Mutual Inhibition of the Insulin Absorption-Enhancing Properties of Dodecylmaltoside and Dimethyl-β-Cyclodextrin Following Nasal Administration

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Purpose. To determine if a nasal insulin formulation containing two distinct absorption-enhancing agents exhibits an additive or synergistic increase in the rate of systemic insulin absorption.

Methods. The pharmacokinetics and pharmacodynamics of insulin absorption were measured in hyperglycemic anesthetized rats following nasal insulin administration with formulations containing two different types of absorption-promoting agents, dimethyl-β-cyclodextrin (DMBCD) and dodecylmaltoside (DDM).

Results. When either DDM (0.1–0.5%) or DMBCD (1.0–5.0%) was added to the nasal insulin formulation, a significant and rapid increase in plasma insulin levels was observed, with a concomitant decrease in blood glucose concentration. A combined preparation containing 0.25% DDM (0.005 M) and 2.5% DMBCD (0.019M), however, failed to cause an increase in plasma insulin levels or a decrease in blood glucose concentration. Increasing concentrations of DDM added to an insulin formulation with a fixed DMBCD concentration caused a decrease, rather than an increase, in systemic absorption of insulin.

Conclusions. Mixing DMBCD and DDM resulted in mutual inhibition of their ability to enhance systemic absorption of insulin following nasal delivery. The results are consistent with the formation of an inclusion complex between DDM and DMBCD which lacks the ability to enhance nasal insulin absorption.

KEY WORDS: nasal insulin; dodecylmaltoside; dimethyl-β-cyclodextrin; inclusion complex; pharmacokinetics; blood glucose.

INTRODUCTION

Cyclodextrins are cyclic oligosaccharides, composed of 6 or more glucose units with a characteristic central cavity, that have the ability to form inclusion complexes with hydrophobic molecules (1). The most extensively studied cyclodextrins are α-, β-, and γ-cyclodextrins, which consist of six, seven, and eight glucopyranose units, respectively. Both natural and modified cyclodextrins are presently used in pharmaceutical formulations to increase drug solubility and dissolution, and enhance drug absorption by means of molecular encapsulation. Certain cyclodextrins have been found useful in enhancing nasal absorption of peptide drugs including insulin (2). Among the cyclodextrin derivatives studied as nasal absorption promoters, dimethyl-β-cyclodextrin (DMBCD) was found to be the most effective, while α-cyclodextrin was less effective and β- and γ-cyclodextrin had negligible effects on insulin absorption (2). The mechanism of action that produces increased nasal absorption of peptide drugs is not clear. Cyclodextrins may protect peptide drugs from enzymatic degradation by molecular encapsulation or directly deactivate proteolytic enzymes; in particular, dimethyl-β-cyclodextrin may have a direct effect on the membrane and enhance drug absorption by binding with, and/or extracting/removing membrane components that serve as a barrier to insulin transport; or cyclodextrins may interact directly with hydrophobic side chains on the peptide drug molecules and change their inherent aggregability or permeability across a phospholipid bilayer (3–5). Loftsson and Jarvinen report that the ability of cyclodextrins to interact with biological membranes is greatly reduced when their cavity is occupied by a lipophilic substrate (6).

Many other potential absorption promoters besides the cyclodextrins have been investigated (7–11). Studies in this laboratory have shown that alkylglycosides such as dodecylmaltoside, dodecylsucrose, and tetradecylmaltoside are potent enhancers of insulin absorption following either ocular or nasal delivery (12,13). Dodecylmaltoside (DDM), a nonionic alkylglycoside containing the disaccharide maltose, glycosidically linked to a twelve carbon alkyl chain, significantly increased nasal absorption of insulin when used even at very low concentrations (0.06–0.25%). The mechanism of action of DDM is not clear. It appears that DDM has a direct effect on the epithelium, rather than on the multimeric insulin molecule, since it proved equally effective as an enhancer of the nasal absorption of multimeric regular human insulin and fast-acting monomeric lyspro insulin (14).

Currently, it is not known if DMBCD will form an inclusion complex with DDM, and whether or not such a complex is still biologically active as an absorption enhancer. This study was designed to determine if DDM and DMBCD could be formulated together to provide greater insulin absorption at lower concentrations of surfactants than obtained with either absorption promoter alone.

EXPERIMENTAL

Materials

DDM and DMBCD were purchased from Anatrace Corp. (Maumee, OH) and Sigma Chemicals (St Louis, MO), respectively. Regular human insulin (Humulin® 100 Units/ml) was obtained from Eli Lilly & Company (Indianapolis, IN).

