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EFFECTIVE INTESTINAL ABSORPTION OF INSULIN IN DIABETIC RATS USING ENTERIC COATED CAPSULES CONTAINING SODIUM SALICYLATE

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ABSTRACT

The absorption of insulin manifested as percent reduction of blood glucose was evaluated after placement of capsules containing 4.6 units of the drug and 20 mg of sodium salicylate as an absorption promoter in the rats stomach. The capsules were coated with either Eudragit L100 or Eudragit S100 to deliver insulin in different regions of small intestine of the rats as they are pH dependent. The data obtained after administration of the capsules were compared with that after intraperitoneal injection of 1 U of insulin and also after administration of coated capsules containing insulin alone. The administration of insulin capsules containing sodium salicylate result in a significant ($p<0.01$) increase of the hypoglycemic effect over the 5 h period of the experiments. They produced the same hypoglycemic effect as I.P. injection at 5 h point. The areas under the % blood glucose reduction curves produced were 363.5, 221.7 and 236.5% h for I.P. injection and capsules coated with Eudragit L100 and Eudragit S100, respectively. The relative bioavailabilities of capsules to I.P. injection were 13.26 and 14.15% for those coated with Eudragit L100 and Eudragit S100, respectively. Enteric coated capsules of insulin alone caused no glucose reduction.

INTRODUCTION

Discovery of insulin by Banting and Best (1) was one of the greatest triumph of the twentieth century medicine and was translated into a lifesaving remedy. Although a new era had dawned for the diabetic patient, there remained problems to be surmounted, one of which is the insulin administration route. Troubles of

repeated injections, physiological stress, costs, risks, infections, inability to handle and inconvenience all made it highly desirable to find an alternative route for administration. The oral administration of insulin has long been the hope of clinicians. Insulin being a protein suffers from incomplete oral bioavailability due to several reasons of which (a) resistance of the mucosal membrane to its penetration, (b) degradation of insulin by proteases in the luminal cavity and the cells lining the mucosa, and (c) rapid clearance of the administered dose from the site of deposition. The first reason is overcomed by use of penetration enhancers (2,3). The second reason where insulin is degraded by proteolytic enzymes (4-7) could be solved by formulation approach where insulin is housed with a delivery system not only to protect the drug from contact with the luminal protease but also to release the drug only upon reaching a favorable area for its absorption. Over the years several formulations have been explored to protect insulin from proteolysis in the GIT, including water-in-oil-in-water emulsion (8-10), liposomes (11-13), nanoparticles (14), soft gelatin capsules coated with polyacrylic polymers (15) and water-in-oil-microemulsion (16).

The aim of this work is to formulate and test in diabetic rats, the bioavailability of insulin from capsules containing sodium salicylate as an absorption enhancer and coated with pH-dependent Eudragit L100 or Eudragit S100 to protect capsules against gastric degradation and deliver insulin at different parts of small intestine of low protease activity.

MATERIALS AND METHODS

Materials:

Crystalline insulin 23U/mg was purchased from Fluka Chemicals (AG, CH-9470 Buchs, Switzerland). Eudragit L100 and Eudragit S100 from Rohm Pharma (GmbH, Darmstadt, Germany). Isopropyl alcohol from E. Merck (Darmstadt, Germany). Ether and acetone from BDH Limited (Poole, England). Isophane insulin injection BP 100U/ml (Nova Industri A/S Bagsvaerd, Denmark) was purchased from the market.

Dosage Form Design:

The oral dosage form design is based on the incorporation of either 0.2 mg insulin (4.6 U) alone or a physical mixture of 0.2 mg insulin and 20 mg sodium salicylate into each (5/7 mm) hard gelatin capsule. The capsules placed in a coating pan rotated at 50 rpm were coated by spraying with 10% solution of either Eudragit S100 or Eudragit L100 in acetone or mixture of acetone and isopropyl alcohol (1:1 v/v), respectively. The coated capsules were dried using a stream of hot air (50-60^{o}C). The enteric coated capsules were tested for their pH dependent release, where the Eudragit L100 and Eudragit S100 coated capsules released their

contents at pH's 6 and 7 respectively indicating the intact nature of the film coatings.

