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Effect of additives on insulin absorption from intratracheally administered dry powders in rats

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Abstract

The lungs are useful for administration of macromolecules, which are poorly absorbed from the intestine. In the present study, we prepared several dry powder formulations of insulin using a spray drying technique to examine the effect of additives on insulin absorption. The bioavailability of insulin was estimated from the change in the plasma glucose level. The bioavailability of insulin from dry powder with no additive exceeded that obtained from pH 7.4 solution. The absolute bioavailability of insulin administered as a solution with 1.4 mg/dose of bacitracin or 1.0 mg/dose of Span 85 was almost 100%. The bioavailability of dry powder with 0.42 mg/dose of bacitracin was 20% that of the solution with 1.4 mg/dose of bacitracin. The insulin dry powder with 0.21 mg/dose of Span 85 showed a bioavailability less than that for the insulin solution with 0.1 mg/dose of Span 85. Bacitracin and Span 85 were not as effective in dry powder as in solution in the present study. While citric acid was more effective in dry powder that in solution to increase the hypoglycemic effect. The pH 5.0 and pH 3.0 solutions containing 0.19 mg of citric acid in 0.1 ml showed absolute bioavailabilities of 43% and 57%, respectively, while the bioavailabilities for dry powders containing 0.025 and 0.036 mg/dose citric acid were 42% and 53%, respectively. In addition, the hypoglycemic effect of dry powders continued for a longer period and remained at 240 min with the dry powders, while it disappeared at 180 min with the solutions. When the insulin dry powder containing 0.036 mg/dose of citric acid was administered, the lactate dehydrogenase activity, a sensitive indicator of acute toxicity to lung cells, in bronchoalveolar lavage was as low as that for saline administration, suggesting citric acid is a safe additive. Thus, citric acid appears to be a safe and potent absorption enhancer for insulin in dry powder.

Keywords: Insulin; Dry powder; Citric acid; Pulmonary absorption; Enhancer; Lactate dehydrogenase

1. Introduction

The lungs have been used as sites for drug administration for the local treatment of respiratory organ diseases such as bronchial asthma. In addition, increasing attention has been paid to the lungs as a site for administration of drugs includ-

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ing macromolecules, which are poorly absorbed from the intestine. There have been a number of basic studies in which peptide solutions were administered to animals via the lungs. These studies showed that macromolecules, which are hardly absorbed from small intestine, could be absorbed after intratracheal administration (Yoshida et al., 1979; Patton et al., 1994). However, the bioavailability was not high enough to promise effective and economical systemic therapy with macromolecular drugs. It has been reported, from pulmonary administration experiments with peptide solutions, that the bioavailability is improved by adding bile acids, surfactants, enzyme inhibitors, and so on (Takada et al., 1980; Yamamoto et al., 1993; Morita et al., 1994; Yamamoto et al., 1997).

Pressurized metered-dose inhalers (MDI), nebulizers, and dry powder inhalers (DPI) are the three main delivery systems used for aerosol inhalation in humans (Timsina et al., 1994). Among these, DPI appears to be the most promising for future use because the device is small and relatively inexpensive, no propellants are used, and breath-actuation can be used successfully by many patients with poor MDI technique (Newman et al., 1994; Timsina et al., 1994). Edwards et al. (1997) reported that the pulmonary absorption of insulin formulated in dry powders was improved by controlling the physical characteristics of dry powders to give a proper size distribution and low density. As Kobayashi et al. (1996) showed for calcitonin, adding an absorption enhancer to dry powder may be another method to improve bioavailability of macromolecules for DPI. However, few studies on the effect of additives on peptide absorption from dry powders have been reported. In the present study, we prepared several dry powder formulations of insulin to examine the effect of additives on insulin absorption after intratracheal administration of the dry powders in rats.

2. Materials and methods

2.1. Materials

Insulin from bovine pancreas was supplied by Sigma Chemical Co. (28.0 U/mg; St. Louis, MO). Mannitol, bacitracin, sorbitan trioleate (Span 85), citric acid, and Triton-X 100 were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other reagents used were of analytical grade.

2.2. Preparation of dosage forms

2.2.1. Insulin solutions

Insulin was suspended or dissolved in a phosphate-buffered saline (PBS, pH 7.4) or 0.01 M citrate buffer solution (pH 3.0 or 5.0), respectively (5.0 U/ml). The additives, Span 85 (0.10% or 1.0%) and bacitracin (1.4%), were dissolved in the PBS solution.

