, since this approach could simulate *in vivo* liver perfusion despite a major difference of the perfusion buffer solution from blood. Although propranolol has been reported to be rather extensively (approximately 80–95%) bound to blood plasma or serum protein in both man [13–17] and experimental animals such as monkey, dog and rat [5, 6, 14, 17], it has been generally proposed that the elimination of drugs having a high hepatic extraction ratio, such as propranolol or lidocaine, is insensitive to the changes in plasma or serum protein binding but essentially sensitive to the perfusion rate [18, 19]. Therefore, any protein such as bovine serum albumin was not added to the perfusion medium in the present experiments, for the purpose of calculating the intrinsic clearance for the unbound drug directly.

In 7-week-old rats, the *in vitro* clearance (CL_{perf}) when the liver was perfused was found to increase with the perfusion rate [1.0 to 2.0 ml·min^{-1}·(g liver)$^{-1}$], suggesting that the lower perfusion flow rate (Q) limits the hepatic clearance (namely "perfusion-limited" hepatic elimination). The lack of the effect of increasing perfusion flow rate from 2.0 to 3.0 ml·min^{-1}·(g liver)$^{-1}$ might be due to the substantial decrease of the actual flow rate in the elimination phase upon perfusing at 3.0 ml·min^{-1}·(g liver)$^{-1}$. The reason for this decrease in the terminal phase remains unclear. The present flow dependence was almost consistent with the data reported by Branch *et al.* [20]. Recently, Keiding and Steiness [21] have also demonstrated the flow dependence of propranolol elimination in perfused rat liver during steady-state infusion into a recirculating medium but suggested that their data were consistent with the "sinusoidal perfusion model" [22] rather than with the "venous equilibrium model" [23]. Assuming that CL_{perf} is a function of the perfusion flow (Q) and the extraction ratio (E) of the drug (i.e. $CL_{perf} = Q \cdot E$), the extraction ratio under the present *in vitro* perfusion condition was estimated to be approximately 0.6, which was appreciably smaller than the previous *in vivo* extraction ratio [5]. Linearity of the present *in vitro* hepatic elimination with the initial perfusate propranolol level was verified up to 2.5 μg/ml (i.e. 250 μg/liver as the initial dose), and saturation kinetics seemed to be involved at the higher perfusate drug levels. Our previous *in vivo* results obtained after the intraportal infusion in 7-week-old rats also showed significant saturation kinetics in the hepatic elimination of propranolol at dose levels of 5.0 mg/kg and higher [24].

Under the present conditions where both flow rate [2.0 ml·min^{-1}·(g liver)$^{-1}$] and initial drug concentration (1.0 μg/ml) of the perfusate were fixed, a significant age-dependence was observed in the *in vitro* hepatic clearance. A relatively rapid metabolic rate in 5- and 7-week-old rats resulted in higher hepatic clearance than those in other age groups. A similar age-dependence profile was obtained when the clearance was expressed per body weight

($ml \cdot min^{-1} \cdot kg^{-1}$). However, the latter *in vitro* hepatic clearance was found to be considerably lower (by approximately 10–60%) than the previous *in vivo* clearance ($ml \cdot min^{-1} \cdot kg^{-1}$) data [5] despite showing almost similar age-dependence. This strongly suggests that there may be some extrahepatic elimination of propranolol after i.v. administrations [5] or some experimentally inevitable limitations to produce these differences between *in vivo* and *in vitro* results. For the former, pulmonary clearance might possibly be involved in the first-pass elimination of propranolol following i.v. dosing (unpublished data by K. Iwamoto *et al.*), since the renal clearance has been known to be almost insignificant [5]. On the other hand, absence of oxygenated blood cells might be unfavorable for the liver to extract propranolol avidly. Thus, an unexpectedly lower extraction ratio (about 0.6 in 7-week-old rats) than that (about 0.9) obtained in the *in vivo* study might explain the substantial difference described above. However, the present *in vitro* results obtained at a fixed flow rate [$2.0\ ml \cdot min^{-1} \cdot (g\ liver)^{-1}$] could suggest that the significant age-dependence in the hepatic clearance might be attributed to a possible age-dependence in the extraction ratio, $E = CL_{int}/(CL_{int} + Q)$, where CL_{int} is intrinsic hepatic clearance [25]. Therefore, the estimate for CL_{int} was found to decreased from approximately 3.0 to $0.22\ ml \cdot min^{-1} \cdot (g\ liver)^{-1}$ in 7- to 104-week-old rats.

In conclusion, the present *in vitro* study where the rat liver was perfused with propranolol has strongly suggested that the intrinsic clearance (hepatic metabolic function) as well as the perfusion flow rate may be the major determinant in its age-dependent elimination and it also supported our previous *in vivo* results demonstrating a significant age-dependence in the hepatic clearance of this drug [5].

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