



Nasal absorption of mixtures of fast-acting and long-acting insulins

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ABSTRACT

Mixtures of fast-acting and long-acting insulins were administered nasally to anesthetized, hyperglycemic rats in the presence and absence of tetradecyl- β -D-maltoside (TDM). The fast-acting analogs, aspart insulin, lispro insulin and glulisine insulin, were all rapidly absorbed from the nose when applied individually with 0.125% TDM (T_{\max} = 15 min). One long-acting insulin analog, glargine insulin, was also absorbed from the nose when applied individually in the presence of 0.125% TDM (T_{\max} = 60 min). The other long-acting insulin analog, detemir insulin, was not soluble when formulated with 0.125% TDM. A series of mixtures (1:1) of the three rapid-acting insulins and long-acting glargine insulin were formulated with 0.125% TDM and applied nasally. The pharmacokinetic and pharmacodynamic profiles of the insulin mixtures reflected the additive contributions of both the rapid-acting and the long-acting insulins. These results support the possibility of formulating certain insulin mixtures in tandem to provide nasal insulin products that match the needs of patients with diabetes mellitus better than those currently available.

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1. Introduction

Patients with diabetes mellitus require precise and timely administration of insulin to maintain normal glycemic control. Continuous subcutaneous insulin infusion (CSII) pumps provide a steady flow of insulin, but require the purchase of expensive equipment and vigilant patient oversight. CSII is further complicated by the short and sometimes variable length of time a device will remain patent after insertion. Occlusion of CSII lines then creates a hazardous situation in which patients are not receiving adequate insulin delivery. Subcutaneous injections of insulin remain the most widely used approach for insulin delivery. Fast-acting and long-acting analogs of insulin have allowed many patients to achieve improved glycemic control, with fast-acting insulin injections before each meal to cover food intake and one or possibly two injections of long-acting insulin to cover insulin requirements between meals and overnight. The currently available fast-acting insulins (lispro insulin, aspart insulin and glulisine insulin) have distinctly quicker onsets of action compared to regular insulins used previously. Unfortunately, many patients still encounter great difficulty in successfully utilizing injections of these insulins to maintain a glycemic status near normal. Additionally, many Type 2 diabetic patients are averse to beginning insulin therapy because of a reluctance or fear of performing subcutaneous injections. The fact that the fast-acting and long-acting insulin analogs currently

available cannot be mixed, and injected together, mandates more injections per day, and this creates a still higher barrier for patients with Type 1 or Type 2 diabetes to overcome.

To circumvent the problems associated with subcutaneous injections or CSII, alternate routes of insulin administration have been explored, but to date these have been found to present barriers to insulin absorption of varying intensities. The pulmonary route of delivery can be utilized without the aid of any absorption-enhancing agent, albeit with a modest bioavailability of approximately 10% of the insulin administered. An inhaled insulin product was developed, tested and shown to be effective. Unfortunately, issues with patient acceptance and pulmonary health, variability of insulin absorption among smokers, and a requirement for monitoring pulmonary function, all contributed to the decision to withdraw this product. While this was certainly a disappointing episode, it provided the proof-of-concept that, with improved technology and refined formulations, an insulin product could be administered by a non-invasive route. This realization is further evidenced by the fact that another inhaled insulin product is in development. Other routes of insulin delivery under consideration include oral, buccal, transdermal and nasal delivery.

The nasal route of delivery, like all alternate routes, offers some potential advantages and disadvantages. The primary challenge to any nasal insulin product is to achieve substantial and reproducible bioavailability from the nasal cavity, a system that is naturally designed to warm and moisten air, and to remove particulate matter, not designed to absorb drugs. Despite the nearly absolute impermeability of the nasal passage to insulin under basal conditions, there is a remarkable increase in insulin absorption when

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a very small concentration of an alkylglycoside surfactant agent, with the appropriate size and hydrophilic/hydrophobic balance, is added to the formulation. Alkylglycoside surfactants that consist of an alkyl chain 12–14 carbons in length, and a disaccharide, such as dodecyl- β -D-maltoside (DDM), tetradecyl- β -D-maltoside (TDM) or dodecylsucrose, produce a transient increase in the nasal permeability to a wide variety of both peptide (Pillion et al., 1998, 2002; Arnold et al., 2004) and non-peptide (Arnold et al., 2002) drugs. It is important to note that alkylglycosides with side chains shorter than 10 carbons in length, or those linked to a monosaccharide, fail to have this effect on the nasal cavity. Studies with cultured epithelial cells and with intact animals provide evidence that the alkylglycosides increase both paracellular and transcellular insulin absorption (Arnold et al., 2004; Ahsan et al., 2003).