2.2.2. Insulin dry powders

Dry powders were prepared by a spray drying technique using mannitol as a carrier. The following standard operating conditions were used for spray drying with an SD-1000 spray-drier (EYELA, Tokyo): an inlet temperature of 90°C, a drying air flow rate of 0.75 m^3/min, a solution feed rate of 5 ml/min, and an atomizing air pressure of 100 kPa. Operating under these conditions resulted in an outlet temperature from 63 to 69°C. The dry powders are referred to by the formulation codes listed in Table 2 in this report. The dry powder MI was prepared by spray drying a 0.25% insulin suspension containing 5% mannitol. The dry powders MIS0.1, MIS1.0, and MIB were prepared using the same insulin suspensions with 0.10% Span 85, 1.0% Span 85, and 1.4% bacitracin, respectively. MIC0.1 and MIC0.2 were manufactured with 0.25% insulin solutions containing 5% mannitol and citric acid (0.10% and 0.20%, respectively).

2.3. Evaluation of particle size distribution and particle shape

The particle size distribution was measured with a laser micron sizer LMS-30 (Seishin Enterprise Co., Ltd., Tokyo, Japan) based on laser diffraction. We dispersed the dry powder into a laser beam directly from the apparatus used for intratracheal administration (Fig. 1). An electron

microscope (Hitachi, Tokyo) was used to observe the particle shape.

2.4. Intratracheal administration of insulin solutions and dry powders in rats

Male Sprague–Dawley rats weighing 200–220 g were anaesthetised with pentobarbital (50 mg/kg, i.p.) and secured on their backs on a board during the experiments. The trachea was exposed and a 3.5 cm length of PE-240 polyethylene tubing was inserted to a depth of 0.6 cm through an incision made between the fifth and sixth tracheal rings caudal to thyroid cartilage according to the method of Enna and Schanker (1972). A PE-50 tubing cannula was made in the carotid artery for blood sampling.

Then 100 μl of insulin solution in a microsyringe was administered into the rat trachea according to the method of Enna and Schanker (1972).

Insulin dry powder was administrated as previously reported (Okamoto et al., 2000) using an apparatus described in Fig. 1. In brief, the dry powder was put in the body of a needle and dispersed in the rat trachea by releasing air compressed in a syringe by opening a three-way stopcock connecting the needle and the syringe. The amount of dry powder administered was calculated by subtracting the needle weight after administration from that before administration.

2.5. Assay for plasma glucose

Blood samples (200 μl) were collected before administration and at 30, 60, 90, 120, 150, 180, 210 and 240 min after insulin administration and centrifuged at 4°C to separate plasma. The plasma glucose level was measured with a glucose assay kit Glucose CII Test Wako (Wako Pure Chemical Industries, Ltd.) based on the mutarotase-GOD method. The change in plasma glucose level (ΔGLC) expressed in percentage/unit was calculated using the following equation:

$$\Delta\mathrm{GLC} = \left(\frac{\mathrm{GLC}_t - \mathrm{GLC}_0}{\mathrm{GLC}_0}\right) \Big/ \mathrm{dose} \times 100 \qquad (1)$$

where GLC_0 and GLC_t mean plasma glucose concentrations at time 0 and at time t, respectively. The change in plasma glucose level was normalized by the dose because the doses were different from administration to administration.

The area under the curve (AUC) and the area under the first moment curve (AUMC) for ΔGLC with respect to time from 0 to 240 min were calculated by the trapezoidal rule. Mean effect time (MET) was calculated using AUC divided by AUMC.

2.6. Assay for insulin

Insulin was measured using a high performance liquid chromatography (HPLC) system (Shimadzu Co., Kyoto), composed of a pump (LC-10ADvp), diode array detector (SPD-M10Avp), column oven (CTO-10ASvp), and LC work station (CLASS-LC10). The mobile phase was a 72:28 mixture of 0.1 M ammonium sulfate buffer (pH 2.3) and acetonitril at a flow rate of 1.2

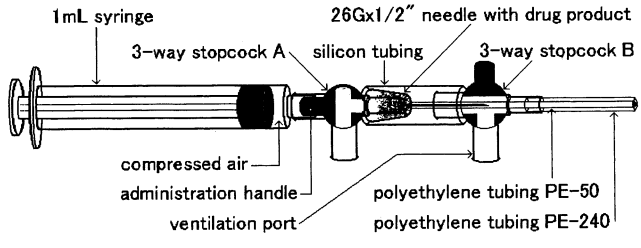

Fig. 1. Apparatus for intratracheal administration.

ml/min. The column was a Shodex Asahipak ODP-50 6D (4.6 × 150 mm, 5 μm) (Showa Denko, Ltd., Tokyo) heated at 36°C. Ultraviolet absorption was measured at 214 nm. The injection volume was 10 μl.

