Evaluation of Drug Absorption After Intrapulmonary Administration Using *Xenopus* Pulmonary Membranes: Correlation with *In Vivo* Pulmonary Absorption Studies in Rats

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INTRODUCTION

Recently, the pulmonary route appears promising for the delivery of drugs, since various drugs which were poorly absorbed from the gastrointestinal tract are well absorbed from the lungs due to the large surface area of the alveolar epithelium and the short distance of the air-blood exchange pathway (1,2). Thus, many researchers have examined the pulmonary absorption of drugs by an *in vivo* pulmonary absorption experiment. However, *in vivo* absorption experiment was not usually suitable to characterize the absorption pathway or absorption mechanisms of drugs, because the pulmonary epithelial surface of mammals is relatively inaccessible due to the anatomic complexity of the multiply branched airways of their lungs. Therefore, it is necessary to establish an *in vitro* pulmonary absorption experiment for evaluating the transport mechanisms of drugs.

In vitro transport across alveolar epithelium can be studied with primary cultures of mammalian alveolar epithelia cells (3), or in isolated perfused lungs, usually from rat or dog. Both approaches have the potential advantage of utilizing mammalian systems, which may more closely mimic human physiology. However, considerable similarity exists between *Xenopus* lung and mammalian lung, including similar alveolar cell morphology and overall dimensions of the air-blood barrier (4,5), active Na^+ and amino acid absorption (6,7), surfactant composition (8), high transepithelial resistance, and presence of large- and small-radius pore populations of similar dimensions and relative frequency (9).

Primary cell culture is a more labor-intensive system than use of amphibian lung tissue, requiring isolation of fresh cells each week owing to their relatively short-term viability. Perfused lungs possess some of the advantages of *in vitro* systems for controlling experimental parameters such as perfusion medium composition, but accurate sampling of the alveolar fluid and uniform deposition of administered compounds are as difficult to achieve as *in vivo*. On the other hand, *Xenopus* lung has a simpler structure than that of mammals, therefore, it is possible mounting this tissue on Ussing chamber.

From these standpoints, we previously examined the effects of various absorption enhancers on the permeability of phenol red using *Xenopus* pulmonary membrane, and revealed that this system is effective method for screening the effectiveness of various additives (10). In this study, to establish the convenient system estimating the *in vivo* drug absorption, the transport of a number of different drugs across *Xenopus* pulmonary membrane was studied, and the results were compared with absorption data from rat *in vivo* experiments (2,11–17).

MATERIALS AND METHODS

Chemicals

Caffeine, aminophylline, chloramphenicol, dexamethasone and p-aminohippuric acid (PAH) were obtained from Nacalai Tesque, Japan. Theophylline, phenol red, sulfanilic acid and cyanocobalamin were obtained from Wako Pure Chemicals, Japan. Fluorescein isothiocyanate dextrans (FDs) with average molecular weights of 4kDa (FD4), 10kDa (FD10), and 70kDa (FD70) were purchased from Sigma Chemical Co., MO. All other chemicals were of analytical grade.

Animals

Female South African clawed frogs (*Xenopus laevis*) were obtained from Shimizu Laboratory Supplies, Japan, and were kept in tap water at room temperature. Females are preferred to males because females are larger than males and the lung tissues of females are easier to mount.

Tissue Preparation

Frogs (50–60 g) were anesthetized by ether, and the lungs exposed by a ventral incision. Lungs were excised by severing the tracheoglottis and placed in Ringer solution (110.0 mM NaCl, 2.4 mM $KHCO_3$, 1.0 mM Ca-D-gluconate, 1.0 mM $MgSO_4$, 10.0 mM HEPES, pH 7.4). For preparation of a planar sheet of tissue, the lung was incised along the pathway of the large pulmonary artery, and if necessary, connecting septa were cut to unfold the lung sac. And the tissue was washed gently with Ringer solution. The lungs were mounted in Ussing chambers. The tissue was bathed with amphibian Ringer solution on both sides at room temperature. The reservoir was gassed continuously with 95% O_2 and 5% CO_2 in order to mix each solution and maintain the membrane viability. The entire system was maintained at room temperature throughout the experiment. The membrane viability in this study was monitored by measuring the electrophysiological parameters, such as transmembrane resistance and short-circuit current. These parameters were not changed during the experimental period. These findings suggest that the membrane viability was maintained during the experimental period.