The effects of alkylglycosides on the permeability of the nasal cavity *in vivo* are transient, with maximal effectiveness observed immediately after application and gradual reversal over the next 2–4 h. From a clinical perspective, this pattern of transient loosening of the permeability barrier of the nose overlaps with the most likely pattern of use for a nasal fast-acting insulin product, i.e. the nasal insulin would be taken just before meals and produce an immediate hypoglycemic response. Animal experiments have shown that the pharmacokinetic profiles of regular insulin and the fast-acting analog lispro insulin were similar when applied nasally in the presence of TDM, with very rapid absorption of insulin ($T_{\max} = 15$ min) and return to baseline insulin values within 1–2 h (Pillion et al., 1998). The long-acting insulin analog, glargine insulin, gave different results when applied nasally in the presence of alkylglycosides (Arnold et al., *in press*). Glargine insulin showed a slower onset ($T_{\max} = 60$ min) and an extended duration of absorption and action.

This study is designed to address the following two questions:

- (1) Can a fast-acting insulin analog be mixed with a long-acting insulin analog and be formulated in the presence of an alkylglycoside?
- (2) If so, can the mixture be applied nasally and absorbed into the circulation to produce a blended pharmacokinetic/pharmacodynamic profile that would be suitable for treating a patient with diabetes mellitus?

2. Materials and methods

2.1. Animals

Studies were performed in Sprague–Dawley male rats (200–450 g) obtained from Charles River Laboratories (Charlotte, NC). Standard laboratory food and tap water were available *ad libitum*.

The animal study was 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.

2.2. Materials

Glargine insulin (Lantus[®]) and glulisine insulin (Apidra[®]) were obtained from Sanofi/Aventis (Bridgewater, NJ). Detemir insulin (Levemir[®]), aspart insulin (Novolog[®]), NPH insulin (Novolin N[®]), and regular insulin (Novolin R[®]) were all obtained from Novo Nordisk Pharmaceuticals, Inc. (Princeton, NJ). Lispro insulin (Humalog[®]) was obtained from Eli Lilly Corp. (Indianapolis, IN). Tetradecyl- β -D-maltoside (TDM) was purchased from Anatrace, Inc. (Maumee, OH). Xylazine HCl (100 mg/ml) was obtained from Vedco Inc. (St. Joseph, MO). Ketamine HCl (100 mg/ml) was

obtained from Lloyd Laboratories (Shenandoah, IA). Isoflurane was obtained from Minrad Inc. (Bethlehem, PA). Heparin (1000 U/ml) was obtained from Elinks-Sin Inc. (Cherry Hill, NJ). A human insulin specific radioimmunoassay kit was obtained from Millipore Corp. (St. Louis, MO).

2.3. Analytic procedures

Rats were anesthetized with 4% isoflurane, initially at a flow rate of 3–4 L/min, and then anesthesia was maintained, when necessary, with 1–2% isoflurane at a flow rate of 1–2 L/min. Rats were then removed from isoflurane and injected with ketamine (100 mg/kg) and xylazine (10 mg/kg) intramuscularly to induce hyperglycemia. Anesthesia was maintained with additional ketamine/xylazine as needed throughout the experiment. Sixty minutes after the first administration of ketamine/xylazine, blood was collected from the tail for the determination of basal glucose and insulin levels. Nose drops (0.02 ml) containing 1 Unit of insulin were administered to the right nares of anesthetized rats in the supine position using a pipettor with a disposable plastic tip. Rats then received a second 0.02 ml dose of the nasal formulation containing 1 Unit of insulin applied to the right nares 2 min later. The rats were turned over to the prone position 2 min later. This experimental protocol has been utilized to prevent airway obstruction that can occur if nose drops are applied to both nares at the same time and to eliminate leakage of the formulation from the nose. Nose drops were formulated by mixing one volume of insulin (U-100) with one volume of either 0.9% NaCl or with 0.25% TDM in 0.9% NaCl. In some experiments, phosphate buffered saline was used in place of 0.9% NaCl. No differences were observed in the experiments utilizing phosphate buffered saline. Glucose levels were measured in drops of blood taken from the tip of the rat tail using a glucose meter (Glucometer Elite[™], Bayer Corp., Elkhart, IN) at various times after the administration of insulin. The upper limit of the glucose meter was 600 mg/dL. Insulin levels were measured using a commercially available human insulin RIA kit. Plasma samples were prepared by collecting rat blood from the tip of the tail in tubes containing 5 Units of heparin.

