Dodecylmaltoside-Mediated Nasal and Ocular Absorption of Lyspro-Insulin: Independence of Surfactant Action from Multimer Dissociation

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INTRODUCTION

Insulin Delivery and Absorption-Enhancers

Insulin administration by the nasal or ocular route has been a goal for many years, but previous efforts have been thwarted by problems with poor absorption in the absence of an absorption-enhancer, and irritation of the nasal mucosa in the presence of such an enhancer (1–4). Clinical trials have shown that nasal administration of regular human insulin in the presence of an enhancer was efficacious, but significant nasal irritation was observed (5,6). Many compounds have been tested for their efficacy as absorption-enhancers in the search for a non-irritating formulation for nasal insulin delivery (7,8). The alkylglycosides are particularly promising candidates as absorption-enhancers because they are nonionic surfactants that are effective at low concentrations (0.03–0.125%) and they can be metabolized to simple non-toxic metabolites (9–12). The alkylglycosides, including dodecylmaltoside, were found to be particularly effective at promoting systemic insulin absorption following either nasal (9,10) or ocular (10,11) delivery.

Mechanism of Dodecylmaltoside Action

Despite the accumulation of considerable experimental evidence concerning the efficacy of alkylglycosides as absorption-enhancers, their mechanism of action remains uncertain. One possibility is that the alkylglycosides promote a loosening of tight junctions between epithelial cells that comprise the permeability barrier of the nasal mucosa, allowing for more unrestricted paracellular movement of peptide drugs. A second possible mechanism of action is that the surfactant agents favor the formation of insulin monomers over the larger and less readily absorbed hexameric and dimeric forms of regular insulin. It had not been possible to test this hypothesis previously, since all regular human insulin formulations contained multimeric insulin.

Lyspro-Insulin

Lyspro-insulin is a modified form of human insulin recently made available for the treatment of diabetes mellitus (Humalog; Eli Lilly Co). Lyspro-insulin retains a monomeric configuration, even when formulated at high concentrations, unlike regular human insulin, which forms hexamers (13). Lyspro-insulin has a more rapid onset of action than regular insulin following subcutaneous injection and the differences noted in the pharmacokinetic profiles of lyspro-insulin and regular insulin are consistent with more rapid absorption of the monomer into the circulation from the site of delivery. The pharmacokinetic and pharmacodynamic profiles of lyspro-insulin delivered nasally or ocularly have not been reported.

RESEARCH DESIGN AND METHODS

Animals

Studies were performed in Sprague-Dawley male rats obtained from Charles River Laboratories. Rats were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg), injected intramuscularly, and anesthesia was maintained with additional ketamine/xylazine as needed throughout the experiment.

Materials

Lyspro-insulin (Humalog) and NPH insulin (HumulinN) were obtained from Eli Lilly Co, Indianapolis, IN. Regular human insulin (NovolinR) was purchased from Novo Nordisk Pharmaceuticals Inc, Princeton, NJ. Dodecylmaltoside was purchased from Anatrace, Inc., Maumee, OH.

Analytic Procedures

Nosedrops and eyedrops were formulated on the day of the experiment by mixing one volume of insulin (U-100) with an equal volume of alkylglycoside prepared in phosphate-buffered saline (pH = 7.4). NPH insulin remained turbid following the addition of dodecylmaltoside. Nosedrops (0.02 mL) were administered to the left nares of anesthetized rats in the supine position, using a pipettor with a disposable tip, at time 0 and again after 5 minutes. Rats were turned over two minutes later. Ocular administration of insulin was carried out in the same fashion, except that the eyedrops (0.02 mL in each eye) were administered only at time 0 and rats were in the prone position. Glucose levels were measured in drops of blood 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. Representative blood samples were collected for serum glucose determination by a glucose oxidase spectrophotometric assay to validate the experimental results obtained with the glucose meter (4). Serum insulin levels were measured concomitantly in samples of rat blood collected from the tails of anesthetized animals using a human insulin-specific radioimmunoassay kit (Linco Research, Inc., St. Louis, MO).

Statistical Analysis

Results were evaluated using the Student t-test and considered significantly different if $P < 0.05$. All data are expressed as mean ± S.E.M.

