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COMMUNICATIONS

Effects of bovine serum albumin and recirculation rate on the uptake of propranolol by rat perfused lung

KIKUO IWAMOTO, JUN WATANABE*, HINA YONEKAWA*, *Department of Pharmacy, Shimane Medical University Hospital, Izumo 693, *Department of Biopharmaceutics, Faculty of Pharmaceutical Sciences, Nagoya City University, Nagoya 467, Japan*

Abstract—Lungs isolated from 7-week-old rats were perfused with pH 7·4, oxygenated Krebs-Ringer bicarbonate buffer solution containing 2·5 μg mL^{-1} of propranolol and 3 to 5% BSA at the recirculation rate of 4 to 16 mL min^{-1}, at 37°C for 60 min. The extent of propranolol metabolism after 60 min was less than 2·3% of the initial load under any in-vitro perfusion condition. Therefore, the amount disappearing from the perfusion medium was considered as being predominantly that taken up by tissue. Under all experimental conditions, perfusate drug level declined bi-exponentially with time. Apparent in-vitro pulmonary clearance of propranolol was not affected by the increase of BSA level from 3 to 5%. When the perfusate BSA level was fixed at 3%, the lowest recirculation rate (4 mL min^{-1}) yielded the smallest clearance (about 0·15 mL min^{-1} g^{-1}) but almost constant clearance value (about 0·40 mL min^{-1} g^{-1}) was obtained at the rate ranging from 8 to 16 mL min^{-1}. The tissue to medium concentration ratio of propranolol, after the perfusion with 3 to 5% BSA at the rate of 8 to 16 mL min^{-1}, was approximately 35, whereas that with 3% BSA at 4 mL min^{-1} was reduced to about 20. The findings suggest evidence for flow-dependent in-vitro pulmonary clearance of propranolol.

Propranolol is extensively extracted from the circulation by a first-pass through rat lungs after intravenous administration and this is age-dependent (Iwamoto et al 1987, 1988). However, the mechanism and kinetics of this extraction (or uptake) have not been clarified. Well-designed in-vitro lung perfusion experiments may enable possible mechanisms or kinetics to be predicted for the pulmonary clearance of drugs by modifying key physiological or biochemical conditions. A trend towards saturable accumulation (or uptake) kinetics of propranolol has been proposed in the lungs isolated from the rats of 200–300 g, perfused with an artificial medium containing 4·5% bovine serum albumin (BSA) at the rate of 10 mL min^{-1} (Dollery & Junod 1976). Plasma albumin levels exhibit specific age-dependent change in rats (Iwamoto et al 1985). While a similar but less marked age-dependence was shown by the apparent lung blood flow measured by the hydrogen gas clearance method (Iwamoto et al 1988).

The present work was set out to examine the effects of BSA level in artificial perfusion medium and its recirculation rate on the in-vitro clearance of propranolol by the lungs isolated from 7-week-old rats.

Materials and methods

Preparation and perfusion of isolated lungs. Male Wistar rats, 7 weeks old (210–235 g), were anaesthetized with urethane (800 mg kg^{-1} i.p.) after an overnight fasting. Immediately after the tracheotomy with catheterization, positive-pressure ventilation (about 8 and 2 cm H_2O peak inspiratory and expiratory pressure, respectively) with warmed (37°C), humidified room air was started at approximately 70 breaths min^{-1} using an animal respirator. Lungs were exposed by a midline thoracotomy and the isolated rat lungs were prepared according to Gillespie et al (1984). The pulmonary artery and vein were cannulated with PE-205 tubing (o.d. 2·08 mm, i.d. 1·57 mm). Single-pass perfusion of the lungs was immediately started with warmed, pH 7·4 Krebs-Ringer bicarbonate buffer solution containing 3, 4 or 5% bovine serum albumin (BSA, fraction V, fatty acid free, Sigma Chemical Co., St. Louis, USA) and oxygenated with 95%O_2–5%CO_2, at the rate of 8 mL min^{-1} by a peristaltic pump. During continuous perfusion, the lungs were isolated from the rats.

