Pulmonary Delivery of Salmon Calcitonin Dry Powders Containing Absorption Enhancers in Rats

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Purpose. To evaluate the effects of absorption enhancers in dry powders and in liquids, pulmonary absorption of salmon calcitonin (sCT) in various formulations was measured.

Methods. The dry powder of sCT was prepared by a freeze-drying method with a jet mill. After intratracheal administration of sCT dry powder and liquid (solution) preparations to rats, plasma sCT levels and calcium levels were measured.

Results. After intratracheal administration without absorption enhancers, sCT in the dry powder and in the liquid were absorbed nearly to the same degree. Absorption enhancers (oleic acid, lecithin, citric acid, taurocholic acid, dimethyl-β-cyclodextrin, octyl-β-D-glucoside) were much more effective in the dry powder than in the solution. The reason may be that the enhancers added to the dry powder dissolved at high concentrations in a trace volume of the fluid lining the alveolar epithelium.

Conclusions. The present results suggest that the pulmonary absorption of peptides and proteins can be greatly improved by formulating them into dry powders with smaller amounts of enhancers than in liquid dosage forms.

KEY WORDS: pulmonary absorption; salmon calcitonin; dry powder; absorption enhancer.

INTRODUCTION

Pulmonary delivery of peptides and proteins has attracted increasing attention, because it may be an important new route of noninvasive systemic administration (1-5). Studies done in small animals on pulmonary absorption of peptides and proteins in the form of clinically usable powders (1, 2, 4, 5) are particularly interesting. Macromolecules administered by inhalation of solutions, aerosols, and powders are known to lead to differences in patterns of absorption and deposition of drugs in the lung (1,2,5,6).

Transpulmonary administration of peptides and proteins can be expected to lead to higher rates of systemic absorption than other noninasive routes, because the alveolar epithelium where absorption can take place is thin, and it has a large surface area. However, transpulmonary administration of high molecular weight compounds is almost always incomplete, because of absorption barriers in the alveolar epithelium. Absorption enhancers can increase the bioavailability of peptides and proteins, as we reported from a study of the pulmonary absorption of salmon calcitonin (sCT) in rats (3). The effects of enhancers have generally been studied by coadministration with a drug in solution, but little has been reported on their effect when administered in a dry powder (4). Here we report the effects of various enhancers on pulmonary absorption of sCT in vivo, and compare the absorption of sCT in solution to the absorption of sCT in a dry powder form.

MATERIALS AND METHODS

Chemicals

The following commercially available chemicals were used: salmon calcitonin (sCT) from Bachem; sodium oleate and purified egg yolk lecithin (lecithin) from Nippon Oil & Fats, Co.; FITC-labeled dextran (F-150, MW 149800; FITC-dextran), bovine serum albumin (BSA, fatty acid-free) from Sigma Chemical Co.; and *n*-octyl-β-D-glucoside, sodium taurocholate and dimethyl-β-cyclodextrin (DMβCD), from Wako Pure Chemical. Ind. Ltd.

Dosage Forms

sCT solution. sCT was dissolved in and diluted with saline solution.

sCT dry powder. sCT was dissolved in a lactose solution prepared with purified water. The enhancer to be used, if any, was then added, after which the solution was lyophilized. The lyophilizate was micronized in a jet mill to give the dry powder for administration. The mean size of the particles was measured with an AEROSIZER™ Mach II (Amhert Process Instruments), and was found to be 2.1±1.4 μmϕ (mean ± SD). Particles smaller than 4 μmϕ accounted for 95% of the powder preparation. At doses of 1 and 20 μg/kg, sCT contents of the dry powder 5 mg/dose were 0.3 and 6 μg, respectively.

Animals and Test Procedures

Eight-week-old male Sprague-Dawley rats were used. The trachea was cannulated under anesthesia, as reported previously (3). A microsyringe was used to instill 100 μl of sCT solution through the endotracheal cannula. sCT dry powder was administered as follows: An endotracheally cannulated rat was fixed in a supine position; an administration device (Fig. 1) was attached to the external opening of the cannula; and 5 mg of the dry powder put in a plastic tip taken from a 100 μl pipette, and an air pump was used to force the powder into the trachea. the opening of the Y-shaped connector opposite to that connected to the drug tip was closed with a finger, and the electromagnetic valve was opened for 0.3 sec. The volume of air provided was 3 ml. Immediately after the insufflation, the finger was released to allow air to escape from the lungs, and then the device was detached. Observation of the lung tissue through a stereoscopic microscope revealed no damage referable to this method of insufflation.

For reference, 0.2 ml of sCT solution without enhancers was intramuscularly injected into the femoral muscle.