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RESULTS

Nasal Administration of Lyspro-Insulin

No significant decrease in blood D-glucose levels was observed in anesthetized rats following nasal or ocular administration of 2 Units of lyspro-insulin formulated in saline (Fig. 1). In contrast, nasal or ocular administration of 2 Units of lyspro-insulin formulated with 0.125% dodecylmaltoside caused a rapid and significant decrease in blood D-glucose levels (Fig. 1). The decreased blood D-glucose concentration was observed within 20 minutes and was sustained for the remainder of the experiment (120 minutes).

Comparison of Lyspro-Insulin and Regular Insulin Administered Nasally

A direct comparison of the pharmacodynamic profiles of nasal formulations containing regular insulin and lyspro-insulin, in the presence of 0.125% dodecylmaltoside, was undertaken (Fig. 2). There was a rapid and sustained decrease in blood D-glucose levels with both formulations, with the two pharmacodynamic profiles virtually superimposable (Fig. 2). By comparison, nasal administration of NPH insulin in the presence of 0.125% dodecylmaltoside yielded similar results and failed to produce a more sustained hypoglycemic response than nasal administration of lyspro-insulin or regular insulin (not shown). The rate of insulin absorption was measured directly by radioimmunoassay in these same animals (Fig. 3). Insulin levels increased significantly within 10 minutes of nasal administration and maximal levels of insulin were observed 20 minutes after delivery with both types of insulin. Administration of nosedrops containing lyspro-insulin formulated with dodecylmaltoside caused a slightly greater and more rapid increase in serum insulin levels than administration of nosedrops containing regular insulin (Fig. 3). In the absence of dodecylmaltoside, no significant insulin absorption was observed (not shown).

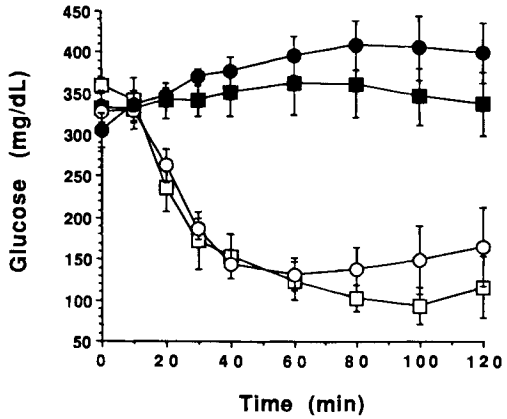

Fig. 1. Nasal and ocular administration of lyspro-insulin with or without dodecylmaltoside. Rats were anesthetized with xylazine and ketamine to induce hyperglycemia. At time 0, 0.02 mL nosedrops containing 50 Units/mL lyspro-insulin, formulated in saline (closed squares) or in saline plus 0.125% dodecylmaltoside (open squares) were instilled into the left nares. After 5 minutes, the administration of nosedrops was repeated. The total amount of insulin administered was 2 Units/rat. Eyedrops containing lyspro-insulin formulated in saline (closed circles) or in saline plus 0.125% dodecylmaltoside (open circles) were administered to both eyes (0.02 mL/eye) at time 0. Blood D-glucose levels were measured in drops of blood collected from the tip of the tail at timed intervals. Data represent the mean ± S.D. of triplicate determinations.

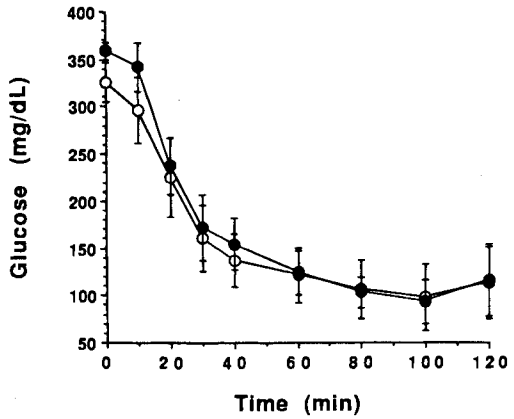

Fig. 2. Comparison of insulin bioavailability following nasal administration of regular insulin and lyspro-insulin. Anesthetized rats received nosedrops containing 2 Units of either regular human insulin (open circles) or lyspro-insulin (closed circles) formulated in saline plus 0.125% dodecylmaltoside, as described in Fig. 1. Blood D-glucose levels were measured in drops of blood collected from the tip of the tail at timed intervals. Data represent the mean ± S.D. of triplicate determinations.

