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Human Insulin Absorption from the Intestine in Diabetic Rats

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ABSTRACT

The ability of human insulin to produce hypoglycemia in streptozocin-diabetic rats (average weight 500 g) was investigated. A simple solution of human insulin (insulin in hypotonic buffer solution) was administered intraduodenally. Rats received a dose of insulin of either 200 U/kg, 400 U/kg, or 600 U/kg. The hypoglycemic response was most significant when 600 U/kg solution of insulin was administered. The 200 U/kg dose was of two forms; one form was a solution of insulin with a concentration of 25 U/ml, 4 ml, and the other was a solution of insulin with a concentration of 50 U/ml, 2 ml. The dose of 25 U/ml, 4 ml produced a more significant hypoglycemic response than that of 50 U/ml, 2 ml. Carrier-insulin systems were also introduced intraduodenally in streptozocin-diabetic rats. The results indicate that the carrier systems without insulin had glucogenic effect. Despite this glucogenic effect, carrier-insulin systems at 200 U/kg dose were as effective as that of 600 U/kg of insulin solution. We conclude that insulin absorption from the intestine can cause a significant hypoglycemic state if given in a large enough dose, even without the presence of other absorption enhancers in the dosage form.

INTRODUCTION

Since the discovery of insulin by Doctors Charles H. Best (University of Toronto, Canada) and Fred G. Banting (Western University, London, Ontario) (1), type I diabetes mellitus in man has been treated with repeated subcutaneous injections of insulin. Recently, human insulin, a product of recombinant DNA technology, was introduced to the market; estimated sales in 1991 of one product containing human insulin was reported to be $ 400 milion (2).

Several studies examined liposomes as oral delivery systems for insulin (3-10). Liposomes are believed to protect insulin from enzyme action when insulin is encapsulated inside the liposomes (11). Other forms of delivery systems, such as nanoparticles (12) and microspheres (13) have also been suggested as potential oral delivery systems for insulin. One study (14) reported intraduodenal administration of w/o/w emulsion systems in normal and alloxan-induced diabetic Wistar rats; there was a significant reduction in blood glucose levels after the administration of emulsion-insulin in normal but not in diabetic rats. We have recently reported (15) the use of erythrocyte-membrane systems for the intraduodenal delivery of human insulin in rats. In our study there was a significant

reduction in blood glucose concentrations after the administration of ghosts-insulin, vesicles-insulin, and liposomes-vesicles-insulin systems as compared to controls (i.e., carrier-free insulin).

Insulin absorption from the G.I. tract was also found to be facilitated by the presence of intestinal enzyme inhibitors (16-18). Administering insulin orally to normal and diabetic human subjects with absorption enhancers such as surfactants has been investigated. Absorption of insulin was evident from the decrease seen in blood glucose level (19). A recent publication showed that administering a simple solution of insulin (400 U/kg) in nondiabetic rats into the duodenum/jejunum or jejunum/ileum areas caused a decrease in blood glucose level and was more pronounced with the jejunum/ileum administration (20). This suggests high doses of a simple solution of insulin placed in the intestine resulted in significant hypoglycemia. However, the effect of administering insulin intraduodenally with no absorption enhancers or modifications into diabetic animals has not been fully illucidated.

This paper reports experiments with high doses of human insulin (200, 400, and 600 U/kg) free or associated with either erythrocyte-ghosts (EG) or liposomes-vesicles (LEV) as carrier systems administered intraduodenally in streptozocin-induced diabetic male Wistar rats.

MATERIALS AND METHODS

Materials

Human insulin (Humulin R, Eli Lilly) was purchased from N.C. Mutual, North Carolina. All other chemicals were from Sigma Chemical Company, St. Louis, Missouri. Human erythrocytes were obtained from the American Red Cross, North Carolina.

Preparation of Dosage Forms

A- Preparation of erythrocyte-ghosts-insulin suspension (EG-INS):

EG suspensions were prepared according to a previously described method (21). The association of human insulin with 1 ml of ghosts suspension was determined by incubating 1 ml of ghosts suspension with a volume (1, 2, or 3 ml) of insulin solution (100 U/ml) and the volume was made to 4 ml with hypotonic buffer solution. The incubation was done at 37°C for 24 hr. The amount of insulin associated with ghosts was determined by a method previously reported (22). Table 1 presents the amount of insulin associated with 1 ml of ghosts under the conditions stated for the incubation.

