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Research Paper

Inhaled Insulin is Better Absorbed When Administered as a Dry Powder Compared to Solution in the Presence or Absence of Alkylglycosides

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Purpose. This study was performed to investigate the safety of alkylglycosides administered via the respiratory route and to compare the pulmonary absorption profiles of insulin administered as dry powder inhaler and inhaler solution.
Methods. The safety of a series of alkylglycosides with varying alkyl chain lengths was studied by measuring the enzymatic activities in the bronchoalveolar lavage (BAL) fluid of rat lungs. Pulmonary formulations of insulin plus octylmaltoside were administered either as solution or lyophilized dry powder to anesthetized rats, and absorption of insulin was assessed by measuring plasma insulin and glucose levels. The physical characterization of the dry powder formulation was performed using scanning electron microscope (SEM) and Fourier transform infrared spectrophotometer (FTIR).
Results. The BAL analysis showed that there was a gradual increase in the amount of lung injury markers released with the increase in the hydrophobic chain length of alkylglycosides. The pulmonary administration of lyophilized dry powder of insulin plus octylmaltoside or its solution counterpart showed that the bioavailability of powder formulation was about 2-fold higher than that of the formulation administered as solution. The SEM studies showed a subtle difference in the surface morphologies of formulation particles after lyophilization. FTIR data showed minor interactions between the peptide and excipients upon lyophilization.
Conclusions. Of the alkylglycosides tested, octylmaltoside was least toxic in releasing lung injury markers. Octylmaltoside-based dry powder insulin formulations were more efficacious in enhancing pulmonary insulin absorption and reducing plasma glucose levels compared with the formulations administered as a solution.

KEY WORDS: alkylglycosides; bronchoalveolar lavage; dry powder; inhalation; insulin.

INTRODUCTION

Dry powder inhalers (DPIs) offer several advantages over other pulmonary drug delivery systems that include enhanced drug stability, greater accuracy in dosing, breath actuated delivery, and improved patient compliance (1). More importantly, DPIs are preferred delivery systems for protein and peptide drugs that are susceptible to degradation upon extended storage in aqueous solution. However, one of the limitations of pulmonary delivery of large molecular weight drugs is the requirement that they be coadministered with a group of reagents called absorption promoters. Surfactants, cyclodextrins, and amphiphilic polymers are widely used to promote absorption of macromolecules across the mucosal routes including pulmonary route of administration (2). The most common practice for preparing and studying absorption enhancer-based formulation of peptide drugs is to mix the drug and absorption enhancer in an aqueous solution and then administer the formulation by an aerosolizer or spraying device (3–5). However, little information is available with regard to the efficacy of absorption enhancers present in dry powder formulations of protein and peptide drugs. More specifically, it remains largely unknown if absorption enhancer-based formulations administered as a DPI are as efficacious as that administered as an inhaled solution. Furthermore, reports on the relative rates of absorption of peptide drugs administered as DPIs and inhaler solutions are rather conflicting. For example, the bioavailability of human granulocyte colony-stimulating factor administered as a DPI was found to be lower than that administered as an inhaled solution (6). On the other hand, no differences in the bioavailabilities of several therapeutic proteins including calcitonin-, insulin-, and thyrotropin-stimulating hormone were observed when administered as DPI and inhaled solution (7).

Previously, we have shown that the pulmonary absorption of insulin can be increased by formulating the drug in an aqueous solution of a group of absorption enhancers called alkylglycosides (5). Alkylglycosides are a relatively newer class of nonionic surfactants consisting of disaccharides such

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as maltose or sucrose attached to alkyl chains of variable length. These agents have shown promise as absorption enhancers of protein and other macromolecules after delivery via the respiratory route (8,9). In earlier studies, efficacy of alkylglycosides as absorption enhancers was tested by administering aqueous solution of alkylglycoside plus peptide formulation via the respiratory routes. Currently, it is unknown if there are differences between the efficacies of alkylglycoside-based pulmonary formulation of insulin administered as a DPI and that administered as an inhaled solution.

On the other hand, it is of significant importance to investigate the biochemical changes that may occur in the lungs because of chronic administration of a surfactant-based pulmonary formulation of peptide drugs. Surfactants increase the paracellular or transcellular transport of a drug by loosening of cell–cell tight junctions or solubilizing cell membrane components (10). However, the mechanisms of action of these agents may compromise the integrity of lung epithelial barrier. A relatively simple method for screening the safety of absorption enhancers for pulmonary drug delivery in animal models is the analysis of bronchoalveolar lavage (BAL) fluid. The pulmonary toxicity studies using BAL fluid can provide important information as to the lung injury at both cellular and enzymatic levels (11,12). Previously, we have reported an acute toxicity study of a C-14 maltoside (14 carbon containing maltoside)-based pulmonary formulation of insulin (13). However, there are no data as to the safety of these agents upon chronic administration via the lungs. It has been shown previously that the efficacy of alkylglycosides in enhancing nasal absorption of insulin increases with the increase in hydrophobic chain length (8). Nevertheless, it is not known if the hydrophobic chain length of alkylglycosides plays any role in the release of injury markers in the lungs because of chronic administration of these agents.

Furthermore, the knowledge of physical characteristics of proteins, absorption enhancers, formulation carriers, and their interactions in a dry powder formulation is important to further optimize the formulation for pulmonary delivery. Traditionally, the carrier of choice for DPIs has been lactose, although alternative carriers such as mannitol, glucose, and sorbitol have also been proposed (14). Lactose offers several advantages as a carrier for pulmonary formulations, including its well-established safety profiles (15), good flow properties, ease of availability, and relatively low price. Furthermore, the adhesional properties of lactose carriers used in DPI formulations have also been well investigated (16). However, it is important to know the interactions between the absorption enhancer, alkylglycosides, and the carrier, lactose, to design an alkylglycoside-based pulmonary formulation of insulin.

