# Transport of Drugs Across the Xenopus Pulmonary Membrane and Their Absorption Enhancement by Various Absorption Enhancers

**Shigeki Okumura,[1] Yoshimi Fukuda,[1] Kanae Takahashi,[1] Takuya Fujita,[1] Akira Yamamoto,[1,2] and Shozo Muranishi[1]**

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***Purpose.*** The permeability of drugs across the Xenopus pulmonary membrane and the effects of various absorption enhancers on their absorption were examined using an *in vitro* Ussing chamber technique.
***Methods.*** Phenol red and fluorescein isothiocyanate-labeled dextrans (FDs) with different molecular weights were chosen as water-soluble model drugs. Absorption enhancers used in this study were *N*-lauryl-β-D-maltopyranoside (LM), linoleic acid-HCO60 mixed micelle (MM), sodium glycocholate (Na-GC), sodium caprate (Na-Cap), sodium salicylate (Na-Sal) and disodium EDTA (EDTA).
***Results.*** The permeability of drugs gradually decreased with increasing their molecular weights, and the absorption of phenol red significantly increased by these absorption enhancers. Among these additives, LM, MM and Na-Cap appeared to be more effective for enhancing the permeability of drugs than the others. Furthermore, we plotted the logarithm of apparent permeability coefficient (Papp) of these drugs against the logarithm of their molecular weights. There exists a good correlation between these parameters. We measured transmembrane resistance(Rm) of Xenopus pulmonary membrane during the transport experiment to examine the membrane integrity. The average Rm value was about 700 $\Omega \cdot cm^2$, and this value was maintained for 3 hr.
***Conclusions.*** This method is useful for estimating the transport characteristics of drugs across the pulmonary membrane.

**KEY WORDS:** drug transport; Xenopus pulmonary membrane; absorption enhancer; transmembrane resistance.

## INTRODUCTION

The bioavailability by the oral administration of peptide and protein drugs is generally poor. Consequently, non-oral routes, such as the buccal, nasal , rectal and pulmonary routes, are being investigated as alternative routes for the systemic delivery of these peptides (1). Of various alternative routes, the pulmonary route is promising for delivering these drugs, since various drugs which are poorly absorbed from enteral and other mucosal sites are well absorbed from the lung due to its large absorptive surface area and the short distance of the air-blood exchange pathway.

We have examined the pulmonary absorption of drugs including peptides and proteins by an *in situ* pulmonary absorption experiment (2–6). However, the transport characteristics and mechanisms of these drugs have not been fully clarified by this *in situ* absorption method. Therefore, it is necessary to establish an *in vitro* pulmonary epithelial system for evaluating the transport of drugs, but the complex architecture of the mammalian lung precludes mounting planer sheets of tissue in flux chambers. Recently, Wall *et al.* developed a method to evaluate the pulmonary transport of drugs using an amphibian lung as a model of the mammalian lung (7). The Xenopus lung has a simpler structure than that of the mammalian and morphologically and physiologically resembles the mammalian lung (7,8), including: 1) similar composition and dimensions of the air-blood barrier, 2) active $Na^+$ absorption, with passive $Cl^-$ secretion, 3) high transepithelial electrical resistance and 4) surfactant production by pulmonary epithelial cells (7). The above reasons allow this tissue to be mounted in Ussing chambers to study transport of peptides and other model compounds.

In this study, phenol red and fluorescein isothiocyanate-labeled dextrans (FDs) with different average molecular weights were chosen as model drugs and the transport characteristics of these compounds across the Xenopus pulmonary membrane were examined by an *in vitro* Ussing chamber method. We also examined the effects of various absorption enhancers on the transport of phenol red across these membranes. Furthermore, we described the correlation of data between *in situ* absorption studies, as reported previously and the present *in vitro* transport studies.

