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Effect of age on the hepatic clearance of propranolol in rats

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The effect of age on hepatic clearance of propranolol was investigated in male Wistar rats (3 to 104 weeks old). Pharmacokinetic analysis of the plasma propranolol data obtained after i.v. dosage (1·0 mg kg^{-1}) indicated that both the elimination rate constant and the volume of distribution decreased with age between weeks 5 and 11, after when the distribution volume remained almost constant. The elimination rate constant was reduced consistently with age after 15 weeks. Total body clearance of the drug was reduced extensively with age between weeks 5 and 11 (94 to 43 ml min^{-1} kg^{-1}) and decreased gradually thereafter. The plasma free fraction of propranolol also decreased with age, falling from more than 20% in 3 to 5 weeks-old rats to about 10% in rats of 52 to 104 weeks. In immature rats the renal clearance was approximately 5 to 12% of the total body clearance. Intrinsic hepatic clearance for unbound propranolol also decreased with age between weeks 7 and 24. As expected from the evidence that propranolol has a high extraction ratio, the extent of its hepatic clearance was significantly dependent on the liver blood flow. These data suggest that the age-dependent decrease in hepatic clearance of propranolol is largely due to a reduction of the elimination rate that might be accompanied by the age-dependent decrease in liver blood flow.

Among age-dependent changes, those that are directly sensitive to age include hepatic metabolic rate and/or activity and renal function. Although differences in systemic clearance, distribution volume and elimination half-life vary with age for many drugs (Ritschel 1982), no consistent pattern has emerged (Crooks et al 1976; Triggs & Nation 1975; Vestal 1978).

Propranolol is considered a good model to test the effects of ageing on the hepatic activity to clear highly extracted drugs. Castleden et al (1975) described the effect of age on plasma levels of propranolol in man; other reports (Vestal et al 1979; Castleden & George 1979; Feely et al 1981) have shown that its total body clearance decreases with age.

We have investigated the effect of postnatal development and ageing on the hepatic clearance of propranolol in male Wistar rats of from 3 to 104 weeks old. Plasma levels following i.v. and oral dosage were compared and analysed, plasma protein binding and renal clearance were determined, and liver blood flow was also measured.

MATERIALS AND METHODS

Materials

Propranolol hydrochloride (±-racemate) was donated by Sumitomo Chemical Industry Co., Osaka, Japan. Spectrapor membrane tubing (Type 2, MW 12000–14000), used for equilibrium dialysis experiments, was purchased from Spectrum Med. Ind. Inc., Los Angeles, USA. A hydrogen monitor (PHG-300, M.T.-Giken Co., Nagoya, Japan) was used for measuring liver blood flow; it included a wire or needle electrode (Pt–Pt black H_2-electrode) with an Ag–AgCl electrode as reference. All other chemicals were of analytical grade.

Animals

Male Wistar rats, 3 (58–89 g), 5 (111–148 g), 7 (206–240 g), 11 (354–387 g), 15 (375–412 g), 24 (448–525 g), 36 (547–613 g), 52 (586–780 g), 75 (711–782 g) and 104 (786–894 g) weeks old were used. Each was used once. To measure plasma levels of propranolol, 3 to 5 rats in all age-groups were cannulated in the jugular vein one day before the experiment, as previously reported (Iwamoto et al 1982) and fasted overnight (at least 15 h). Three to 5 rats among the same age-groups were lightly anaesthetized with urethane (800 mg kg^{-1} i.p.) for the measurement of liver blood flow.

Measurement of plasma propranolol after intravenous and oral administration to rats

Unanaesthetized rats in each age-group were given 1·0 mg kg^{-1} of propranolol (as base) either intravenously via the cannula or orally by gastric intuba-

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tion. Blood samples (7 or 8 samples, about 0·12 ml for 3 to 5 week old and 0·25 ml for 7 to 104 week old rats) were withdrawn from the jugular vein cannula over 120 min, and placed in chilled centrifuge tubes containing 1 unit of heparin and centrifuged at 300 rev min^{-1} for 10 min. The plasma so obtained was used for analysis of unchanged propranolol (50 or 100 µl) determined with spectrofluorometric method of Vervloet et al (1977) as modified by Iwamoto & Watanabe (1984); the assay is specific for propranolol in rat plasma.

