Pulmonary Absorption of Insulin Mediated by Tetradecyl-β-Maltoside and Dimethyl-β-Cyclodextrin

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Purpose. To determine if tetradecyl-β-maltoside (TDM) and dimethyl-β-cyclodextrin (DMβCD) enhance pulmonary absorption of insulin and to investigate if they do so by a reversible action on respiratory epithelium.

Methods. Insulin formulated with saline, TDM, or DMβCD was administered intratracheally, after laryngoscopic visualization, as a spray to anesthetized rats. Reversibility studies were conducted in intact rats by administering insulin at different time points after administration of TDM or DMβCD. The pharmacodynamics and pharmacokinetics of insulin formulations were assessed by measuring plasma glucose and plasma insulin concentrations.

Results. When insulin formulated with increasing concentrations (0.06–0.25%) of TDM or DMβCD were administered to anesthetized rats, there was a concentration-dependent decrease in plasma glucose and increase in plasma insulin concentrations. The relative bioavailability of insulin formulations containing TDM was higher (0.34–0.84%) than that of formulations containing DMβCD (0.19–0.48%). When insulin was administered 120 min after an agent was administered, in the reversibility study, no significant change in plasma glucose and insulin levels occurred compared to control.

Conclusions. Both TDM and DMBCD enhance pulmonary absorption of insulin, with TDM being more efficacious than DMβCD in enhancing insulin absorption via pulmonary administration. The effects of TDM and DMβCD on respiratory epithelium are reversible, and the epithelium reestablishes its normal physiologic barrier 120 min after exposure to these agents.

KEY WORDS: insulin absorption; tetradecyl-β-maltoside; dimethyl-β-cyclodextrin; bioavailability; reversibility study.

INTRODUCTION

Over the past few decades, inhaled insulin has been proposed as a potentially useful alternative to subcutaneously administered insulin (1–3). The pulmonary route has emerged as a route of choice for noninvasive delivery of protein and peptide drugs because of (a) relatively lower extracellular enzymatic activity, (b) large absorptive surface area (100 m^2), (c) extensive vasculature, (d) thin layer of alveolar epithelium (0.1–0.2 μm), and (e) short distance of air–blood exchange pathway (4–6). Because pulmonary delivery of insulin results in rapid absorption, insulin administered via this route has better control over postprandial hyperglycemia in comparison to subcutaneously administered insulin. It has been shown in humans that inhaled insulin is more rapidly absorbed (T_{max} 5–60 min) than injectable insulin (T_{max} 60–180 min) (2). Although newly developed Lyspro® insulin was shown to control after-meal hyperglycemia more efficaciously and expeditiously than regular insulin (7,8), this preparation still needs to be administered as an injection when food intake occurs. In addition, use of subcutaneous insulin is associated with discomfort, and there is a perceived notion that noninvasive insulin administration could improve the management of type I and type II diabetes mellitus. This would allow frequent dosing to more precisely control blood glucose levels. However, the primary obstacle to the pulmonary delivery of insulin is the relative impermeability of the drug when formulated without an absorption enhancer. Many absorption promoters have been investigated to obtain a clinically acceptable bioavailability of insulin administered via the pulmonary route (9–11). In particular, insulin formulated with different surface-active agents has been delivered in the form of solution or powder via the pulmonary route (9,10). Although many surfactants were found to be efficacious in enhancing pulmonary insulin absorption, none has yet satisfied the stringent requirements that must be imposed on a chemical that needs to be applied multiple times a day for the entire lifetime of a diabetic patient.

More recently, Pillion and his colleagues showed that alkylglycosides could be used effectively at extremely low concentrations to enhance nasal or ocular absorption of insulin, calcitonin, and glucagon (12–14). Chemically, alkylglycosides are disaccharides such as maltose or sucrose or monosaccharides such as glucose, attached to alkyl chains of variable length. Of the alkylglycosides tested, tetradecyl-β-maltoside (TDM), an alkylglycoside containing a 14-carbon alkyl chain attached to a maltose ring, has been shown to be the most efficacious in enhancing the nasal insulin absorption (15). Because alkylglycosides are nonionic in nature, metabolized to simple carbohydrates and alcohols, and inexpensive to produce, these excipients have shown promise as absorption promoters for nonparenterally administered peptide drugs (16).