## MATERIALS AND METHODS

### Chemicals

Phenol red (Wako Pure Chemical, Japan), fluorescein isothiocyanate-labeled dextrans (FDs) with average molecular weights of 4 kDa (FD4), 10 kDa (FD10), and 70 kDa (FD70) and sodium glycocholate (Na-GC) were obtained from Sigma Chemical Co., MO. *N*-Lauryl-β-D-maltopyranoside (LM), linoleic acid (extra pure grade) and HCO60 were kindly supplied by Japan Fine Chemical Co., Japan, Nippon Oil & Eats, Japan and Nikko Chemical, Japan, respectively. Sodium caprate (Na-Cap) was from Tokyo Kasei Kogyo, Japan. Sodium salicylate (Na-Sal) and disodium EDTA (EDTA) were from Nacalai Tesque, Japan. All other chemicals were of analytical grade.

### Tissue Preparation

The pulmonary membrane used in these experiments was obtained from a female South African clawed frogs (*Xenopus laevis*). 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 ice-cold Ringer's solution. The lungs were cut open to form a sheet and the luminal side washed gently with Ringer's solution. The lungs were mounted in Ussing chambers by the method of Wall et al (7). The tissue was bathed with 2.5 ml amphibian Ringer's 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) on both sides at 37°C. 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 37°C throughout the experiment.

[1] Department of Biopharmaceutics, Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607, Japan.
[2] To whom correspondence should be addressed.



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### Electrophysiological Parameters

The transepithelial potential difference (PD) with reference to the mucosal bathing and short circuit current (Isc) were measured between two salt agar bridge (3% agar in 150 mM NaCl) connected via each electrode to a short circuit amplifier. These parameters were measured at every ten minutes. Transmembrane resistance was calculated from the PD and Isc value according to Ohm's law.

### Permeation Studies

Tissue was equilibrated for 20–30 min after mounting in chambers prior to transport studies. After the equilibration period, 2.5 ml of Ringer's solution was added to the reservoir bathing the serosal side. An equal volume of 2.5 mg/ml drug solution was added to the mucosal side. At predetermined times up to until 3 hr, 200μl of solution was sampled from the serosal side and immediately replaced by an equal volume of buffer solution. These samples were analyzed on a spectrophotometer (HITACHI U-2000) or a spectrofluorometer (HITACHI F-2000).

### Analysis

The concentrations of phenol red were determined on a spectrophotometer using the absorbance wavelength of 560 nm. The concentrations of FDs were determined on a spectrofluorometer using the exitation and emission wavelengths of 495 and 512 nm, respectively. The slope was obtained from the linear portion of the permeation profiles (90, 120, 150 min) and apparent permeability coefficient (Papp) was calculated by the following equation.

$$Papp = dX_R/dt \cdot /(A \cdot C_0)$$

where Papp is the apparent permeability coefficient (cm/sec), $X_R$ is the amount of phenol red (mg), A is the diffusion area (0.2826 $cm^2$) and $C_0$ is the initial concentration of phenol red (2.5 mg/ml) on the donor side.

## RESULTS

### Transport of Drugs with Different Molecular Weights Across the Xenopus Pulmonary Membrane

Figure 1 shows the permeation profiles of four kinds of drugs with different molecular weights across the Xenopus pulmonary membrane. After a short lag time, the transport of these drugs from the donor side to the receptor side was linear over the entire time period. Phenol red, with a low molecular weight, exhibited the highest permeability among these four drugs, and the cumulative amount up to 3 hr was 0.220%. The permeability of these drugs gradually decreased with increasing molecular weight. This suggested that the permeability of drugs across the Xenopus pulmonary membrane was dependent on their molecular weights.

Figure 2 indicates the relationship between the logarithm of the Papp of drugs up to 3 hr and the logarithm of their molecular weights. There was a linear correlation between these parameters with a correlation coefficient of 0.996 and the logarithm of Papp of these drugs up to 3 hr were inversely related to the logarithm of their molecular weights.