2.7. Assay for lactate dehydrogenase activity in bronchopulmonary lavage

We administered 100 μl of PBS, 100 μl of Triton-X 100 solutions in PBS (0.25%), or insulin dry powders containing citric acid, Span 85, or bacitracin through the rat trachea. The rats were sacrificed 24 h after administration by exanguination from the aorta and the lungs were washed three times with 10 ml of PBS to obtain bronchopulmonary lavage. The activity of lactate dehydrogenase (LDH) in the supernate in the lavage centrifuged at 1500 rpm at 4°C for 5 min was determined using an assay kit LDH CII (Wako Pure Chemical Industries, Ltd.) based on the nitrotetrazolium blue colorimetry.

2.8. Statistical analysis

Statistical differences in ΔGLC, AUC, MET, and LDH activity were examined using a one-way ANOVA followed by least significant difference test. The significance level was set at $P < 0.05$.

3. Result

3.1. Intratracheal administration of insulin solutions

Fig. 2 shows the ΔGLC–time curves after the intratracheal administration of bovine insulin (0.5 U/rat) in 100 μl of PBS (pH 7.4) or citrate buffer (pH 5.0 or 3.0). In the absence of Span 85, bacitracin, or citric acid, a slight hypoglycemic effect was obtained. However, we observed a significant and elongated hypoglycemic effect after insulin administration with these additives. The plasma glucose level was decreased quickly using the pH 3.0 citrate buffer; however, the effect disappeared in 180 min. On the other hand, coadministration with 1% Span 85 (1.0 mg/dose) or 10 mM bacitracin (1.43 mg/dose) elongated the hypoglycemic effect.

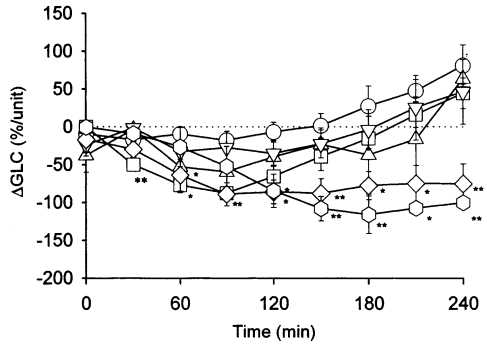

Fig. 2. Effect of additives on the change in the plasma glucose level (ΔGLC) after intratracheal administration of insulin solutions (0.5 unit/dose) in rats. (○) PBS (pH 7.4); (△) 10 mM citrate buffer (pH 5.0, citric acid 1.9 mg/dose); (□) 10 mM citrate buffer (pH 3.0, citric acid 1.9 mg/dose); (▽) 0.1% Span 85 (0.10 mg/dose) in PBS; (◇) 1.0% Span 85 (1.0 mg/dose) in PBS; (⬡) 10 mM bacitracin (1.4 mg/dose) in PBS. Error bar represents S.E. for three or four rats. Statistical significance: *$p < 0.05$ and **$p < 0.01$ compared with PBS.

Table 1 summarizes the AUC and MET values for intratracheal (0.5 U/rat) and intravenous (0.1 U/rat) administration of insulin solutions. The AUC for intratracheal administration of pH 7.4 solution was one-eighth of that for intravenous administration, while the AUC value obtained after intratracheal administration of insulin with 1.0% Span 85 or 10 mM bacitracin dissolved in pH 7.4 PBS was almost equivalent to that obtained after intravenous administration of the control solution, suggesting the usefulness of the additives dissolved in solution. The MET value was also elongated with these additives, indicating that absorption of insulin was prolonged. The effect of Span 85 was dependent on its concentration. Citrate buffer solution increased the AUC value; however, the absolute bioavailability was less than 60% even at pH 3.0. The MET values with citrate were shorter than those with 1% Span 85 or 10 mM bacitracin, suggesting citrate in solution was short acting among the additives examined. The absorption of insulin was pH dependent and the lower pH was preferable.

3.2. Preparation of dry powders containing insulin

The spray drying technique reproducibly yielded dry powders comprised of fine and spherical particles except for those containing citric acid (Fig. 3). The mean diameters were 5–7 μm (Table 2). The yield of dry powders was about 50% except for MIS 1.0 (Table 2). The theoretical potencies of insulin dry powders were ca. 1.3 unit/mg. The potencies for MIC0.1 and MIC0.2 agreed well with the theoretical potencies (Table 2). However, those for MI, MIS0.1, MIS1.0, and MIB were 2/3 to 1/4 of the theoretical values. These powders were successfully dispersed from the apparatus shown in Fig. 1 for dry powder administration.