On the other hand, cyclodextrins are a distinct family of chemical reagents that contain six, seven, or eight monosaccharide units in a cyclized ring with a central cavity that can accommodate other chemicals. A limited number of cyclodextrin derivatives stimulate transmucosal absorption of peptide drugs, and others have no effect (17). Importantly, methylated cyclodextrins, such as dimethyl-β-cyclodextrin (DMβCD), strongly accelerate transmucosal insulin absorption, whereas unmodified β-cyclodextrin has little effect on insulin absorption (18). It is believed that both DMβCD and TDM enhance absorption of insulin by different mechanisms. Cyclodextrins enhance transmucosal absorption of insulin by formation of an inclusion complex with insulin or by direct action on the membrane. The latter may involve removal of membrane proteins, complexation with different membrane components, or inhibition of proteolytic enzyme activity (18,19). However, it is speculated that TDM acts by loosening cell–cell tight junctions (unpublished data).

Currently, it is not known if alkylglycosides enhance pulmonary absorption of insulin; neither is it known if TDM, the most potent alkylglycoside, is as efficacious as DMβCD in enhancing pulmonary insulin absorption. This study is designed to test the hypothesis that TDM enhances pulmonary

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absorption of insulin and that it is as potent as DMβCD in enhancing absorption of insulin via the pulmonary route. In addition, to compare and contrast the efficacy of these reagents as absorption promoters, we investigated the duration of action of both agents on the epithelial membrane of the respiratory tract.

MATERIALS AND METHODS

Materials

Tetradecyl-β-D-maltoside (TDM) was purchased from Calbiochem-Novabiochem Corp. (La Jolla, CA), dimethyl-β-cyclodextrin (DMβCD) from Sigma-Aldrich Inc. (St. Louis, MO), and regular human insulin, Novolin®, from Novo Nordisk Pharmaceuticals Inc. (Princeton, NJ). A fiberoptic laryngoscope was a product of Welch Allyn (Skaneateles Falls, NY). A small animal intratracheal aerosolizer (MicroSprayer™) was purchased from PennCentury Inc. (Philadelphia, PA).

Preparations of Formulations

Stock solutions of TDM (1%) and DMβCD (1%) were prepared by dissolving the reagents in normal saline and stored at 4°C for 30 days or less. Stock solutions older than 30 days were discarded. It was previously determined that there were no differences in drug absorption when formulations containing reagents that had been stored for 30 days were used compared to formulations containing freshly prepared TDM or DMβCD solutions. The concentrations of TDM and DMβCD used in insulin formulations were 0.06, 0.125, and 0.25%.

Pulmonary Absorption Studies

Male Sprague-Dawley rats (Charles River Laboratories, Charlotte, NC) weighing 250–350 g were used for pulmonary absorption studies. On the day of the experiment, 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 during the experiments. Formulations were administered 50–60 min after initial dose of anesthetic agents. For intratracheal insulin administration, the upper incisors of anesthetized animals were tied with a rubber band, and the animal was held vertically by hanging to a burette stand. The tongue of the animal was pulled aside with blunt forceps and the inside of the mouth was illuminated with a fiberoptic laryngoscope. While the tongue was kept pressed toward the lower jaw by a restrainer attached to the laryngoscope, the trachea was visualized by maneuvering the laryngoscope. Once the trachea was exposed, the MicroSprayer® tube attached to a syringe (Merit Medical Systems, Inc., South Jordan, UT) was inserted into the trachea and pushed gently inward until the tube reached the bifurcation. Formulations containing insulin (1.25 U/kg) plus saline, TDM, or DMβCD were then quickly sprayed into the trachea. The amount of formulation administered was 80–115 μl depending on animal body weight. The rat was removed from the stand, placed on the working table, and serial blood sampling was accomplished from the tip of the tail at time 0 (immediately before administration of any formulations) and 5, 10, 20, 30, 40, 60, 90, and 120 min after drug administration. At each time point, ~300 μl of blood was collected in a heparinized microcentrifuge tube, stored on ice until the plasma was separated by centrifugation (1320 × *g* for 5 min), and stored at –20°C until further analysis.

Plasma glucose concentrations were measured using a glucose kit (Infinity™ Glucose Reagent, Sigma Diagnostics Inc., St. Louis, MO), the principle of which is based on the hexokinase method and measures glucose concentration with linearity in the range 0–650 mg/dl. Plasma insulin concentrations were determined using a human insulin-specific radioimmunoassay (RIA) kit (Linco Research Inc., St. Charles, MO).