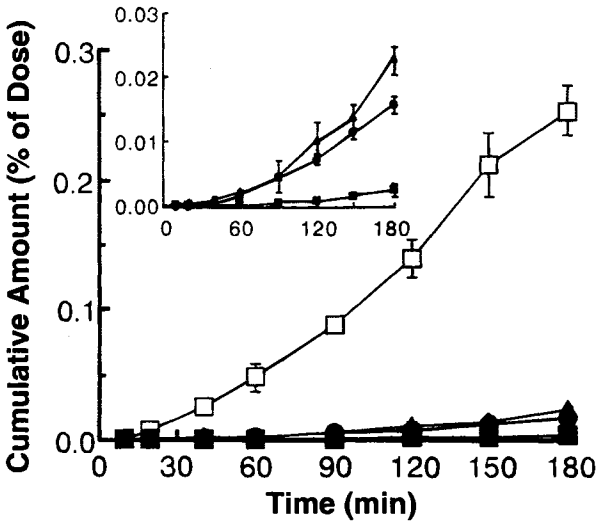


**Fig. 1.** Permeation profiles of water soluble compounds with various moleculer weights across the Xenopus pulmonary membrane. Each point represents the mean ± S.E. of 3–4 experiments. Keys: Phenol red (□); FD4 (▲); FD10 (●); FD70 (■).

### Effects of Various Absorption Enhancers on the Transport of Phenol Red Across the Xenopus Pulmonary Membrane

Figure 3 shows the effects of various absorption enhancers on the permeation profiles of phenol red across the Xenopus pulmonary membrane. Table I summarizes the cumulative amounts and Papp values of phenol red across the Xenopus pulmonary membrane in the presence of various absorption enhancers. LM, MM and Na-Cap significantly increased the transport of phenol red, whereas we did observe a slight increase in its transport in the presence of Na-GC. However, Na-Sal and EDTA exerted no significant increase in the permeability of phenol red. The rank order of enhancing effects of absorption

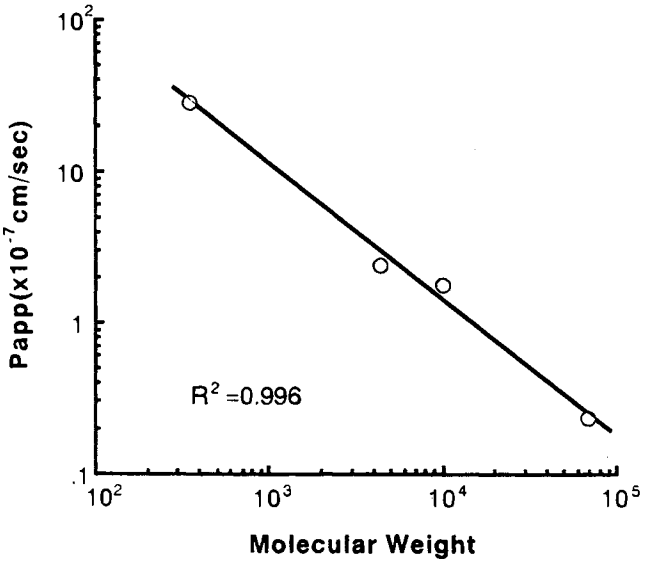


**Fig. 2.** The relationship between the logarithm of Papp of drugs and their molecular weights. Each point represents the mean ± S.E of 3–4 experiments.