Measurement of plasma protein binding of propranolol

To 1 ml control plasma obtained immediately after jugular vein cannulation in rats not given propranolol, was added 0·5 or 1·0 µg propranolol and plasma was dialysed against 1 ml of Krebs-Ringer bicarbonate buffer (pH 7·4, oxygenated with 95% O_2–5% CO_2) using Spectrapor membrane tubing at 37 °C. Equilibrium was attained within 4 h after which, drug concentrations in both inner and outer phases were determined as described above. There was no apparent volume change in inner or outer phase after the equilibrium. Total plasma protein level was determined by the method of Lowry et al (1951) with bovine plasma albumin (Fraction V, Sanko Pure Chemicals, Tokyo, Japan) as standard.

Determination of renal clearance of propranolol

Other groups of cannulated and unanaesthetized rats (n = 3 or 4) 3, 5, 7, 15, 36 and 75 weeks old were given propranolol, 1 mg kg^{-1}, and housed individually in metabolic cages. Urination was induced with a cotton pad wetted with ether. Urine samples were collected at 30 min intervals for 2 h and blood sampling was at the midpoints of the urine collection intervals. Propranolol in the diluted urine sample was determined as for plasma level except that 0·2 ml of the diluted urine was used.

Measurement of liver blood flow

An abdominal midline incision (2 to 3 cm) was made in lightly anaesthetized rats. A needle or wire H_2-electrode was then placed into the liver tissue and after giving a trace amount of hydrogen gas to the rats, by inhalation, the elimination-time profile for hydrogen gas circulating in the liver tissue was monitored with PHG-300 H_2 gas monitor. Liver blood flow was estimated from the hydrogen gas clearance data according to Wodick et al (1978). Flow data representing one liver was obtained from the average value of three to five determinations (coefficient of variation ranged from 3·7–8·1%).

Data analysis and statistical quantification

Plasma concentration-time curves were analysed according to least-squares regression analysis program MULTI (Yamaoka et al 1981) for mono- or bi-exponential decline. The best fit of the data was achieved by weighting with the reciprocal of either the plasma concentration or its square. Significant difference was quantified by Student's *t*-test.

RESULTS AND DISCUSSION

Total body clearance of propranolol after i.v. dosing

Tables 1 and 2 summarize the pharmacokinetic parameters, which were estimated according to mono- or bi-exponential equations, for plasma propranolol in 3- to 104-week-old rats. The elimination rate constant decreased consistently with age from 5 to 104 weeks, while the volume of distribution was reduced dramatically with age from 3 to 11 weeks remaining almost constant. The AUC increased with age.

The effect of age on total body clearance of propranolol is shown in Fig. 1. The total body clearance (ml min^{-1} kg^{-1}) decreased extensively with age from 5 to 11 weeks and then decreased gradually. The direct cause in the reduction appears to be the age-dependent decreases in both elimination rate constant and distribution volume which

Table 1. Pharmacokinetic parameters for propranolol given intravenously (1·0 mg kg^{-1}) to rats 3 to 104 weeks old.

	Mean ± s.e.[a] for rats[b] in age of (weeks)								
Parameter	3	5	11	15	24	36	52	75	104
k_{el} (10^{-2} min^{-1})	2·62 ± 0·43	3·14 ± 0·25	2·20 ± 0·08	2·07 ± 0·09	1·56 ± 0·04	1·24 ± 0·12	1·16 ± 0·13	0·96 ± 0·09	0·83 ± 0·10
V_d (1 kg^{-1})	3·35 ± 0·26	2·99 ± 0·20	1·86 ± 0·14	2·07 ± 0·09	1·87 ± 0·14	2·39 ± 0·18	2·50 ± 0·20	2·10 ± 0·21	2·11 ± 0·23
$t_{\frac{1}{2}}$ (min)	26·50 ± 6·20	22·10 ± 3·80	31·50 ± 1·00	33·50 ± 1·40	44·40 ± 1·00	55·90 ± 8·80	59·70 ± 9·20	72·00 ± 8·10	83·40 ± 9·90
AUC (µg min ml^{-1})	11·40 ± 2·10	10·60 ± 1·10	24·50 ± 1·70	23·30 ± 1·20	36·80 ± 3·40	33·80 ± 4·10	34·50 ± 4·70	49·50 ± 4·70	57·10 ± 7·00

[a] With weight, $W(i) = 1/(C_i)^2$.
[b] n = 3, 4, 3, 4, 4, 4, 4, 3 and 3 for 3, 5, 11, 15, 24, 36, 52, 75 and 104 weeks, respectively.

