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Effect of age on the uptake of propranolol by perfused rat lung

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Organs other than the liver possess some ability to eliminate and/or extract drugs or chemicals from the circulation [1]. Some compounds are metabolized in the lungs, whereas others are removed from the circulation and accumulated in the tissue [1]. Extensive first-pass pulmonary elimination (i.e. uptake with high capacity) of propranolol after intravenous administration of 1–10 mg/kg to rats has been reported recently [2]. Furthermore, the first-pass pulmonary clearance and the extraction ratio of the drug tend to increase with age between weeks 3 and 7 and to decrease thereafter [3]. This unique but distinct age-dependence of the first-pass pulmonary clearance of propranolol is not primarily related to the lung blood flow but may be accompanied by possible age-related differences (i.e. from immaturity to senescence) in the uptake capacity and/or affinity [2–4]. However, neither detailed mechanisms nor kinetics of the age-dependent pulmonary elimination (or extraction) of propranolol were investigated. Our previous report, in which the *in vitro* lung perfusion method was used to recirculate, at 8 ml/min, Krebs–Ringer bicarbonate buffer solution (pH 7.4), that contained propranolol and 3% bovine serum albumin (BSA), through the isolated lung of 7-week-old rats, suggested the approximate magnitude of the pulmonary uptake capacity of the drug [4]. This *in vitro* perfusion method also enabled us to examine the effect of age on the pulmonary elimination kinetics of propranolol.

The present work, therefore, was designed to examine the effect of age (both growth and senescence) on the pulmonary clearance of propranolol in 3- to 104-week-old male Wistar rats by analyzing the perfusate drug concentration–time curves after *in vitro* lung perfusion with the drug.

Materials and methods

Materials. *dl*-Propranolol (PPL) hydrochloride was donated by I.C.I.-Pharma, Ltd. (Osaka, Japan). Bovine serum albumin (BSA, fraction V) was purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.) All other chemicals and reagents, including *n*-heptane and isoamyl alcohol (Wako Pure Chemicals Co., Nagoya, Japan) used for the extraction of unchanged PPL from the perfusate or perfused lung tissue, were of analytical grade.

Animals. Male Wistar rats, 3 (55–75 g), 5 (105–135 g), 7 (215–230 g), 11 (350–380 g), 15 (385–410 g), 24 (455–490 g), 52 (605–685 g) and 104 (790–845 g) weeks old, were used throughout the experiments. All rats, purchased from the Shizuoka Laboratory Animal Farm (Hamamatsu, Japan), were housed in a specific pathogen-free room where the relative humidity was kept between 50 and 60% at 22–24° with normal light–dark cycles. The rats were fasted overnight and anesthetized with urethane (800 mg/kg, i.p.) before use. In all the rats used, a macroscopic observation of the lung preparation did not reveal any development of pulmonary infection due to long-term housing. Plasma pH, which is known to be sensitive to alveolar hypoxia caused by most pulmonary infections [1], was also normal, ranging from 7.37 to 7.39.

Perfusion of isolated rat lung. Preparation and perfusion of isolated rat lung were carried out by slightly modifying the previous method [5]. The anesthetized rat was tracheotomized by catheterization and then given positive-pressure ventilation (about 6 to 9 cm and 1.5 to 2 cm H_2O peak inspiratory and end expiratory pressure respectively) into the trachea with warmed (37°), humidifed room air at approximately 70 breaths/min using an animal respirator. Anticoagulation of the animals, exposure of the lungs, and cannulation of the pulmonary artery and vein were carried out as reported previously [4], except that slightly tapered PE-205 tubing was used for the 3-week-old rats. Single-pass perfusion of the lung was started immediately with the Krebs–Ringer buffer solution mentioned previously being oxygenated with 95% O_2–5% CO_2 and perfused at 8 ml/min [4, 5] by a peristaltic pump. During continuous perfusion, the heart and lungs were removed *en bloc*. After ligation to restrict flow through the lungs from the artery to the vein, the buffer solution was replaced by fresh drug solution prepared at 2.5 μg/ml in the same buffer solution, and the heart lung preparation was then mounted in the warmed, humidified chamber of the perfusion apparatus at time zero, with the perfusate recirculating at the same rate as above [4, 5]. An aliquot (0.1 ml) of the perfusate was withdrawn periodically over 60 min from the upper reservoir chamber of the apparatus and used for analysis. Perfusate pH was found to be 7.38 to 7.40 immediately

after perfusion for 60 min. The perfused lung was blotted thoroughly for measurement of its wet weight and then homogenized with control buffer solution (1 to 10).