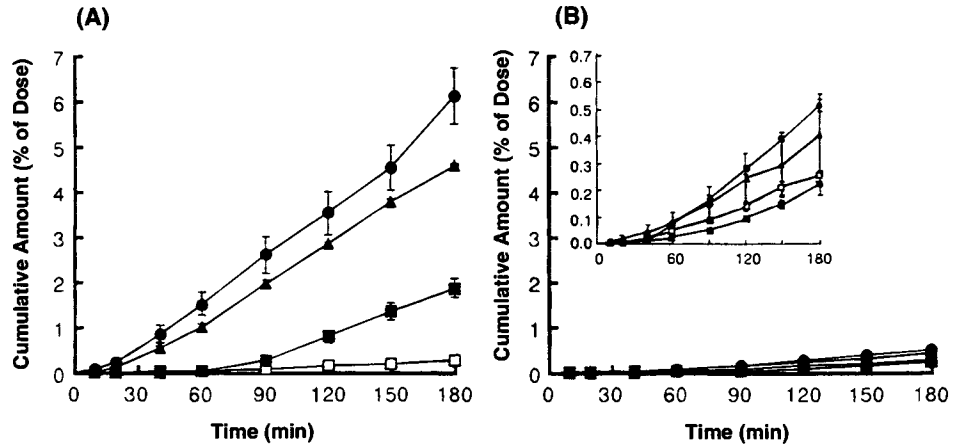


**Fig. 3.** Effects of various absorption enhancers (10 mM) on permeation of phenol red across the Xenopus pulmonary membrane. Each point represents the mean ± S.E. of 3–5 experiments. Keys: (A) Control (□); 10mM LM (●); 10mM Na-Cap (■); 10mM MM (▲); (B) Control (□); 10mM Na-GC- (●); 10mM EDTA (■); 10mM Na-Salt (▲).

enhancers was similar to the results of *in situ* rat pulmonary absorption experiments, as reported previously (3).

Table I shows the cumulative amounts and Papp values of phenol red across the Xenopus pulmonary membrane in the presence of various concentrations of LM. The enhancing effect of LM on the permeability of phenol red did not depend on its concentration. A significant increase in the Papp value was observed in the presence of various concentrations of LM and the Papp value in the presence of 1 mM LM was almost equal to the value in the presence of 5 mM and 10 mM LM. Furthermore, 1 mM LM increased the Papp value to the same extent as 10 mM MM.

### Correlation Between Percent Absorption of Drugs Following Intrapulmonary Administration to Rats and Their Transport Across the Xenopus Pulmonary Membrane in the Presence of Various Absorption Enhancers

Figure 4 illustrates the relationship between the cumulative amount of phenol red across the Xenopus pulmonary membrane and the absorption percentages of phenol red after intrapulmonary administration to rats. The absorption percentages of phenol red from the lung in rat were estimated by a deconvolution method, as reported previously (3). There is a correlation between these two parameters with a correlation coefficient of 0.893.

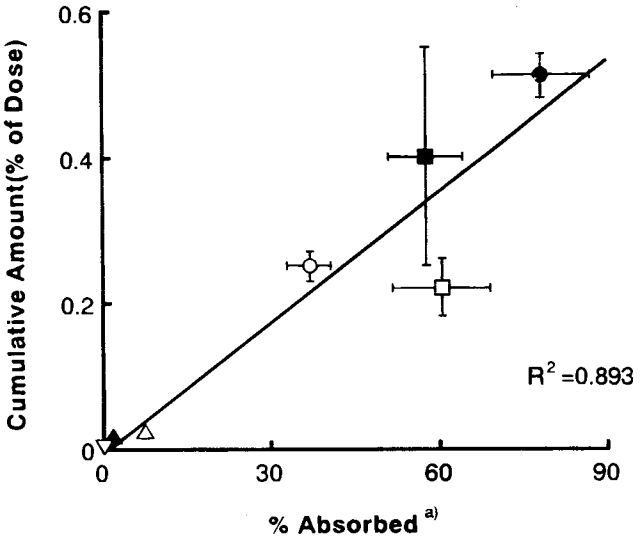


**Fig. 4.** Correlation between percent absorption of drugs following intrapulmonary administration to rats and their transport across the Xenopus pulmonary membrane in the presence of various absorption enhancers. Each point represents the mean ± S.E. of 3–6 experiments. Keys: phenol red (○); phenol red + Na-GC (●); phenol red + Na-Sal (■); phenol red + EDTA (□); FD-4 (△); FD-10 (▲); FD-70 (▽). a); Data were cited from (3).

**Table 1.** Cumulative Amounts and Papp Values of Phenol Red Across Xenopus Pulmonary Membrane in the Presence of Various Absorption Enhancers

| | Concentration | Cumulative amount in the receptor side up to 180 min (% of Dose) | Papp ($\times 10^{-6}$cm/sec) |
|---|---|---|---|
| Control | | 0.25 ± 0.02 | 2.83 ± 0.57 |
| LM | 1mM | 3.81 ± 0.21*** | 42.60 ± 3.12*** |
| | 5mM | 5.42 ± 0.23*** | 51.17 ± 0.52*** |
| | 10mM | 6.22 ± 0.62** | 47.57 ± 11.20* |
| MM | 10mM | 4.59 ± 0.02*** | 44.74 ± 0.29*** |
| Na-Cap | 10mM | 1.86 ± 0.23** | 26.56 ± 0.34** |
| Na-GC | 10mM | 0.51 ± 0.03** | 5.57 ± 0.54* |
| Na-Sal | 10mM | 0.40 ± 0.15 | 3.51 ± 1.44 |
| EDTA | 10mM | 0.22 ± 0.04 | 2.32 ± 0.54 |