The animal experiment reported here was reviewed by

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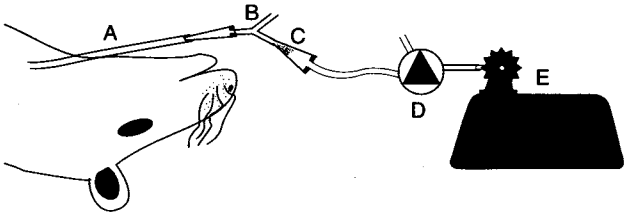

Fig. 1. Device designed for delivery of dry powder to rat lungs. A: polyethylene tube for cannulation; B: Y-shape polyethylene tube; C: dry powder put in a plastic tip taken from a 100 μl pipett; D: electromagnetic valve; and E: air pump (Minipon®, Kaburagi Kagaku K.K.).

the Animal Care and Use Committee of Hoechst Japan Ltd., and conducted according to the guideline "Principle of Laboratory Animal Care" (NIH publication #85-23, revised 1985).

Assay of Salmon Calcitonin

Samples of blood (100 μl each) were collected from the tail vein at various times during the first 6 hr after sCT was administered, and then centrifuged at 4°C to separate plasma. Plasma Ca levels were measured with a Calcium Test Wako C (Wako Pure Chemical Ind., Ltd.). The ratios of plasma Ca levels after administration to that just before administration were calculated to draw the Ca reduction curve. The areas (0-6 hr) between the Ca reduction curve for each experimental group and the curve for the control (saline solution alone) were calculated by the trapezoidal method. The result was called the area of Ca reduction (ACR), and was used as an index of the pharmacological activity of sCT (3).

sCT was assayed in duplicate by the ELISA method using the mouse anti-sCT monoclonal antibody (Affinity Reagents, Inc.), rabbit anti-sCT antibody (Peninsula Laboratories, Inc.) and purified peroxidase-labeled goat anti-rabbit IgG (Kirkegaard & Perry Laboratories Inc.).

Alveolar Deposition of FITC-Labeled Dextran

The solution and dry powder of FITC-dextran were prepared in a manner similar to that of sCT. Immediately after the FITC-dextran was given, the lungs were excised. The right lung was separated into 3 lobes (upper, middle and lower lobes), and the left lung into 2 lobes (upper and lower lobes) equally at the middle point of the long axis. Each lobe was homogenized in 2 ml of saline solution and centrifuged at 3000 × g. Then the fluorescence intensity in the supernatant was measured. The FITC-dextran content of each lobe was calculated, to estimate the FITC-dextran deposition ratio.

RESULTS

Absorption of sCT from Dry Powder and from Solution

Pulmonary absorption of the dry powder sCT was markedly greater with oleic acid, lecithin, or citric acid than without an enhancer (Fig. 2a). The maximum concentration of sCT was highest for oleic acid, followed by citric acid, and then lecithin. When compared to absorption without an enhancer, these substances enhanced absorption by factors of

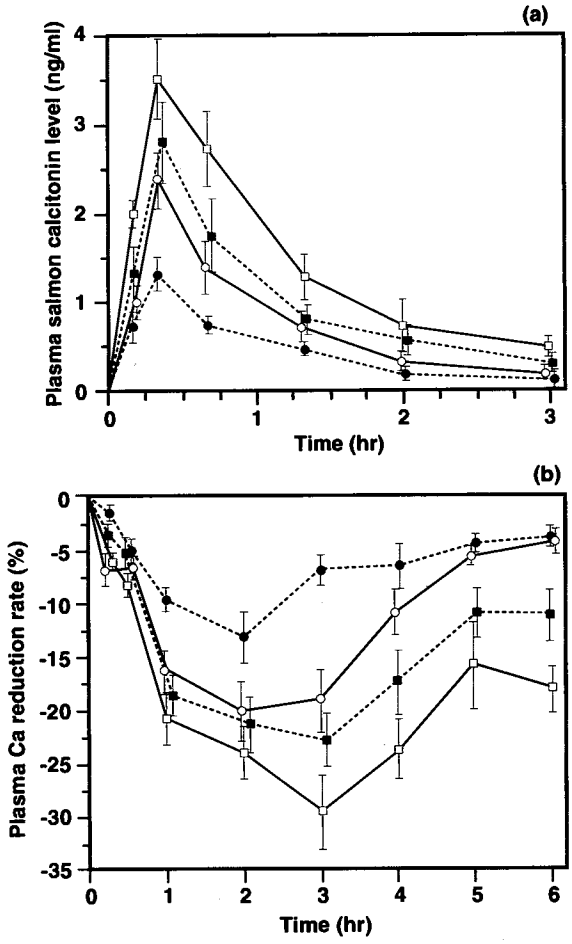

Fig. 2. Pulmonary absorption of salmon calcitonin from dry powders in rats. Values shown are means ± S.E. (n=4~6) of plasma levels of salmon calcitonin (a) and plasma calcium reduction ratios (b). Effects of three enhancers are shown (250 μg/5 mg dry powder/dose). Open squares: oleic acid; closed squares: citric acid; open circles: lecithin; closed circles: none. The doses of salmon calcitonin were 20 μg/kg (a) and 1 μg/kg (b).