Pharmacokinetic analysis of perfusate PPL concentration. PPL concentration in the perfusate or the lung homogenate prepared in control pH 7.4 buffer solution was analyzed by slightly modifying the method which was reported previously for plasma drug levels [6]. For analyzing unchanged PPL, an aliquot (0.1 ml) of a perfusate sample or tissue homogenate was subjected to duplicate extractions with 5 ml of *n*-heptane containing 1.5% isoamyl alcohol and with 3 ml of 0.1 N HCl. Fluorometric determination was then carried out with the aqueous phase [6]. Perfusate PPL concentration(C)–time curves were analyzed according to the least-squares regression analysis program MULTI [7] for bi-exponential decline ($C = Ae^{-\alpha t} + Be^{-\beta t}$). Apparent *in vitro* clearance (CL_{perf}) was estimated according to the equation [4, 8]. CL_{perf} = (initial load)/(AUC·lung weight), where the initial load was 87.5 μg/ml and $AUC = A/\alpha + B/\beta$. The cumulative quantities of metabolites in both perfusate and perfused lung tissue were determined by the difference of the total amount of PPL in the perfusate (including the periodical samples) and the tissue homogenate after the perfusion for 60 min from the initial load [4]. The extent of PPL bound to BSA in control perfusate, which was obtained by recirculating the fresh buffer solution containing 3% BSA through the lungs of control rats for 60 min, was evaluated by the equilibrium dialysis method that was carried out at the initial drug concentration of 2.5 μg/ml in the same way as reported previously [9].

Results

Effect of age on the perfusate PPL concentration–time curve. Figure 1 shows the perfusate PPL concentration–time curves obtained in 3-, 7- and 52-week-old rats. In all age groups tested, the perfusate drug level declined bi-exponentially with time. Three-week-old rats showed the slowest elimination, whereas the lungs of 7-week-old rats exhibited the highest elimination rate ($P < 0.01$ between 3 and 7 weeks). The extent of propranolol metabolism evaluated after 60 min of perfusion was less than 2.5 ± 0.7% of the initial load in 3- to 104-week-old rats. Another assay by high performance liquid chromatography analysis showed that, in 7-week-old rats, only 2.3% of the initial load was converted to 4-hydroxy PPL and its conjugates (unpublished data). The extent of drug bound to BSA in control perfusate was almost 85% (ranging from 84.4 ± 3.1 to 85.6 ± 4.2%) and did not show any age-related change.

Effect of age on apparent pulmonary clearance of PPL in vitro. The apparent pulmonary clearance from the perfusion medium, CL_{perf}, of PPL in 3- to 104-week-old rats is shown in Fig. 2. As expected from the results in Fig. 1, 3-week-old rats showed the lowest pulmonary clearance (0.092 ± 0.028 ml/min/g), whereas 7-week-old rats yielded the highest clearance (0.486 ± 0.095 ml/min/g, $P < 0.01$ from 3 weeks). The clearance decreased consistently with age from 7 to 52 weeks (0.214 ± 0.061 ml/min/g, $P < 0.05$ from 7 weeks) but exhibited no further change thereafter. Wet weight of the perfused lung which was used for the calculation of CL_{perf} ranged from 0.739 ± 0.086 to 2.95 ± 0.14 g in 3- to 104-week-old rats.

Relationship between in vivo *pulmonary clearance and* CL_{perf}. The above specific age-dependence in CL_{perf} is very similar to that reported for *in vivo* pulmonary clearance, CL_p, after an intravenous dose (2.5 mg/kg) to 3- to 104-week-old rats [3]. In Fig. 3, CL_p values (Y, ml/min/kg) are plotted against CL_{perf} values (X, ml/min/g) in 3- to 104-week-old rats, showing a fairly good correlation (where $r = 0.967$, $P < 0.01$) expressed as $Y = 39.6X + 1.44$.