DISCUSSION

The delivery of insulin remains a critical problem in the lives of millions of persons suffering from diabetes mellitus. Alternate routes of insulin delivery, including nasal or ocular formulations, would provide a valuable alternative to patients with limited access to, or problems in using, multiple daily subcutaneous injections of insulin. Moreover, even patients who successfully take insulin injections routinely would welcome

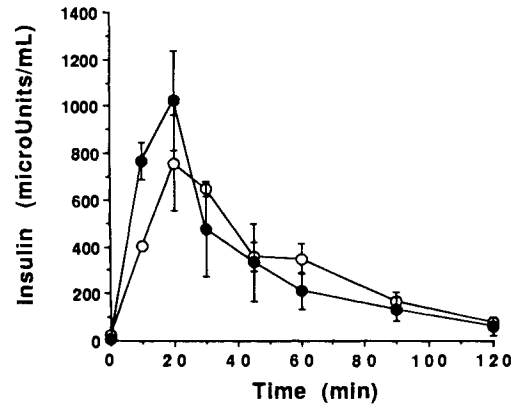

Fig. 3. Comparison of immunoreactive insulin levels following nasal administration of regular insulin and lyspro-insulin. Serum immunoreactive insulin levels were measured in blood samples collected from the animals described in Fig. 2 Anesthetized rats received nosedrops containing 2 Units of either regular human insulin (open circles) or lyspro-insulin (closed circles) formulated in saline plus 0.125% dodecylmaltoside. Data represent the mean ± S.D. of triplicate determinations.

the availability of a convenient, portable, non-invasive insulin delivery system for use at certain times during the day, such as school, workplace, recreation and mealtimes. The possibility of developing nasal or ocular insulin formulations that are both efficacious and non-irritating have driven considerable research into the properties that control insulin absorption following topical application. Dodecylmaltoside has been studied as a potential excipient for insulin formulations in experiments with rodents and a diabetic dog (9–11). This alkylglycoside is non-ionic, readily metabolized to innocuous products, and effective at enhancing insulin absorption at exquisitely low concentrations. Rodents were used in the current experiments because they allow for a comparison of these results with data in the literature and because insulin bioavailability can be determined accurately as a result of anesthesia-induced hyperglycemia. Limitations of the rodent experiments include the lack of ability to directly extrapolate the results to larger animals.

The data presented in Fig. 1 provide direct evidence that the monomeric form of insulin can not readily pass across the nasal mucosa in the absence of an absorption-enhancer. Monomeric lyspro-insulin behaved exactly the same as multimeric regular insulin in these studies. Furthermore, the rate of lyspro-insulin absorption in the presence of dodecylmaltoside was similar to, and just slightly faster than, the rate of regular insulin absorption; maximal serum levels of insulin were obtained 20 minutes after nasal delivery (Fig. 3).

These results are inconsistent with the hypothesis that dodecylmaltoside acts to enhance regular insulin absorption by favoring a shift from the hexameric to monomeric form. The data support the alternative hypothesis, i.e. that dodecylmaltoside favors a loosening of cell-cell junctions and thereby allows more extensive paracellular movement of both lyspro-insulin and regular insulin. This conclusion is also consistent with the observation that dodecylmaltoside increased the absorption of glucagon applied nasally (10), since dodecylmaltoside could increase paracellular movement of glucagon across the nasal mucosa without any impact on the size of monomeric glucagon. It was somewhat disappointing to determine that nasal or ocular formulations of lyspro-insulin in saline are ineffective, from the perspective that successful nasal or ocular insulin delivery of lyspro-insulin formulated simply in saline would be the safest vehicle for chronic multiple daily insulin administration. Nonetheless, this information can provide insight into the development of safe, effective insulin formulations that contain alkylglycosides as excipients.

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