2.7, 2.2, and 1.8, respectively. Changes in plasma Ca levels reflected the changes in plasma sCT levels (Fig. 2b).

Without an enhancer, there was little difference in the area under the curve (AUC) or in the ACR between the dry powder and the solution (Table I). The effect of absorption enhancers in the dry powder was concentration-dependent. In the solutions, the effects of the enhancers was weaker than their effects in the dry powders, with the exception of DMβCD.

Enhancement by oleic acid of pulmonary absorption of sCT was greater in the dry powder than in the solution, particularly at low concentrations (Fig. 3).

Alveolar Deposition of FITC-Labeled Dextran

The total deposition of FITC-dextran in the lung did not differ significantly between the dry powder and the solution (Table II. With both forms, about 60% of the FITC-dextran given was found in the middle and lower lobes of the right lung.

Table I. Effect of Absorption Enhancers on the Pulmonary Absorption of Salmon Calcitonin from Dry Powder and Solution in Rats

Enhancer	Amount of enhancer (μg/dose)	Dry powder		Solution	
		AUC (%)[a]	ACR (%)[b]	AUC (%)[a]	ACR (%)[b]
None		29 ± 5[c]	34 ± 7	25 ± 6	30 ± 9
Oleic acid	25	43 ± 7*	58 ± 10[c]	29 ± 9	34 ± 8
	250	80 ± 12[d]	120 ± 25[c,d]	40 ± 11	52 ± 16
Dimethyl-β-cyclodextrin	25	35 ± 9[c,d]	39 ± 12	29 ± 8	31 ± 10
	250	60 ± 18[c]	70 ± 20[c]	60 ± 12[c]	76 ± 15[c]
Lecithin	25	41 ± 12	46 ± 10	27 ± 9	30 ± 11
	250	53 ± 13[c,d]	67 ± 16[c,d]	38 ± 9	42 ± 10
Taurocholic acid	25	32 ± 8	36 ± 10	27 ± 5	31 ± 8
	250	54 ± 10[c,d]	61 ± 13[c,d]	34 ± 10	45 ± 9
Octyl-β-D-glucoside	25	30 ± 6	34 ± 9	26 ± 8	30 ± 10
	250	47 ± 9[c]	57 ± 14[c]	34 ± 12	45 ± 14
Citric acid	25	41 ± 14	49 ± 13	27 ± 9	30 ± 8
	250	63 ± 12[c,d]	66 ± 14[c,d]	38 ± 7	42 ± 11

Dose of salmon calcitonin: 1 μg/kg.
Values shown are Means ± SE (n=4~6).
[a] Area under the curve.
[b] Area of calcium reduction as a percentage of the value measured after intramuscular injection of salmon calcitonin at 1 μg/kg.
[c] Between control group (without enhancer) and group given enhancer.
[d] Between group given dry powder and group given solution.
* Statistically significant difference ($P < 0.05$).

DISCUSSION

Komada *et al.* (4) and Niven *et al.* (5) reported that pulmonary absorption of some peptides and proteins can be better with solutions than with dry powders. By contrast, in the present experiment, we found no such difference in deposition or absorption when the drugs were prepared without absorption enhancers (Table I, II). This difference in results may be explained by differences in the particle size and in the devices used to deliver the dry powder, which could lead to differences in the amounts and sites of drug deposition.

Reports on alveolar deposition after dry powder insufflation have been inconsistent (1, 2, 5, 6). The data in Table 2 on deposition of FITC-dextran in the lung are consistent with those reported by Okumura et al. (1). Furthermore, preferential delivery to the right lung is consistent with the reports of Niven (5) and Byron (6). Like in some other studies (1, 5), in the present experiment the drug was not inhaled during spontaneous ventilation but was artificially forced to into the lungs. Insufflation of the doses used in this study is believed to have no adverse effect on lung tissue or permeability (8).