Animals:

Twenty four male sprague Dawley rats weighing 300-350 g were induced diabetic by intraperitoneal injection of streptozotocin (80 mg/kg). The rats were starved for 20 h before the experiment and received water ad libitum during the experiment. The diabetic rats were divided into four groups of six each. The first group was given Eudragit S100 enteric coated capsules containing insulin alone. The second group was given intraperitoneal injection of 1 U of insulin. The last two groups were given either Eudragit L100 or Eudragit S100 coated capsules containing insulin and sodium salicylate.

Operating Procedures and Capsule Placement:

Under ether anaesthesia, an incision approximately 4 cm long was made 2-3 cm lateral to the abdominal midline and 3-4 cm caudal to the sternum. The stomach was exposed though the incision. The capsule was placed in the fundus. The stomach was then tied securely with purse-string suture. The abdominal muscles of the incision were closed with gut sutures and the skin incision was also closed with silk sutures.

Blood Sampling:

While rats are under transient ether anaesthesia 0.5 ml blood was collected by cardiac puncture before capsule placement and at 30, 60, 120, 180, 240 and 300 min afterwards. Blood glucose was then determined by an enzymatic colorimetric assay procedure (God-Perid method) (17), using DMS 90 UV visible spectrophotometer at wavelength 436 nm.

RESULTS AND DISCUSSION

Table 1 shows that the gastric placement of hard gelatin capsules containing 4.6 U of crystalline insulin and 20 mg of sodium salicylate and coated with 10% solution of Eudragit L100 to diabetic rats resulted in a significant ($p<0.01$) reduction of blood glucose level from a mean±standard deviation (X±S.D.) of 324.50±17.12 mg/100 ml to 238.40±15.49 (26.5%) in the first 30 min. The reduction continued to increase and the blood glucose level dropped to 116.53±13.16 mg/100 ml (64.1%) after 5 h. While, the administration of the same capsules coated with Eudragit S100 resulted also in a significant ($p<0.01$) drop of the blood glucose levels of the diabetic rats from 309.37±12.98 to 259.16±12.84 mg/100 ml (16.2%) after 30 min., and continued to decrease to reach a value of 107.29±4.24 mg/100 ml (65.3%) after 4 h before it started to rise to 122.50±6.66 mg/100 ml (60.4%) in the fifth

TABLE 1

Blood Glucose Levels in Diabetic Rats After I.P. Injection of 1 U of Insulin, Gastric Placement of Insulin (4.6 U) Capsules Coated With 10% Solution of Eudragit S100, Insulin (4.6 U) and Sodium Salicylate (20 mg) Capsules Coated With 10% Solution of Either Eudragit S100 or Eudragit L100.

Time (min.)	I.P. Injection	Insulin Capsules Coated With S100	Insulin+Na Salicylate Capsules Coated With S100	Insulin+Na Salicylate Capsules Coated With L100
		Blood Glucose (mg/100 ml)		
0	312.49±20.03	321.70±12.11	309.37±12.98	324.50±17.12
30	84.37±06.03	354.37±04.81	259.16±12.84	238.40±15.49
60	75.13±04.83	368.69±03.01	187.50±17.67	236.25±10.21
120	53.12±04.37	364.68±04.65	150.25±18.89	177.37±17.13
180	71.87±04.68	384.76±10.47	148.75±06.35	167.50±11.78
240	75.00±04.14	314.22±09.23	107.29±04.24	122.50±12.96
300	100.00±04.72	305.35±14.06	122.50±06.66	116.53±13.16

hour. The capsules of insulin alone gave no reduction in blood glucose indicating that insulin is insignificantly absorbed in absence of absorption promoter.