The studies reported in our manuscript have been carried out in accordance with the declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institute of Health.

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Permeation Studies

Tissue was equilibrated for 20–30 min after it was mounted in chambers prior to the transport studies. After an equilibration period, 2.5 ml of Ringer solution was added to the reservoir bathing the pleural side of the pulmonary membrane. An equal volume of test solution was added to the alveolar side of the pulmonary membrane. At predetermined times up until 120 min, 200 μl of solution was sampled from the pleural side and immediately replaced by an equal volume of buffer solution.

Analysis

The concentrations of compounds were determined by the following methods, respectively. Caffeine, aminophylline, theophylline, dexamethasone and phenol red were determined on a spectrophotometer (HITACHI U-2000). FDs were determined on a spectrofluorometer (HITACHI F-2000). Sulfanilic acid, chloramphenicol, PAH and cyanocobalamin were determined by reversed phase HPLC. The slope was obtained from a linear portion of the permeation profiles and the apparent permeability coefficient (Papp) was calculated by the following equation:

$$\text{Papp} = dX_R/dt \cdot 1/(A \cdot C_0)$$

where Papp is the apparent permeability coefficient (cm/sec), X_R is the amount of compounds (mg), A is the diffusion area (0.2826 cm^2), and C_0 is the initial concentration of compounds on the donor side (mM).

The percent absorbed was calculated by the following equation:

$$\% \text{ absorbed} = \%_{max} \{1 - \exp(-ka \cdot t)\} \quad (1)$$

where $\%_{max}$ is the maximal absorption percent, *i.e.*, 100%, *ka* is the absorption rate constant derived from the previous reports (1/min) (2,11–17), t is the time (min).

RESULTS AND DISCUSSION

In this study, a large number of compounds with different physicochemical properties were used. Table 1 summarizes the results obtained from the *in vitro* transport experiments or *in vivo* absorption experiments (2,11–17). The absorption rate constant (*ka*) of compounds spanned more than 5 orders, and their molecular weights differed by up to 400-fold (the molecular weight of FD70 is almost 400-fold larger than that of sulfanilic acid). The absorption of the compounds after intrapulmonary administration in rats varied from nearly 0 to 100 %. All drugs were transported across *Xenopus* pulmonary membrane, therefore, we calculated the permeability coefficients of these drugs. Table 1 shows that apparent permeability coefficient (Papp) values of compounds ranging from 0.023 × 10^{-6} cm/sec (FD70) to 37.8 × 10^{-6} cm/sec (caffeine).

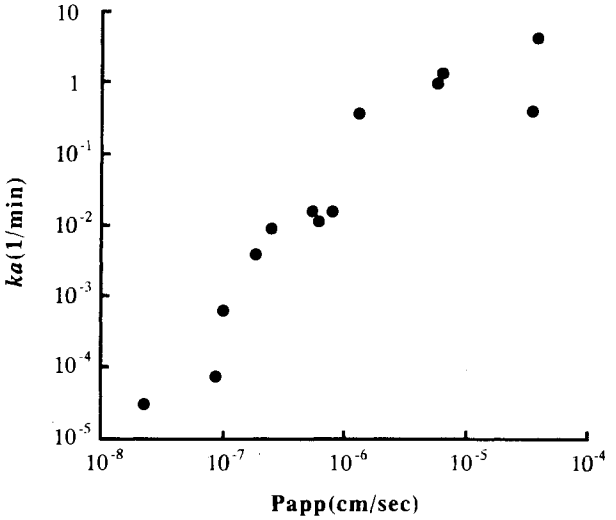

Fig. 1. Relationship between the absorption rate constants of various compounds in rat lungs and their apparent permeability coefficients in *Xenopus* pulmonary membranes. Each point represents the mean of at least three experiments.