2.4. Statistical analysis

In nasal drug delivery studies, the maximal concentration (C_{\max}) of insulin and the time to maximal concentration (T_{\max}) were determined directly from the pharmacokinetic profile. The area under the curve (AUC_{0-180}) was determined via the linear trapezoidal rule. Results are presented as mean \pm standard error. Statistical significance was determined with Student's *t*-test (SigmaStat Software, SPSS, Inc., Chicago, IL). Differences with a *p* value of less than 0.05 were considered significant.

3. Results

Previous studies have shown that rats anesthetized with ketamine/xylazine become hyperglycemic and hypoinsulinemic within 60 min (Pillion et al., 1994; Saha et al., 2005, 2006). This experimental system allows an exquisitely sensitive platform in which to study the absorption of exogenous human insulin delivered nasally. In this study, seven different forms of insulin were compared. Nasal administration of three different fast-acting insulin analogs, in the presence of 0.125% TDM, caused a rapid and significant increase in plasma insulin levels (Fig. 1A). Maximal insulin levels were obtained within 15 min for all three insulin analogs and insulin levels returned to baseline within 120 min. Two of the three fast-acting insulin analogs, lispro and aspart, were absorbed more effectively ($C_{\max} = 907 \pm 65$ μ U/ml and 1073 ± 45 μ U/ml, respectively) than

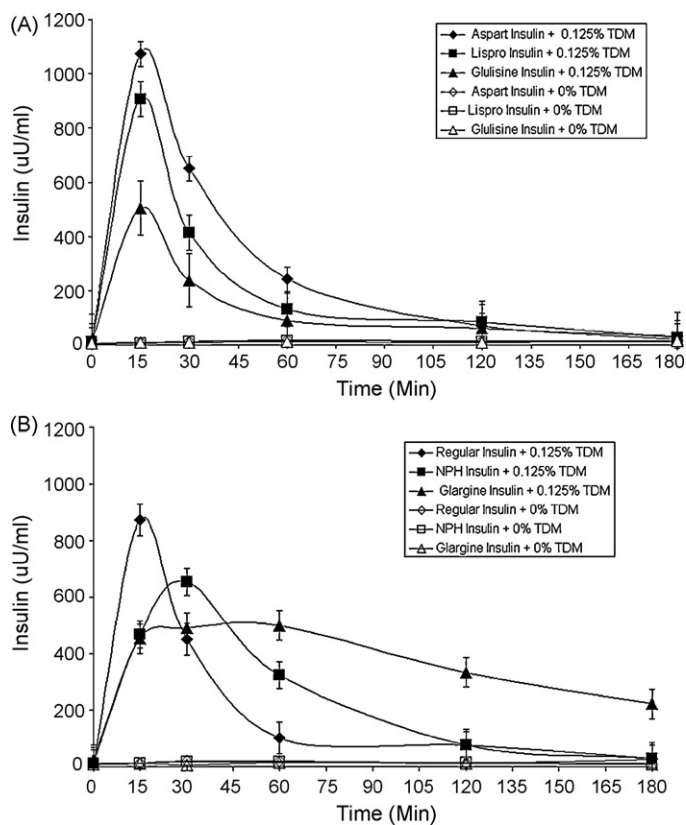


Fig. 1. Plasma insulin levels in rats that received 2 Units of various fast-acting insulins (A) or regular or long-acting insulins (B) with or without 0.125% TDM. Each data point represents the mean \pm SEM of 3–6 animals.

glulisine ($C_{\max} = 505 \pm 99 \mu\text{U/ml}$). None of the three fast-acting insulin analogs were absorbed when formulated without TDM. Three other forms of insulin (regular, NPH and glargine insulins) were also absorbed from the nasal cavity when formulated with 0.125% TDM (Fig. 1B). Like the fast-acting insulins shown in Fig 1A, none of these three forms of insulin were absorbed in the absence of TDM. The T_{\max} for NPH insulin (30 min) and glargine insulin (60 min) were different than the T_{\max} for regular insulin and the fast-acting insulin analogs (15 min). Glargine insulin applied nasally in the presence of 0.125% TDM caused a much greater increase in plasma insulin levels 180 min after administration than the other forms of insulin tested.

The other long-acting form of insulin, detemir insulin, formed a cloudy mixture when formulated with 0.125% TDM. When this cloudy mixture was applied to rats nasally, under the same condi-

tions as those described above for the other six insulin products, no measurable absorption of insulin was detected.