Discussion

In vitro lung perfusion studies have received much attention recently, since many drugs or environmental chemicals are known to be taken up or accumulated extensively by the lung *in vivo*. Several recent studies employing lung perfusion experiments have evaluated some possible mechanisms or kinetics of pulmonary elimination of isoproterenol [10, 11] and isosorbide dinitrate [12] in rabbits, verapamil [5], *leu*- and *met*-enkephalins [13] and PPL [4, 14] in rats, and doxorubicin in dogs [15]. However, there have been no reports clarifying either kinetics or age-dependent changes in pulmonary drug elimination by an *in vitro* perfusion technique. The present *in vitro* perfusion experiments, which were carried out in almost the same way as the previous methods [4, 5, 14], enabled us to explain the specific *in vivo* age-dependence in the first-pass pulmonary elimination of PPL, which exhibited an increase and decrease in the pulmonary clearance in rats aged from 3 to 7 and from 7 to 104 weeks respectively [3].

The perfusate PPL level in the present study, which

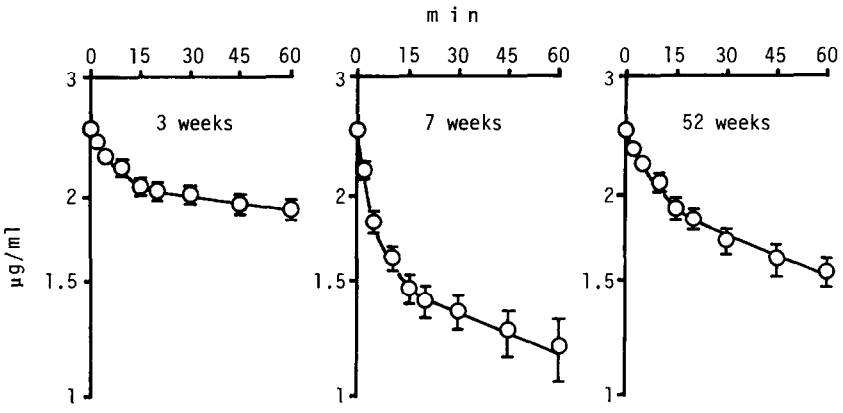

Fig. 1. Time-course of perfusate propranolol levels (μg/ml) when 3-, 7- or 52-week-old rat lungs were perfused with propranolol. The drug (2.5 μg/ml) prepared in pH 7.4 Krebs–Ringer bicarbonate buffer solution (35 ml) containing 3% BSA, oxygenated with 95% O_2–5% CO_2, was recirculated at a rate of 8 ml/min and 37°. Each point is the mean ± SD of four rats. The solid line indicates the computer-fitted bi-exponential curve weighted with the reciprocal of the perfusate drug concentration.

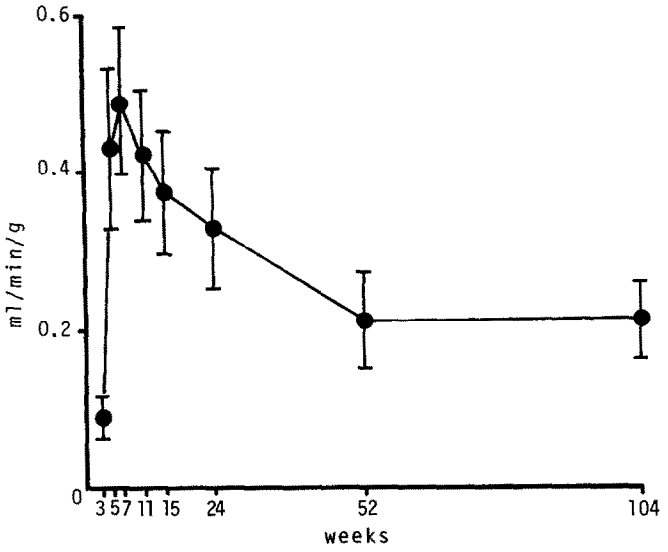

Fig. 2. Effect of age (weeks) on pulmonary perfusion clearance (ml/min/g) of propranolol in rats. The perfusion conditions were the same as those described in the legend of Fig. 1. The perfusion clearance (CL_{perf}, ml/min/g) was estimated by the equation, CL_{perf} = (initial load)/(AUC·lung weight), where the initial load refers to the initial amount of the drug in whole perfusate (i.e. 87.5 μg). Each point is the mean ± SD of four rats. Significant differences were found at 3 weeks vs other weeks ($P < 0.01$) and at 52 or 104 weeks vs 5 to 15 weeks ($P < 0.05$).