Note: Each value represents the mean ± S.E. of 3–5 experiments. (***); $p < 0.001$, (**); $p < 0.01$, (*); $p < 0.05$, compared with the control.

### Transmembrane Resistance of Xenopus Pulmonary Membrane

To examine the integrity of membrane during the experiment, we measured the transmembrane resistance (Rm) of the Xenopus pulmonary membrane in the presence or absence of

absorption enhancers. Figure 5 shows the time course of Rm values of the Xenopus pulmonary membrane mounted in Ussing chamber for 3 hr. The average Rm value in the absence of absorption enhancers was about 700 $\Omega \cdot cm^2$ after equilibration, and was maintained at this level for 3 hr. However, the Rm value markedly decreased in the presence of 1 mM and 10 mM LM. Na-Cap also decreased the Rm value, but its effect was less than 1 mM and 10 mM LM. On the other hand, a slight decrease in the Rm value was observed in the presence of Na-GC.

## DISCUSSION

In the present study, we examined the transport of drugs across the Xenopus pulmonary membrane and found that there exists a linear relationship between the permeability of drugs (Papp) and their molecular weights. This result is fairly correlated with the previous finding of Enna and Schanker that the absorption rate constants of saccharides and urea from rat lung were in the same order as the diffusion coefficients of the compounds, although they proposed three different populations of pore sizes in the air-blood barrier (9). Similarly, Ohtani *et al.* and Morita *et al.* reported that the absorption of drugs from the lungs gradually decreased with increasing molecular weights (2,3). The above *in situ* pulmonary absorption findings of rats were in good agreement with the present *in vitro* transport findings obtained in the Xenopus lung by the Ussing chamber.

Previously, Schanker *et al.* reported that the absorption of phenol red from the lung was concentration dependent and was inhibited by the coadministration of various organic anions, suggesting the carrier-mediated transport of phenol red across the pulmonary membrane (10). However, in this study, this carrier-mediated transport of phenol red is unlikely, since we used 2.5 mg/ml (about 7mM) as the initial concentration of phenol red, which was much higher than that in the case of Schanker's experiment (0.01 mM–1mM).

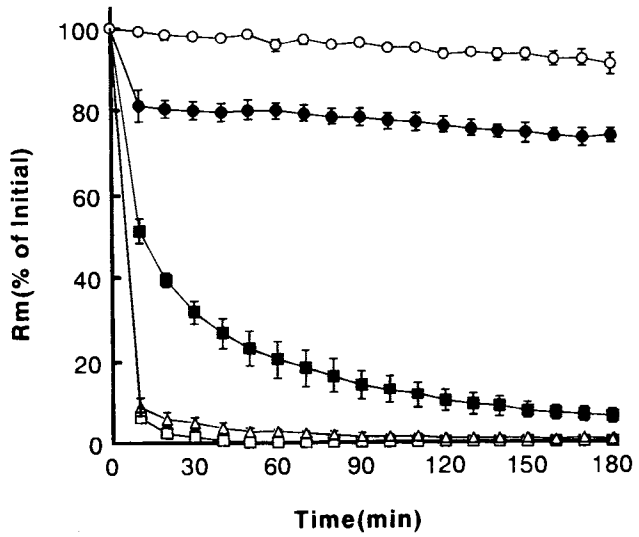


**Fig. 5.** Effects of various absorption enhancers on tissue electrical resistance (Rm) of Xenopus pulmonary membrane. Each point represents the mean ± S.E. of 3–4 experiments. Keys: Control (○); 10mM Na-GC (●); 10mM Na-Cap (■); 1mM LM (△); 10mM LM (□).