Preparation of Nasal Formulations

DDM or DMBCD stock solutions were prepared by dissolving the excipients in normal saline and were stored for 30 days or less at 4°C. On the day of an experiment, the stock solutions were used to prepare the desired concentrations of the excipients. The concentrations used for DDM were 0.1% (2 mM), 0.25% (5 mM), 0.5% (10 mM), and 1% (20 mM), and the concentrations for DMBCD were 1.0% (7.5 mM), 2.5% (19 mM), 3.75% (28 mM), and 5.0% (38 mM). It should be noted that all concentrations of DDM used in this study were

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above its critical micelle concentration, which is 0.17 mM (15). The nasal formulations were prepared by mixing regular human insulin (100 Units/ml) with the appropriate concentrations of DDM, or DMBCD, or a combination of both, to achieve a final mixture that contained 25 Units/ml insulin. No differences were observed in experimental results when the order in which DDM, DMBCD, and insulin were added to the combined nasal formulation was altered.

Absorption Studies in Rats

Nasal absorption studies were performed in Sprague-Dawley male rats obtained from Charles River Laboratories (Charlotte, NC). Rats were anesthetized by intramuscular injection of a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) and anesthesia was maintained with additional xylazine/ketamine as needed throughout the experiment.

The nasal formulations were instilled 45–60 min after the initial dose of anesthetic agents, to allow time for the blood insulin levels to decrease and blood glucose levels to increase (250–400 mg/dl) prior to insulin administration. Nasal formulations were instilled as nosedrops (20 μl) administered to the left nare of the anesthetized rats in the supine position at time zero, using a pipetter with a disposable plastic tip. The total amount of insulin delivered to each rat was 0.5 Unit. Blood glucose levels were measured in blood collected from the tip of the rat tail using a glucose meter (Glucometer Elite, Bayer Corp., Elkhart, IN) at time zero and at 5–20 min intervals for 120 min following nasal insulin delivery. The value of blood glucose at time zero ranged from 250 to 350 mg/dl and was normalized for each experiment at 100%. The blood glucose content at various times thereafter was calculated as a percentage of this initial value in each animal. There was a tendency for blood glucose values to increase by 50–100 mg/dl over the course of a 2-h experiment in animals that did not receive any insulin or in animals that received nasal insulin formulated in saline without any excipients. This tendency toward hyperglycemia, caused by blockade of endogenous insulin release, was reflected in data obtained from the rats that received insulin formulated in saline at time zero.

Concomitantly, blood samples were collected from the tip of the tails of anesthetized animals in heparinized tubes for determination of plasma insulin levels. Plasma was separated and stored at −20°C until assayed for insulin content. A human insulin specific radioimmunoassay kit (Linco Research, Inc., St. Charles, MO) was used to measure exogenous human insulin in the rat at various times after nasal insulin delivery. The areas under the curve for plasma insulin and blood glucose profiles were determined by the trapezoidal rule.

The animal studies were conducted according to the principles outlined in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

RESULTS AND DISCUSSION

Rats anesthetized with xylazine plus ketamine exhibited reduced insulin secretion and subsequent hyperglycemia. Nasal administration of 0.5 Unit of regular human insulin to hyperglycemic anesthetized rats was ineffective in lowering blood glucose levels when the insulin was formulated in saline (Fig. 1A). When DDM was added to the nasal insulin formu-