The perfusion buffer solution was completely replaced by a 2-min single-pass of drug (propranolol hydrochloride, ICI Pharma, Ltd., Osaka, Japan) solution prepared at 2·5 μg (as base) mL^{-1} in the same buffer solution. The lungs were then mounted in the warmed, humidified, air-tight chamber of the perfusion apparatus which was equipped with essentially the same devices as those reported for rabbit lungs (Brazzell et al 1982). At time zero, the pump was adjusted to recirculate the lungs with 35 mL of the same fresh drug solution as described above at the rate of 4, 8, 12 or 16 mL min^{-1}. An aliquot (0·1 mL) of the perfusate was withdrawn periodically over 60 min and used for analysis. Perfusate pH was found to be kept at 7·38 to 7·41 in any preparation after 60 min, showing no evidence for hypoxia of the isolated tissue. The perfused lung was rinsed with the fresh buffer solution, blotted thoroughly with filter paper for the measurement of its wet weight and then homogenized with control buffer solution (1 to 10 mL).

The extent of propranolol bound to 3 to 5% BSA in the control Krebs-Ringer bicarbonate buffer solution was determined by the same equilibrium dialysis method as reported previously (Iwamoto et al 1985).

Extents of metabolism and uptake of propranolol by perfused lungs. Cumulative amount of metabolites in both perfusate and tissue homogenate after 60 min was determined by the difference of the total amount of the intact drug remained in both samples from the initial load, 87·5 μg at time zero.

Tissue to medium (T/M) concentration ratio (μg g^{-1}/μg mL^{-1}) was also estimated for the intact drug after a 60-min perfusion.

Assay of propranolol. Propranolol concentration in the perfusate sample, tissue homogenate or inner or outer phase after the equilibrium dialysis was analysed by slightly modifying the method of Iwamoto & Watanabe (1985).

Pharmacokinetic analysis of perfusate drug levels. Perfusate propranolol concentration (C)-time curve was analysed accord-

Correspondence to: K. Iwamoto, Department of Pharmacy, Shimane Medical University Hospital, Izumo 693, Japan.

ing to least-squares regression analysis program MULTI (Yamaoka et al 1981) for bi-exponential decline expressed as $C = Ae^{-\alpha t} + Be^{-\beta t}$, where A, B, α and β are hybrid pharmacokinetic parameters. The best fit was achieved by weighting with the reciprocal of C. Apparent in-vitro clearance by perfusion per lung weight (CL_{perf}) was estimated by the equation (Iwamoto et al 1985, 1986). CL_{perf} = (Initial load)/(AUC · lung weight), where AUC is the area under the perfusate concentration-time curve $(A/\alpha + B/\beta)$. All of the present calculations were done on data obtained from each rat (not pooled).

Results and discussion

Effects of BSA and recirculation rate on the removal of propranolol from the perfusate. Isolated rat lungs were perfused with 2·5 μg mL^{-1} of propranolol and 3 to 5% BSA at the rate of 4 to 16 mL min^{-1}. Under any experimental condition, the extent of propranolol metabolism after the perfusion for 60 min was less than 2·3% of the initial load. This almost insignificant extent of propranolol metabolism by the perfused lung was consistent with our previous in-vivo results (Iwamoto et al 1987, 1988). As perfusion experiments with medium containing less than 3% of BSA resulted immediately in massive oedema, probably due to lower osmotic pressure, BSA at 3% or higher was considered to be pre-requisite to the normal physiological condition for the isolated, perfused rat lungs. Previous workers (Dollery & Junod 1976; Brazzell et al 1982; Gillespie et al 1984) have also employed 3 or 4·5% BSA in the perfusate for rat and rabbit lungs.

Under the present experimental conditions with 3 to 5% BSA at a recirculation rate of 4 to 16 mL min^{-1}, each time course for perfusate propranolol concentration showed a bi-exponential decline with time, which was similar to that for plasma levels reported previously in-vivo (Iwamoto et al 1987, 1988). Almost identical perfusate drug level-time courses were obtained with 3, 4 and 5% BSA when recirculated at 8 mL min^{-1}. These three BSA levels did not make any difference to pharmacokinetic parameters estimated for the perfusate propranolol as summarized in Table 1. The extent of propranolol bound to 3, 4 and 5%

Table 1. Effect of BSA on pharmacokinetic parameters for propranolol after perfusion with the initial load of 87·5 μg (35 mL of 2·5 μg mL^{-1}) at 8 mL min^{-1} in isolated rat lungs.

