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Propranolol uptake with high capacity by rat perfused lung

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Abstract—Lung isolated from 7-week-old rats was perfused with pH 7·4 Krebs-Ringer bicarbonate buffer solution (35 mL) containing 1 to 100 $\mu g\ mL^{-1}$ of propranolol and 3% BSA at the recirculation rate of 8 $mL\ min^{-1}$. Almost parallel bi-exponential drug concentration-time curves were obtained at the initial load lower than 10 $\mu g\ mL^{-1}$, whereas relatively slow, mono-exponential decline was found after perfusion at 100 $\mu g\ mL^{-1}$. Pharmacokinetic analysis for the perfusate propranolol concentration-time curves when loaded at 1 to 10 $\mu g\ mL^{-1}$ yielded almost comparable values for the pulmonary perfusion clearance ($0{\cdot}387 \pm 0{\cdot}092$ to $0{\cdot}486 \pm 0{\cdot}095$ $mL\ min^{-1}\ g^{-1}$). In contrast, this parameter was significantly reduced at 100 $\mu g\ mL^{-1}$ ($0{\cdot}113 \pm 0{\cdot}042$ $mL\ min^{-1}\ g^{-1}$). The present findings suggest a trend towards saturation kinetics in the in-vitro pulmonary clearance of propranolol.

In our previous reports, it has been suggested that the first-pass pulmonary elimination of propranolol after the intravenous administration to rats may be driven predominantly by the rapid and extensive uptake by the lung (Iwamoto et al 1987) and may have a particular age-dependence (Iwamoto et al 1988a). However, the detailed mechanism and kinetics of pulmonary propranolol clearance, including the magnitude of its capacity (i.e. saturation kinetics) to extract the drug from the circulation, were not clarified yet in any animal species. This was simply because high enough dose to yield a saturation of its pulmonary clearance was not accessible in-vivo (Pang et al 1982; Roth 1984; Iwamoto et al 1987).

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Well-designed in-vitro organ perfusion experiments may enable us to predict some possible mechanisms or kinetics for the dose- or age-dependent organ clearance of drugs by modifying physiological or pharmacological condition such as flow rate or initial drug level in the perfusate. Our previous work has proposed the prerequisite of the in-vitro perfusion condition for examining propranolol uptake by the rat lung, i.e. to recirculate the isolated organ with pH 7·4 Krebs-Ringer bicarbonate buffer solution containing 3% bovine serum albumin at the flow rate of 8 $mL\ min^{-1}$ (Iwamoto et al 1988b).

The present work was, therefore, designed to estimate an approximate magnitude of the in-vitro pulmonary capacity of 7-week-old rats to clear propranolol from the perfusate by analysing the perfusate drug concentration-time curves after the various initial loads.

Materials and methods

Materials. Propranolol hydrochloride (racemate) was donated by I.C.I-Pharma, Ltd. (Osaka, Japan). Bovine serum albumin (BSA, fraction V) was purchased from Sigma Chemical Co. (St. Louis, USA). Spectrapor membrane tubing (Type 2, MW 12000–14000), used for equilibrium dialysis of propranolol bound to BSA, was purchased from Spectrum Med. Ind. Inc. (Los Angeles, USA). All other chemicals including n-heptane and iso-amylalcohol used to extract unchanged propranolol from its metabolites were of analytical grade.

Perfusion of isolated lung with propranolol. Male Wistar rats, 7 weeks old (215–235 g) which had been fasted overnight were anaesthetized with urethane (800 mg kg^{-1} i.p.). Immediately after catheterization into the trachea, positive-pressure ventilation with warmed (37°C), humidified room air was initiated in the same way as reported previously (Iwamoto et al 1988b). The lung was exposed gently by the midline thoracotomy after anticoagulating the animal. Cannulations into pulmonary artery and vein were then provided with PE-205 tubing (i.d. 1·57 mm; o.d. 2·08 mm, Intramedic, Clay Adams, Parsippany, USA) and single-pass lung perfusion was initiated with pH 7·4 Krebs-Ringer bicarbonate buffer solution containing 3% BSA and oxygenated with 95%O_2–5%CO_2 at the flow rate of 8 mL min^{-1} by a peristaltic pump.

The perfusate was replaced by the same buffer solution containing propranolol at 1, 2·5, 5, 10 or 100 $\mu g\ mL^{-1}$ for 2 min after the lung tissue was carefully isolated from the rat. The isolated lung was then placed into the air-tight lung chamber of the perfusion apparatus (Iwamoto et al 1988b) which was equipped with essentially the same devices as those reported for rabbit lung (Brazzell et al 1982). The fresh buffer solution (35 mL) containing the drug at 1 to 100 $\mu g\ mL^{-1}$ was initiated to recirculate through the lung at 8 mL min^{-1}. Periodic sampling (0·1 mL) of the perfusate over 60 min, measuring the perfusate pH and the organ wet weight and homogenizing the organ after 60 min perfusion, were carried out in the same way as reported previously (Iwamoto et al 1988b).