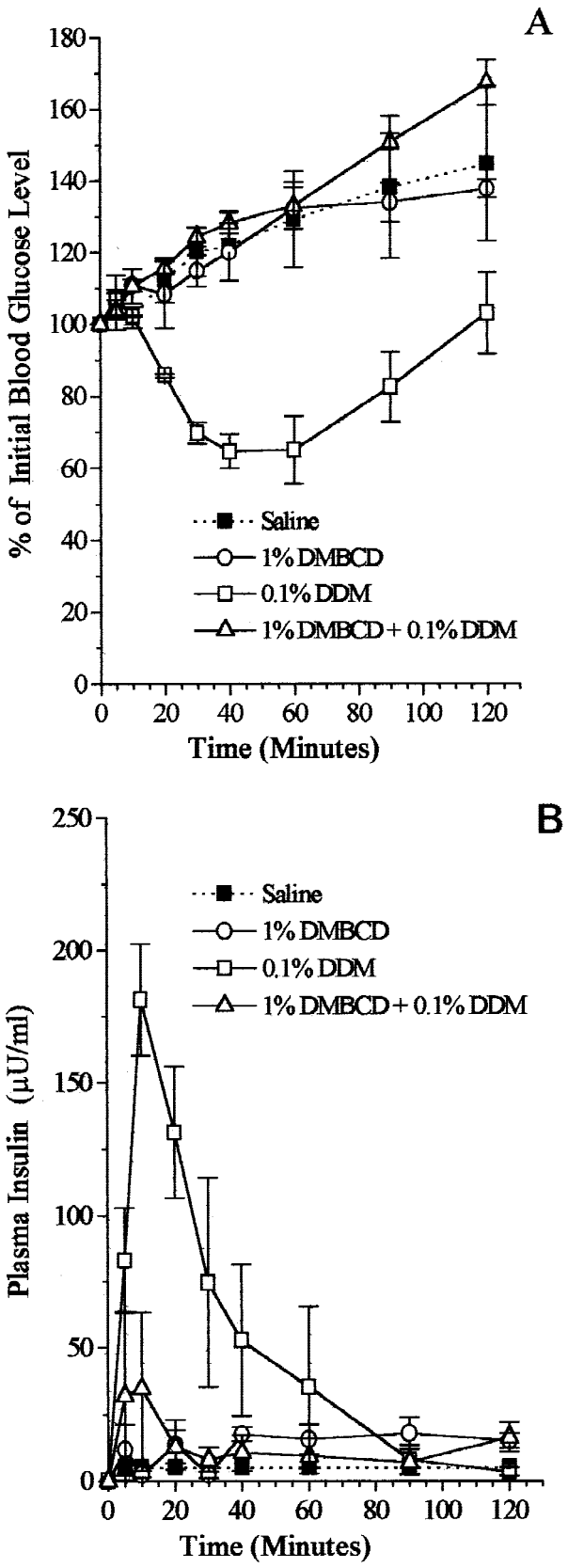

Fig. 1. Change in the blood glucose (A) and plasma insulin (B) concentrations after nasal administration of regular insulin in saline or in the presence of either 1% DMBCD, 0.1% DDM, or a combination of 0.1% DDM and 1% DMBCD. Data represent mean ± standard error of the mean; $n = 3$.

lations at a concentration of 0.1%, the blood glucose content was significantly decreased (Fig. 1A). The results are consistent with the systemic uptake of insulin from the nose in a biologically active form in the rats that received the nasal insulin formulated with 0.1% DDM. Nasal formulations containing insulin plus 1% DMBCD failed to produce a decrease in blood glucose concentration (Fig. 1A). A nasal formulation containing insulin with both of the excipients together was completely ineffective at promoting a reduction in blood glucose levels (Fig. 1A). Concomitant changes in plasma insulin levels in these animals are presented in Fig. 1B. The administration of nasal insulin formulations containing only saline failed to cause an increase in plasma insulin content, while the administration of formulations containing 0.1% DDM caused a rapid and transient increase in plasma insulin levels. A formulation containing 1% DMBCD plus insulin failed to increase plasma insulin levels. In agreement with the pharmacodynamic data reported in Fig. 1A, a formulation containing both 0.1% DDM and 1% DMBCD plus insulin produced only a small increase in plasma insulin content (Fig. 1B). When formulations containing either 0.25% DDM or 2.5% DMBCD plus insulin were administered nasally, insulin absorption was enhanced (Fig. 2). The ability of this higher concentration of DMBCD to enhance the bioavailability of insulin from a nasal formulation was consistent with reports from Merkus *et al.* (16) and Irie *et al.* (17). The effect of 0.25% DDM to enhance insulin bioavailability was also consistent with earlier studies from this laboratory (12,13). However, a formulation containing both 0.25% DDM and 2.5% DMBCD plus insulin was not effective at enhancing insulin absorption, as evidenced by a failure to produce a reduction in blood glucose levels or an increase in plasma insulin level (Fig. 2).

When insulin formulations containing 0.5% DDM or 5% DMBCD or both were tested (Fig. 3), the response to the excipients was different, despite the fact that the DDM:DMBCD ratio was held constant. Rats that received a formulation containing either 0.5% DDM or 5% DMBCD plus insulin had a significant reduction in blood glucose levels (Fig. 3A) and increase in plasma insulin levels (Fig. 3B). Rats that received a formulation containing both 0.5% DDM and 5% DMBCD plus insulin showed a reduction in blood glucose concentrations (Fig. 3A) and an increase in plasma insulin levels (Fig. 3B). Again, the pharmacokinetic and pharmacodynamic data presented in Fig. 3 are internally consistent with each other; animals that received nasal insulin formulations containing either 0.5% DDM and/or 5.0% DMBCD plus insulin demonstrated a reciprocal fall in blood glucose levels and an increase in plasma insulin levels.