	BSA level (%)		
Parameter[a]	3	4	5
A (μg mL^{-1})	0·750 ± 0·291[b]	0·816 ± 0·188	0·845 ± 0·202
B (μg mL^{-1})	1·74 ± 0·29	1·68 ± 0·25	1·65 ± 0·33
α (min^{-1})	0·194 ± 0·053	0·187 ± 0·041	0·209 ± 0·036
β (min^{-1})	0·0096 ± 0·0034	0·0091 ± 0·0027	0·0099 ± 0·0031
$t_{1/2\beta}$ (min)	72·1 ± 28·6	76·2 ± 27·7	70·1 ± 24·8
AUC (μg min mL^{-1})	181 ± 44	189 ± 41	171 ± 38
Lung weight (g)	1·24 ± 0·13	1·20 ± 0·15	1·28 ± 0·11
CL_{perf} (mL min^{-1} g^{-1})	0·399 ± 0·067	0·389 ± 0·089	0·399 ± 0·060

[a] Estimated from each rat by weighting with 1/(perfusate concn).
[b] Mean ± s.d. of four rats.

BSA in the perfusion medium was 83·4 ± 3·6, 88·2 ± 3·2 and 90·3 ± 2·9%, respectively. Thus, the substantial change of the unbound fraction (17 to 10% with 3 to 5% BSA) did not affect the disappearance rate of propranolol from the perfusate, yielding almost equivalent pulmonary clearance of the drug, approximately 0·40 mL min^{-1} g^{-1}. This result suggests that there may be no contribution of 'protein-mediated' mechanism in the pulmonary clearance of propranolol in rats.

Perfusate BSA level was then fixed at 3% to examine the effect of recirculation rate on the removal of propranolol by rat lungs.

Table 2. Effect of recirculation rate on pharmacokinetic parameters for propranolol after the initial load of 87·5 μg with 3% BSA in isolated rat lungs.

	Recirculation rate (mL min^{-1})		
Parameters[a]	4	12	16
A (μg mL^{-1})	0·743 ± 0·296[b]	1·08 ± 0·38	0·911 ± 0·316
B (μg mL^{-1})	1·78 ± 0·22	1·41 ± 0·27	1·58 ± 0·38
α (min^{-1})	0·0754 ± 0·0317[c]	0·138 ± 0·028	0·163 ± 0·039
β (min^{-1})	0·0043 ± 0·0019[c]	0·0087 ± 0·0023	0·0089 ± 0·0036
$t_{1/2\beta}$ (min)	160 ± 47	80·0 ± 27·4	77·5 ± 25·1
AUC (μg min mL^{-1})	422 ± 101	171 ± 31·9	192 ± 40·6
Lung weight (g)	1·34 ± 0·16	1·35 ± 0·15	1·25 ± 0·17
CL_{perf} (mL min^{-1} g^{-1})	0·155 ± 0·049	0·379 ± 0·090	0·394 ± 0·073

See parameter values at 8 mL min^{-1} in Table 1 (with 3% BSA).
[a] Estimated from each rat by weighting with 1/(perfusate concn.).
[b] Mean ± s.d. of four rats.
[c] Significantly different from the estimates at other rates ($P < 0·05$).

Table 2 summarizes the pharmacokinetic parameters for propranolol when the lungs were perfused with 2·5 μg mL^{-1} of the drug and 3% BSA at 4 to 16 mL min^{-1}. The lowest flow yielded the smallest values for both α and β ($P < 0·05$). In contrast, there was no significant difference in either the perfusate drug level-time course or each resultant pharmacokinetic parameter value among the other recirculation rates, 8, 12 and 16 mL min^{-1} (Table 1, Table 2). The decrease in the recirculation rate from 8 (or higher) to 4 mL min^{-1} reduced the clearance to about 0·15 mL min^{-1} g^{-1} but there was no difference in the clearance among the rates from 8 to 16 mL min^{-1}. It was, therefore, suggested that the apparent clearance of propranolol by the rat isolated lung might be 'flow-limited' in the region lower than 8 mL min^{-1}. This critical rate or slightly higher one (10 mL min^{-1}), which is considered to approximately correspond with the in-vivo lung plasma flow (Bischoff et al 1971), has also been employed in the previous studies (Dollery & Junod 1976; Gillespie et al 1984) employing the rats of almost the same size (i.e. the same age) as that in the present experiments.