Binding of propranolol to perfusate BSA. The extent of binding of propranolol to 3% BSA in the control perfusate was determined by the same equilibrium dialysis method as reported previously (Iwamoto et al 1985) using Spectrapor membrane tubing at 37°C. One mL of the inner phase (pH 7·4 Krebs-Ringer bicarbonate buffer solution containing 3% BSA) spiked with 1 to 100 $\mu g\ mL^{-1}$ of propranolol was dialysed against 1 mL of BSA free buffer solution spiked with the same drug level as that in the inner phase. Equilibrium was attained within 4 h, after which the drug concentrations in both inner and outer phases were determined.

Analytical procedures. Propranolol concentration in the perfusate sample, tissue homogenate or inner or outer phase after the equilibrium dialysis was analysed according to the method reported previously (Iwamoto & Watanabe 1984). Perfusate drug concentration (C)-time curve was analysed according to the least-squares regression analysis program MULTI (Yamaoka et al 1981) for bi- or mono-exponential decline, i.e. $C=Ae^{-\alpha t}+Be^{-\beta t}$ or $C=A'e^{-\alpha' t}$ where A, B, α and β are hybrid parameters. AUC was estimated by the equation, $AUC=A/\alpha+B/\beta$ or $AUC=A'/\alpha'$ and perfusion clearance (CL_{perf}) by the equation, CL_{perf}=(initial load)/AUC × lung weight), where the initial load was 35 to 3500 μg (1 to 100 $\mu g \times 35$ mL), as reported previously (Iwamoto et al 1988b). Amount of metabolites in both perfusate and tissue homogenate at 60 min was determined by the difference of the total amount of the unchanged drug remained in both samples from the initial load (35 to 3500 μg) at time zero.

Results and discussion

Effect of propranolol concentration on its pulmonary elimination by rat perfused lung. Effect of the initial propranolol concentration on its disappearance from the recirculating (at 8 mL min^{-1}) perfusate by 7-week-old rat lung is shown in Fig. 1. Almost parallel bi-exponential time-courses for propranolol disappearance were found in the perfusates with 1 to 10 $\mu g\ mL^{-1}$, whereas relatively slow, mono-exponential decline was observed at 100

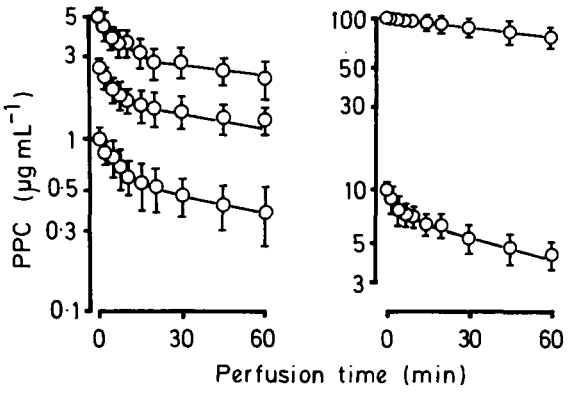

FIG. 1. Effect of initial perfusate propranolol concentration (PPC) on its elimination by the perfused 7-week-old rat lung. The lung was perfused from its artery to vein with Krebs-Ringer bicarbonate buffer solution (pH 7·4, 35 ml as the initial volume) containing 1, 2·5, 5, 10 or 100 $\mu g\ mL^{-1}$ (as the initial concentration) of the drug and 3% BSA, oxygenated with 95%O_2-5% CO_2 at the recirculation rate of 8 mL min^{-1} and 37°C. Each point is the mean ± s.d. of four rats. The solid line indicates the computer-fitted bi-exponential or mono-exponential curve weighted with the reciprocal of the perfusate drug concentration.

$\mu g\ mL^{-1}$. The initial drug concentration range from 1 to 10 $\mu g\ mL^{-1}$ referred to that measured in the plasma levels immediately after the bolus intravenous administration with 1 to 10 mg kg^{-1} to 7-week-old rats (Iwamoto et al 1987). The present results obtained with this relatively low initial concentration range, therefore, were consistent with the previous in-vivo results which did not show any saturation in the pulmonary elimination kinetics and/or clearance after the i.v. dosage with 1 to 10 mg kg^{-1} (Iwamoto et al 1987). Terminal elimination half-life at 100 $\mu g\ mL^{-1}$ was approximately 1·8 times those obtained at other lower concentrations. This may be due to the limited capacity for the lung to eliminate propranolol from the perfusate containing relatively high drug levels. The extent of propranolol metabolism for the 60 min perfusion was found to be less than 2·5% of any initial load.