Table 2. Pharmacokinetic parameters for propranolol given intravenously (1·0 mg kg^{-1}) to rats 7 weeks old.

Parameter	mean ± s.e.[a] (n = 5)
A (μg ml^{-1})	0·473 ± 0·025
B (μg ml^{-1})	0·319 ± 0·008
α (10^{-2} min^{-1})	37·0 ± 3·04
β (10^{-2} min^{-1})	1·92 ± 0·07
k_{10} (10^{-2} min^{-1})	4·42 ± 0·19
k_{12} (10^{-2} min^{-1})	18·4 ± 1·9
k_{21} (10^{-2} min^{-1})	16·1 ± 1·1
V_1 (l kg^{-1})	1·26 ± 0·05
V_2 (l kg^{-1})	1·45 ± 0·19
$t_{1/2\beta}$ (min)	36·1 ± 1·3
AUC (μg min ml^{-1})	17·9 ± 0·9

[a] With weight, W(i) = 1/(C_i).

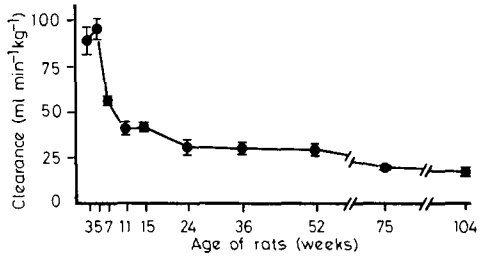

FIG. 1. Effect of age on total body clearance of propranolol in rats (n = 3 to 5). Propranolol was given intravenously (1·0 mg kg^{-1}) to rats. Each point with vertical bar represents the mean data point with standard deviation.

were observed in young rats (5 to 11 weeks). The elimination rate constant seems to be a factor affecting total body clearance in all age-groups. It decreased consistently with age from 5 to 104 weeks (Tables 1, 2), suggesting that the hepatic elimination of propranolol would also be reduced with age after the onset of maturity.

Effect of age on plasma protein binding of propranolol

Table 3 represents the effect of age on the free fraction in rat plasma initially spiked with propranolol at 0·5 or 1·0 μg ml^{-1}. There was no significant difference in the percentage of free fraction between these two plasma levels for any age-group. The plasma free fraction (f) decreased rapidly with age from 3 to 11 weeks and gradually thereafter, suggesting that there may be relatively higher binding capacity in the older rats. This tendency is considered to be consistent with that found in the volume of distribution (Tables 1, 2). Since there was no significant difference in total plasma protein levels in variously aged rats, it could be that the older rats keep relatively high plasma α_1-acid glycoprotein levels, as Sager et al (1981) have demonstrated in man.

The sensitivity of organ clearance of a drug to changes in binding within blood plasma or serum depends on its unbound clearance (Gibaldi & Perrier 1982). If the hepatic extraction ratio is relatively high, such as that of propranolol or lignocaine, elimination becomes perfusion rate-limited and the clearance will be relatively insensitive to the changes in binding (Gibaldi & Perrier 1982; Rowland 1984). Evans et al (1973) have demonstrated that, for propranolol, increased binding is accompanied by decreases in drug half-life and distribution volume. It has been proposed that the renal clearance of a drug with a low extraction ratio will be sensitive to the changes in protein binding (Levy 1980; Rowland & Tozer 1980). Though it has been empirically proved and it is generally believed that there may be no extra hepatic elimination of propranolol when it is given to normal healthy subjects or animals, the extensive increase in the free fraction (f) of propranolol observed in rats of 5 weeks or less (Table 3) led us to examine the possibility of renal clearance in immature and aged rats.

Renal clearance of propranolol in rats 3 to 75 weeks old

The renal clearance value and its ratio to the total body clearance in six different age-groups are shown in Table 4. The renal clearance of propranolol as expected, was insignificant in rats older than 7 weeks, while in rats younger than 5 weeks, it was found to play a small, but significant, role in the total body clearance (approximately 5 to 12% of total). The present clearance data show large fluctuations,

Table 3. Plasma free fraction (f) of propranolol in rats (n = 4) 3 to 104 weeks old.