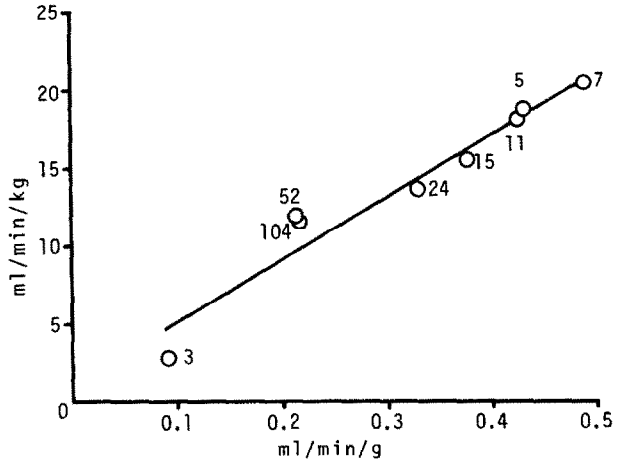

Fig. 3. Correlation between *in vivo* pulmonary clearance (CL_p, ml/min/kg) and *in vitro* perfusion clearance (CL_{perf}, ml/min/g) of propranolol in rats. CL_p values have been estimated in a previous report [3]. The numbers indicate the age (weeks) of the rats. The solid line represents the regression line for this correlation ($r = 0.967$, $P < 0.01$) between both mean values as $Y = 39.6X + 1.44$, where Y and X represent CL_p and CL_{perf} respectively.

simulated the plasma drug level after a previous bolus i.v. injection at 2.5 mg/kg [2, 3], showed a bi-exponential decline with time in all age groups (Fig. 1). This kinetic aspect is consistent with previous *in vivo* elimination kinetics which show a bi-exponential time-course [2, 3]. Furthermore, the small extent of *in vitro* pulmonary metabolism of PPL (less than 2.5% of the initial load) is also comparable to previous results *in vivo* [2, 3]. It has also been reported that little or no metabolism of PPL takes place under similar *in vitro* experimental conditions [14]. In view of these results, it is logical, therefore, to assume that the lung does not metabolize much PPL but accumulates (or extracts from the circulation) the drug extensively. Tissue/medium concentration ratios (T/M ratios) at 5 and 60 min after perfusion were fairly large, e.g. approximately 15 and 35, respectively, in 7-week-old rats. These ratios were found to be almost comparable to those reported previously [14].

Apparent pulmonary clearance from perfusion medium *in vitro*, CL_{perf}, of PPL exhibited almost the same age-dependence as the pulmonary clearance *in vivo*, CL_p, suggesting that immaturity and reduction of the pulmonary ability to extract this drug affect relatively young and senescent animals respectively (Fig. 2). The initial drug concentration (2.5 μg/ml) was considered low enough to yield a linear elimination kinetics in all age groups [2, 4]. Therefore, the specific age-dependence in the present *in vitro* pulmonary clearance may not be mainly related to its capacity. A fixed recirculation rate (8 ml/min) may give relatively high and low perfusion of the lungs of younger

and aged rats respectively. However, our previous report has indicated an incomplete correlation between CL_p and apparent lung blood flow in 3- to 104-week-old rats [3]. Another perfusion study in 7-week old rats also suggested an absence of flow dependence of either CL_{perf} or T/M ratio at 8–16 ml/min (unpublished data). Thus, the apparent pulmonary clearance in the aged rats (probably 52 weeks or older) may be a little underestimated due to a possible reduction in the flow per gram of tissue.

There is a fairly good correlation, however, between the previous *in vivo* CL_p (ml/min/kg) and the present *in vitro* CL_{perf} (ml/min/g). This suggests that there may be some age-related specificity (per unit weight) of the lung to extract PPL, such as a possible age-related affinity of a particular tissue component(s) of the lung to bind the drug. Other simple *in vitro* tissue uptake (or binding) experiments would be favored to clarify this point.

In conclusion, the present results from the *in vitro* lung perfusion experiments completely support the specific age-dependence in the previous *in vivo* pulmonary clearance, suggesting that some age-related changes in the affinity of the lung tissue to extract (or accumulate) PPL may play an important role in the age-dependent pulmonary clearance of this drug.

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Department of Pharmacy KIKUO IWAMOTO*
Shimane Medical University Hospital
Izumo 693, Japan

Faculty of Pharmaceutical Sciences JUN WATANABE
HINA YONEKAWA
Nagoya City University
Mizuho-ku, Nagoya 693, Japan

* Author to whom correspondence should be addressed.

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