All studies were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Reversibility Studies

In vivo reversibility studies were performed as described earlier (20,21). For these experiments, formulations were administered in two phases. In the first phase, 100 μl of a formulation containing 0.25% of one of the reagents, without insulin, was administered to the trachea at time 0. In the second phase, a formulation containing only the drug insulin (1.25 U/kg) was administered immediately or at 60 or 120 min after the first phase of administration. In these experiments, blood samples were collected at 0, 5, 10, 20, 30, 40, 60, 90, and 120 min as described above. Plasma glucose and insulin concentrations were determined as described above.

Pharmacokinetic/Pharmacodynamic Analysis

Standard noncompartmental pharmacokinetic analysis (Kinetica®, Version 4.0, InnaPhase Corp., Philadelphia, PA) was performed for plasma insulin–time profiles. The area under the curve ($AUC_{0\to\infty}$) for plasma insulin–time curves and $AUC_{0\to120}$ for blood glucose–time curves were calculated by the trapezoidal method. The area above the curve ($AAC_{0\to120}$) for the blood glucose–time curve was then obtained by subtracting the $AUC_{0\to120}$ of the plasma glucose curve from the total area of the quadrilateral. The value of plasma glucose at time zero was normalized to 100%. The percentage total reduction in initial plasma glucose concentration from 0 to 120 min ($\%TRG_{0\to120}$) was calculated as follows:

$$\%TRG_{0\to120} = \frac{AAC_{0\to120}}{AUC_{0\to120}} \times 100.$$

The area under the first moment curve ($AUMC_{0\to\infty}$) for plasma insulin-time profile was estimated from a plot of the product of plasma insulin concentration and time (c × t) vs. time. The mean residence time (*MRT*) was calculated by dividing $AUMC_{0\to\infty}$ by $AUC_{0\to\infty}$. The relative bioavailability (F') was assessed by comparing the $AUC_{0\to\infty}$ for plasma-insulin time curve obtained after pulmonary administration to that obtained after subcutaneous administration.

Statistical Analysis

Bioavailability values for different formulations were compared with paired *t* test or one-way ANOVA. When the

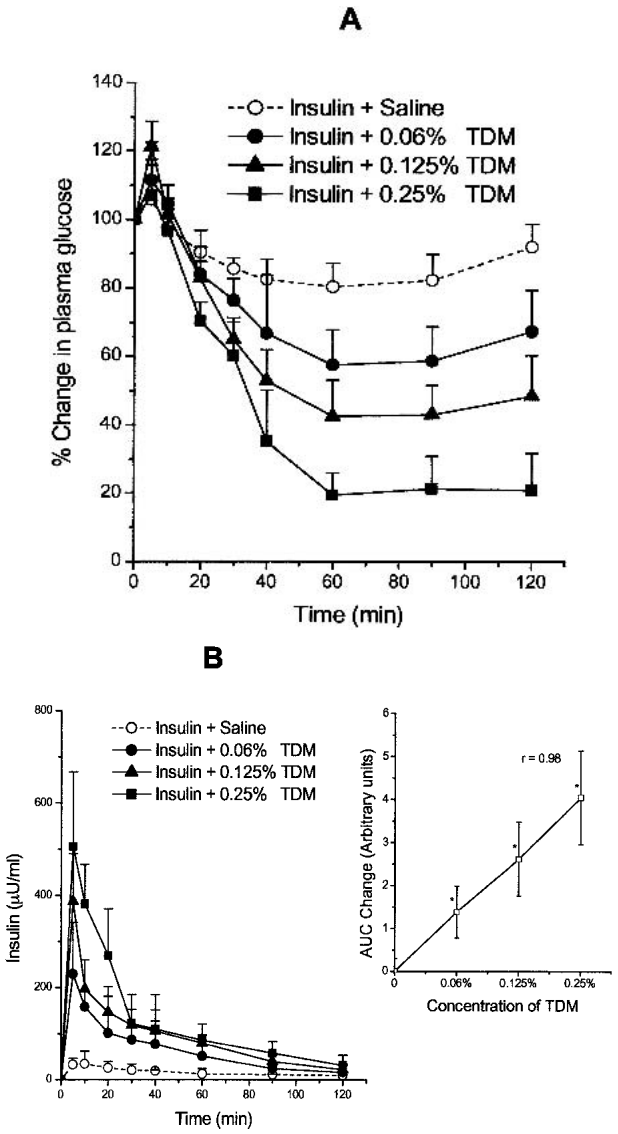

Fig. 1. Changes in plasma glucose (A) and plasma insulin (B) after intratracheal administration of insulin in saline or in the presence of increasing concentrations of TDM. Inset shows changes in $AUC_{0\to\infty}$ for plasma insulin-time curve with increasing concentrations of TDM. Data represent mean ± SD, n = 3–5.

differences in the means were significant, *post-hoc* pair wise comparisons were conducted using Newman-Keuls multiple comparison tests (GraphPad Prism version 3.03, GraphPad Software, San Diego, CA).