The data in Fig. 1 have been used to determine the total amount of insulin absorbed from the nasal cavity (AUC_{0-180}) for each of the insulin analogs (Table 1). It should be noted that glargine insulin levels had not returned to baseline values 180 min after nasal administration. Hence, the values reported for glargine insulin absorption in Table 1 under-represent total glargine insulin absorption when compared to each of the other forms of insulin tested. The total absorption of glulisine insulin (AUC_{0-180}) and the maximal concentration of glulisine insulin (C_{\max}) were consistently less than those observed for the other insulin analogs. Nasal delivery of aspart insulin generated the highest concentration of insulin (C_{\max}) immediately following administration, while nasal delivery of glargine insulin produced the greatest bioavailability of insulin (AUC_{0-180}) (Table 1). Blood glucose levels were also measured in parallel in the experiments described above to determine the bioavailability of the various insulin analogs, to determine the time course of insulin action following nasal delivery, and to confirm that intact biologically active insulin, rather than a partially degraded but immunologically competent fragment was delivered to the circulation. All three of the fast-acting insulin analogs, formulated with 0.125% TDM and applied nasally, produced a rapid and substantial decrease in blood glucose concentrations compared to the same formulations lacking 0.125% TDM (Fig. 2A). Of the three fast-acting insulin analogs formulated with 0.125% TDM, glulisine insulin produced the smallest hypoglycemic response, consistent with the insulin absorption data presented in Fig. 1A and Table 1. The effects of all three fast-acting insulin analogs on the glycemic levels of the rats diminished after 180 min, again consistent with insulin absorption data presented in Fig. 1A. In Fig. 2B, the effects of regular insulin, NPH insulin, and glargine insulin nose drops formulated with and without 0.125% TDM are presented. Unlike the other five forms of insulin tested, long-acting glargine insulin produced a reduction in blood glucose concentration that was still robust after 180 min. Hence, the total hypoglycemic response to glargine insulin is under-represented when the AUC_{0-180} data are compared to the other insulins. Additional experiments are required to define the duration of this effect. When the total hypoglycemic responses to the six forms of insulin were compared directly, glulisine insulin produced the smallest response when applied nasally, whereas regular insulin and glargine insulin produced the largest responses (Table 2).

As described above, detemir insulin formed a cloudy mixture when formulated with 0.125% TDM. In order to test the impact of TDM on the nasal absorption of detemir insulin, a different experimental protocol was required. In these experiments, the nasal passages were pre-treated with or without 0.125% TDM. Nose drops containing 2 Units of detemir insulin formulated in 0.9% NaCl were

Table 1
Effect of tetradecyl- β -D-maltoside (TDM) on the nasal absorption of various insulins.

Conditions		Plasma insulin		
0.125% TDM (+/-)	Type of insulin	AUC_{0-180} ($\mu\text{U/ml min}$)	C_{\max} ($\mu\text{U/ml}$)	T_{\max} (min)
(-)	Regular insulin ^a	N/D	N/D	N/D
(+)	Regular insulin	$33,398 \pm 2500^b$	874 ± 56^b	15
(+)	Lispro insulin	$41,820 \pm 3300^b$	907 ± 62^b	15
(+)	Aspart insulin	$55,238 \pm 4600^{b,c}$	$1073 \pm 45^{b,c}$	15
(+)	Glulisine insulin	$25,335 \pm 1200^{b,c}$	$505 \pm 99^{b,c}$	15
(+)	NPH insulin	$41,790 \pm 3900^b$	$653 \pm 48^{b,c}$	30
(+)	Glargine insulin	$67,170 \pm 4800^{b,c}$	$500 \pm 52^{b,c}$	60

Rats received nose drops containing 2 Units of insulin with or without 0.125% TDM. Plasma insulin levels were determined at various times up to 180 min after nasal insulin administration. The AUC values were measured by the trapezoidal rule and compared to the AUC values observed with regular insulin. Data represent mean \pm SEM ($n = 3-6$). N/D = not detectable.

^a Similar results were obtained when other insulins were tested in the presence of 0% TDM.

^b Significantly different than the same insulin formulated with 0% TDM ($p < 0.05$).

^c Significantly different than regular insulin formulated with 0.125% TDM ($p < 0.05$).