These results can best be explained by an interaction between the two excipients (e.g., the formation of an inclusion complex in which the central cavity of cyclodextrin can associate with the hydrophobic alkyl chain of dodecylmaltoside) that prevents either agent from interacting with insulin and/or the nasal mucosal membrane and therefore, prevents either of them from enhancing the absorption of insulin. It has been reported that surfactants, including dodecyltrimethylammonium bromide and dodecylethylammonium bromide, can form inclusion complexes with different cyclodextrins (18–23). In the presence of cyclodextrins, the critical micelle concentrations of surfactants have been found to be shifted to higher values (22,23), presumably because some of the surfactant molecules were bound to cyclodextrins, while the con-

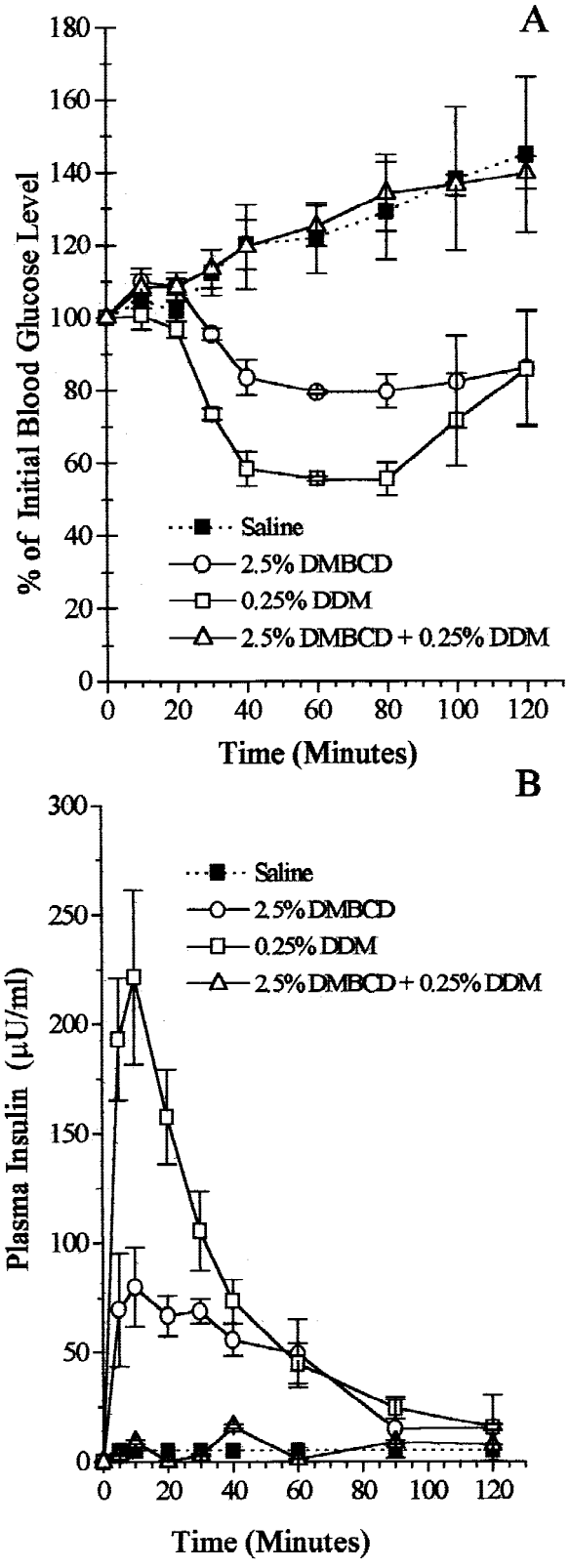

Fig. 2. Change in the blood glucose (A) and plasma insulin (B) concentrations after nasal administration of insulin formulated either in saline or in the presence of either 2.5% DMBCD or 0.25% DDM or a combination of both 0.25% DDM and 2.5% DMBCD. Data represent mean ± standard error of the mean; $n = 3$.