RESULTS AND DISCUSSION

As reported previously, rats anesthetized with xylazine/ketamine display hyperglycemia as a result of impaired insulin release (22). Pulmonary administration of 1.25 U/kg of regular human insulin was ineffective in lowering initial plasma glucose level when insulin was formulated in saline Fig. 1A.A When 0.06% TDM was added to the pulmonary insulin formulations, the plasma glucose concentration significantly decreased ($p < 0.05$). Additionally, when the concentration of TDM was increased from 0.06% to 0.25% in the formulations, there was a further decrease in plasma glucose level. The time to reach percent minimum plasma glucose level ($t\%MG$) for all formulations that contained insulin plus TDM was 60 min. Table I. Further, $\%TRG$ was highest when 0.25% TDM was used in the formulation (Fig. 1A). The results are consistent with the systemic uptake of insulin from the respiratory tract in a biologically active form in rats that received insulin formulations containing TDM. In fact, the effect of xylazine/ketamine anesthesia to diminish pancreatic insulin release would cause a steady increase in plasma glucose over the 120-min experiment if insulin had not been absorbed from the respiratory tract. In agreement with the decrease in plasma glucose, the plasma insulin was markedly increased when insulin was formulated with TDM at a concentration as low as 0.06% ($p < 0.05$). In contrast, little or no insulin was absorbed when it was formulated in saline (Fig. 1B). A plot of $AUC_{0\to\infty}$ values for plasma insulin–time curve against the concentration of TDM confirms a dose-dependent effect of TDM on pulmonary insulin absorption (inset, Fig. 1B). Likewise, the $AAC_{0\to120}$ values for plasma glucose–time curves for the formulations containing TDM were found to increase with increasing TDM concentrations (Table I). However, when the TDM concentration was further increased from 0.25% to 0.5% (data not shown), no additional increase in $AAC_{0\to120}$ or $AUC_{0\to\infty}$ was observed ($p > 0.05$). This suggests that TDM caused maximum insulin absorption at a concentration of 0.25%. In fact, it has been reported that absorption enhancers produce maximum increase in insulin absorption at a certain concentration above which no further absorption increase is observed. This may be attributed to maximum loosening of cell–cell tight junctions or saturation of insulin transport, which is believed to be receptor mediated (23,24), or saturation of the interaction between insulin and TDM.

Pharmacokinetic parameters of insulin resulted in a substantial increase in C_{max} with the increasing concentrations of TDM ($p < 0.05$) Table II. The T_{max} for insulin formulations containing TDM was reduced from 10 min (control) to 5 min.

Table I. Hypoglycemic Effects of Intratracheally Administered Insulin Formulated with Saline or with Different Concentrations of TDM or DMβCD

		Formulations containing insulin plus TDM			Formulations containing insulin plus DMβCD		
		Concentration of TDM			Concentration of DMβCD		
	Insulin alone	0.06%	0.125%	0.25%	0.06%	0.125%	0.25%
$\%TRG_{0\to120}$	18.7 ± 4.26	47.3 ± 8.92	68.9 ± 7.45	81.8 ± 10.3	37.7 ± 11.59	58.5 ± 7.29	72.5 ± 9.31
$\%MG$	80.7 ± 5.71	58.0 ± 8.82	42.4 ± 10.8	19.6 ± 5.0	61.9 ± 8.17	45.7 ± 10.2	36.5 ± 6.35
$t\%MG$ (min)	60	60	60	60	90	90	90
$AAC_{0\to120}$ ($\mu U \cdot min \cdot ml^{-1}$)	1890 ± 980	3850 ± 1230	5290 ± 1980	8470 ± 1758	3090 ± 1020	4430 ± 1420	5570 ± 2060

Table II. Pharmacokinetic Parameters of Intratracheally Administered Insulin Formulated with Saline or with Different Concentrations of TDM or DMβCD

