Effects of Various Absorption Promoters on Pulmonary Absorption of Drugs with Different Molecular Weights

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The effects of various absorption promoters on the pulmonary absorption of drugs with different molecular weights were examined in rats. Phenol red and fluorescein isothiocyanate-labeled dextran (FDs) with various molecular weights were used as model drugs and the absorption promoters used in this study were sodium glycolate, sodium salicylate, ethylenediaminetetraacetic acid disodium salt (Na2-EDTA) and sodium caprate, all at a concentration of 1%. Of these absorption promoters, sodium glycolate and sodium caprate appeared to be more effective for enhancing the pulmonary absorption of these drugs than sodium salicylate and Na2-EDTA. Furthermore, it was indicated that there is the optimal molecular weight to which each absorption promoter gives the largest enhancing effect on the pulmonary absorption of drugs.

Keywords phenol red; pulmonary absorption; absorption promoter; macromolecular absorption

Introduction

It is well known that bioavailability by the oral administration of peptide and protein drugs is generally poor. This has been attributed to their extensive proteolytic degradation in the gastrointestinal tract and the impermeability of the intestinal mucosa due to their high hydrophilic characteristics and large molecular weights. Consequently, non-oral routes, such as the buccal, nasal, rectal, vaginal and pulmonary, routes, are being investigated as alternative routes for the systemic delivery of these peptides. Among those explored, the pulmonary route would seem to be a promising alternative for delivering peptide and protein drugs due to the feasibility of the intratracheal administration and relatively good absorption characteristics. However, the bioavailability of these drugs with relatively high molecular weights from the pulmonary route is still poor when compared with the parenteral route. Therefore, absorption promoters are required to promote the pulmonary absorption of these peptides and proteins. Recently, Niven and Byron reported that surfactants such as oleic acid, oleyl alcohol and Span 85 can produce an increase in the transfer rate of disodium fluorescein, a model compound, from the airways of the isolated rat lung. In addition, Ohtani et al., demonstrated that the effects of various absorption promoters such as linoleic-acid-surfactant mixed micelles (MM), N-lauryl-beta-D-maltopyranoside (LM), diethylmaleate and disodium ethylenediaminetetraacetic acid (Na2-EDTA) on the pulmonary absorption of fluorescein isothiocyanate dextran (FDs) were dependent on the molecular weights of FDs. However, it has not been fully investigated whether the effect of other typical absorption promoters such as bile salts, fatty acids and chelating agents would be dependent on the molecular weight of drugs. For nasal absorption, our previous report indicated that Na-glycolate, Na-caprate and Na2-EDTA showed the highest promoting effect to a drug with an approximate molecular weight of 10000. In this study, phenol red and FDs with average molecular weights of 4000, 10000 and 70000, were chosen as model drugs and the relationship between the molecular weights of these drugs and the enhancing effects of various absorption promoters on the pulmonary absorption were examined in rats.

Materials and Methods

Chemicals Phenol red (Nacalai Tesque, Japan), fluorescein isothiocyanate-labeled dextran (FDs) with average molecular weights of 4000 (FD-4), 10000 (FD-10) and 70000 (FD-70) (Sigma Chemical Company, MO, U.S.A.) were obtained commercially. Sodium glycolate (Sigma Chemical Company, MO, U.S.A.), ethylenediaminetetraacetic acid disodium salt (Na2-EDTA, Nacalai Tesque Inc., Japan) and sodium caprate (Tokyo Chemical Industry, Japan) were obtained commercially. All other chemicals were of analytical grade.

Preparation of Drug Solutions Dosing solutions containing FDs were prepared in Krebs-Ringer bicarbonate buffer (pH 7.4) to yield a final concentration of 1 mg/100 μl. In the case of phenol red, the drug solution was prepared in an isotonic phosphate buffer (PBS) to yield the same concentration as in the case of FDs, since phenol red was hardly dissolved in Krebs-Ringer bicarbonate buffer (pH 7.4). In certain experiments, the dosing solutions were added with addition of absorption promoters such as Na-glycolate, Na2-EDTA, Na salicylate and Na caprate to yield a final concentration of 1%, since these absorption promoters have usually been used at this concentration in previous reports.

Pulmonary Absorption of Drugs To investigate the pulmonary absorption of phenol red and FDs, male Wistar rats, weighing 180 to 230 g, were anesthetized by means of an intraperitoneal injection of sodium pentobarbital and prepared surgically according to the method of Emna and Schanker. Shortly, after the trachea was exposed through a ventral midline incision in the neck, and a 2.5 cm length of polyethylene tubing (i.d. 1.5 mm o.d. 2.3 mm) was inserted through an incision between the fourth and fifth tracheal rings caudal to the thyroid cartilage to a depth of 0.6 cm. One hundred μl of the drug solution was administered intratracheally through a tube inserted in the trachea by a 250 μl glass syringe. After administration of the drug solution, 500 μl of blood sample were periodically collected from the cannula inserted into the carotid artery for up to 2 h.