3.3. Intratracheal administration of insulin dry powders

Fig. 4 shows the effect of additives on change in plasma glucose level after intratracheal administration of insulin dry powders. Table 3 lists the AUC and MET values. The insulin dry powders elongated the hypoglycemic effect of insulin compared with the insulin solutions with the same additive except for Span 85. In contrast to the findings obtained with insulin solutions, citric acid was the most effective for increasing insulin absorption from dry powder. The effect of citric acid on increasing the hypoglycemic effect was dose-dependent. With 0.036 mg/dose of citric acid (MIC0.2), the blood glucose level was rapidly decreased after administration and the effect continued for the experimental period. The absolute bioavailability was greater than 50%. Span 85 (MIS0.1 and MIS1.0) did not increase the insulin effect regardless of its concentration. Bacitracin (MIB) showed a slow onset of insulin effect.

3.4. Lactate dehydrogenase activity in bronchopulmonary lavage

The LDH activities in bronchopulmonary lavage after administration of the insulin dry powders MI, MIC0.2, MIS1.0, and MIB were compared with those observed after administration of insulin solutions in PBS with or without 0.25% Triton-X 100 (Fig. 5). The LDH activities with all the dry powders were significantly less than those with Triton-X 100, positive control, and were as low as the PBS, a negative control, except for MIB.

4. Discussion

There have been several studies comparing pulmonary delivery of macromolecules formulated in solution and dry powder. Niven et al. (1994)

Table 1
AUC and MET for intratracheal administration of insulin solutions in rats

Route[a]	Formulation	$AUC_{0\rightarrow240}\times10^{-3}$ (%/unit min)[b,c]	$MET_{0\rightarrow240}$ (min)[b,d]	Additive (mg/dose)	*n*
IV	PBS (pH 7.4)	18.1 ± 2.4	50 ± 6	–	4
IT	(a) PBS (pH 7.4)	2.29 ± 0.77	70 ± 15	–	4
IT	(b) 10 mM citrate buffer (pH 5.0)	7.75 ± 1.78	100 ± 14	0.19	4
IT	(c) 10 mM citrate buffer (pH 3.0)	10.3 ± 1.7	90 ± 10	0.19	4
IT	(d) 0.1% Span 85 in PBS	7.36 ± 3.11	109 ± 12	0.10	3
T	(e) 1.0% Span 85 in PBS	16.6 ± 3.7	133 ± 3	1.0	4
IT	(f) 10 mM bacitracin in PBS	16.1 ± 1.2	145 ± 15	1.4	3

[a] IV – intravenous (insulin 0.1unit/dose), IT – intratracheal (insulin 0.5 unit/dose).

[b] Mean ± S.E.

[c] Statistical significance ($P<0.05$) was observed for (a vs c), (b vs e), (b vs f), (d vs e), and (d vs f). Statistical significance ($P<0.01$) was observed for (a vs e) and (a vs f).

[d] Statistical significance ($P<0.05$) was observed for (a vs d), (b vs f), and (c vs e). Statistical significance ($P<0.01$) was observed for (a vs e), (a vs f), and (c vs f).