This study seeks to fill the above gaps in the knowledge of alkylglycoside-based pulmonary formulation of peptide drugs, and therefore, it is designed to (1) screen the safety of alkylglycosides for pulmonary drug delivery by monitoring the release of lung injury markers in BAL fluid; (2) investigate the relative efficacies of alkylglycoside-based DPI and inhaled solution of a widely used peptide drug, insulin; and (3) study the interactions between the drug, carrier, and absorption enhancer.

MATERIALS AND METHODS

Recombinant human insulin (expressed in yeast) was obtained from Sigma-Aldrich Inc. (St. Louis, MO, USA). *n*-Octyl-β-D-maltoside (C-8), *n*-decyl-β-D-maltoside (C-10), *n*-dodecyl-β-D-maltoside (C-12), and *n*-tetradecyl-β-D-maltoside (C-14) were purchased from Anatrace Inc. (Maumee, OH, USA). Reagents for lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) determination were obtained from Pointe Scientific, Inc. (Lincoln Park, MI, USA). *N*-Acetylglucosaminidase (NAG) assay kit was purchased from Equal Diagnostics (Exton, PA, USA). Protein quantitation kit was obtained from Pierce (Rockford, IL, USA). Sodium dodecyl sulfate (electrophoresis purity reagent) was purchased from BioRad Laboratories (Hercules, CA, USA). Alpha-lactose monohydrate was a product of Fisher Scientific (Fair Lawn, NJ, USA).

Bronchoalveolar Lavage Studies

For this set of experiments, the rats were divided into six groups, four rats in each group, to receive six different treatments. Four groups of animals received 4.6-mM concentration of different alkylglycosides (C-8 to C-14 maltosides), whereas the fifth and sixth groups received saline as a negative control and 4.6 mM of sodium dodecyl sulfate solution as a positive control. The preparations (100 μl) were administered pulmonarily for 7 days as reported earlier (5), and on the eighth day, animals were reanesthetized, and the BAL fluid analysis was performed as described earlier (13). Briefly, the respiratory apparatus was exposed by a midlevel incision in the thoracic cavity. The lungs were surgically removed after exsanguination by severing the abdominal aorta, and wet lung weight was recorded. The lungs were lavaged by instillation of 5-ml aliquot of normal saline into the trachea, left in the lungs for 30 s, withdrawn, reinstilled for additional 30 s, and finally withdrawn. The fluid obtained was centrifuged at $500 \times g$ for 10 min, and the supernatant was collected for analysis of total protein content, LDH, ALP, and NAG activity. The concentrations of these enzymes in the lavage fluid were determined by using commercial assay kits, and activities of these enzymes are expressed as IU/l.

Preparation of Pulmonary Insulin Formulations

To prepare an insulin solution without absorption enhancer, the drug was initially suspended in saline and then solubilized with the addition of 0.1 N HCl. The pH of the solution was adjusted to 7.4 with the addition of 0.1 N NaOH. Insulin solution formulation that contained C-8 maltoside was prepared by mixing a stock solution of C-8 maltoside with lactose and insulin solution. The concentration of maltoside in the solution was 4.6 mM. The dry powder formulations were prepared by lyophilizing insulin solution in the presence or absence of C-8 maltoside and lactose. The lyophilized powder obtained was then ground uniformly before being used for pulmonary absorption studies. The lyophilization of various solutions was carried out at a temperature of −50°C and pressure of 120 mT in EZ-DRY (FTS Systems Inc., Stone Ridge, NY, USA) for 16 h.

Pulmonary Absorption Studies

Male Sprague–Dawley rats (Charles River Laboratories, Charlotte, NC, USA) weighing between 275 and 325 g were used for the pulmonary absorption studies. On the day of the experiment, the animals were anesthetized by an intramuscular injection of a mixture of xylazine (20 mg/ml) and ketamine (100 mg/ml). Anesthesia was maintained with additional doses of the anesthetic solution as needed throughout the experiments. At 50–60 min after the initial anesthesia, pulmonary formulations (100 μl) were administered as reported previously (5). Briefly, the tongue of the anesthetized animal was pulled aside with a pair of blunt forceps, and the inside of the mouth was illuminated with a fiber optic laryngoscope. Once the trachea was located, a MicroSprayer® tube (Penn Century Inc., Philadelphia, PA, USA) attached to a syringe was inserted into the trachea, and the formulation in the syringe was quickly sprayed. Pulmonary administration of lyophilized dry powder was performed in a similar way using a dry powder insufflator (Penn Century Inc.). Dose response studies were conducted by administering increasing doses (0.375, 0.75, and 1.5 U) of insulin solution or lyophilized dry powder or their formulation with C-8 maltoside and/or lactose. The rats were placed on their stomach after the administration of formulations, and blood sampling was performed from the tip of the tail at time 0 (immediately before administration) and 5, 10, 20, 30, 40, 60, 90, and 120 min after the drug administration. About 200 μl of blood was collected in heparinized microcentrifuge tubes, placed in an ice bucket until the plasma was separated by centrifugation (1320 × *g* for 5 min), and stored at −20°C until analysis. The plasma glucose levels were measured using a glucose kit (Infinity™ Glucose Reagent, Sigma), and plasma insulin levels were determined using a human insulin-specific radioimmunoassay kit (Linco Research Inc., St. Charles, MO, USA). All the animal studies were conducted in accordance with the principles outlined in the *Guide for the Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.

Pharmacokinetic/Pharmacodynamic Analysis

Standard noncompartmental pharmacokinetic analysis (Kinetica, Version 4.0, InnaPhase Corp., Philadelphia, PA, USA) was performed for the plasma insulin–time profiles. $AUC(In)_{0\rightarrow\infty}$ for plasma insulin–time curves and $AUC(Gl)_{0\rightarrow120}$ for blood glucose–time curves were estimated by the trapezoidal method. The value of plasma glucose at time zero was normalized to 100%.