Recently, absorption enhancers were used to improve the pulmonary absorption of drugs including peptides and proteins (4,5). In this study, we examined the effects of various absorption enhancers on the transport of phenol red across the Xenopus pulmonary membrane. We found that LM, MM and Na-Cap markedly enhanced the transport of phenol red across the membrane, while a slight increase in its transport was observed in the presence of Na-GC.

LM, an alkylsaccharide, has been found to have absorption enhancing activity in the gastrointestinal tract (11). Concerning the *in situ* pulmonary absorption studies, we previously demonstrated that LM improved the pulmonary absorption of FDs and insulin (2,4). These findings indicate that LM is a suitable absorption enhancer for enhancing the pulmonary absorption of drugs in both *in situ* and *in vitro* systems.

MM is known to improve the absorption of various poorly absorbable drugs in the gastrointestinal tract (12). Our previous findings suggest that the increase in membrane permeability caused by the fatty acid is associated with the disorder of the interior of the membrane and interaction between the incorporated fatty acid and polar head group of the phospholipid (12). More recently, a sulfhydryl-related substance was found to be involved in the permeability-enhancing effect of oleic acid (13). Therefore, MM may increase the transport of phenol red in the same manner as the intestine, although the mechanism by which MM acts in the lungs is still unknown.

Na-Cap is known to enhance the transcellular permeability by causing the membrane perturbation by interacting with the protein region in the gastrointestinal membranes, and to enhance the paracellular permeability by some structural change in the tight junction (14). Previously, we found that Na-Cap were effective for enhancing the pulmonary absorption of phenol red by an *in situ* pulmonary absorption experiment (3), which was in good agreement with the present finding.

Na-GC has also been found to improve the absorption of phenol red from lung, as reported previously (3). In this study, the transport of phenol red was slightly improved by the addition of Na-GC. It was reported that bile salts removed the epithelial cells and thereby improving the poorly absorbable drugs (15). Thus, this action of bile salts may be related to the enhancement effect of Na-GC on the pulmonary absorption of phenol red in both *in vivo* and *in vitro* systems.

We observed no increase in the absorption of phenol red in the presence of EDTA or Na-Sal. This finding is correlated with finding that these enhancers did not significantly increase the absorption of phenol red and FDs in an *in situ* pulmonary absorption experiment (3). However, these enhancers were effective for improving the absorption of poorly absorbable drugs in the gastrointestinal tract (12). This difference in the effectiveness of the enhancers between the lung and intestine may be partly explained by morphological differences, varying sensitivity of enhancers and application of enhancers for different drugs.

We found a linear relationship between *in situ* absorption experiments and *in vitro* transport studies, although we used different species in each experiment. This suggested that the *in vitro* transport studies using the Xenopus pulmonary membrane reflects the *in situ* pulmonary absorption in rats and this experiment was effective for evaluating the transport of drugs in the presence or absence of absorption enhancers.

The present study showed that the Rm value at a steady state was about 700 $\Omega \cdot cm^2$ in the absence of absorption enhancers. This value was consistent with the values previously reported by Wall *et al.*, 701 $\pm$ 25 $\Omega \cdot cm^2$ (7). Thus, this finding suggested that the integrity of membrane was maintained during the transport studies.

We observed a significant decrease in the Rm value in the presence of LM, whereas Na-GC slightly decreased the Rm value as compared with the control. This decrease in the Rm value was fairly correlated with the result of transport studies in the presence of LM or Na-GC. Changes in Rm value can be used on a structural change of tight junction. Therefore, these absorption enhancers especially LM may open the tight junction of the Xenopus pulmonary membrane and increase the transport of phenol red.

In conclusion, the present study indicated that a molecular weight dependent decrease in the transport of drugs was observed in the *in vitro* experiments using Xenopus pulmonary membranes. LM, MM and Na-Cap appeared to be more effective for enhancing the transport of phenol red across the Xenopus pulmonary membrane than Na-GC, EDTA and Na-Sal. Furthermore, this *in vitro* method can be useful to evaluate the absorption of drugs from the lungs, since these exists a good correlation between the present results and the *in situ* pulmonary absorption results.

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