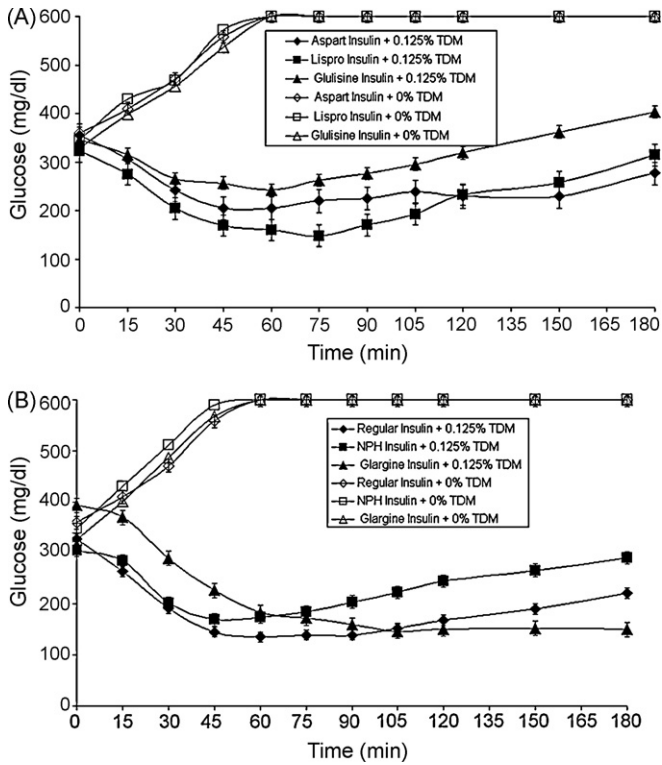


Fig. 2. Blood glucose levels in rats that received 2 Units of various fast-acting insulins (A) or regular or long-acting insulins (B) with or without 0.125% TDM. Each data point represents the mean \pm SEM of 3–6 animals.

administered 15 min later. For comparison, two other forms of insulin, fast-acting aspart insulin and long-acting glargine insulin, were run in parallel. Under these experimental conditions, all three forms of insulin were absorbed from the nasal cavity when applied 15 min after 0.125% TDM, but not when applied after 0.9% NaCl (Fig. 3). However, nasal delivery of aspart insulin produced a robust increase in plasma insulin concentrations, whereas glargine insulin and detemir insulin produced progressively smaller responses. Plasma insulin concentrations remained elevated for a longer duration when glargine insulin was applied nasally 15 min after 0.125% TDM. Blood glucose concentrations were also obtained in these experiments to determine the time course of insulin action following nasal delivery and to confirm that a biologically active form of insulin had been delivered to the circulation (Fig. 4). Fast-acting aspart insulin produced a rapid and more substantial decrease in blood glucose when applied 15 min after 0.125% TDM than the long-

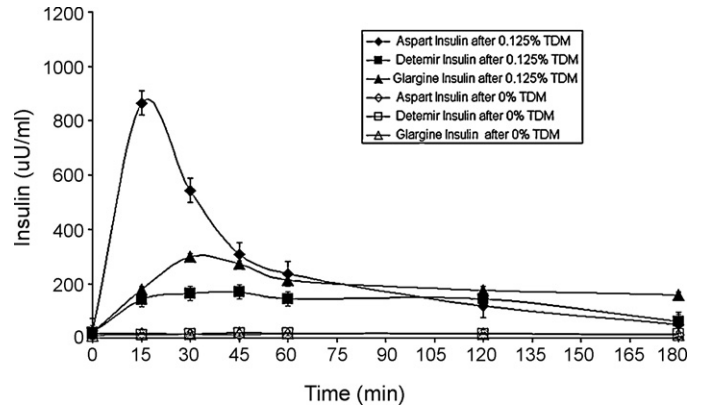


Fig. 3. Plasma insulin levels in rats that received 2 Units of various insulins 15 min after the administration of 0% or 0.125% TDM. Each data point represents the mean \pm SEM of 3–6 animals.

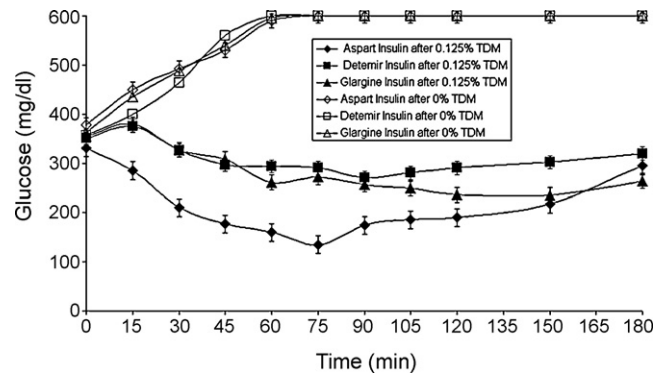


Fig. 4. Blood glucose levels in rats that received 2 Units of various insulins 15 min after the administration of 0% or 0.125% TDM. Each data point represents the mean \pm SEM of 3–6 animals.

acting insulin analogs, detemir and glargine. All of these changes in blood glucose concentrations were consistent with the insulin absorption data in Fig. 4.