	Insulin alone	Formulations containing insulin plus TDM			Formulations containing insulin plus DMβCD		
		Concentration of TDM			Concentration of DMβCD		
		0.06%	0.125%	0.25%	0.06%	0.125%	0.25%
C_{max} ($\mu U \cdot ml^{-1}$)	34.2 ± 27.7	229 ± 97.0	366 ± 113	505 ± 161	102 ± 28.9	167 ± 31.8	254 ± 58.3
T_{max} (min)	10	5	5	5	5	10	10
$AUC_{0\to\infty}$ ($\mu U \cdot min \cdot ml^{-1}$)	4330 ± 1130	10300 ± 4220	15600 ± 4900	2560 ± 11600	5970 ± 1380	8330 ± 2790	14200 ± 6290
K_a* (min^{-1})	0.008 ± 0.001	0.012 ± 0.004	0.019 ± 0.008	0.02 ± 0.007	0.009 ± 0.002	0.011 ± 0.004	0.014 ± 0.003
MRT (min)	185 ± 37.2	49.7 ± 11.4	48.2 ± 8.65	53.6 ± 13.7	82.1 ± 15.2	107 ± 24.6	70.6 ± 18.2
F'	0.14 ± 0.03	0.34 ± 0.08	0.52 ± 0.11	0.84 ± 0.15	0.19 ± 0.04	0.29 ± 0.08	0.48 ± 0.13

* Values have been exchanged with elimination rate constant based on the absorption rate–limited disposition of pulmonary delivered insulin (11).

There was little or no significant change in absorption rate constant K_a or *MRT* with changing TDM concentration. Overall, the experimental data suggest that TDM is an effective absorption promoter of intratracheally delivered insulin. Further, the efficacy of TDM as an absorption promoter for intratracheally administered insulin agrees with its ability to enhance absorption of peptide drugs such as insulin, glucagon, and calcitonin administered nasally and ocularly (12–14).

Data presented in Fig. 2 depict changes in plasma glucose and plasma insulin that occurred over 120 min following intratracheal administration of insulin plus saline or insulin plus DMβCD formulations. Addition of 0.06% DMβCD to insulin formulations produced a significant decrease in plasma glucose and increase in plasma insulin compared to that obtained when insulin was formulated with saline ($p < 0.05$). As with TDM, when the concentration of DMβCD was increased from 0.06 to 0.25%, a more pronounced and concentration-dependent decrease in plasma glucose and increase in insulin concentration were observed. A plot of $AUC_{0\to\infty}$ values vs. concentration of DMβCD added to insulin formulations shows a positive correlation with an increase in the enhancer concentration (inset, Fig. 2B). The C_{max} value obtained reached a maximum when 0.25% DMβCD was incorporated in the formulation (Table II). However, T_{max} values for all formulations containing 0.125% or 0.25% DMβCD were the same as that obtained for the control formulation. As observed with TDM, there were no significant changes in K_a or *MRT* with changes in DMβCD concentration.

It is worthwhile to note that one of the major challenges to insulin delivery is the reproducibility in the amount of insulin delivered. The data presented in this study showed that the amount of insulin delivered was fairly reproducible. For example, in case of 0.25% TDM formulations, the coefficients of variation for C_{max} and relative bioavailability were 31% and 18%, respectively. Similarly, the coefficients of variation for DMβCD formulation were in the acceptable range. The low variability observed in this study was probably because the drug was administered as a fine mist directly into the lung after laryngoscopic visualization of the trachea. However, variability in insulin delivery is not unexpected. Others have also reported a wide variation in the percentage of instilled dose reaching the lungs from nebulizers (25). For a reproducible insulin delivery to the lungs, variables that need to be standardized include drug concentration, droplet size distribution, animals' breathing pattern, and state of an-