Statistical Analysis

The changes in enzyme activities in BAL fluid and bioavailability parameters after different treatments were compared using one-way ANOVA. When the differences in the means were significant, *post hoc* pairwise comparisons were carried out using Newman–Keuls multiple comparison tests. For all the statistical tests performed, the level of significance (α) was set at 0.05.

Scanning Electron Microscope Analysis

Scanning electron microscope (SEM) images of different excipients in the formulation were obtained in Hitachi S-808 scanning electron microscope (Freehold, NJ, USA). The samples were prepared on a conductive, double-sided adhesive tape and then sputter-coated with gold under argon at an atmospheric pressure of 50 mPa.

Fourier-Transform Infrared Spectroscopy

Infrared spectra were obtained with a Nexus 470 Fourier-transform infrared (FTIR; Thermo Nicolet Corporation, Madison, WI, USA). The spectra were recorded under automatic atmosphere suppression mode, and the number of scans and resolution were 32 and 4, respectively. The samples were analyzed between wave numbers 4000 and 600 cm^{-1}. A background spectrum was collected before running each sample.

RESULTS AND DISCUSSION

Bronchoalveolar Lavage Studies

The BAL fluid analysis was used to assess the biochemical changes that may occur in the lungs after 7 days of treatment with alkylglycosides. Although there are reports that shows data on the toxicity of absorption enhancers used in dry powder formulations for pulmonary delivery (17,18), there are little or no data on the effects of repeated dosing of absorption enhancers on the respiratory route. In fact, most of the studies were performed after administration of a single dose of a given agent. A single dose study used to assess the pulmonary injury is not of much relevance to a formulation that needs to be administered on a chronic basis. In this study, short-term effects of alkylmaltosides and the role of their alkyl chain length on the lungs were investigated by determining the wet lung weight and enzyme levels in the lung after administration of alkylmaltosides for 7 days.

The wet lung weight of the animals showed a gradual increase with an increase in the alkyl chain length of the glycosides (Fig. 1a). However, with the exception of the positive control, sodium dodecyl sulfate (SDS), lung weights of none of the glycoside-treated rats were significantly different from that of saline-treated rats ($p > 0.05$). The levels of LDH, ALP, and NAG activities in the BAL fluid analysis revealed a concomitant increase with the increase in chain length of the alkylglycosides tested (Fig. 1b–d). However, increases in LDH and ALP enzyme levels for C-12 and C-14 maltosides and SDS-treated rats were significantly higher compared with saline-treated rats ($p < 0.05$). For NAG levels in the BAL fluid, the increase in enzyme activity was significant only for C-14 maltoside and SDS-treated group. The total protein content in the lavage fluids showed a significant increase in C-10, C-12, and C-14 maltoside-treated groups, with the positive control showing the maximum protein activity (Fig. 1e).

The BAL fluid analysis has been used as a useful tool for *in vivo* screening of pulmonary injury in experimental