Figure 1 indicated the relationship between the logarithm of the *ka* and the logarithm of the Papp. There was a linear correlation between these two parameters with correlation coef-

Table 1. Summary of the Physiocochemical Properties and Absorption Characteristics of Various Drugs

Drugs	Papp[a] (×10^{-6}cm/sec)	ka(1/min)	% abs.	M.W.	log PC[b]
Caffeine (11)	37.8	4.55	100	194	1.5
Dexamethasone (12)	34.0	0.408	100	392	—
Aminophylline (11)	6.32	1.33	100	420	−0.6
Theophylline (11)	5.71	0.950	100	180	−0.5
Chloramphenicol (13)	1.31	0.365	100	323	−0.6
Sulfanilic acid (14)	0.80	0.0154	60.3	173	−3.9
Mannitol (15,18)	0.61	0.0107	47.3	182	—
PAH (14)	0.54	0.0154	60.3	194	−5.5
Phenol red (16)	0.25	0.0089	41.3	354	−2.0
Cyanocobalamin (17)	0.18	0.00385	20.6	1355	—
FD4 (2)	0.10	0.000617	3.6	4400	—
FD10 (2)	0.09	0.000073	0.44	9300	—
FD70 (2)	0.023	0.000031	0.19	69000	—

Note: PAH: p-aminohippuric acid.
[a] Our data represent the mean of at least three experiments.
[b] Chloroform/buffer partition coefficient at pH 7.4.

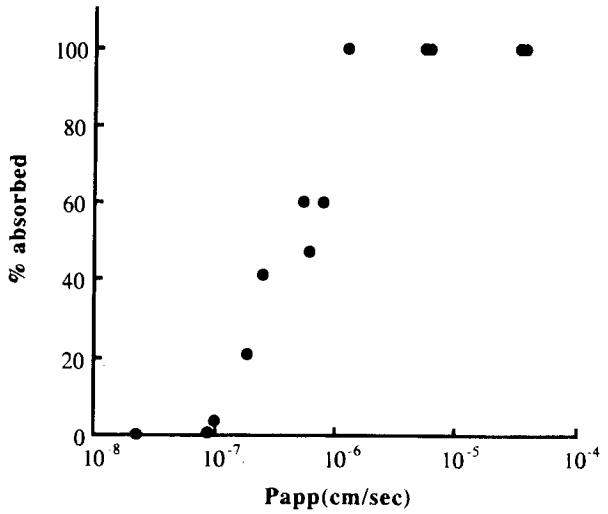

Fig. 2. Relationship between the percent absorbed of various compounds in rat lungs and their apparent permeability coefficients in *Xenopus* pulmonary membranes. Each point represents the mean of at least three experiments.

ficient of 0.924. This correlation was expressed as the following equation:

$$\log ka = 1.75 \log \text{Papp} - 1.46 \quad (2)$$

Similarly, we investigated the relationship between the Papp value and the percent absorbed through rat lungs (Figure 2). A good correlation was obtained when the percent absorbed was expressed as a function of the Papp values. Drugs that are completely absorbed (100%) in rats had Papp values of approximately $> 1 \times 10^{-6}$ cm/sec.

Figure 3 shows the correlation between the % absorbed obtained from the equation (1) in METHODS and predicted % absorbed calculated by substituting the equation (2) for the equation (1). There was a linear correlation between the two

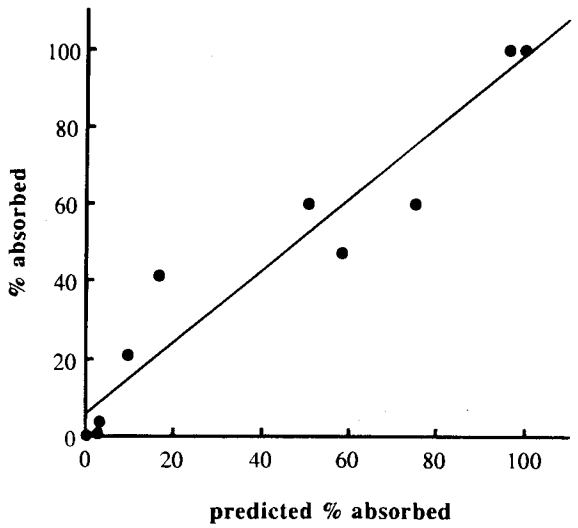

Fig. 3. Relationship between the actual extent of percent absorbed of various compounds and their predicted percent absorbed. The straight line represents the linear regression equation: $Y = 0.93X + 5.3$ ($R^2 = 0.95$).

parameters with correlation coefficient of 0.948, and this result also suggests the possibility of predicting the pulmonary absorption of drugs by measuring their permeability across *Xenopus* pulmonary membrane.