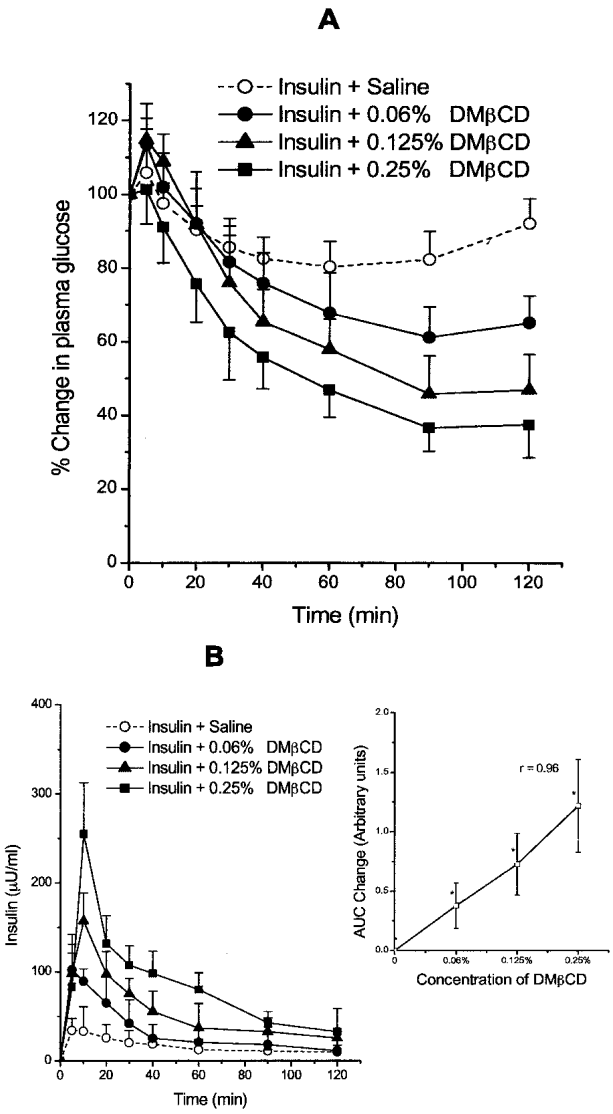

Fig. 2. Changes in plasma glucose (A) and plasma insulin (B) after intratracheal administration of insulin in saline or in the presence of increasing concentrations of DMβCD. Inset shows changes in $AUC_{0\to\infty}$ for plasma insulin-time curve with increasing concentrations of DMβCD. Data represent mean ± SD, n = 3–5.

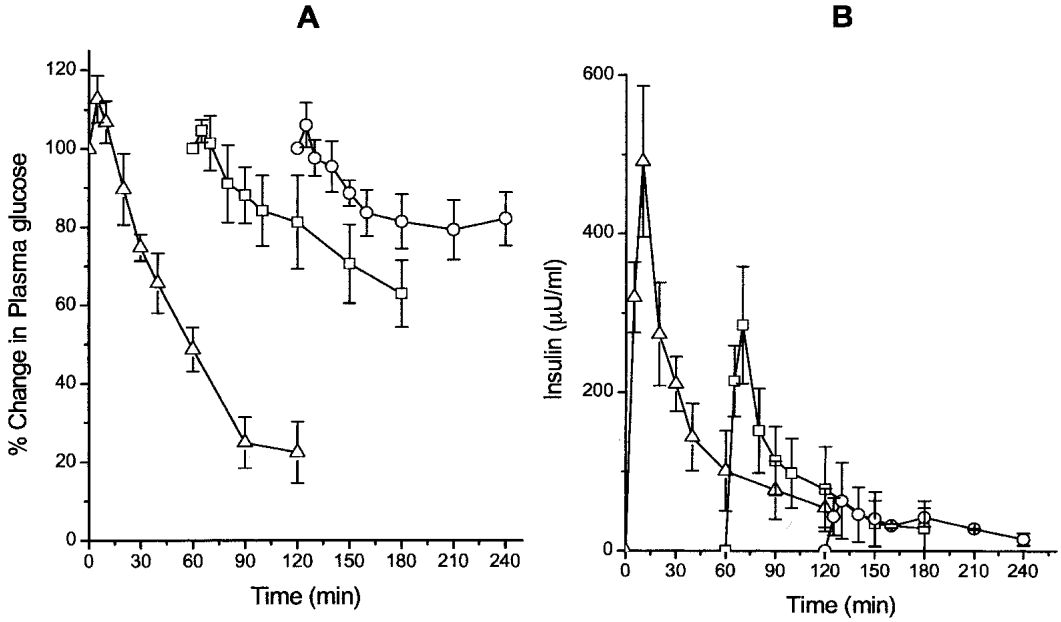

Fig. 3. Changes in plasma glucose (A) and plasma insulin (B) following administration of 1.25 U/kg of insulin at time zero (△), 60 (□), or 120 (○) min after administration of 0.25% TDM. Data represent mean ± SD, n = 3–5.

esthesia. Future studies will be directed to investigate the influence of these parameters for TDM- and DMβCD-based pulmonary formulations of insulin.