Dose-dependent pulmonary perfusion clearance of propranolol. Pulmonary clearance of propranolol was estimated from pharmacokinetic analysis of the above results shown in Fig. 1. Fig. 2 summarizes the effect of the initial propranolol level on its pulmonary clearance. There was no significant difference in the perfusion clearance values estimated at the initial drug levels from 1 to 10 $\mu g\ mL^{-1}$, ranging from 0·387 ± 0·092 to 0·486 ± 0·095 mL $min^{-1}\ g^{-1}$. In contrast, the perfusion clearance was substantially reduced to 0·113 ± 0·042 mL $min^{-1}\ g^{-1}$ when the lung was perfused with 100 $\mu g\ mL^{-1}$ of the drug. This concentration-dependence was almost the same as that reported on the initial accumulation (up to 5 min) of propranolol in the rat lung which had been perfused under the similar experimental conditions (Dollery & Junod 1976). When the present in-vitro perfusion clearance value at 1 to 10 $\mu g\ mL^{-1}$ of propranolol was normalized by relation to the body weight of the animal, it corresponded to only about one-eighth of the in-vivo pulmonary clearance (Iwamoto et al 1987). This discrepancy might be largely due to the isolation of the organ and the significant difference of the perfusion medium from the intact, circulating blood in spite of the optimum flow rate and an adequate BSA level as have been employed for the lung perfusion with other drugs in rats and rabbits (Brazzell & Kostenbauder 1982; Brazzell et al 1982; Gillespie et al 1984). However, the present results show a trend towards saturation of the pulmonary

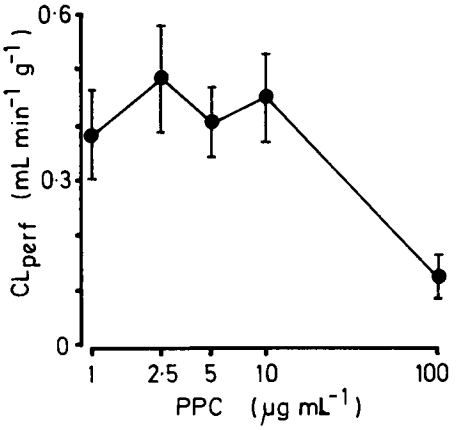

FIG. 2. Effect of initial perfusate propranolol concentration (PPC) on its pulmonary perfusion clearance by the 7-week-old rat lung. The perfusion condition was the same as that described in Fig. 1. The perfusion clearance (CL_{perf}) was estimated by the equation, CL_{perf} = (Initial load)/AUC·lung weight), where the initial load refers to the initial amount of the drug in the whole perfusate (35 to 3500 μg) and AUC by the equation, $AUC = A/\alpha + B/\beta$ or $AUC = A'/\alpha'$. Each point is the mean ± s.d. of four rats. Significant difference was found at 100 μg mL^{-1} as compared with other conditions ($P < 0.05$).

perfusion clearance of propranolol over the concentration range from 10 to 100 μg mL^{-1} and support qualitatively previous in-vivo findings, which suggested an absence of any saturation kinetics in the drug's pulmonary elimination after the i.v. dosage with doses of 1 to 10 mg kg^{-1}. Furthermore, the capacity of the perfused rat lung to take up propranolol may be extremely high compared with uptake reported in other organs such as liver (Iwamoto & Watanabe 1985; Iwamoto et al 1985).

Table 1. The effect of the initial propranolol concentration on its binding to BSA in the perfusate. [a] One mL of the control perfusate (pH 7·4 Krebs-Ringer bicarbonate buffer solution containing 3% BSA an inner phase) spiked with propranolol (1 to 100 μg mL^{-1}) was dialysed against 1 mL of BSA-free control buffer solution spiked with the same initial drug concentation as that of the inner phase using Spectrapor membrane at 37°C for 4 h.

Initial drug concn (μg mL^{-1})	Bound fraction ± s.d. (%)[a] (n = 4)
1	86·3 ± 5·2
2·5	85·1 ± 4·6
5	82·3 ± 6·7
10	80·1 ± 5·3
100	67·2 ± 5·8

Extent of propranolol binding to BSA in the perfusate. The extent of propranolol bound to 3% BSA in the perfusate was evaluated at each initial concentration as shown in Table 1. At a relatively low concentration (i.e. to 10 μg mL^{-1}), the extent of protein binding tended to decrease with the initial concentration. Increase in the initial drug concentration to 100 μg mL^{-1} significantly reduced the extent of binding (to 67·2 ± 5·8% $P < 0.05$). However, some possible effect such as an enhancement of the perfusion clearance at 100 μg mL^{-1} due to the significant increase in the unbound drug fraction might be far smaller than the saturation effect, yielding a net reduction in the perfusion clearance as shown in Fig. 2.

In conclusion, the present in-vitro data are compatible with the previous in-vivo results in that no saturation of pulmonary uptake of propranolol occurs up to drug concentration of 10 μg mL^{-1}. This implies that the saturation of pulmonary uptake of the drug may occur in-vivo at concentrations above 10 μg mL^{-1} if these are achievable.

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