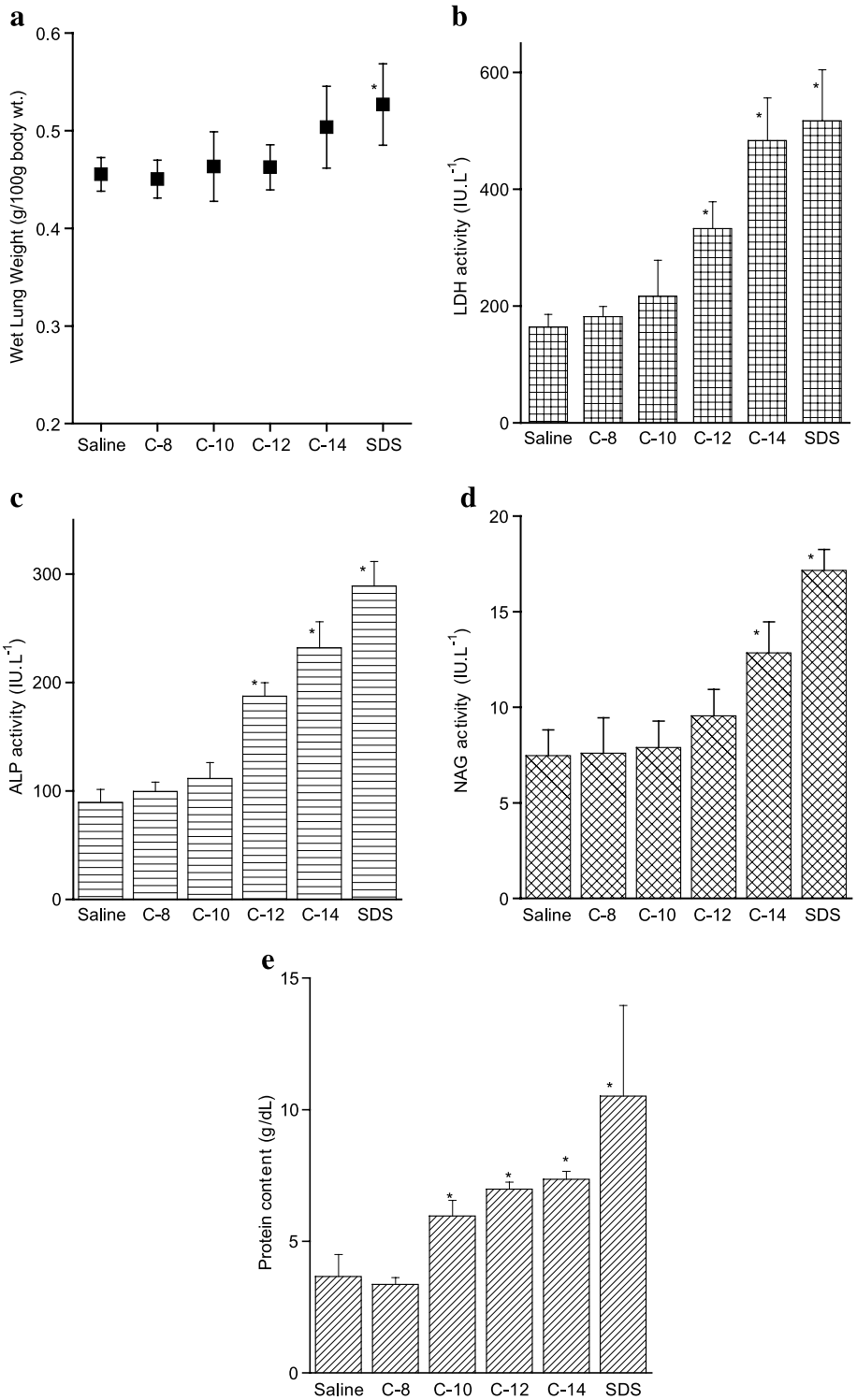

Fig. 1. (a) Corrected lung weights; activities of (b) lactate dehydrogenase (LDH); (c) alkaline phosphatase (ALP); (d) *N*-acetylglucosaminidase, NAG; and (e) total protein content in bronchoalveolar lavage fluid, following chronic administration of 4.6 mM concentration of alkylglycosides. C-8, C-10, C-12, and C-14 are octyl-, decyl-, dodecyl-, and tetradecylmaltoside, respectively. SDS = sodium dodecyl sulfate. Data represent mean ± SD. $n = 4$.

animals (19). The detection of LDH activity in BAL fluid as an indicator of lung injury upon instillation of a surfactant showed that the LDH levels increased with the increase in amount of instilled surfactant (20). ALP, a membrane-bound enzyme, has been considered as a marker for type II pneumocyte proliferation in response to type I cell damage (11). The presence of lysosomal enzymes such as NAG in the BAL fluid has been associated with increased phagocytic activity

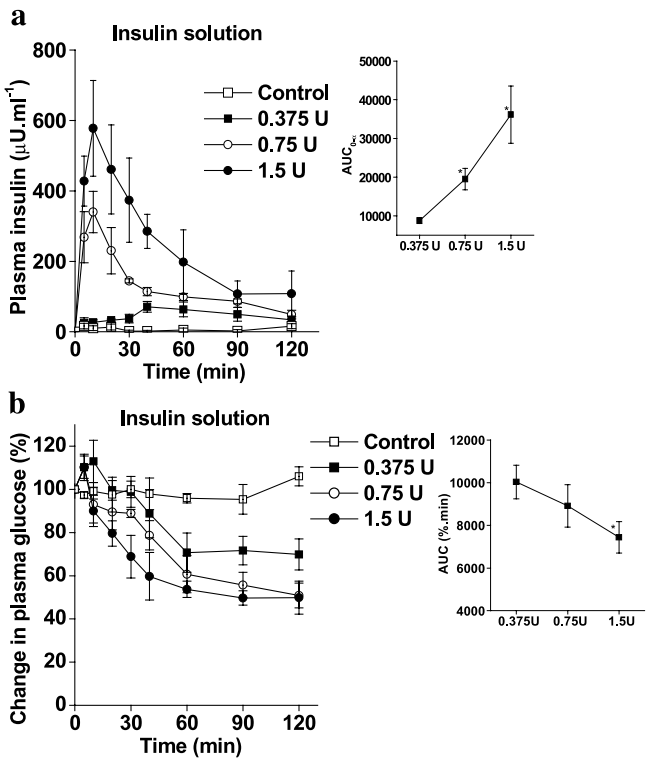

Fig. 2. Changes in (a) plasma insulin and (b) plasma glucose after pulmonary administration of increasing doses of insulin solution. Inset shows area under the curve (AUC) changes in (a) and (b) with increasing doses of insulin. Data represent mean ± SD. $n = 4$.

or toxicity and lysis of phagocytic cells in the lung (11). The total protein content in BAL fluid is a marker of alveolar–capillary barrier permeability, and total protein in BAL fluid from animal studies has shown to be a sensitive marker of inflammation (21).

The data presented above clearly suggest that the levels of injury markers released by different agents vary depending on alkyl chain of the agent administered. These differences can perhaps be best explained by critical micelle concentration and micelle size of the agents. It has been proposed that the micelle size of a surfactant increases with the square of the alkyl chain length (22). Therefore, we hypothesize that with the increase in the size of the micelles, the longer chain glycosides are likely to accommodate more of the membrane components in their micellar structure than the shorter chain glycosides and thereby may compromise the epithelial cell membrane integrity to a larger extent. These propositions are consistent with the findings in the present study in that we found a gradual increase in the release of enzymes in BAL fluid with the increase in chain length of alkylglycoside. In contrast, the C-8 maltoside showed the least increase in enzyme levels compared to saline control. In terms of the safety of alkylglycosides, shorter chain glycosides, such as C-8 maltoside, could be relatively safe compared with longer chain glycosides when used as absorption enhancers for pulmonary delivery of peptide and protein drugs. However, it must be recognized that in this study, rats were treated with the absorption enhancers for 7 days. Although the data show that C-8 maltosides were relatively safe compared to longer chain agents, further studies are required to establish its safety for long-term use in a pulmonary formulation of insulin that needs to be administered for the entire lifetime of a diabetic patient.