Initial concn. (μg ml^{-1})	f × 100, Mean ± s.d. (%)[a] for rats in age of (weeks)									
	3	5	7	11	15	24	36	52	75	104
0·5	22·9 ± 0·7	20·9 ± 0·8	19·1 ± 1·9	14·8 ± 1·3	14·1 ± 0·9	14·1 ± 1·0	12·4 ± 0·6	11·9 ± 0·8	10·3 ± 0·9	10·4 ± 0·6
1·0	21·3 ± 0·9	20·2 ± 0·9	18·4 ± 1·4	12·8 ± 1·6	12·3 ± 1·6	12·1 ± 1·9	10·7 ± 1·3	10·1 ± 1·2	10·4 ± 1·0	10·8 ± 1·7

[a] One ml of control plasma spiked with propranolol (0·5 or 1·0 μg ml^{-1}) was dialysed against 1 ml of Krebs-Ringer bicarbonate buffer solution using Spectrapor membrane tubing at 37 °C.

Table 4. Renal clearance (Cl_r) and its ratio to total body clearance (Cl_r/Cl_s) in rats 3 to 75 weeks old.

	Mean ± s.d. for rats[a] in age of (weeks)					
	3	5	7	15	36	75
Cl_r (ml min^{-1} kg^{-1})	4·5 ± 1·8	11·3 ± 4·1	0·02 ± 0·01	0·00 ± 0·00	0·01 ± 0·01	0·00 ± 0·00
Cl_r/Cl_s[b]	0·051	0·121	0·00	0·00	0·00	0·00

[a] n = 4, 3, 3, 3, 3 and 4 for 3, 5, 7, 15, 36 and 75 weeks, respectively.
[b] Ratio of mean renal clearance to mean total body clearance.

Table 5. AUC, systemic availability (F) and intrinsic hepatic clearance (Cl_{int}) for propranolol following oral administration (1·0 mg kg^{-1}) to rats 3 to 104 weeks old.

	Mean ± s.d. for rats[a] in age of (weeks)									
Parameter	3	5	7	11	15	24	36	52	75	104
AUC[b] (μg min ml^{-1})	1·44 ± 0·70	1·58 ± 0·65	1·45 ± 0·21	2·72 ± 0·42	2·52 ± 0·40	4·42 ± 1·03	4·76 ± 2·24	4·85 ± 3·24	4·61 ± 1·02	4·70 ± 0·78
F[c]	0·13 ± 0·07	0·15 ± 0·02	0·08 ± 0·02	0·11 ± 0·01	0·11 ± 0·01	0·12 ± 0·03	0·14 ± 0·07	0·14 ± 0·10	0·09 ± 0·02	0·08 ± 0·01
Cl_{int}[d] (ml min^{-1} kg^{-1})	n.e.[e]	n.e.	3670 ± 920	2870 ± 790	3000 ± 770	1780 ± 560	1800 ± 780	1870 ± 990	1990 ± 380	2050 ± 340

[a] n = 4, 4, 5, 4, 5, 4, 4, 4, 3 and 3 for 3, 5, 7, 11, 15, 24, 36, 52, 75 and 104 weeks, respectively.
[b] Calculated by the trapezoidal rule and extrapolation to infinite time.
[c] Calculated by the equation, $F = AUC_{p.o.}/AUC_{i.v.}$.
[d] Calculated by the equation, $Cl_{int} = Dose_{p.o.}/(AUC_{p.o.} \cdot f)$, where the mean value for f obtained at 0·5 and 1·0 μg ml^{-1} of propranolol (see Table 3) was used.
[e] Not estimated.

probably due to the relatively long sampling interval (30 min) for urine, the mean renal clearance value was accordingly subtracted from the corresponding total body clearance value to estimate the hepatic clearance. Here, it was assumed that there was no renal clearance of propranol in rats older than 7 weeks.