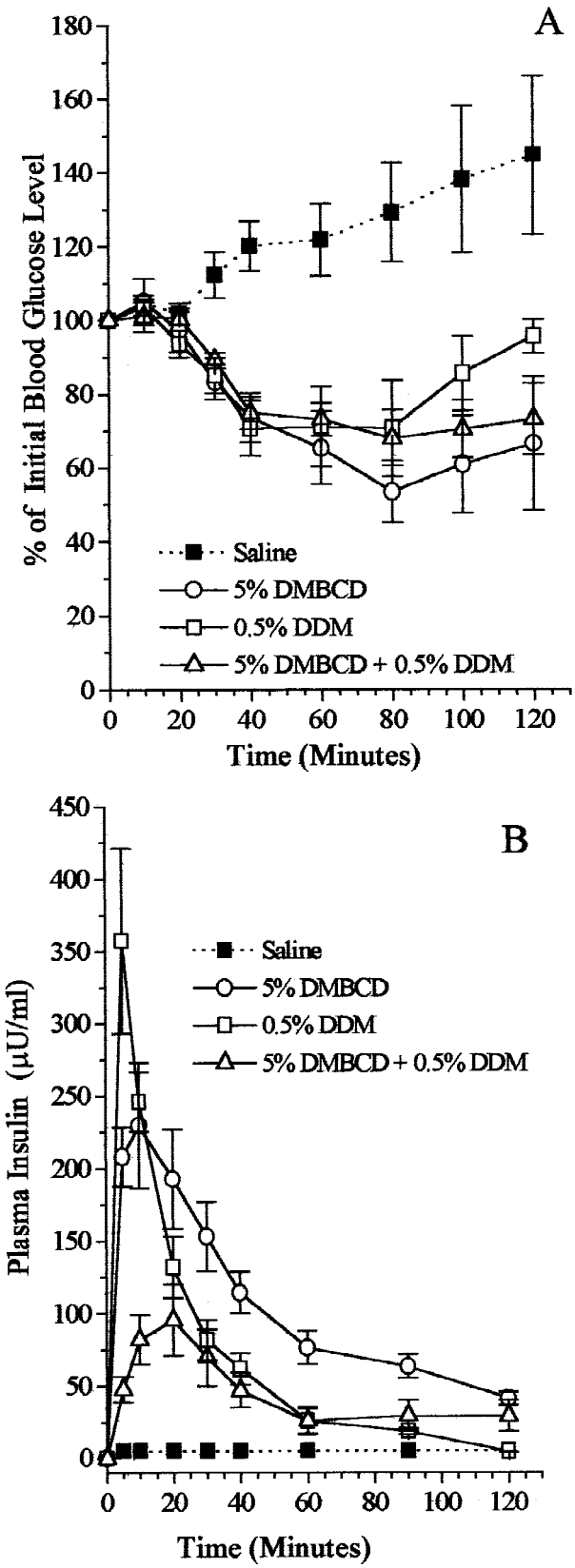

Fig. 3. Change in the blood glucose (A) and plasma insulin (B) concentrations in rats after nasal administration of insulin formulated in saline, 5% DMBCD, 0.5% DDM, or a combination of 0.5% DDM and 5% DMBCD. Data represent mean ± standard error of the mean; $n = 3$.

centration of free surfactant monomers remained basically the same or slightly increased or decreased depending on the chemical nature of the surfactant (20,21). It has also been reported that surfactant micelles can break up due to the formation of surfactant/cyclodextrin inclusion complexes as the association constant of the surfactant/cyclodextrin is higher than that of micelle formation from monomers (21). The formation of inclusion complexes between DDM and different parent cyclodextrins (α-, β-, γ-) has been reported previously (24), but no direct experimental evidence regarding an interaction between DDM and DMBCD has been published.

The precise nature of the interaction between cyclodextrins and surfactants with long alkyl chains has been studied (25–27). For example, α-cyclodextrin can form inclusion complexes with polyethylene glycol (PEG) and produce compounds called polyrotaxanes, where several cyclodextrin molecules are threaded on the linear PEG chain (27). A similar relationship may develop between DDM and DMBCD. If several DMBCD molecules were threaded on the alkyl chain of DDM, the resulting complex may be inactive when included in a nasal insulin formulation, since the central cavity of DMBCD would be occupied.

Another possible mechanism of action whereby DMBCD may enhance insulin absorption is by shifting the equilibrium of multimeric insulin from hexameric to dimeric and monomeric. Shao *et al.* (28), for example, have reported that certain cyclodextrins can alter circular dichroism spectra of insulin in vitro. Their results are consistent with the conclusion that DMBCD can favor the dissociation of insulin hexamers to dimers or monomers and at least two other reports have suggested that certain cyclodextrins can modify the multimeric configuration of insulin by interacting with hydrophobic amino acid chains (29,30). This is one potential mechanism whereby DMBCD could promote nasal absorption of insulin (28). However, direct measurement of the absorption of hexameric and monomeric insulin following nasal administration to rodents in the absence of any absorption enhancer failed to show any significant absorption of either form of insulin (14). This finding would necessitate some additional action of DMBCD, besides insulin multimer dissociation, to enhance systemic absorption of insulin following nasal adminsitration.