EG-INS suspensions were prepared by adding the appropriate amount of insulin dissolved in solution to 1 ml of ghosts suspension and the final volume was made to 4 ml with hypotonic solution. The mixture was then incubated at 37°C for 24 hr. This final mixture was then used for the in vivo experiments. All preparations were made fresh prior to the day of the experiment.

B- Preparation of liposomes-vesicles-insulin (LEV-INS):

LEV suspensions were prepared according to a method previously published (22). LEV-INS suspensions were made by mixing one ml of LEV suspension with a volume (1, 2, or 3 ml) of insulin solution (100 U/ml) and enough hypotonic solution to make 4 ml. The mixture was then incubated at 37°C for 24 hr. Table 2 presents the amount of human insulin associated with one ml of LEV suspension.

Animals and Treatments

A total of 100 male Wistar rats weighing on average 500.7 ± 43.8 g (mean ± s.d.) were used in this investigation. Diabetes was

TABLE 1
Amount of Human Insulin Associated With One ml of Ghosts Suspension Following an Incubation at 37°C for 24 hr. Approximately 30 % of the Initial Amount Added was found Associated With Ghosts. Total Volume of Sample was 4 ml.

Initial Amount of Insulin (U)	Amount Associated (U)
100	27.17 ± 1.48 (6)[a]
200	63.00 ± 3.24 (6)
300	83.32 ± 5.21 (6)

a) Mean ± S.E. (Number of Observations).

TABLE 2
The Amount of Human Insulin Associated With One ml of Liposomes-Vesicles Following Incubation at 37°C for 24 hr. Total Volume of Sample was 4 ml.

Initial Amount of Insulin (U)	Amount Associated (U)
100	12.94 ± 4.67 (6)[a]
200	25.51 ± 12.78 (6)
300	58.41 ± 30.47 (6)

a) Mean ± S.E. (Number of Observations).

induced by a single i.v. injection of streptozocin (12). Diabetes was confirmed by a blood glucose concentration of 324.53 ± 54.13 (100) (mean ± s.d, (number of rats)). All animals were fasted overnight (16-18 hr) prior to the day of experiment (water was provided ad libitum). Anesthesia was induced using pentobarbital sodium 80 mg/kg i.p. and maintained with an hourly i.m. dose of 20 mg/kg. After a midline incesion was made, a dose of insulin (carrier-insulin suspension 100, 200, or 300 U or insulin solution 100, 200, or 300 U) or a control dose (carrier-free-insulin or saline) in a volume of 4 ml was introduced into the duodenum followed by one ml of saline. Rats were placed in a human infant incubator (Isolette, Air-Shields, Inc., Hatboro, PA) so that body temperature was maintained throughout the experiment. Blood glucose level was determined from tail blood prior to administration and at 0.5, 1, 2, 3, 4, and 5 hr post administration. Rats were sacrificed by exsanguination at the end of the experiment. Blood samples (one drop from tail blood) were assayed for glucose using a glucometer (One Touch II, Lifescan Inc., Milpitas, CA).

Data Analysis

A repeated measures technique (split-plot method) (23) was used to compare treatment groups with their controls and among each other. A significant difference among the treatments indicates the efficacy of the treatment in delivering insulin to the systemic circulation.

TABLE 3
Percent of Initial of Blood Glucose Concentration (Mean ± S.E.) After Intraduodenal Administration of Insulin Solution (25 U/ml, 4 ml) and (50 U/ml, 2 ml) in Rats.

Time (hr)	Treatments		
	25 U/ml (4 ml) n = 16[b]	50 U/ml (2 ml) n = 11	P[a]
0.5	85.21 ± 4.52	88.46 ± 4.69	n.s.[c]
1	81.21 ± 6.10	101.21 ± 4.49	0.023
2	85.78 ± 8.88	100.72 ± 5.88	n.s.
3	84.57 ± 9.33	123.85 ± 8.18	0.017
4	85.87 ± 9.50	124.83 ± 9.13	0.009
5	84.32 ± 9.30	118.64 ± 6.86	0.008

a) Based on t-Test. b) Number of Rats. c) Not Significant.