To determine if mixtures of fast-acting and long-acting insulins could be prepared with TDM and used successfully in nasal insulin delivery studies, long-acting glargine insulin (1 Unit) was mixed with either aspart insulin, lispro insulin or glulisine insulin (1 Unit) and applied to rats nasally in the presence or absence of 0.125% TDM (Fig. 5). All three formulations were clear and remained clear for several weeks when stored at 4 °C. Absorption of insulin was observed with all three mixed insulin formulations

Table 2
Effect of TDM on the hypoglycemic response to various insulins.

Conditions		Blood glucose	
0.125% TDM (+/–)	Type of insulin	AUC _{0–180} (mg/dL \times min)	Blood glucose at 60 min after insulin administration (mg/dL)
(–)	Regular insulin ^a	93,728 \pm 2600	600 \pm 10 ^b
(+)	Regular insulin	32,745 \pm 2400 ^c	135 \pm 22 ^c
(+)	Lispro insulin	39,863 \pm 3200 ^c	160 \pm 27 ^c
(+)	Aspart insulin	43,470 \pm 4300 ^{c,d}	205 \pm 13 ^{c,d}
(+)	Glulisine insulin	55,335 \pm 4000 ^{c,d}	242 \pm 15 ^{c,d}
(+)	NPH insulin	41,730 \pm 3100 ^{c,d}	174 \pm 19 ^c
(+)	Glargine insulin	36,345 \pm 3600 ^c	183 \pm 26 ^c

Rats received nose drops containing 2 Units of insulin with or without 0.125% TDM. The blood glucose levels were measured at various times up to 180 min after nasal insulin administration. The AUC values were measured by the trapezoidal rule and compared to the AUC values observed with regular insulin. Basal glucose levels ranged from 303 mg/dL to 393 mg/dL. Data represent mean \pm SEM ($n = 3–6$).

^a Similar results were obtained when other insulins were tested in the presence of 0% TDM.

^b Upper limit of glucometer = 600 mg/dL

^c Significantly different than the same insulin formulated with 0% TDM ($p < 0.05$).

^d Significantly different than regular insulin formulated with 0.125% TDM ($p < 0.05$).

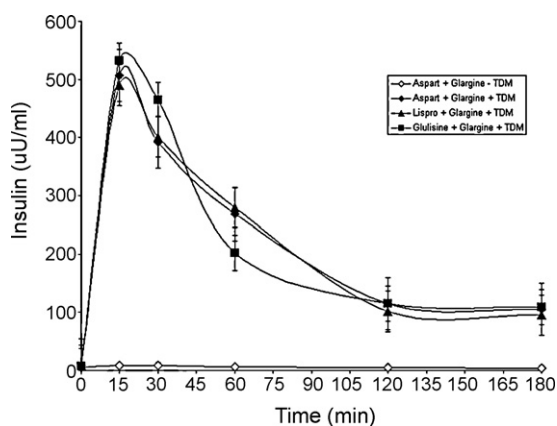


Fig. 5. Plasma insulin levels in rats that received 2 Units of a mixed insulin formulation with 0.125% TDM. Each data point represents the mean \pm SEM of 3–6 animals.

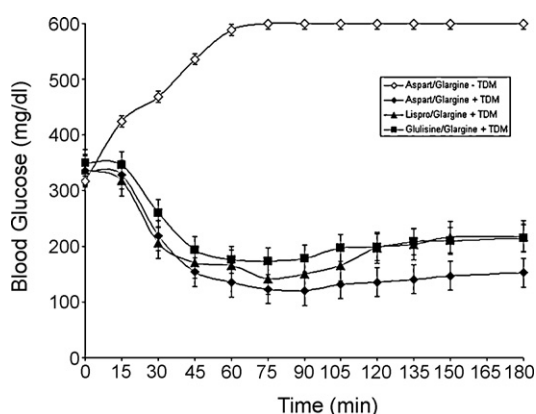


Fig. 6. Blood glucose levels in rats that received 2 Units of a mixed insulin formulation with 0.125% TDM. Each data point represents the mean \pm SEM of 3–6 animals.