The results presented in this manuscript agree with the data reported by Jabbal-Gill *et al.* (31) in which it was observed that insulin formulated with hydroxypropyl-β-cyclodextrin plus lysophosphatidyl choline or glycodeoxycholate was not absorbed as well as insulin formulated with lysophosphatidyl choline or glycodeoxycholate alone. However, the data in Figs. 1–3, are the first to demonstrate that two biologically active reagents, DDM and DMBCD, mutually inhibit each other's activity *in vivo*. The results also agree with the in vitro study by Veiga and Ahsan (32,33). The authors showed that the solubility and rate of dissolution of tolbutamide in an aqueous solution of either β-cyclodextrin or sodium dodecyl sulfate was greater than the solubility and rate of dissolution obtained from an aqueous solution of both of the agents together. Based on these results, it was concluded that β-cyclodextrin could form an inclusion complex with either tolbutamide or sodium dodecyl sulfate.

In nasal formulations containing either 0.1% DDM and 1% DMBCD, 0.25% DDM and 2.5% DMBCD, or 0.5%

DDM and 5% DMBCD, the molecular stoichiometry of DDM:DMBCD is approximately 1:4. At this molar ratio, much or all of the total DDM could be associated with DMBCD in inclusion complexes, while the excess DMBCD could remain available to modulate the rate of insulin absorption. If the DDM-DMBCD inclusion complexes were incapable of increasing insulin absorption, then the mixture would not enhance insulin absorption unless the concentration of excess, unbound DMBCD was great enough to have an effect. The dose response relationship for unbound DMBCD as a promoter of nasal insulin absorption can be gleaned from the experimental results in Figs 1–3. The actual amount of uncomplexed DMBCD available, when higher concentrations of DMBCD and DDM are mixed, is not known, but the results of nasal absorption studies can provide some insight as to the stoichiometry of the DDM-DMBCD interactions.

A series of molecular models (Fig. 4A) can be used to visualize the possible interaction of these excipients. These models are patterned after those proposed by Ceccato *et al.* (27) to depict the interaction of α -cyclodextrin with polyethylene glycol. Model A (Fig. 4A) represents a 1:1 complex of DDM:DMBCD. If this complex were formed, approximately one-fourth of the available DMBCD and all of the DDM would interact with each other. The remaining three-fourths of the DMBCD would be free and available to enhance insulin absorption. Model B (Fig. 4A) represents a DDM:DMBCD complex with a 1:2 stoichiometry. If such a complex were formed, one-half of the available DMBCD and all of the available DDM would be complexed, while one half of the DMBCD would be free. Model C (Fig. 4A) represents a complex composed of DDM:DMBCD with a stoichiometry of 1:3. If this type of complex were formed, three-fourths of the DMBCD and all of the DDM would be complexed, while one-fourth of the DMBCD would be free. None of these models are mutually exclusive and at equilibrium, a mixture of two or three types of complexes could occur. These models were used to predict the outcome of a series of experiments in which nasal insulin formulations were prepared with varying concentrations of DDM, while holding the concentration of DMBCD constant at 5%. The AUC for blood glucose and plasma insulin concentrations, presented in Figs 1–3 and for two additional formulations (0.25% DDM + 5% DMBCD and 1% DDM + 5% DMBCD) are presented in Table I. The data presented in this table clearly demonstrate that though the addition of DDM alone to a nasal insulin formulation produced a dose dependent increase in insulin absorption, there was a dose-dependent decrease in insulin absorption, when DDM was added to formulations containing insulin and 5% DMBCD. Whereas 5% DMBCD alone increased the AUC for plasma insulin from 0.6 μ U × min/ml × 10^3 to 13.0 μ U × min/ml × 10^3, addition of 1% DDM to the formulation containing 5% DMBCD caused a reduction in AUC for insulin absorption to 3.1 μ U × min/ml × 10^3 (Table I). These results are consistent with the models presented in Fig. 4A, if one assumes that DDM/DMBCD complexes are not active at enhancing insulin absorption. The data are not consistent with a model in which DDM-DMBCD complexes are fully active in promoting nasal insulin absorption.

