Pharmacodynamics of Insulin Following Intravenous and Enteral Administrations of Porcine-Zinc Insulin to Rats

Robert J. Schilling1,2 and Ashim K. Mitra1,3

Received August 28, 1991; accepted January 20, 1992

Previous work from this laboratory showed site-dependent variations in the apparent permeability of insulin as measured using the everted rat gut sac technique, with the greatest permeability in the distal jejunum and the lowest in the duodenum (5). To quantify better the rate and extent of insulin absorption from the small intestine, closed-loop in situ experiments were performed in nondiabetic rats. Results correlated with the everted gut sac technique in that the absolute bioavailability determined in situ was higher for insulin solution administered to the more distal region of the intestine (0.133%) than that absorbed from an earlier portion of the intestine (0.059%). While the difference in regional bioavailability was not significant (P = 0.08), the blood glucose response showed highly significant differences (P = 0.0015), with severe and prolonged hypoglycemia resulting from insulin delivered to the distal jejunum/proximal ileum. Insulin administered iv followed a two-compartment pharmacokinetic model. Whole-body elimination rate constants were similar for both iv and enteral insulin. Although therapeutic quantities of insulin were absorbed from the distal small intestine, absorption enhancers would be necessary to decrease the dose of insulin required.

KEY WORDS: insulin; intravenous; enteral; pharmacokinetics; bioavailability; pharmacodynamics.

INTRODUCTION

The treatment of Type I diabetic patients (and some Type II diabetics) requires subcutaneous insulin injections, normally once or twice each day. For more intensive treatment, and better metabolic control, diabetics may receive as many as four injections daily. For chronic administrations, the parenteral route is not the ideal means for insulin delivery.

Oral administration, if possible, not only will be the most acceptable from a patient compliance standpoint but also will deliver insulin to the liver via the portal vein exactly as would be observed with endogenous insulin. Therefore, oral delivery would more closely mimic natural insulin secretion from the pancreas. Studies in human, insulin-dependent diabetics who also lacked pancreatic digestive enzymes have shown that insulin delivered to the upper portion of the small intestine was absorbed in small quantities based on plasma insulin levels and/or blood glucose depression (1,2). Others have also directly shown the ability of intact, active insulin to be absorbed in small amounts from a particular area of the small intestine (3,4). We have proposed (5) that regional differences in the small intestine (6) might affect the overall extent of insulin absorption and thus could influence the choice and design of a dosage form for oral insulin delivery.

Previous work from this laboratory has discussed the site-dependent intestinal mucosal permeability of insulin (5). The regions of the small intestine with optimal permeability of insulin were the distal jejunum, followed by the ileum. Moreover, brush border metabolism of intact insulin was found to be negligible or absent. Based on these in vitro results, studies were performed using a more physiologically normal in situ procedure to evaluate better whether greater insulin absorption and blood glucose response resulted from delivery to more distal as compared to more proximal regions of the small intestine.

The present report describes in detail the pharmacokinetics and pharmacodynamics of insulin following intravenous administration of porcine-zinc insulin to rats. This report also describes the compartmental and noncompartmental approaches to the evaluation of in situ intestinal absorption and bioavailability of insulin and corresponding blood glucose effects. These studies are useful for examining the results of insulin delivery to different regions of the small intestine and were necessary for establishing a baseline to compare the effects of absorption enhancers on insulin absorption at a particular site (which will be described in subsequent reports).

MATERIALS AND METHODS

Materials

Crystalline porcine-zinc insulin (Lot No. 009HC7; potency, 28 U/mg) was kindly donated by Eli Lilly and Company (Indianapolis, IN). The buffer solutions were prepared with analytical reagent-grade chemicals. A sterile 0.9% sodium chloride solution for intravenous use (Abbott Laboratories, North Chicago, IL) was used for the replacement of blood volume taken during sampling. Heparin solutions were prepared by diluting 1000 USP U/ml heparin sodium injection, USP (Lyphomed, Rosemont, IL), with saline to a final concentration of 10 U/ml.

Preparation of Insulin Solution

Buffer components are known to influence the intestinal passage of water and drugs (7,8). Studies involving in situ intestinal absorption of theophylline recommended normal saline as the medium, however, a diluted phosphate buffer also did not significantly alter absorption (8). Since insulin needs to be dissolved initially in pH 2 hydrochloric acid for solubilization purposes, adjustment of the administering solution to neutral pH was necessary. Since pH 2 is too acidic for intestinal studies and the isoelectric point of insulin is near pH 5.5, the function of the administering solution, pH 7.4 phosphate-buffered saline (PBS), was to present insulin.

1 Department of Industrial and Physical Pharmacy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907.
3 To whom correspondence should be addressed.
in solution composed predominantly of saline and having a neutral pH. With only a low buffer capacity, this solution was not intended or expected to maintain a given pH once in the intestinal lumen, as buffers are believed to have little, if any, effect on the micro-pH at the membrane interface (7,8) and the ability of the rat small intestine to alter the bulk pH of more concentrated buffers toward neutral pH has been observed (7). The buffered saline employed throughout the intestinal absorption experiments was thus prepared by diluting a 0.0667 M to 0.01 M phosphate. Osmolality was measured with an Osmette S Model 4002 osmometer (Precision Systems, Inc., Natick, MA) and was adjusted with sodium chloride to a final value of 225–230 mOsm/kg.