There are a number of physicochemical factors influencing the pulmonary absorption of xenobiotics, for example, molecular weight, and lipophilicity. Previously, we reported that the permeability of drug across *Xenopus* pulmonary membrane was inversely related to their molecular weight, *i.e.*, the permeability of drug gradually decreased with increasing molecular weight. Our present results were consistent with these previous studies. Taylor reported that the absorption of drugs from the lungs was dependent on the lipophilicity of drugs, and the absorption of drugs whose apparent partition coefficients (logPC) calculating from chloroform/buffer pH 7.4 partitioning data were approximately < -2 were generally very slow (19). The drugs used in our present study had various logPC values. For example, xanthine derivatives, such as caffeine and theophylline have higher logPC values and are more lipophilic than sulfanilic acid or PAH whose logPC value are below -2. Therefore, the permeability and absorption of caffeine were higher than those of PAH, although they have almost same molecular weights.

Artursson and Karlsson mentioned the possibility of evaluating the gastrointestinal absorption of drugs in humans using Caco-2 cell monolayers, colon adenocarcinoma cell line derived from humans (20). They reported that a good correlation was observed between the percent absorbed in humans and Papp across the Caco-2 cell monolayers, and drugs which had Papp value of approximately $> 1 \times 10^{-6}$ cm/sec were completely absorbed in humans. Therefore, Caco-2 monolayers are an effective and convenient system to estimate the drug absorption in humans. Although the properties of *Xenopus* lung differed from Caco-2 cell monolayers (for example, *Xenopus* lung has higher transepithelial resistance than Caco-2 cell monolayers), we showed that compounds which had Papp values more than certain values were absorbed completely in both *Xenopus* lung and Caco-2 cells.

In summary, our present data suggested that the pulmonary absorption of drugs can be evaluated by the results obtained in this study using a calibration curve. Therefore, the absorption of drugs from the lung could be estimated by the *in vitro* transport studies using *Xenopus* pulmonary membrane.

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REFERENCES

1. T. Ohtani, M. Murakami, A. Yamamoto, K. Takada, and S. Muranishi. *Int. J. Pharm.* **77**:141–150 (1991).
2. T. Morita, A. Yamamoto, M. Hashida, and H. Sezaki. *Biol. Pharm. Bull.* **16**:259–262 (1993).
3. L. G. Dobbs, M. C. Williams, and R. Gonzalez. *Biochim Biophys. Acta.* **970**:146–156 (1988).
4. Y. Okada, S. Ishiko, S. Daido, J. Kim, and S. Ikeda. *Acta. Tub. Jpn.* **11**:63–72 (1962).
5. C. Meban. *J. Anat.* **114**:235–244 (1973).
6. H. Fischer, W. V. Driessche, and W. Clauss. *Am. J. Physiol.* **256**:C764–C771 (1989).

7. K. J. Kim. *Respir. Physiol.* **81**:29–40 (1990).
8. M. Hallman and L. Gluck, *J. Lipid Res.* **17**:257–262 (1976).
9. E. D. Cradall and K. J. Kim, *J. Appl. Physiol.* **50**:1263–1271 (1981).
10. S. Okumura, Y. Fukuda, K. Takahashi, T. Fujita, A. Yamamoto, and S. Muranishi. *Pharm. Res.* **13**:1247–1251 (1996).
11. E. Arakawa and S. Kitazawa. *Chem. Pharm. Bull.* **35**:2038–2044 (1987).
12. J. A. Burton and L. S. Schanker. *Steroids* **23**:617–624 (1974).
13. J. A. Burton and L. S. Schanker. *Proc. Soc. Exp. Biol. Med.* **145**:752–756 (1974).
14. S. J. Enna and L. S. Schanker. *Am. J. Physiol.* **223**:1227–1231 (1972).
15. S. J. Enna and L. S. Schanker. *Am. J. Physiol.* **222**:409–414 (1972).
16. S. J. Enna and L. S. Schanker. *Life Sci.* **12**:231–239 (1973).
17. L. S. Schanker and J. A. Burton. *Proc. Soc. Exp. Biol. Med.* **152**:377–380 (1976).
18. D. A. Wall, D. Pierdomenico, and G. Wilson. *J. Control. Rel.* **24**:227–235 (1993).
19. G. Taylor. *Adv. Drug Del. Rev.* **5**:37–61 (1990).
20. P. Artursson and J. Karlsson. *Biochem. Biophys. Res. Comm.* **175**:880–885 (1991).