For the purpose of distinguishing between the three models presented in Fig 4A, it is useful to compare the results obtained with formulations containing DDM plus DMBCD to results obtained with formulations containing DMBCD alone. Fig. 4B was constructed using plasma insulin AUC data obtained with nasal insulin formulations containing DMBCD alone (1%, 2.5%, and 5%), presented in Figs. 1–3, plus an additional data point using a formulation containing insulin

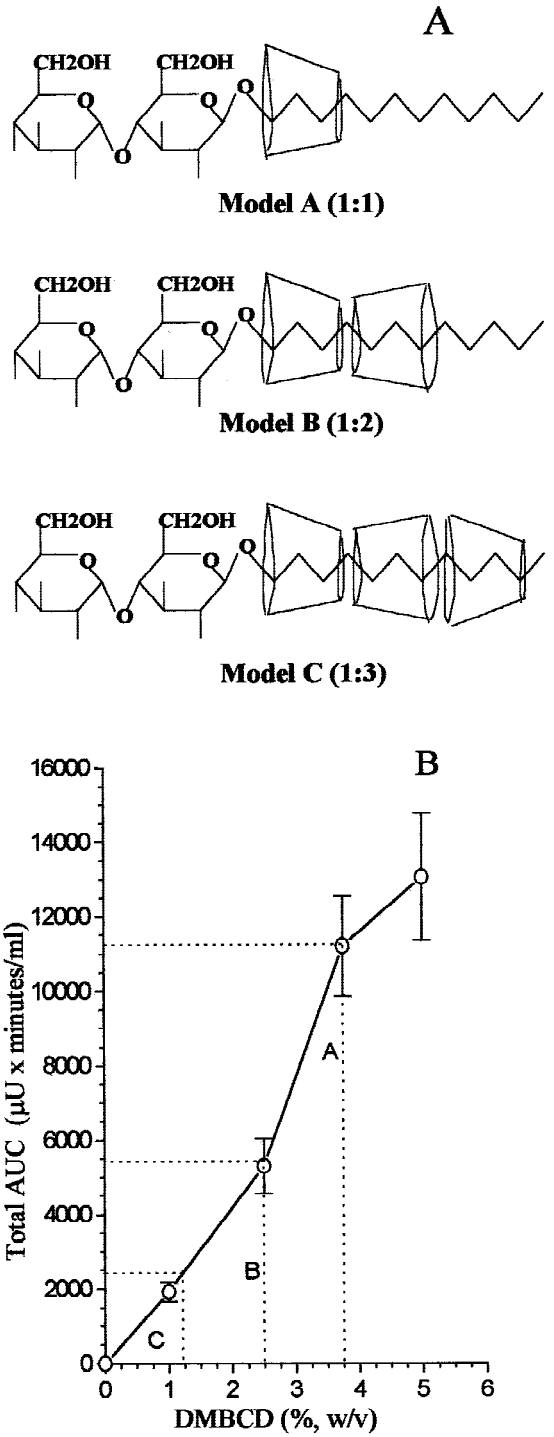

Fig. 4. (A) Proposed models for DDM and DMBCD complexes. (B) Total AUC of blood plasma insulin-time curves with increasing concentrations of DMBCD. Cumulative AUC data from plasma insulin-time curves from experiments in which insulin was formulated with 0, 1, 2.5, 3.75, and 5.0% DMBCD alone were compiled and total AUC for each concentration is presented ± SEM. (solid line). The corresponding predicted total AUC for the plasma insulin-time curves for a solution containing 5% DMBCD plus 0.5% DDM according to Models A, B, C are presented (dashed lines).

Table I. AUC Values from Blood Glucose-time Curves and Plasma Insulin-Time Curves

Formulation		$AUC_{0-120min}$ Blood glucose-time curve[a]	$AUC_{0-120min}$ Plasma insulin-time curve[b]
DMBCD (%)	DDM (%)		
0	0	17.0 ± 0.8	0.6
1	0	17.1 ± 0.5	1.9 ± 0.2
2.5	0	11.6 ± 0.8	5.3 ± 0.7
5	0	8.0 ± 0.1	13.0 ± 0.5
0	0.1	11.2 ± 0.9	5.8 ± 0.6
0	0.25	9.7 ± 0.8	8.7 ± 1.0
0	0.5	11.1 ± 0.1	8.0 ± 0.9
1	0.1	18.8 ± 0.5	1.7 ± 0.2
2.5	0.25	17.4 ± 0.6	0.8 ± 0.1
5	0.5	10.2 ± 0.6	5.6 ± 0.6
5	0	8.0 ± 0.1	13.0 ± 0.5
5	0.25	8.2 ± 0.5	11.3 ± 2.0
5	0.5	10.2 ± 0.6	5.6 ± 0.6
5	1	16.1 ± 0.03	3.1 ± 0.2

[a] AUC values decrease as the blood glucose levels decrease (expressed in arbitrary units).
[b] AUC values increase as the insulin absorption increases (expressed in $\mu U \times min/ml \times 10^3$).