that contained TDM, but not with formulations that lacked TDM. All three mixed insulin formulations containing TDM produced rapid insulin absorption from the nasal cavity ($T_{\max} = 15$ min), but insulin concentrations remained elevated for more than 180 min after administration, a pattern that reflected the combined uptake profiles of fast-acting and long-acting insulins. Of note, the absorption of aspart insulin and lispro insulin was not greater than the absorption of glulisine insulin when each fast-acting analog of insulin was mixed with glargine insulin, a result that was different from the results of experiments depicted in Fig. 1A, where the short-acting insulins were tested individually. Importantly, at extended time points after administration of the insulin mixtures ($T = 60$ min, 120 min and 180 min), insulin was present in the plasma at levels far above baseline ($>100 \mu\text{U/ml}$). No such residual insulin was observed in the experimental data presented in Fig. 1A when short-acting insulin analogs were tested individually, but it was observed in Fig. 1B when glargine insulin was tested individually. Blood glucose data from these experimental animals are presented in Fig. 6. The data are consistent with the insulin absorption results shown in Fig. 5. Animals displayed a rapid fall in blood glucose and a sustained hypoglycemic effect when any of the three mixtures were applied nasally in the presence of TDM.

4. Discussion

The results described above provide two seminal pieces of information:

1. All three forms of fast-acting insulin can be mixed with one long-acting form of insulin, glargine insulin, and formulated with TDM. The other long-acting form of insulin, detemir insulin, could not.
2. All three of the mixed insulin formulations, containing a fast-acting insulin analog and glargine insulin plus TDM, produced a blend of rapid and extended insulin absorption and corresponding hypoglycemic effects when applied nasally.

The nasal insulin absorption data obtained in the experiments utilizing mixtures of a fast-acting insulin analog and glargine insulin formulated with TDM are most directly interpreted as follows: the blended response to the insulin mixtures described in Figs. 5 and 6 include two overlapping and additive events. The initial peak of insulin absorption ($T_{\max} = 15$ min) is provided primarily by the absorption of the fast-acting insulin analog. This initial peak of fast-acting insulin absorption is followed by a prolonged period of glargine insulin absorption. This interpretation is consistent with the results obtained when the fast-acting forms of insulin and the long-acting glargine insulin were tested individually (Figs. 1 and 2).

Previous studies have shown that the concentration of TDM used in these studies, i.e. 0.125%, provided a robust increase in regular human insulin absorption with minimal toxicity to the cells that line the nasal cavity (Arnold et al., 2004). At a concentration of 0.25% or 0.50% TDM, slightly more insulin absorption was observed, while at a concentration of 0.06% TDM, incrementally less insulin absorption was observed.

Nasal administration of regular human insulin in the presence of 0.06% and 0.125% TDM resulted in 44% and 55% relative bioavailability respectively when compared to subcutaneous administration of regular insulin (Arnold et al., 2004; Arnold, 2004). By comparison, nasal administration of regular insulin in the presence of 0.25% and 0.50% TDM resulted in 64% and 77% relative bioavailability when compared to subcutaneous administration of regular insulin. At a concentration of 0.50% TDM, changes in the appearance of the cells that line the nasal cavity became more evident than the minor changes observed at a concentration of 0.125% TDM (Arnold et al., 2004).

In all of the nasal insulin delivery experiments described in this report, nasal insulin was administered in the form of liquid nose drops, applied from a stock solution by a pipettor. It is anticipated that nasal delivery of insulin formulations using a spray device, to instill small droplets of solution throughout the nasal cavity, will increase the surface area contacted by the insulin and increase the overall amount of insulin absorbed.

The results obtained in cell culture experiments and whole animal experiments are consistent with the hypothesis that the alkylglycoside causes a temporary perturbation of the nasal barrier to drug absorption. Earlier studies have shown that alkylglycosides increase both paracellular transport and transcellular transport of insulin (Ahsan et al., 2003; Arnold et al., 2004). Substitution of DDM for TDM provided essentially identical results.

While it is possible that an interaction takes place between molecules of the alkylglycoside surfactant and insulin in the nasal formulation prior to administration, direct evidence that this type of interaction directly alters insulin absorption is lacking. Formulations containing 0.125% TDM plus a mixture of glargine insulin with any of the three fast-acting insulins remained clear even after storage for several weeks. Alkylglycosides such as TDM and DDM can stabilize protein formulations and increase their effective shelf-life (Maggio, 2006). Hence, in these experiments, TDM may serve a dual role, to both stabilize the mixtures of short-acting and long-acting insulins, and also to serve as an absorption-enhancing agent. This remarkable duality of actions provides an important and unique role for TDM and DDM in the development of an effective stable nasal insulin formulation, whether it contains a

single form of insulin or a mixture of fast-acting and long-acting insulins.