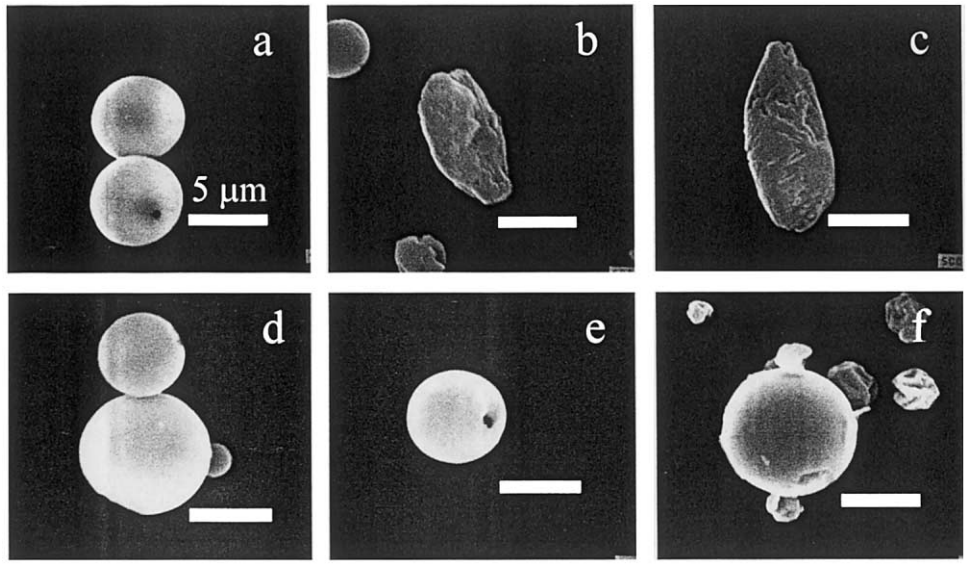

Fig. 3. Scanning electron micrographs of insulin dry powders. Dry powders were prepared by spray drying aqueous solutions containing mannitol and insulin. (a) MI; (b) MIC0.1; (c) MIC0.2; (d) MIS0.1; (e) MIS1.0; (f) MIB.

examined pulmonary absorption of human granulocyte colony-stimulating factor. However, they found less bioavailability for a dry powder than that for a solution. Komada et al. (1994) reported that bioavailability of calcitonin, insulin, thyrotropin stimulating hormone, follicle stimulating hormone, and human chorionic gonadotropin from dry powders was not greater than that from solutions at pH 7.0. They also used citrate as an additive for insulin dry powder. Although adding citrate to dry powder increased bioavailability of insulin 2.7-fold compared with the dry powder without citric acid, it was about half of that for a pH 3 solution.

The improved pulmonary absorption of macromolecules from dry powder was reported by Kobayashi et al. (1996). They showed that pulmonary absorptions of salmon calcitonin from solution and dry powder with no additive were comparable, while adding oleic acid, lecithin, taurocholic acid, octyl-β-D-glucose, or citric acid increased the absorption of calcitonin from dry powder more than from solution. This suggested that the additives are useful for improving pulmonary absorption of macromolecules from dry powders. However, the comparison between effect of the additives on absorption from solution and dry powder has not been enough.

We prepared insulin dry powders by a spray drying technique. The potency assay revealed that the potencies of MI, MIS0.1, MIS1.0, and MIB were less than the theoretical values calculated by the compositions of the insulin suspensions used for spray drying. When citric acid was used as an additive, insulin was dissolved to make clear solutions because of the lowered solution pH. The potencies of MIC0.1 and MIC0.2 were not decreased by spray drying, suggesting that dry powders can be manufactured without insulin potency loss by using insulin solution instead of suspensions. Spray drying a solution may produce small particles having a uniform composition. While the composition of small particles manufactured from a suspension may be uneven because the insulin content would depend on the size of insulin particle. It was likely that MI, MIS0.1, MIS1.0, and MIB particles of high insulin content were exhausted and those of a low insulin content were yielded as products. No insulin degradation during spray drying was observed by the HPLC analysis. We estimated the doses of additives shown in Table 3 supposing that the additive/

Table 2
Yield, insulin potency, and particle size distribution of spray-dried formulations

Formulation code	Yield[a] (%)	Insulin potency (unit/mg)	D10 (μm)	D50 (μm)	D90 (μm)
MI	44.2	0.82	2.6	5.8	12
MIC0.1	44.6	1.29	3.1	7.1	15
MIC0.2	55.5	1.29	2.7	6.6	15
MIS0.1	46.7	0.51	2.3	5.1	10
MIS1.0	12.4	0.35	2.1	5.4	17
MIB	55.6	0.82	2.4	5.9	13

[a] Yield = (spray dried weight)/(recovered weight).

mannitol weight ratio in the dry powders would be the same as that in the insulin solutions or suspensions used for spray drying. This may be reasonable because all the additives and mannitol were dissolved in the insulin solutions or suspensions and the additive/mannitol weight ratio would not be changed by spray drying process.

In the present experiment, the bioavailability of insulin was estimated from the area under the ΔGLC–time curve. The bioavailability of insulin from dry powder with no additive was 2.1 fold that from pH 7.4 solution. The MET value for dry powder was twice that for the solution because the hypoglycemic effect remained at 240 min with the dry powder, while it disappeared at 180 min with the solution. This suggests that the bioavailability calculated for the 240 min data underestimates the bioavailability for dry powder and that the dry powder was much more effective than the solution.