T/M ratio of propranolol after the perfusion. The amount of propranolol that disappeared from the perfusate was thought to refer to predominantly that taken up by the lung tissue, since the amount of metabolites was almost insignificant in both tissue and medium. Table 3 summarizes the effects of perfusate BSA level and recirculation rate on the extent of pulmonary uptake (T/M ratio) of propranolol after the perfusion with 2·5 μg mL^{-1} of the drug. Any BSA level from 3 to 5% at the fixed recirculation rate, 8 mL min^{-1}, yielded almost constant T/M ratio of approximately 35. When BSA level was fixed at 3% and the recirculation rate was decreased to 4 mL min^{-1}, the T/M ratio was reduced to about 20. However, there was no significant

Table 3. Effects of BSA level and recirculation rate on the extent of propranolol uptake (T/M ratio) by isolated, perfused rat lungs.

	T/M ratio[a] at recirculation rate (mL min^{-1})			
BSA level (%)	4	8	12	16
3	20·3 ± 4·1[b]	35·2 ± 6·9	32·6 ± 7·4	34·7 ± 7·0
4	n.d.[c]	34·6 ± 7·1	n.d.	n.d.
5	n.d.	35·1 ± 6·6	n.d.	n.d.

[a] (μg g^{-1})/(μg mL^{-1}).
[b] Mean ± s.d. of four rats.
[c] Not determined.

difference in the T/M ratio among the rates ranging from 8 to 16 mL min^{-1}. The present flow-dependence of pulmonary uptake and/or clearance of propranolol might not be due to any unphysiological effect of the lowest flow, since there was, for example, no evidence for either hypoxia or oedema of the tissue perfused at 4 mL min^{-1}.

From the present results, it was suggested that the in-vitro pulmonary clearance of propranolol might not be dependent on the extent of plasma protein binding but be largely dependent on the change in the lung blood flow.

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References

Bischoff, K. B., Dedrick, R. L., Zaharko, D. S., Lonstreth, J. A. (1971) Methotrexate pharmacokinetics. J. Pharm. Sci. 60: 1128–1133.

Brazzell, R. K., Smith, R. B., Kostenbauder, H. B. (1982) Isolated perfused rabbit lung as a model for intravascular and intrabronchial administration of bronchodilator drugs I: Isoproterenol. Ibid. 71: 1268–1274.

Dollery, C. T., Junod, A. F. (1976) Concentration of (±)–propranolol in isolated, perfused lungs of rat. Br. J. Pharmacol. 57: 67–71.

Gillespie, M. N., Felder, T. B., Blanford, S. L., Reinsel, C. N., Kostenbauder, H. B. (1984) Pulmonary disposition and pharmacodynamics of verapamil. J. Cardiovas. Pharmacol. 5: 802–807.

Iwamoto, K., Watanabe, J. (1985). Avoidance of first-pass metabolism of propranolol after rectal administration as a function of the absorption site. Pharm. Res. 2: 53–54.

Iwamoto, K., Watanabe, J., Aoyama, Y. (1987) High capacity for pulmonary first-pass elimination of propranolol in rats. J. Pharm. Pharmacol. 39: 1049–1051.

Iwamoto, K., Watanabe, J., Aoyama, Y. (1988) Age-dependent pulmonary first-pass elimination of propranolol in rats. Ibid. 40: 135–137.

Iwamoto, K., Watanabe, J., Araki, K., Deguchi, N., Sugiyama, H. (1985) Effect of age on the hepatic clearance of propranolol in rats. Ibid. 37: 466–470.

Iwamoto, K., Watanabe, J., Satoh, M., Deguchi, N., Sugiyama, H. (1986) Age-dependent propranolol clearance in perfused rat liver. Biochem. Pharmacol. 35: 1149–1152.

Yamaoka, K., Tanigawara, Y., Nakagawa, T., Uno, T. (1981) A Pharmacokinetic analysis program (MULTI) for microcomputer. J. Pharm. Dyn. 4: 879–885.