Pulmonary Absorption Studies

The pharmacokinetics and pharmacodynamics of insulin solution and dry powder formulations were studied by measuring plasma insulin levels and monitoring concomitant changes in plasma glucose after pulmonary administration in anesthetized rats. When solutions containing increasing doses of insulin (0.375, 0.75, and 1.5 U) were administered, there was an increase in insulin absorption with the increase in the dose (Fig. 2a). The $AUC(In)_{0\rightarrow\infty}$ values for all doses of insulin tested were significantly higher than that of the saline

Table I. Pharmacokinetic and Pharmacodynamic Parameters Following Pulmonary Delivery of Recombinant Insulin Solution or Dry Powder

		Insulin solution, dose of insulin (U)				Insulin dry powder, dose of insulin (U)		
	Saline	0.375	0.75	1.5	Lactose	0.375	0.75	1.5
Pharmacokinetic parameters								
C_{max} (μU ml^{-1})	16.1 ± 7.7	70.6 ± 11.5	365 ± 28.2	577 ± 135	6.2 ± 5.3	133 ± 32.9	551 ± 140	932 ± 121
T_{max} (min)	20	40 ± 23	6.6 ± 2.8	10	5	20	15 ± 7.0	20
$AUC(In)_{0\rightarrow\infty}$ (μU min ml^{-1})	733 ± 102	8781 ± 763	19513 ± 2767	36180 ± 3370	343 ± 205	14133 ± 1045	28585 ± 2498	43657 ± 7389
F(fr)	–	0.064 ± 0.005	0.07 ± 0.01	0.065 ± 0.006	–	0.11 ± 0.007	0.10 ± 0.01	0.079 ± 0.013
Pharmacodynamic parameters								
$AUC(Gl)_{0\rightarrow120}$ (% min)	12600 ± 496	10035 ± 787	8920 ± 995	7443 ± 734	12101 ± 225	9617 ± 956	7143 ± 624	5898 ± 521
%MG[a]	–	69.9 ± 7.19	50.9 ± 5.7	49.7 ± 3.2	–	72.7 ± 9.33	44.3 ± 5.3	31.7 ± 12.3
t%MG[b] (min)	–	120	120	90	–	90	120	120

Data represent mean ± SD. $n = 4$.
AUC = area under the curve.
[a] Percent minimum plasma glucose value.
[b] Time to reach percent minimum plasma glucose value.

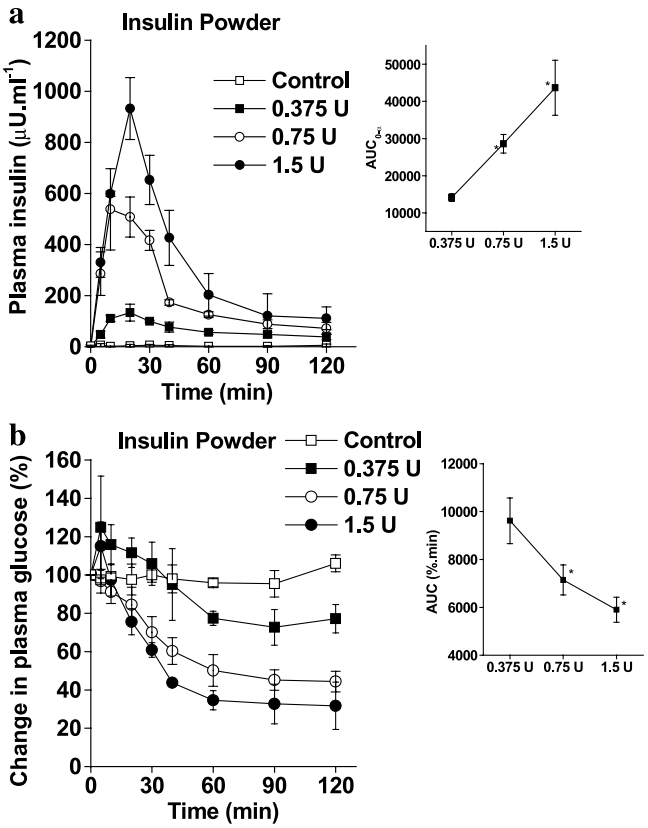

Fig. 3. Changes in (a) plasma insulin and (b) plasma glucose after pulmonary administration of increasing doses of insulin powder. Inset shows AUC changes in (a) and (b) with increasing doses of insulin. Data represent mean ± SD. $n = 4$.

control ($p < 0.05$) and showed an almost linear trend with the increase in dose (inset, Fig. 2a). The pharmacokinetic profiles of insulin solution absorption after pulmonary delivery are presented in Table I. In agreement with insulin data, the plasma glucose values showed a significant reduction with increasing doses of insulin solution (Fig. 2b). Furthermore, there was a gradual decrease in $AUC(Gl)_{0-120}$ values for plasma glucose–time profiles with the increasing doses of insulin (inset, Fig. 2b). Similar to the delivery of insulin solution, administration of different doses of insulin powder led to a gradual increase in the peptide absorption from the lungs (Fig. 3a). The $AUC(In)_{0\rightarrow\infty}$ values for insulin powder showed a linear increase with the increasing dose (inset, Fig. 3a and Table I). The $AUC(In)_{0\rightarrow\infty}$ values for all the doses of peptide powder tested were significantly different from each other and from that of the lactose control ($p < 0.05$). The decrease in plasma–glucose values was in agreement with the increase in insulin absorption after pulmonary delivery. With increase in the dose of insulin powder, there was a decline in plasma glucose levels (Fig. 3b). Furthermore, the AUC $(Gl)_{0-120}$ values for dry powder insulin showed a decrease with the rise in dose of insulin (inset, Fig. 3b). However, when the absorption profiles of insulin solution and that of dry powder were compared, the increase in insulin absorption from dry powder was significantly higher than that for insulin administered as a solution. Interestingly, there was not much difference between the absorption profiles when the dose of insulin was 1.5 U (Figs. 2a and 3a). This anomaly is perhaps because of the saturability of insulin absorption from the lung after dry powder delivery, and as a result, 1.5 U powder insulin dose did not cause further increase in insulin absorption compared to that of solution formulation.