In another experiment, the same amount of drug was injected intravenously into rats, and the blood sample was similarly collected.

Analytical Method The blood sample was separated by centrifugation at 9000 rpm for 5 min and the plasma (200 μl) was collected and diluted with Krebs-Ringer bicarbonate buffer (pH 7.4) or phosphate buffer (pH 3.4).
The concentrations of phenol red in plasma were determined on a spectrophotometer (Shimadzu, UV-2100) using the absorbance wavelength of 560 nm. The concentrations of FDs were determined on a spectrofluorometer (Shimazu, RF-540) using the excitation and emission wavelengths of 490 and 530 nm, respectively.

The absorption percentages of these drugs were estimated by a deconvolution method of Yamaoka et al.\textsuperscript{16} using the plasma concentration–time course data after the intravenous injection. There exists a linear relationship between dose and AUC over the range of 0–2 mg in this experiment.

**Results and Discussion**

Figure 1 shows the plasma concentration–time curves of four kinds of drugs with different molecular weights after intratracheal administration of each drug to the rats. Phenol red, with a low molecular weight (354), was well absorbed from the rat lung, and the peak plasma concentration of phenol red reached as much as 6.0 μg/ml about 90 min after intratracheal administration. When FDs were administered intratracheally to rats, the plasma concentrations of FDs seemed to decrease as the molecular weight of the drug increased, and the peak plasma concentrations of the drugs were not observed for up to 2 h. Consequently, the absorption percentages of each drug up to 2 h were calculated by the deconvolution method. The absorption percentages calculated were as follows: 37.0% for phenol red, 7.2% for FD-4, 2.0% for FD-10, 0.37% for FD-70. The logarithm of the absorption percentages of drugs up to 2 h were plotted against the logarithm of their molecular weights. As shown in Fig. 2, there exists a linear correlation between these parameters with a correlation coefficient of 0.981, and it can be seen that absorption percentages up to 2 h were inversely related to the molecular weights of the compounds. This result was fairly correlated with the report of Enna and Schanker and Ohtani et al., who showed that the absorption rate constants of various kinds of drugs from rat lung were dependent on their molecular weights.\textsuperscript{13,15}

In our previous report, it was also indicated that the nasal absorption of phenol red and FDs gradually decreased with increasing molecular weights of the compounds and that the absorption percentage for each compound in 2 h is 32.9% for phenol red, 2.2% for FD-4, 1.2% for FD-10 and 0.05% for FD-70, respectively.\textsuperscript{14} These results indicated that the absorption percentages of these drugs from the lung as well as the nose were dependent on their molecular weights. Furthermore, the ratios between the pulmonary absorption percentage and the nasal absorption percentage in 120 min were 1.1 for phenol red, 3.3 for FD-4, 1.7 for FD-10 and 7.4 for FD-70, suggesting that the absorption of drugs from the lung is greater than the absorption from the nasal mucosa. These results may be mainly explained by the fact that the pulmonary route has a large surface area and the distance from the surface of the pulmonary epithelium to the blood vessel is shorter (0.1–5 μm) than the nasal mucosa (30–50 μm).\textsuperscript{17}

It was reported that the absorption rate constants of inulin (molecular weight 5250) and dextran (molecular weight 75000) were 0.185 and 0.0249 h\textsuperscript{-1}, respectively.\textsuperscript{15} In contrast, our present study demonstrated that the absorption rate constants of FD-4 and FD-70 were 0.037 and 0.00185 h\textsuperscript{-1}, which were much smaller than the values of inulin and dextran, although these compounds have almost the same molecular weights as FD-4 and FD-70. Furthermore, Folkesson et al. reported that intratracheally instilled bovine serum albumin (molecular weight 67000) into rats were absorbed by 4.1% up to 24 h (absorption rate constant is 0.00174 h\textsuperscript{-1}).\textsuperscript{11} Probably, these different values of absorption rate constants would be due to the different physicochemical characteristics of drugs used in each experiment.

The effects of various absorption promoters on the pulmonary absorption of these drugs were examined.
Figure 4a shows the plasma concentration curves of phenol red in the presence of various absorption promoters. Sodium glycocholate or sodium caprate significantly enhanced the absorption of phenol red, and the peak plasma concentration of phenol red was observed 5 min after intratracheal administration. In contrast, a marginal increase in the plasma concentration of phenol red was observed in the presence of salicylate or Na₂-EDTA. Figure 3b shows the cumulative absorption percentage-time course of phenol red calculated by a deconvolution method. In the control experiment, the absorption percentage of phenol red was linearly increased up to 2 h. Previously, Schanker et al. reported the carrier mediated transport in pulmonary absorption of phenol red, and that the transport maximum for the carrier transport was 1.2 μg/h.¹⁸ However, this transport mechanism is unlikely since our results indicate that 37% of phenol red was absorbed from the lung up to 2 h with an absorption rate of 185 μg/h.