The bioavailability of insulin was affected by adding the additives in the present study. The ΔGLC–time curve depended on both the additives and the formulations used. Both the dry powder and the solution with bacitracin, an enzyme inhibitor, exhibited the hypoglycemic effect of insulin gradually and were still effective at 240 min. The bioavailability of dry powder with 0.42 mg/dose of bacitracin (MIB) was 30% that of the solution with 1.4 mg/dose of bacitracin. The hypoglycemic effect of the insulin solution with 1.0 mg/dose of Span 85 continued for 240 min and the bioavailability of insulin was as large as that for intravenous administration. The effect of the insulin solution with 0.1 mg/dose of Span 85 diminished at 180 min, and the bioavailability decreased to 40%, while insulin dry powder with 0.16 mg/dose of Span 85 (MIS1.0) exhibited a hypoglycemic effect only for 120 min and the absolute bioavailability for 240 min was only 20%. Bacitracin and Span 85 were not as effective in dry powder as in solution in the present study. However, citrate was more effective in dry powder that in solution to increase the hypoglycemic effect. The pH 5.0 and 3.0 solutions containing 0.19 mg/dose of citric acid in 0.1 ml showed bioavailabilities of 43% and 57%, respectively. However, the bioavailabilities for dry powders containing 0.025 mg/dose (MIC0.1) and 0.036 mg/dose (MIC0.2) citric acid were 42% and 53%,

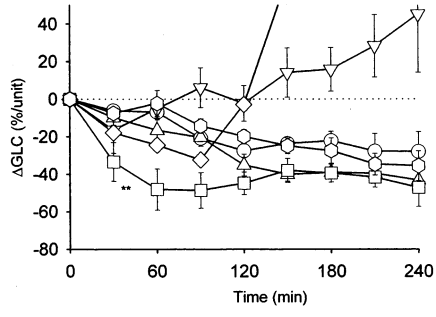

Fig. 4. Effect of the additives on the change in the plasma glucose level (ΔGLC) after intratracheal administration of insulin dry powders with additives in rats. Insulin doses are shown in Table 3. (○) MI; (△) MIC0.1 (citric acid 0.025 mg/dose); (□) MIC0.2 (citric acid 0.036 mg/dose); (▽) MIS0.1 (Span 85 0.033 mg/dose); (◇) MIS1.0 (Span 85 0.16 mg/dose); (⬡) MIB (bacitracin 0.42 mg/dose). Error bar represents S.E. for three or four rats. The error bars for MIS1.0 were not shown. The ΔGLC values for MIS1.0 at 150, 180, 210, and 240 min were above 40% per unit and not shown in the figure. Statistical significance: (*) $P < 0.05$ and (**) $P < 0.01$ compared with MI.

Table 3
AUC and MET for intratracheal administration of insulin dry powder in rats

Route[a]	Formulation code	$AUC_{0\rightarrow240}\times10^{-3}$ (%/unit min)[b,c]	$MET_{0\rightarrow240}$ (min)[b,d]	Insulin (unit/dose)[b]	Additive (mg/dose)	*n*
IT	MI	4.90 ± 0.93	156 ± 12	1.5 ± 0.2	–	3
IT	MIC0.1	7.32 ± 0.67	161 ± 3	1.7 ± 0.5	0.025	3
IT	MIC0.2	10.2 ± 1.75	137 ± 3	1.3 ± 0.3	0.036	3
IT	MIS0.1	1.14 ± 0.50	78 ± 22	1.9 ± 0.5	0.033	3
IT	MIS1.0	3.48 ± 0.76	74 ± 16	1.5 ± 0.3	0.16	3
IT	MIB	4.87 ± 0.71	164 ± 6	2.1 ± 0.0	0.42	4

[a] IT, intratracheal (insulin 0.5 unit/dose).
[b] Mean ± S.E.
[c] Statistical significance ($P<0.05$) was observed for (MI vs MIS0.1), (MIC0.1 vs MIS1.0), and (MIS0.1 vs MIB). Statistical significance ($P<0.01$) was observed for (MI vs MIC0.02), (MIC0.1 vs MIS0.1), (MIC0.2 vs MIS0.1), and (MIC0.2 vs MIB).
[d] Statistical significance ($P<0.01$) was observed for (MI vs MIS0.1), (MI vs MIS1.0), (MIC0.1 vs MIS0.1), (MIC0.1 vs MIS1.0), (MIC0.2 vs MIS0.1), (MIC0.2 vs MIS1.0), (MIS0.1 vs MIB), and (MIS1.0 vs MIB).

respectively. The dry powders lowered the plasma glucose level with smaller amounts of citric acid than the solutions. In addition, the hypoglycemic effect remained at 240 min with the dry powders, while it disappeared at 180 min with the solutions. Citric acid appears to be a potent absorption enhancer for insulin in dry powder.