The pulmonary absorption profiles of C-8 maltoside-based DPI and inhaler solution are presented in Fig. 4. When a lyophilized formulation containing insulin (0.375 U), 4.6 mmol of C-8 maltoside, and lactose was administered pulmonarily, the increase in insulin absorption was significantly higher than that of insulin solution containing C-8 maltoside plus lactose formulation (Table II). The absolute bioavailability of the powder formulation showed almost 2-fold increase compared to that of solution mixture ($F = 0.48 \pm 0.07$ *vs.* 0.25 ± 0.05). Concomitantly, with the increase in exogenous human insulin levels in rat blood, there was a gradual decline in plasma glucose values (Fig. 4). In agreement with the increased plasma insulin levels after the delivery of dry powder formulation, the reduction in plasma glucose levels was more pronounced in the case of dry powder compared to solution (Fig. 4). The administration of lactose or C-8 maltoside plus lactose did not cause any significant reduction in plasma glucose values, thereby suggesting that neither maltoside nor lactose acts as a hypoglycemic agent. These observations suggest that alkylglycosides are more efficacious in enhancing pulmonary absorption of in-

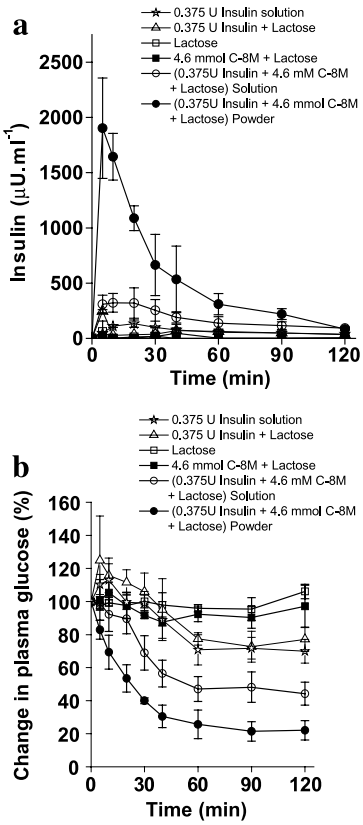

Fig. 4. Changes in (a) plasma insulin and (b) plasma glucose after pulmonary administration of C-8 maltoside (C-8M)-based insulin solution or dry powder formulation. Data represent mean ± SD. $n = 4$.

Table II. Pharmacokinetic and pharmacodynamic parameters of pulmonarily administered solution or dry powder formulations of insulin containing C-8 maltoside

		Insulin, 0.375 U + 4.6 mmol C-8 maltoside + lactose	
	C-8 maltoside + lactose[a]	Solution formulation	Dry powder formulation
Pharmacokinetic parameters			
C_{max} (μU ml^{-1})	15.6 ± 4.06	321 ± 84.6	1902 ± 454
T_{max} (min)	30	10	5
$AUC(In)_{0\rightarrow\infty}$	417 ± 185	66197 ± 8398	134769 ± 4945
F(fr)	–	0.25 ± 0.05	0.48 ± 0.07
Pharmacodynamic parameters			
$AUC(Gl)_{0\rightarrow120}$ (% min)	11222 ± 814	7153 ± 823	4195 ± 709
%MG[b]	–	44.2 ± 6.95	21.4 ± 5.89
t%MG[c] (min)	–	120	90

Data represent mean ± SD. $n = 4$.
AUC = area under the curve.
[a] Combination without insulin.
[b] Percent minimum plasma glucose value.
[c] Time to reach percent minimum plasma glucose value.

sulin when administered as dry powder compared with insulin administered in aqueous solution.

The enhanced absorption of insulin obtained after administration of dry powder formulation compared with that of solution formulation could be attributed to two factors. First, lyophilization of the formulation carrier in the present study has shown to produce particles with a porous morphology (Fig. 5f) compared with nonlyophilized carrier