In the presence of sodium glycocholate or sodium caprate, the absorption percentages of phenol red increased sharply in the early phase of the time course (Fig. 3b). This result suggests that the absorption promoting effects of these promoters occurs only in the initial period.

Figure 4b shows the plasma concentration time profiles of FD-4 in the absence or presence of absorption promoters. Sodium glycocholate and sodium caprate enhanced the absorption of FD-4 in a similar manner as phenol red, but the absorption promoting effect of Na₂-EDTA was more sensitive to FD-4 than phenol red. Plasma FD-4 level in the presence of Na₂-EDTA increased 3 to 4 times higher than the control, and the peak was not seen for up to 2 h. As shown in Fig. 4b, the cumulative absorption percentage-time curves of FD-4 in the presence of sodium glycocholate and sodium caprate were in the same pattern as seen in phenol red whereas the absorption percentage of FD-4 was gradually enhanced with the time up to 2 h by Na₂-EDTA. Na₂-EDTA is known to be a chelating agent to form a chelate compound with calcium ion existing at the tight junction in the membrane, resulting in increased permeability of the paracellular route.¹⁹ Our present result suggests that the enhancing effect of Na₂-EDTA on the paracellular permeability is considerably continuous and irreversible.

We also investigated the enhancing effects of these absorption promoters in FD-10 and FD-70, and the absorption percentages of all the drugs examined in the absence or presence of various absorption promoters were summarized in Table I. All the absorption promoters were effective for increasing the absorption of drugs with different average molecular weights although Na salicylate and Na₂-EDTA were less effective than Na glycocholate and Na caprate.

Figure 5 shows the relationship between the molecular weight of the drugs and the absorption enhancing ratio of various absorption promoters on the pulmonary absorption of drugs. The absorption enhancing ratios were calculated from the absorption percentage in the presence of each absorption promoter/the absorption percentage in the control in 120 min. In the case of the low molecular weight drug (phenol red), the ratio of every promoter was small (1 to 2) and little effect was observed even in the various absorption promoters. This finding may be partly attributed to the fact that phenol red is well absorbed from the lung without any absorption promoter. Sodium glycocholate or sodium caprate was more effective in drugs with relatively high molecular weights than in low molecular weight drugs. In particular, the ratio of the absorption percentage of FD-10 in the presence of sodium caprate has reached as much as 10.5, and a marked promoting effect was observed even in FD-70. Furthermore, the maximal promoting effects of sodium glycocholate and sodium caprate were noted at an average molecular weight of 10000. In the case of Na₂-EDTA, the maximal
effect was seen at an average molecular weight of 4000. In contrast, sodium salicylate was less effective in all drugs used than other absorption promoters (the ratio was less than 2), although this promoter was reported to be useful in the rectal absorption of insulin in dogs.6)

The mechanisms by which these absorption promoters enhance the pulmonary absorption of drugs is still not understood. In the gastrointestinal tract, bile salts have a high capacity for solubilization of phospholipid and the extraction of phospholipid may cause the disruption of epithelial cells.20) In addition, Fasano et al., reported that alteration of tight junctions and increased permeation of lactulose following perfusion of bile salts were observed at low concentrations (below the critical micelle concentration) in rabbit small intestine in vitro.21) It may be possible that these mechanisms may be related to the enhancement of phenol red and FDs absorption from the lung in the present study. Sodium caprate is known to enhance the transcellular permeability by causing membrane perturbation by interacting with the protein region in the membrane, and to enhance the paracellular permeability by some structural change in the tight junction.22,23) Probably, the latter mechanism is more plausible because drugs used in this study are all high hydrophilic compounds. However, the results that the absorption enhancing effects of sodium glycocholate and sodium caprate were more effective than Na₂-EDTA suggest the contribution of the transcellular route as well as the paracellular route.

Our previous study demonstrated that the effects of absorption promoters on the nasal absorption of different sized hydrophilic drugs were dependent on the molecular weights of the drugs.14) Sodium glycocholate, sodium caprate and Na₂-EDTA have showed maximal effects against drug with molecular weight of 4000, and the absorption enhancing ratio was about 5 in every promoter. Compared with the nasal results, the present study in pulmonary route suggests that the pulmonary mucosa is more sensitive to the absorption enhancing agents than the nasal mucosa.

In conclusion, it became clear from the present study that the most suitable molecular weight for enhancing the pulmonary absorption of a drug is in each absorption promoter. These findings are useful basic information when we select the optimal absorption promoter for improving the absorption of peptide and protein drugs from the lung.

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