One of the possible reasons for increased bioavailability for the dry powder with no additive compared with pH 7.4 solution may be saturation of insulin metabolism. Insulin is biodegradable in the rat lung (Lie et al., 1992; Okumura et al., 1992). Some protease inhibitors increase insulin absorption from the lungs (Yamamoto et al., 1993; Fukuda et al., 1995). Since the water volume in the 300 g rat was estimated to be 3–12 μl (Kobayashi et al., 1996), the local insulin concentration after administering dry powder would be much higher than that after administering a 100 μl solution, and the enzyme degrading insulin would be saturated. The action mechanism of bacitracin in solution to increase absorption is inhibition of enzyme degrading insulin. It is likely that adding bacitracin to dry powder showed almost no effect (Fig. 4) because administering insulin with dry powder saturated the enzyme and no additional effect of the enzyme inhibitor was possible.

The reason for the decreased hypoglycemic effect of the insulin dry powder by Span 85 remains to be clarified. A high local concentration of Span 85 would have the increased interaction between Span 85 micelles and insulin and resulted in decreased insulin absorption.

Administering citric acid in dry powder would decrease local pH more than in solution. This will increase insulin monomers at the absorption site (Okumura et al., 1992). Lower pH may change the integrity of the epithelium membrane of the lungs and/or suppress enzyme activity for insulin degradation in alveolar epithelium and phagocytic activity of alveolar macrophages. These may be the possible action mechanisms of citric acid.

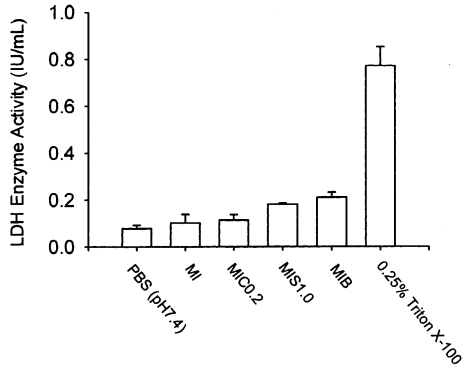

Fig. 5. LDH activity in bronchoalveolar lavage recovered 24 h after administration of PBS, 0.25% Triton-X solution, or insulin dry powders MI, MIC0.2, MIS1.0, and MIB. Statistical significance ($P<0.05$) was observed for (PBS vs MIB), (PBS vs Triton X-100), (MI vs Triton X-100), (MIC0.2 vs Triton X-100), (MIS1.0 vs Triton X-100), and (MIB vs Triton X-100).

If the change in integrity of the epithelium membrane is one of the action mechanisms, the safety of an additive is an important matter of interest. LDH, a cytoplasmic enzyme, should be extracellular in bronchoalveolar lavage fluid only if cell lysis or cell membrane damage has occurred (Henderson, 1984). Henderson et al. (1978) reported that when a lavage was performed in Syrian hamsters using physiological (0.15 M) saline and with saline containing increasing concentrations of Triton-X (from 0.01% to 0.1%), the LDH in the cell-free portion of the lavage fluid increased with increasing Triton-X 100 concentrations. It was reported that LDH activity and the number of cells recovered in the lavage after administration of fine quartz in rat lungs reached a maximum at 24 h (Morgan et al., 1980; Moores et al., 1981). We administered 100 μl of PBS or 0.25% Triton-X solution through the rat trachea. The LDH activity in lavage 24 h after administration was significantly higher with Triton-X 100 than PBS. When the insulin dry powder MIC0.2 was administered, the LDH activity was as low as that for PBS administration, suggesting citric acid is a safe additive as well as a potent absorption enhancer.

5. Conclusions

In the present study, we compared the effect of several additives on the hypoglycemic effect of intratracheally administered insulin as a dry powder or solution. The hypoglycemic effect depended on both the additives and the formulations used. Bacitracin and Span 85 were not as effective in dry powder as in solution in the present study. While citric acid was more effective in dry powder than that in solution to increase a hypoglycemic effect. In addition, when the insulin dry powder containing citric acid was administered, the LDH activity, a sensitive indicator of acute toxicity to lung cells, in bronchoalveolar lavage was as low as that for PBS administration, suggesting citric acid is a safe additive. Thus, citric acid appears to be a safe and potent absorption enhancer for insulin in dry powder.

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References

Edwards, D.A., Hanes, J., Caponetti, G., Hrkach, J., Ben-Jebria, A., Eskew, M.L., Mintzes, J., Deaver, D., Lotan, N., Langer, R., 1997. Large porous particles for pulmonary drug delivery. Science 276, 1868–1871.