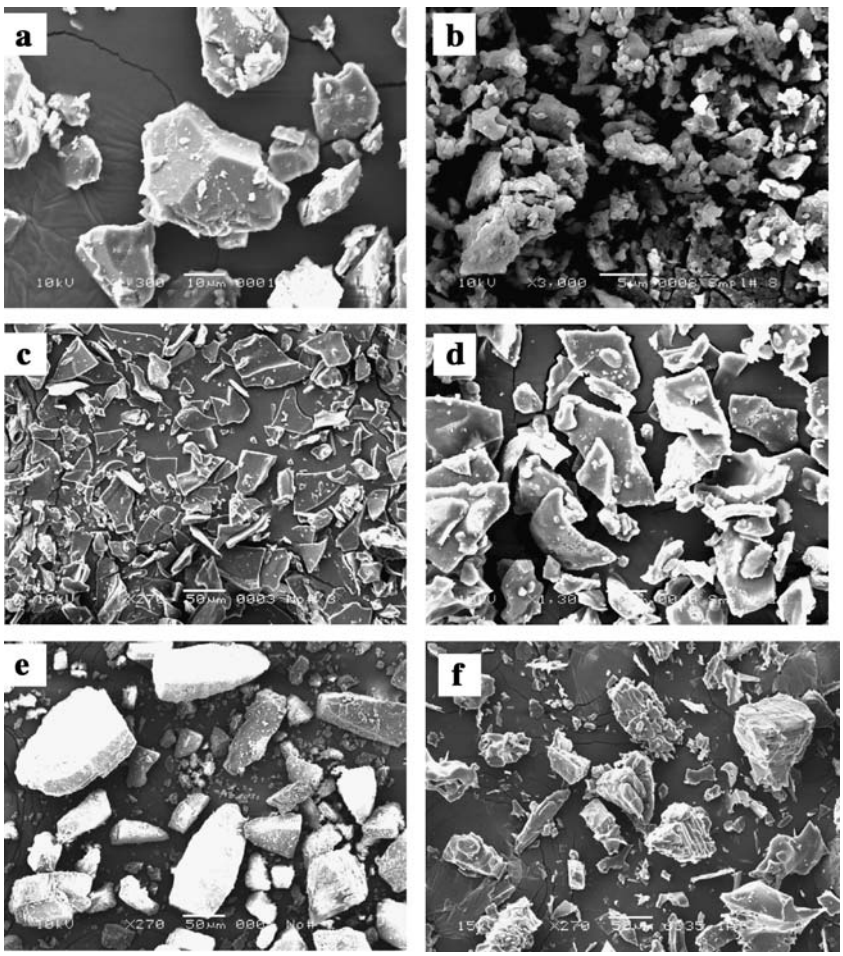

Fig. 5. Scanning electron micrographs of (a) untreated insulin, (b) lyophilized insulin, (c) untreated C-8 maltoside, (d) lyophilized C-8 maltoside, (e) untreated lactose, and (f) lyophilized lactose.

particles (Fig. 5e). The presence of pores within a particle may have led to a decrease in its density, which could have made particles travel longer distance and deposit in the alveolar region. A second explanation for enhanced absorption of DPI could be that for pulmonary delivery using insufflator, a relatively large amount of air (3 ml) is required to force the dry powder through the delivery device for inhalation into rats. This forceful delivery may have caused a deeper lung deposition of the powder, thereby producing increased drug absorption *in vivo* compared to solution delivery. However, such an aliquot of air was not required for administering liquids from the inhalation device.

The results obtained in the present study are consistent with the findings reported by others (23,24) that the dry powders are more efficacious than solution formulation for pulmonary delivery of proteins. Okamoto *et al.* (23) have shown that spray-dried insulin when delivered as a powder was more effective than the spray-dried solution in reducing plasma glucose values in anesthetized rats. Kobayashi *et al.* (24) have shown that when surfactants were incorporated in pulmonary calcitonin formulations, the bioavailability of the dry powder was more than that of the solution formulation.

Scanning Electron Microscopy

The most commonly used method for preparing solid protein pharmaceuticals is lyophilization. This technique has also been employed for reducing particle size of medicinal compounds (25). Scanning electron micrographs of insulin, C-8 maltoside, and lactose show differences in particle size and morphology before and after lyophilization (Fig. 5). The particles of insulin showed a slightly amorphous morphology and reduced size (<5 *vs.* ~10 μm) after lyophilization compared with untreated particles (Fig. 5a and b). The particles of lyophilized C-8 maltoside showed slightly blunt and indistinct edges in appearance in comparison to the sharp and well-defined edges before lyophilization resembling pieces of "broken glass" (Fig. 5c and d). The untreated lactose particles displayed the well-known tomahawk shape with hard surface morphology, whereas the lyophilized particles showed small pores and crevices on their surface (Fig. 5e and f), suggesting the carrier's ability to form good electrostatic, capillary, and/or van der Waals interactions with other formulation excipients (26). However, the contribution of each of these forces to the overall adhesional efficiency of the carrier depends on several factors including contact area between the drug or excipient and lactose, surface and interfacial free energies of formulation particles (27), and environmental conditions (28).

Although particle size of a drug in a DPI should be within the respirable range (<5 μm) for maximum alveolar deposition, particles of the carrier such as lactose are generally outside this range and are mixed with smaller drug particles, which have a larger fraction within the respirable range (29). It should also be recognized that particle interactions are of great significance within a DPI formulation. It is well known that strong interparticulate forces within a powder formulation are the reasons for poor efficiency of a DPI. Although such forces of interaction can be overcome by the forced inhalation methodology employed in rodent studies, in a clinical setting, the inspiratory effort of the patient should be able to overcome these adhesive forces between drug and carrier particles. Moreover, the interparticulate interaction is one of the important aspects of DPI efficiency. The SEM analysis in the present study (Fig. 5f) reveals the carrier's surface morphology conducive to form such interparticle bonding. However, apart from interparticle interactions, several other factors of a DPI formulation including particle density and porosity need to be considered to obtain an optimized formulation capable of deeper lung deposition after pulmonary delivery.

Fourier Transform Infrared Spectroscopy

The process of lyophilization may induce several potential changes in the IR spectra of proteins. In this regard, the FTIR analysis is probably the most widely used technique for studying structural modifications in proteins because of lyophilization (30,31). Because the proportion of insulin and C-8 maltoside in the actual pulmonary formulation was negligible compared to the bulk of the carrier lactose, lyophilized formulations containing 1:1 ratios of insulin/lactose and insulin/C-8 maltoside were prepared to investigate the effects induced to the peptide during lyophilization.

In an IR spectrum of peptides and proteins, the characteristic bands of amide groups are similar to the absorption bands exhibited by secondary amides in general (32). The bands at amide region I and II, which occur at wave numbers 1653 and 1567 cm^{-1}, respectively, have been most frequently used in monitoring the conformational changes in protein molecules. The FTIR analysis in the present study reveals characteristic peaks of amide regions I and II for human insulin obtained from commercial source (Fig. 6a-c). The spectrum of lyophilized insulin/lactose (1:1) system showed two distinct peaks at wave numbers 1656 and 1539 cm^{-1} that correspond to amide regions I and II, respectively (Fig. 6b). There was no change in the position of peak for amide II region, although peak position for amide I region shifted slightly toward lower wave number. In the spectrum of insulin/C-8 maltoside (1:1) system, there was a small shift in regions I and II of amide bands compared with that of untreated insulin (Fig. 6c). Because the observed amide bands are composite complexes representing several overlapping bands such as α-helices, β-structures, turns, and random coils, it remains unknown as to which of these secondary structures of the peptide were affected by lyophilization when combined with C-8 maltoside or lactose. It is worthwhile to mention that a change in the secondary structure of the peptide does not necessarily lead to its reduced biological activity. In many cases, IR-monitored structural changes during lyophilization seemed to be reversible. For example, it was shown that lyophilization caused significant changes in the secondary structures of several model proteins including recombinant human albumin, RNase A, and insulin (33). All these structural changes were reversible upon reconstitution. The reason for the lack of significant changes observed in peptide's secondary structure in the present study could most likely be a result of the presence of lactose or C-8 maltoside in the formulation. This assumption is based on the fact that many sugars/polyols are used frequently as nonspecific protein stabilizers during lyophilization (34,35). Furthermore, of the sugars used, disaccharides seem to be the most