Several new questions are raised as a result of these findings. In the case of nasal delivery of glargine insulin, the precise mechanism involved in the delayed onset and prolonged duration of glargine insulin absorption observed in these experiments is not known. The nasal cavity has a large surface area and is bathed in fluid with a slightly acidic pH. This acidic environment could certainly present an opportunity for localized foci of glargine insulin precipitation to occur. It is not known for certain if long-acting glargine insulin remains in the nose in a depot environment and gradually is released from the depot to be absorbed slowly, as is generally considered to be the case following subcutaneous injections of glargine insulin.

The time course of the reversal of TDM action on nasal permeability to insulin becomes very important when considering how, and if, a patient with diabetes mellitus could utilize nasal formulations that contained both fast-acting and long-acting forms of insulin. Optimally, a patient could take a mixed nasal insulin formulation containing TDM at mealtime. Immediate absorption of fast-acting insulin would occur, to provide appropriate coverage for the glycemic burden consumed at the meal. Thereafter, the effect of TDM on the permeability of the nasal cavity would extend for several hours and allow long-acting insulin to be absorbed and provide extended duration of insulin coverage for at least 3 h after the meal. Additional experimentation will be required to demonstrate that this same scenario holds true in humans and to better define the optimal insulin formulation composition to use (fast-acting and long-acting insulin concentrations and alkylglycoside concentrations can be varied to obtain optimal pharmacodynamic responses).

The rate and extent of absorption of all insulin analogs would be expected to be limited by several factors, including mucociliary clearance of unabsorbed insulin and TDM from the nose, impermeability of the nasal cavity cell surfaces that did not receive an effective dose of TDM because of how and where it was administered, the limited surface area available for absorption, and enzymatic destruction of insulin analogs and TDM, both in the nasal cavity and/or following absorption into the epithelial cells that line the nasal cavity (Newman et al., 1994; Illum, 2003; Pillion et al., 2007). The distinct chemical composition of the insulin analogs may cause one or more of them to behave differently than the others during one or more of the several different stages of the nasal absorptive process.

It has been reported that mixtures of glargine insulin and either lispro insulin or aspart insulin became cloudy, but were still effective, when injected subcutaneously in humans (Kaplan et al., 2004). No increase in pain or adverse reactions was observed in patients receiving the cloudy insulin mixtures. Detemir insulin was not clear when mixed with 0.125% TDM and it could not be absorbed from the nose into the systemic circulation when mixed with TDM. This form of insulin was the only one of seven insulins tested that was not absorbed from the nose in the presence of TDM. NPH insulin was also cloudy, with or without TDM addition, but NPH insulin was absorbed from the nose when mixed with TDM. In some experiments, animals received nose drops with TDM alone and then the insulins were applied 15 min later. Under these conditions, detemir insulin, like aspart insulin and glargine insulin, was absorbed from the nose. Detemir insulin displayed a prolonged duration of action, much like glargine insulin. This type of approach is useful to demonstrate an aspect of TDM action on the nasal cavity, but it is impractical to develop a commercially successful nasal formulation containing detemir insulin if it requires the administration of an alkylglycoside absorption enhancer minutes before a separate application of the insulin. No such requirement exists for a formulation containing glargine insulin.

Recently it was reported that nasal administration of insulin formulated without an absorption-enhancing agent caused a transient increase in insulin levels in the brain, without affecting plasma insulin levels or blood glucose levels (Reger et al., 2008). Remarkably, this treatment produced a significant improvement in short-term learning performance in patients with early signs of Alzheimer's disease (Benedict et al., 2007; Reger et al., 2008). This observation opens the door to increased scrutiny of how insulin can be absorbed from the nasal cavity by one route into the peripheral circulation, and by another route into the central nervous system. Extensive characterization of the optimal insulin formulations to be used in a nasal insulin product for patients with diabetes mellitus and alternate formulations for patients with Alzheimer's disease awaits further studies.

In summary, three fast-acting insulin analogs (aspart, glulisine, and lispro insulins), were absorbed rapidly following nasal delivery in a formulation containing 0.125% TDM. The long-acting insulin analog, glargine insulin, demonstrated a slower rate of absorption and an extended hypoglycemic effect. Mixtures of long-acting glargine insulin with each of the three different short-acting insulins plus 0.125% TDM were successfully absorbed from the nasal cavity. All three mixtures displayed a pharmacokinetic profile that reflected absorption of both the fast-acting and the long-acting forms of insulin.

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