AUC, systemic availability and intrinsic hepatic clearance of propranolol after oral dosing

Table 5 summarizes the effect of age on AUC, systemic availability (F) and intrinsic hepatic clearance of propranolol after oral administration of the same doses as given i.v. (1·0 mg kg^{-1}). The AUC value increased with age from 7 to 36 weeks, while the systemic availability averaged at approximately 12%, and ranged from 8 to 15%. If it is assumed that propranolol is almost fully absorbed from the gastrointestinal tract (Paterson et al 1970; Lo et al 1982) and almost completely metabolized by the liver following oral administration, hepatic clearance of the drug, which may be described as $Dose_{p.o.}/(AUC_{p.o.} \cdot f)$, is approximately equivalent to an intrinsic hepatic clearance (CL_{int}) for the unbound drug. The hepatic extraction ratio, thus, was 0·88, ranging from 0·85 to 0·92. From the results of renal clearance of the drug (Table 4) and each mean f value (Table 3), the intrinsic hepatic clearance (CL_{int}) was estimated for the rats 7 to 104 weeks old. This clearance value decreased by approximately 50% with age from 7 to 24 weeks and remained at an almost constant value thereafter. In addition, these values were approximately 60 to 120 times larger than the hepatic clearance, that is substantially equivalent to total body clearance (Fig. 1). The age-dependent decrease in the intrinsic hepatic clearance of propranolol in rats (Table 5) may mean that the activity of hepatic drug-metabolizing enzymes could be reduced in relatively aged rats compared with that in young rats.

Relation of hepatic clearance of propranolol and liver blood flow

Liver blood flow rate has been estimated traditionally by measuring indocyanin green or bromsulphthalein (Bradley et al 1945) in both man and animals.

Table 6. Liver blood flow (Q_h) in rats 3 to 104 weeks old.

	Mean ± s.d. for rats[a] in age of (weeks)									
	3	5	7	11	15	24	36	52	75	104
Q_h[b] (ml min^{-1} kg^{-1})	55·7 ± 9·9	71·3 ± 4·2	60·1 ± 4·4	50·9 ± 3·3	43·3 ± 3·8	31·5 ± 1·9	15·7 ± 2·8	16·3 ± 3·2	14·8 ± 3·0	13·9 ± 2·8

[a] n = 3, 3, 5, 4, 3, 3, 4, 3, 5 and 4 for 3, 5, 7, 11, 15, 24, 36, 52, 75 and 104 weeks, respectively.
[b] Measured by Hydrogen-Gas Clearance method (Wodick et al 1978).

However, an inert gas washout technique using either ^{85}krypton or ^{133}xenon has been described by Ohnhaus et al (1979), and another method for measuring mean blood flow rate in tissue by application of a hydrogen gas washout technique has been reported by Wodick et al (1978). Although the last technique requires a small midline abdominal incision under light anaesthesia, it is considered to be most convenient for the direct comparison of the liver blood flow in variously aged rats as has been done in the present work (Table 6). Liver blood flow (ml min^{-1} kg^{-1}) was found to decrease extensively with age from 5 to 36 weeks and to remain at an almost constant low value thereafter. These results are consistent with published evidence showing a decline in cardiac output and hepatic blood flow in aged humans (Brandfonbrener et al 1955; Sherlock et al 1950). The present liver blood flow data were considered to be slightly underestimated due to the effects of anaesthesia with urethane, since the reported liver plasma flow rate (6·5 ml min^{-1}) for 200 g (i.e., 7 weeks old) unanaesthetized rats (Bischoff et al 1971) would yield approximately 70 ml min^{-1} kg^{-1} as the liver blood flow.

Mean hepatic clearance (Y) was then plotted against the mean liver blood flow (X) for all ages of rats tested, yielding a relation $Y = 0{\cdot}926X + 6{\cdot}86$, where $r = 0{\cdot}889$ ($P < 0{\cdot}01$). As expected from the evidence that propranolol has a high extraction ratio, the hepatic clearance was largely dependent on the hepatic blood flow. Similar results have been recently demonstrated in dogs yielding an extraction ratio of 0·89 (Branch & Shand 1976). It might be implied that the reduction in hepatic clearance of propranolol with age would be rationally explained by the decrease in blood flow-dependent pharmacokinetic parameters which relate to its extraction and metabolism in the liver.

In conclusion, a significant age-dependent decrease in the hepatic clearance of propranolol in rats may be largely due to the reduction of the elimination rate accompanied by an age-dependent decrease in hepatic blood flow. Intrinsic hepatic clearance was also relatively high in young adult rats, suggesting that uptake and/or metabolic rate in the liver cells would be more rapid in the younger animals. Furthermore, in immature rats, renal clearance plays a significant role in the total body clearance of propranolol.

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