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Analgesic effects of 3-methoxybenzamide in rats

ROBERT SANDERS, RUSSELL E. DILL, ROY L. DORRIS, EDWARD G. MILLER, *Departments of Anatomy, Pharmacology and Biochemistry, Baylor College of Dentistry, Dallas, TX 75246, USA*

Abstract—The i.p. injection of 3-methoxybenzamide (3-MBA) in rats produces a dose-related elevation of the threshold for response to a painful stimulus. Metoclopramide, also a substituted benzamide, has analgesic activity that is attenuated by bromocriptine, a dopamine receptor agonist, and by the narcotic antagonist, naloxone, suggesting involvement of dopamine and opiate receptors in the action of this drug. The involvement of these receptors in the analgesic action of 3-MBA has been examined using L-dopa and naloxone. Neither significantly altered the analgesic action. Although the results are preliminary, the analgesic action of 3-MBA would not seem to occur via opiate or dopamine receptors.

In a study of the tumour-promoting effects of 3-methoxybenzamide (3-MBA) in hamsters, the drug appeared to make the animals insensitive to pain, inducing a state closely resembling general anaesthesia (Miller et al 1986). When the drug was administered to rats, the anesthetic-like state did not ensue, but the animals were lethargic and less sensitive to painful stimuli. Experiments were therefore designed to evaluate this apparent analgesic effect in rats. Since there is evidence for the involvement of opiate and dopaminergic receptors in the analgesic effects of metoclopramide, a substituted benzamide (Ramaswamy & Bapna 1986), the potential roles of these receptors in the analgesic effects of 3-MBA were investigated.

Materials and methods

Male outbred Sprague-Dawley rats, ca 250 g, were used. Tests for analgesia were as described by Swingle et al (1971) using an "Analgesy-Meter" (Varese, Italy), which provides a measure of pain threshold by increasing amounts of pressure applied to the web structure of the paw until the paw is withdrawn. Baseline or predrug responses to paw pressure were determined on forty rats. Subsequently, these rats were divided into five equal groups. Groups I, II, and III were injected i.p. with either 100, 200 or 300 mg kg^{-1} 3-MBA (Aldrich Chemical CO., Milwaukee, WI) prepared in 100% DMSO (Sigma Chemical Co., St. Louis, MO). The response (pain threshold) was measured as before at 0·5, 1·5 and 2·5 h postinjection. Groups IV and V were similarly treated with 100% DMSO or physiological saline, respectively, and tested for pain threshold as before. The means of the measurements of each group at each time interval were compared (using the Student's *t*-test) to the means from the DMSO controls. DMSO is known to produce mild analgesia in animals (Haigler & Spring 1983), thus, the means of the DMSO treated animals were also compared with saline treated animals.

Correspondence to: R. E. Dill, Department of Anatomy, Baylor College of Dentistry, 3302 Gaston Avenue, Dallas, TX 75246, USA.

To test for the possibility that 3-MBA produced its effects via opiate receptors, eight male rats were given 300 mg kg^{-1} 3-MBA as before and the analgesia test applied at 0·5 h post-injection. Immediately following the 0·5 h test, each rat received 0·5 mg kg^{-1} naloxone HCl (DuPont Pharmaceuticals, Manati, Puerto Rico). The analgesia test was repeated at 1·5 and 2·5 h after 3-MBA injection.

To test for dopaminergic involvement in the 3-MBA induced effects, the above procedure was repeated replacing naloxone, with an injection (i.p.) of 50 mg kg^{-1} L-dopa (Sigma Chemical Co.) + 20 mg kg^{-1} carbidopa (kindly provided by Merck Sharp and Dohme, West Point, PA) to increase brain levels of dopamine. The drug combination was prepared in 0·1 M HCl and adjusted to a pH of 5 with 5 M NaOH. Ascorbic acid was added (45 mg mL^{-1}) as an antioxidant. The data from both the naloxone and L-dopa experiments were compared with those previously obtained with 300 mg kg^{-1} 3-MBA alone.

Results

Rats treated with 3-MBA at 300 mg kg^{-1} were lethargic, but, in contrast to the behaviour of hamsters noted earlier, none had a loss of righting reflex or exhibited a state that could be regarded as general anaesthesia. A standard catalepsy test (Morpurgo 1965) gave negative results. The effects of the three doses of 3-MBA are shown in Fig. 1. Consistent with a report by Haigler