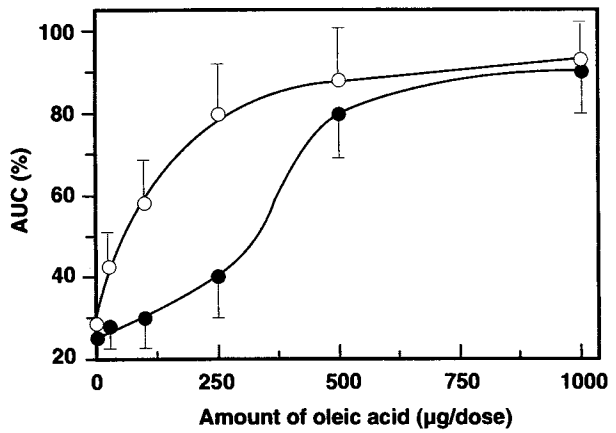

Fig. 3. Effect of oleic acid on pulmonary absorption of dry powders and solutions of salmon calcitonin (1 μg/kg) in rats. Values shown are means ± S.E (n=4~6). AUC (%) is the area under the curve as a percent of the value measured after intramuscular injection of salmon calcitonin at the same dose. Open circles indicate dry powders, and closed circles indicate solutions.

The results reported here show that oleic acid, citric acid, and lecithin can enhance pulmonary absorption when incorporated into a dry powder. One notable result was that at a given concentration, the enhancers were far more potent in the dry powder than in the solution (Table I). The difference of the absorption enhancement between the dry powder and the solution was demonstrated more clearly in AUC rather than in ACR (Table I). It is highly likely that AUC provides more direct detection of the absorption enhancement. Komada et al. (4) reported that citric acid can double the bioavailability of insulin from dry powder, but the effect of citric acid in the solution was not compared.

The fact that enhancers were more effective in the dry powder than in the solution was probably not related to differences in drug deposition or in the amount of the drug delivered, because no difference between the two preparations without enhancers was observed. Instead, it may have been related to differences in the concentration of the enhancer in the fluid that lines the alveolar epithelium. Estimating the volume of this fluid in rat lungs is very difficult. If the absorptive surface area of rat alveolar epithelium is 0.6 m^2 / 300 g body weight (8), then multiplication by the thickness of the fluid, 5-20 nm (9), would give a volume of 3-12 μl. Thus, an enhancer in 100 μl of a solution would not be further concentrated or diluted when it reaches the alveoli. In contrast, an enhancer in a dry powder would dissolve in a

Table II. Deposition of FITC-Labeled Dextran in the Rat Lung After Intratracheal Administration

	Right lung				Left lung			
Preparation	Upper lobe	Middle lobe	Lower lobe	Total	Upper lobe	Lower lobe	Total	Total lung
Dry powder	6.2[a] ± 2.4	35.6 ± 15.8	18.4 ± 7.6	60.2 ± 15.5	6.0 ± 2.4	33.8 ± 8.8	39.8 ± 10.6	50.1[b] ± 6.8
Solution	16.1 ± 7.9	22.8 ± 8.7	22.6 ± 6.9	61.5 ± 12.8	15.5 ± 5.6	23.0 ± 7.2	38.5 ± 11.2	67.2 ± 10.3

The values represent Means ± SE (n = 5).
[a] Relative amount of FITC-labeled dextran delivered to each lobe, expressed as percentage of the sum of the left and right lungs.
[b] Recovery rate expressed as percentage of the initial dose.

small volume of epithelial lining fluid, and the resulting concentration of the enhancer could be tens of times higher than when the drug is delivered in a solution. Such a large difference in the concentration of the enhancer in the fluid lining the alveolar epithelium would explain why the enhancers were so much more effective in the dry powder preparations than in the solutions.

Action mechanisms of the absorption enhancers used in this study are discussed in other literatures. Oleic acid can increase paracellular permeability through a tight junction mechanism that depends on Ca^{2+} and is associated with Ca^{2+} channels (10). Plural mechanisms are reported about taurocholic acid (11, 12, 13). As for citric acid, Hochman (14) proposed that a Ca^{2+}-chelator can cause intracellular changes such as disruption of actin filaments. Citric acid-induced reductions in local pH could also explain its effects on absorption (4). It was proposed that absorption enhancers increase the transport of peptide and protein drugs through tight junctions rather than through cells (14). More study on the mechanisms of absorption enhancement is needed.

In conclusion, the present findings show that formulating a drug and an enhancer into a dry powder can allow the enhancer to exert its effect exactly where it is needed. A variety of dosage forms, including liquids and powders, should be considered when evaluating the effects of absorption enhancers in macromolecular preparations for pulmonary administration. The results reported here are informative for utilization of absorption enhancers for pulmonary administration of peptides and proteins. Future studies should investigate safety aspects of absorption enhancers, especially damage on the alveolar epithelium.

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