Intestinal Absorption with the Closed-Loop Technique

Male Sprague–Dawley rats weighing 175–250 g were fasted for 16–20 hr prior to an experiment. Water was allowed ad libitum. The animals were anesthetized by an intraperitoneal injection of a mixture of 90 mg/kg ketamine and 10 mg/kg xylazine. One-third to one-half of the original dose was administered every 45–60 min thereafter to maintain anesthesia/analgesia. The core body temperature was maintained close to 37°C by placing the animal on a platform above a 40°C water bath with a 100-W light bulb and a reflector above.

Cannulation of the right external jugular vein was performed by inserting a 3-in. piece of Silastic tubing, 0.047-in. O.D. (Dow Corning, Midland, MI). A collar made from a 1-cm piece of PE 200 polyethylene tubing (Becton Dickinson, Parsippany, NJ) was attached to the outer end of the Silastic tubing. Before insertion, the cannula was filled with saline containing 10 U/ml heparin. Microdissecting scissors were used to cut a small opening in the jugular vein, and one tip of a microdissecting forceps, extra delicate, was inserted through the hole to guide the insertion of the cannula toward the heart. Surgical thread underneath the vein was tied around the collar of the cannula to secure it. A 23-gauge needle, filed at the end to smooth out the bevel, was inserted into the cannula and was used with a heparinized 1-ml plastic syringe for the removal of samples.

Next a midabdominal incision was made to expose the small intestine. Intestinal segments were measured with a string to a 15-cm length. Experiments were carried out in individual animals to test for insulin absorption from the distal duodenum/proximal jejunum (beginning 3–4 cm beyond the ligament of Trietz; n = 6) or from the distal jejunum/proximal ileum (beginning 16 cm above the cecum; n = 6). The desired segment was opened at each end and a piece of Tygon tubing, 4-mm o.d., was inserted into the proximal opening. A peristaltic pump (Model 1203, Harvard Apparatus, Millis, MA) was employed to perfuse normal saline through a warming chamber into the intestine to remove any residual gut contents. A total of 30 ml saline was pumped at a rate of 3 ml/min. Each segment was carefully ligated both above and below the incisions to prevent any fluid loss. Air was then pumped through the segment to remove any residual saline. The distal end of the segment was then ligated and the appropriate solution (approximately 0.6 ml) was instilled. The concentration of insulin solutions employed was 4.5 mg/ml, or 126 U/ml, while control experiments utilized an equivalent volume of blank PBS. Finally, the proximal end of the intestinal segment was quickly ligated to form a closed sac, which was carefully returned to its original place inside the peritoneal cavity.

Intravenous Administration

In order to determine the absolute bioavailability of enterally absorbed insulin, intravenous administration was necessary. Insulin (0.25 U/ml) in 0.01 M phosphate-buffered saline was injected at a dose of 0.2 U/kg into the lateral tail vein of fasted, cannulated nondiabetic rats (n = 4). Blood samples (210–230 μl) were obtained at 0, 1, 4, 7, 11, 15, 20, 30, 45, 60, 90, 120, 180, and 240 min postadministration to determine both blood glucose levels and plasma insulin concentrations. Plasma was separated by first collecting the blood samples into heparinized Natelson capillary tubes kept on ice and then centrifuging in a Damon CRU-5000 refrigerated centrifuge (IEC, Needham Heights, MA) at 3000g for 15 min. Plasma samples were quickly frozen in dry ice-acetone and then stored at −20°C until analysis. Blood sample volumes were replaced with the same volume of warm (37°C) saline.

Analytical Methods

Blood Glucose

Blood samples were immediately tested for glucose levels using Chemstrip bG reagent test strips (Boehringer Mannheim Diagnostics, Indianapolis, IN) with an AccuCheck II blood glucose monitor (Boehringer Mannheim Diagnostics). The sample size was approximately 30 μl of whole blood. The precision of the assay was found to be within ±3% (n = 5) and measurable glucose levels ranged from 10 to 500 mg/dl.

Plasma Insulin

Plasma insulin levels were quantitated using Coat-A-Count insulin radioimmunoassay kits (Diagnostic Products Corporation, Los Angeles, CA). The method utilized a solid phase radioimmunoassay technique where a standard amount of 125I-labeled insulin competes with unlabeled sample insulin for binding to insulin-specific antibodies immobilized to the wall of a polypropylene tube. This technique can be used to detect as little as 1 μU/ml insulin and concentrated samples can accurately be measured upon dilution with blank serum provided with the kit.

Frozen plasma samples were thawed at room temperature and then aliquots were pipetted into the insulin-antibody coated tubes and diluted with blank serum as needed to generate a final sample volume of 120 μl. Next 600 μl 125I-labeled insulin was added, followed by vortexing for 30 sec. Duplicate standards were run to generate a standard curve (each time insulin samples were analyzed) by adding 120 μl of insulin standards with 600 μl of 125I-labeled insulin. Following incubation at room temperature for approximately 18 hr, the tubes were decanted and the contents counted for 1 min with a Beckman Model 5500 gamma counter (Fullerton, CA).