The area under the curve (AUC 0-5 hours) from the % blood glucose reduction versus time profile was calculated by means of trapezidal rule for the capsules coated with Eudragit L100 and Eudragit S100 and also after I.P. injection of 1 U of insulin and were found to be 221.69, 236.54 and 363.5% h, respectively. The relative bioavailability of the capsules were 13.26% and 14.15%, respectively compared to that of the I.P. injection. The hypoglycaemic effect of the four treatments expressed as % of initial blood glucose content is shown in Fig. 1. There was no significant difference ($p>0.05$) between % reduction of the blood glucose levels after administration of capsules coated with Eudragit L100 compared to that coated with Eudragit S100. Although the I.P. injection shows rapid and significant hypoglycaemic effect (73% reduction in the first 30 min) it is not considered the optimal therapy since it may result in undesirable pharmacological effects (18). The coated capsules are better as they show more gradual decrease in blood glucose that reach the same value as after I.P. injection in the fifth hour.

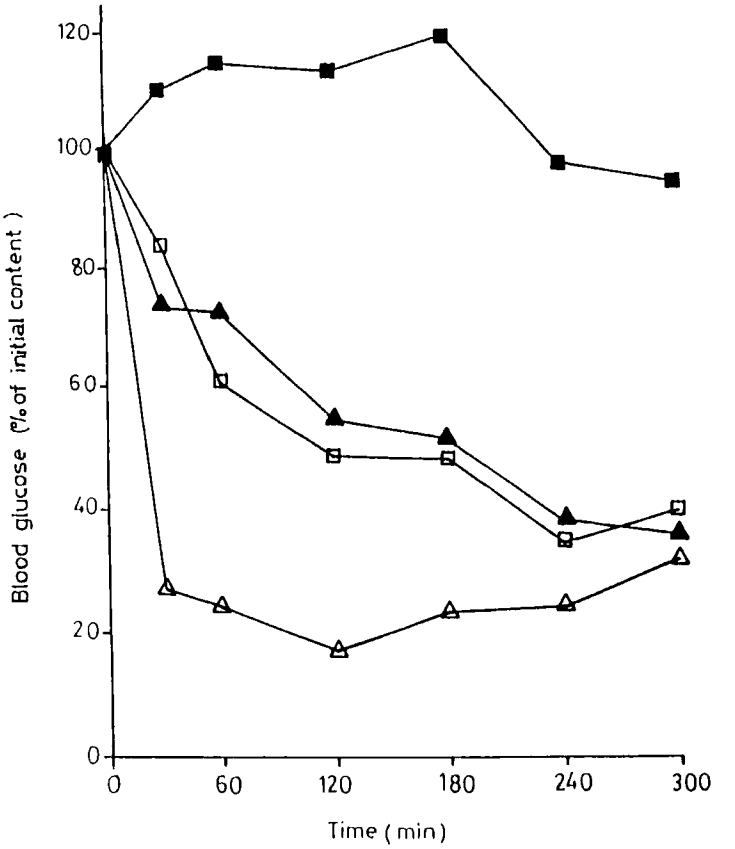

FIGURE 1.

Hypoglycemic effect (% of initial content) of insulin capsules coated with Eudragit S100 (■), insulin and sodium salicylate capsules coated with Eudragit L100 (▲), Eudragit S100 (□), and after I.P. injection (△).

Sodium salicylate was used in this study as it has been used before for rectal promotion of insulin absorption in rats (19), dogs (20) and humans (21) and resulted in a significant decrease in plasma glucose and remarkable increase in plasma insulin levels. Also, 1.5 M sodium salicylate has been shown to increase solubility of insulin 7875 times (22). In addition sodium salicylate stimulates the absorption of insulin though acting on the apical cell membrane (transcellular pathway) and also on the tight junction between cells (paracellular pathway) (23,24). Eudragit L100 and Eudragit S100 were used for coating the capsules in this

study to avoid the insulin digestion which is initiated in the gastric juice by the pepsins which are most active at pH 2-3 and become inactive at pH's above 5. These Eudragits coating are of course unable to further protect the insulin from degradation by the pancreatic proteases in the duodenum and beyond as they dissolve at pH 6 (L) and 7 (S) and also upon contact with brush border or following entry into cells. This may be the reason for the low relative bioavailability from these capsules compared to that after I.P. injection.

On conclusion, sodium salicylate seems to be a suitable absorption promoter for oral insulin absorption. Further studies are needed in different types of animals and in diabetic patients to optimize this enhanced delivery of insulin.

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