To compare and contrast the efficacy and duration of action of TDM and DMβCD for pulmonary insulin absorption, a reversibility study was conducted. Because the mechanism of absorption promoters involves action on the epithelial membrane, reversibility studies may give clues to the toxicity of the agents at the site of administration. In addition, Bagger *et al.* (20) observed that a short duration of action minimizes local toxicity and facilitates a quicker reestablishment of the normal epithelial barrier. The concentration of TDM and DMβCD used in the reversibility study was 0.25%. When insulin (1.25 U/kg) was administered immediately after administration of 0.25% TDM solution, a significant decrease in plasma glucose and increase in plasma insulin were observed compared to control ($p < 0.05$) Fig. 3 and Table III. These plasma glucose and insulin profiles were comparable to that observed when insulin formulated with 0.25% TDM was administered as a mixture to the anesthetized rat during pulmonary absorption studies (Fig. 1). When insulin was delivered at 60 min after TDM administration, there was a moderate decrease in plasma glucose and increase in plasma insulin. However, when insulin was delivered at 120 min after TDM administration, no significant differences in plasma glucose or plasma insulin levels were observed compared to control (Fig. 3). The data for the reversibility study for DMβCD, presented in Fig. 4 and Table III, show a similar trend to that of TDM. Like that of TDM, the effect of DMβCD on the epithelial membrane was reversible in 120 min. These experiments sug-

Table III. Pharmacokinetic and Pharmacodynamic Parameters of Insulin Administered at Time Zero, 60, or 20 Min After Administration of 0.25% of TDM or DMβCD During Reversibility Study

	Time at which insulin was administered after application of TDM			Time at which insulin was administered after application of DMβCD		
	0 min	60 min	120 min	0 min	60 min	120 min
Pharmacodynamic parameters						
$\%TRG_{0\to120}$	80.6 ± 10.1	37.4 ± 9.72	20.2 ± 6.35†	67.9 ± 9.13	38.5 ± 7.62	18.8 ± 4.37†
$\%MG$	22.5 ± 11.8	62.9 ± 8.52	79.2 ± 7.60†	45.8 ± 7.29	61.2 ± 11.3	79.8 ± 12.3†
$t\%MG$ (min)	120	120	90	90	90	90
$AAC_{0\to120}$ (μU · min · ml^{-1})	5706 ± 1790	2578 ± 1123	2014 ± 898†	4841 ± 1224	3334 ± 1067	1899 ± 913†
Pharmacokinetic parameters						
C_{max} (μU · ml^{-1})	491 ± 95.7	284 ± 93.7	62.9 ± 47.7	245 ± 65.9	119 ± 14.5	81.1 ± 34.55
T_{max} (min)	10	10	10	10	10	10
$AUC_{0\to\infty}$ (μU · min · ml^{-1})	23150 ± 10135	12395 ± 5223	4961 ± 1060†	11683 ± 4664	5165 ± 1378	5038 ± 2794†
K_a (min^{-1})*	0.010 ± 0.002	0.017 ± 0.005	0.018 ± 0.004	0.013 ± 0.004	0.009 ± 0.003	0.005 ± 0.001
MRT (min)	79.6 ± 9.21	57.2 ± 10.2	72.0 ± 8.65	76.8 ± 11.7	80.9 ± 10.3	114 ± 20.8

* Values have been exchanged with elimination rate constant based on the absorption rate–limited disposition of pulmonary delivered insulin (11).

† Not significantly different with respect to insulin plus saline formation ($p > 0.05$).