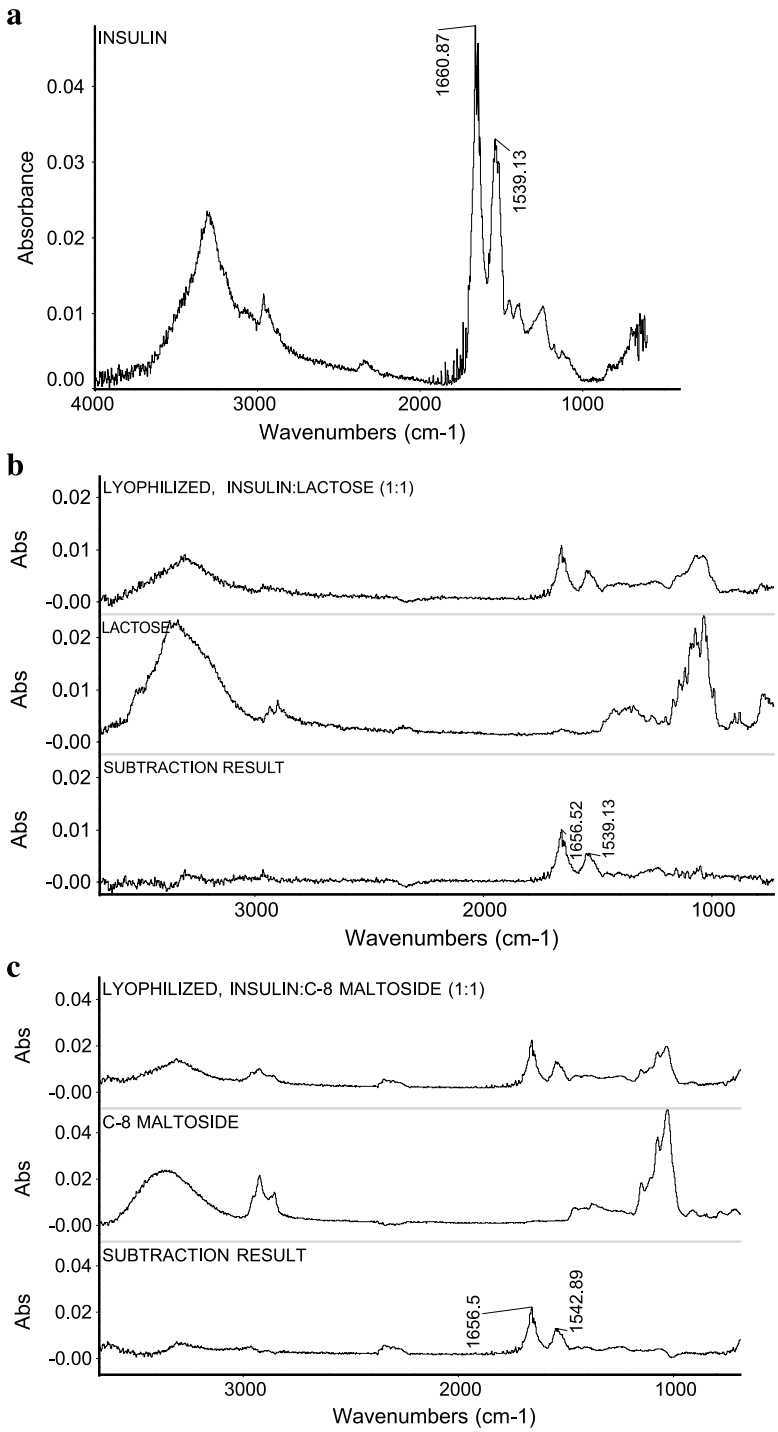

Fig. 6. Fourier transform infrared spectrophotometry analysis of dry powder. (a) Untreated insulin, (b) lyophilized insulin plus lactose (1:1), and (c) lyophilized insulin plus C-8 maltoside (1:1).

effective stabilizers. For instance, the disaccharides trehalose, sucrose, maltose, and lactose were all essentially equivalent to or more effective than monosaccharides such as glucose in stabilizing phosphofructokinase during lyophilization (36). As lactose and C-8 maltoside are disaccharides, it is reasonable to assume that these excipients also served as protein stabilizers during lyophilization apart from rendering their functions *in vivo* as formulation vehicle and absorption enhancer, respectively.

Taken together, the data presented above show that C-8 maltoside-based dry powder formulation of insulin was effective in enhancing pulmonary absorption of peptide drugs. The DPI of insulin plus alkylglycoside was at least twice as effective as inhaler solution in enhancing insulin absorption through the pulmonary route. In fact, this is the first study that reports the use of dry powder of alkylglycoside in peptide drug delivery via the respiratory route. The study also shows that alkylglycosides with shorter hydropho-

bic chain length are relatively safe compared with the alkylglycosides with longer hydrophobic chain. However, safety studies of short-chain alkylglycosides should be performed in higher laboratory animals for a much longer period of time, as most therapeutic protein and peptide drugs are administered on a long-term basis.

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