plus 3.75% DMBCD alone. The resulting dose-response curve can be used to predict the responses expected when formulations containing various amounts of DMBCD and DDM were tested. The concentrations of free DMBCD available in each formulation containing DDM plus DMBCD tested were predicted according to the models presented in Fig. 4A, and the results are presented in Table II. For example, according to Model A (1:1), when 5% DMBCD (38 mM) is mixed with 0.5% DDM (10 mM) at a molar ratio of approximately 4:1, three fourths of the DMBCD (3.75%) would be left uncomplexed and available to enhance insulin absorption. According to this model, the net effect of a mixture of 5% DMBCD plus 0.5% DDM would equal the effect of a formulation of 3.75% DMBCD alone. The data in Table 2 reflects similar calculations for each of the models. The predicted values for free DMBCD can be used to evaluate the validity of each model using the dose response curve in Fig. 4B. For example, the formulation containing 5% DMBCD and 0.5% DDM was predicted by Model A to contain 3.75% free DMBCD, by Model B to contain 2.5% DMBCD, and by Model C to contain 1.25% free DMBCD (Table 2). As described in Fig. 4B, each of these different concentrations of free DMBCD would be expected to produce a different change in plasma insulin AUC. These predicted values are shown in Fig. 5 for each model and compared to actual in vivo

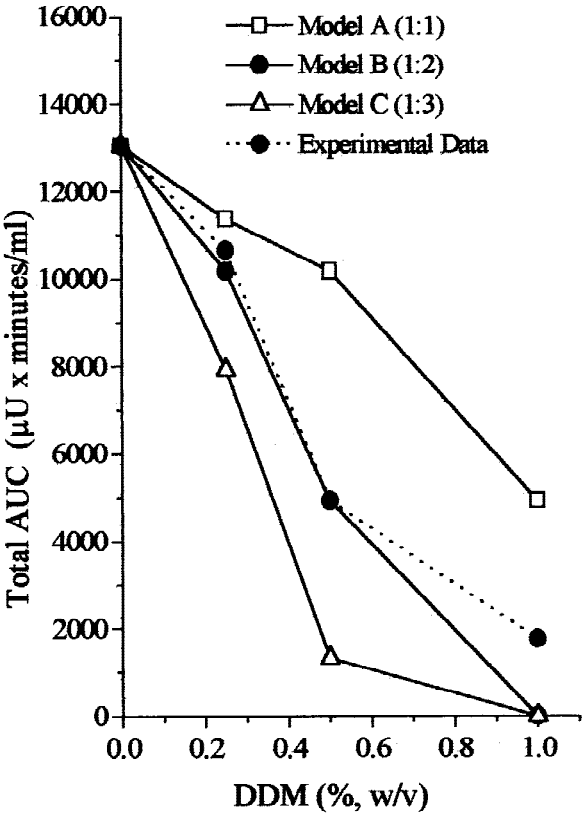

Fig. 5. Predicted and experimental changes in total AUC of plasma insulin-time curves for formulations of insulin containing 5% DMBCD with increasing concentrations of DDM.

experimental results. Of the three models, the change in insulin absorption predicted from Model B, a 1:2 complex of DDM and DMBCD, most closely approximated the actual experimental results obtained. This interpretation requires three assumptions: (1) that the complex formed between DDM and DMBCD is inactive; (2) that only one type of complex, such as 1:1 or 1:2 DDM:DMBCD, represents the bulk of complexes formed, rather than a broad spectrum of complexes containing different proportions of the excipients; and (3) that all of the available DDM will form complexes with DMBCD. Furthermore, it negates the possible contribution of any residual uncomplexed DDM that may exist at equilibrium in the formulations containing 5% DMBCD. The critical micelle concentration of DDM is 0.17 mM and a small amount of residual free surfactant is not expected to contribute significantly to insulin absorption, based on the observation that formulations containing insulin plus extremely low concentrations of DDM (less than 0.5 mM) display no enhancement of insulin absorption.

Table II. Predicted Concentration of DMBCD

Formulations		Model A 1:1 Predicted [Free DMBCD]	Model B 1:2 Predicted [Free DMBCD]	Model C 1:3 Predicted [Free DMBCD]
DDM (%)	DMBCD (%)			
0	5	5	5	5
0.25	5	4.38	3.75	3.13
0.5	5	3.75	2.50	1.25
1	5	2.5	0.00	0.00

In summary, the experimental results are consistent with the hypothesis that dimethyl-β-cyclodextrin and dodecylmaltoside can both accelerate nasal absorption of insulin formulated alone. However, when formulated together, dimethyl-β-cyclodextrin and dodecylmaltoside fail to enhance insulin absorption. The best explanation for these phenomena is that DDM and DMBCD form a molecular complex, which lacks the ability to enhance insulin absorption.

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