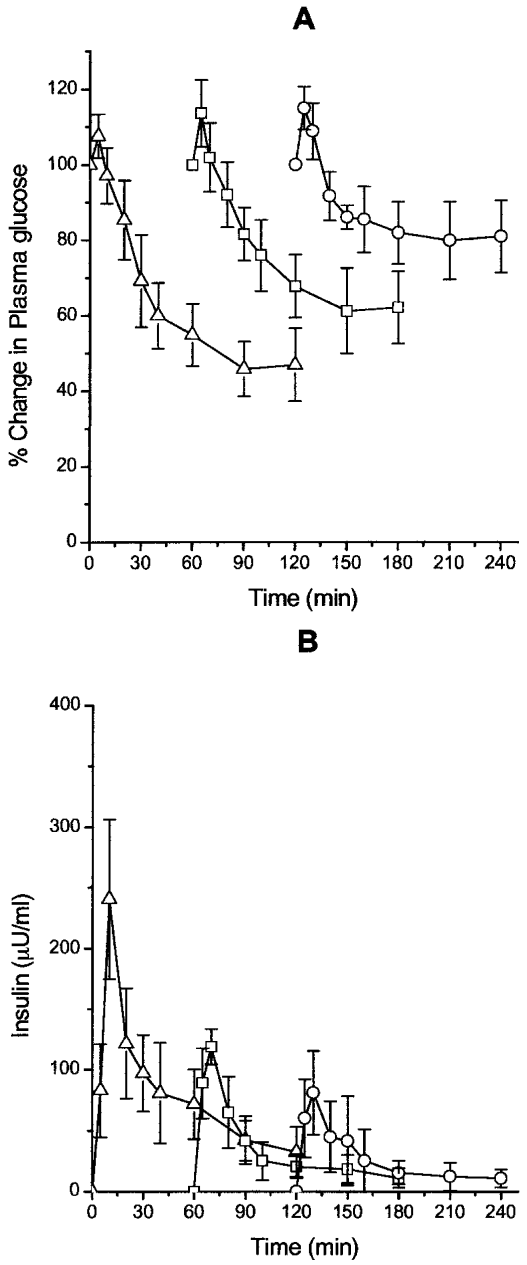

Fig. 4. Changes in plasma glucose (A) and plasma insulin (B) following administration of 1.25 U/kg of insulin at time zero (△), 60 (□), or 120 (○) min after administration of 0.25% DMβCD. Data represent mean ± SD, n = 3–5.

gest that increased permeability produced by TDM and DMβCD is rapidly reversible. The data from reversibility study with TDM are in agreement with the observations by Arnold *et al.* (21) on the effects of TDM on nasal mucosa.

The mechanism by which TDM enhances absorption of insulin is not known. Pillion *et al.* (15) suggested that TDM may accelerate absorption of insulin by two mechanisms: a direct, selective effect on the insulin molecule or a direct interaction with the epithelial membrane that makes it more permeable to insulin. If alkylglycosides caused insulin multimers to dissociate into dimers or monomers, absorption from the respiratory tract could be accelerated as a result of reduction of size of the insulin complex. It can also be hypothesized that micelles formed as a result of the presence of TDM in the formulation may entrap insulin monomers or dimers and help movement of insulin molecule through the hydrophobic bilayer of respiratory epithelium. It should be noted that TDM concentrations used in three formulations were above the critical micellar concentration (0.00054%) of TDM (Anatrace Chemical Catalog, Maumee, OH). However, our reversibility study, mentioned above, showed that prior administration of TDM to the respiratory tract could maintain a residual effect on insulin absorption. This finding suggests that the concept of interaction between an absorption promoter and insulin to form a complex that facilitates the permeability of the latter across the membrane could be negligible with the present route because both insulin and TDM were administered independent of each other in the reversibility study. This study also implies that TDM enhances absorption perhaps by solubilizing membrane components or loosening cell–cell tight junctions.

On the other hand, reports involving use of cyclodextrins in pulmonary protein delivery are limited. However, the selection of a cyclodextrin for this route is narrowed to β-cyclodextrin, hydroxypropyl-β-cyclodextrin, and DMβCD (26). Data obtained in the present study agree with the fact that DMβCD can increase absorption of a peptide drug administered via the pulmonary route (27). *In vitro* studies reported previously (28,29) confirmed that DMβCD directly interacts with the insulin molecule by shifting the equilibrium of multimeric insulin to the dimeric or monomeric form. For example, Shao *et al.* (30) reported that certain cyclodextrins alter the circular dichroism spectra of insulin *in vitro*. This report, taken in conjunction with data from the reversibility study reported herein, suggests that DMβCD may enhance insulin absorption by dual mechanisms: a direct interaction with insulin molecules and direct action on the respiratory epithelium. The latter mechanism is probably accomplished by the formation of a complex between cyclodextrin and membrane components.

In summary, *in vivo* rat studies provide an elegant model for pulmonary insulin delivery. Data obtained in this study were reproducible because the trachea of the anesthetized rats was visualized by a fiberoptic laryngoscope, which reduced the possibility of accidental delivery of drug into the esophagus compared to blind intubations into the trachea (31). Both absorption promoters were found to be effective in enhancing pulmonary insulin absorption and caused increased insulin absorption by acting on the membrane rather than by interacting with insulin. However, TDM was shown to be twice as efficacious as DMβCD in enhancing pulmonary insulin absorption. Reversibility studies demonstrated that acute exposure of TDM or DMβCD is less likely to cause extensive damage or cellular toxicity in respiratory epithelial cells. However, the *in vivo* reversibility study provides rather limited data as to the safety of an absorption promoter for pulmonary drug delivery. More extensive studies are required to determine the cellular toxicities that may result from chronic use of these agents. In the near future, studies will be performed to measure the effects of TDM and DMβCD on the mucociliary clearance of the respiratory apparatus and long-term cellular and biochemical response of the lung from exposure to these relatively new pulmonary absorption promoters.

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