Enna, S.J., Schanker, L.S., 1972. Absorption of saccharides and urea from the rat lung. Am. J. Physiol. 222, 409–414.

Fukuda, Y., Tsuji, T., Fujita, T., Yamamoto, A., Muranishi, S., 1995. Susceptibility of insulin to proteolysis in rat lung homogenate and its protection from proteolysis by various protease inhibitors. Biol. Pharm. Bull. 18, 891–894.

Henderson, R.F., Damon, E.G., Henderson, T.R., 1978. Early damage indicators in the lung. I. Lactate dehydrogenase activity in the airways. Toxicol. Appl. Pharmacol. 44, 291–297.

Henderson, R.F., 1984. Use of bronchoalveolar lavage to detect lung damage. Environ. Health Perspect. 56, 115–129.

Moores, S.R., Black, A., Evans, J.C., Evans, N., Holmes, A., Morgan, A., 1981. The effect of quartz, administered by intratracheal instillation, on the rat lung. I. The cellular response. Environ. Res. 24, 275–285.

Morgan, A., Moores, S.R., Holmes, A., Evans, J.C., Evans, N.H., Black, A., 1980. The effect of quartz, administered by intratracheal instillation, on the rat lung. I. The cellular response. Environ. Res. 22, 1–12.

Morita, T., Yamamoto, A., Takakura, Y., Hashida, M., Sezaki, H., 1994. Improvement of the pulmonary absorption of (Asu1,7)-eel calcitonin by various protease inhibitors in rats. Pharm. Res. 11, 909–913.

Kobayashi, S., Kondo, S., Juni, K., 1996. Pulmonary delivery of salmon calcitonin dry powders containing absorption enhancers in rats. Pharm. Res. 13, 80–83.

Komada, F., Iwakawa, S., Yamamoto, N., Sakakibara, H., Okumura, K., 1994. Intratracheal delivery of peptide and protein agents: absorption from solution and dry powder by rat lung. J. Pharm. Sci. 83, 863–867.

Lie, F.-Y., Kildsig, D.O., Mitra, A.K., 1992. Pulmonary biotransformation of insulin in rat and rabbit. Life Sci. 51, 1683–1689.

Newman, S.P., Hollingworth, A., Clark, R., 1994. Effect of different modes of inhalation on drug delivery from a dry powder inhaler. Int. J. Pharm. 102, 127–132.

Niven, R.W., Lott, F.D., Ip, A.Y., Cribbs, J.M., 1994. Pulmonary delivery of powders and solutions containing recombinant human granulocyte colony-stimulating factor (rhG-CSF) to the rabbit. Pharm. Res. 11, 1101–1109.

Okamoto, H., Aoki, M., Danjo, K., 2000. A novel apparatus for rat in vivo evaluation of dry powder formulations for pulmonary administration. J. Pharm. Sci. 89, 1028–1035.

Okumura, K., Iwakawa, S., Yoshida, T., Seki, T., Komada, F., 1992. Intratracheal delivery of insulin absorption from solution and aerosol by rat lung. Int. J. Pharm. 88, 63–73.

Patton, J.S., Trinchero, P., Platz, R.M., 1994. Bioavailability of pulmonary delivered peptide and proteins: a-interferon, calcitonins and parathyroid hormones. J. Contr. Release 28, 79–85.

Takada, K., Yamamoto, M., Nakae, H., Asada, S., 1980. Enhancement of pulmonary absorption of fluoroscein isothiocyanate-labelled dextran (FITC-dextran) by sodium glycocolate in rats. Chem. Pharm. Bull. (Tokyo) 28, 2806–2808.

Timsina, M.P., Martin, G.P., Marriott, C., Ganderton, D., Yianneskis, M., 1994. Drug delivery to the respiratory tract using dry powder inhalers. Int. J. Pharm. 101, 1–13.

Yamamoto, A., Umemori, S., Muranishi, S., 1993. Absorption enhancement of intrapulmonary administered insulin by various absorption enhancers and protease inhibitors in rats. J. Pharm. Pharmacol. 46, 14–18.

Yamamoto, A., Okumura, S., Fukuda, Y., Fukui, M., Takahashi, K., Muranishi, S., 1997. Improvement of the pulmonary absorption of (Asu1,7)-eel calcitonin by various absorption enhancers and their pulmonary toxicity in rats. J. Pharm. Sci. 86, 1144–1147.

Yoshida, H., Okumura, K., Hori, R., Anmo, T., Yamaguchi, H., 1979. Absorption of insulin delivered to rabbit trachea using aerosol dosage form. J. Pharm. Sci. 68, 670–671.