RESULTS AND DISCUSSION

In a recent report (15), we showed the effect of EG and LEV as carriers for human insulin through the intestinal membrane when administered intraduodenally. In that study, the dose of insulin was fixed to 100 U and the volume of the dose was 2 ml. In the current study the dose of insulin varied from 100 U to 300 U and the volume of the dose was 4 ml. Introducing 100 U in 4 ml (25 U/ml) rather than in 2 ml (50 U/ml) (15) resulted in a much better response in lowering the blood glucose level (Table 3). This result can possibly be explained as follows: (1) a larger volume occupied a larger surface area in the intestine for absorption which increases total absorption for the larger volume and/or (2) the presence of high molecular weight aggregates of insulin in more concentrated solutions than diluted ones which would decrease absorption of higher concentrations (24). To achieve similar effects to that with 4 ml of 25 U/ml, 4 ml of a solution of 50 U/ml needed to be administered. However, increasing the dose to 600 U/kg (i.e., 300 U given in 4 ml to a 500 g rat) resulted in significantly lower blood glucose levels than that seen with 100 U or 200 U of insulin (P = 0.0001). There was an average decrease in blood glucose concentration at its maximum (2 hr postadministration) of 54 mg/dl and 49 mg/dl when the insulin dose was 100 U (200 U/kg) or 200 U (400 U/kg), respectively; a greater reduction in blood glucose concentration (144 mg/dl) occurred when 300 U of insulin was administered (Fig. 1-A). In contrast, in the case of EG-INS or LEV-INS, there was no difference in effect among the doses. An average decrease in blood glucose level from initial of a magnitude of 56 mg/dl, 53 mg/dl, and 65 mg/dl for 100 U, 200 U, and 300 U respectively, was observed after administration of LEV-INS (Fig. 1-B). In a similar fashion EG-INS administration resulted in an average reduction in blood glucose concentration of 35 mg/dl, 21 mg/dl, and 28 mg/dl for 100 U, 200 U, and 300 U, respectively (Fig. 1-C).

The control groups (LEV and EG without insulin) resulted in an increase in blood glucose significantly higher than saline control (P = 0.0001). This indicates that LEV and EG have a possible glucogenic effect. Based on this observation, the actual decrease in blood glucose level should be relative to the control group, since the carrier itself has an opposite action on blood glucose

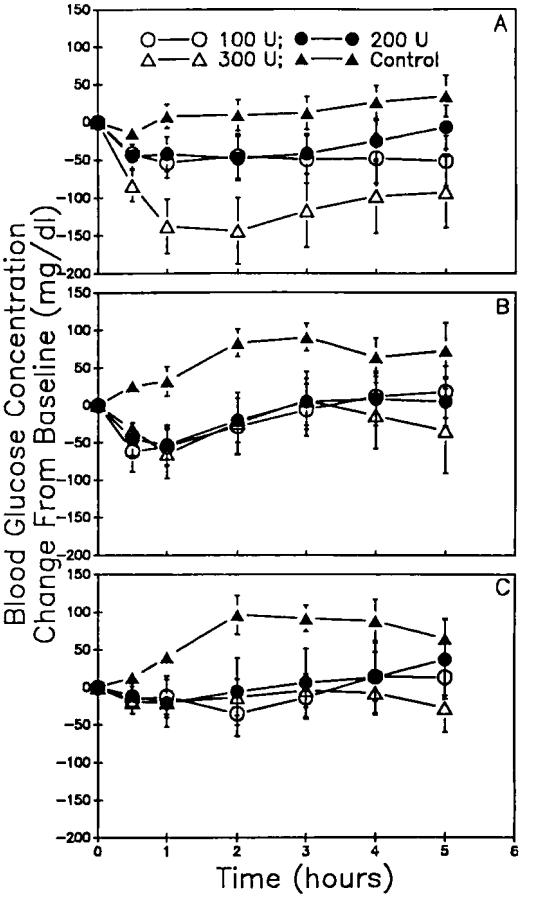

FIGURE 1
Blood Glucose Concentration (mg/dl) (Change From Baseline) vs Time (hr) Profile for A) Insulin Solution (100 U, 200 U, and 300 U) and Saline Administered Intraduodenally, B) Liposomes-Vesicles-Insulin Suspension (100 U, 200 U, and 300 U) and Liposomes-Vesicles Without Insulin Administered Intraduodenally, and C) Erythrocyte-Ghosts-Insulin Suspension (100 U, 200 U, and 300 U) and Erythrocyte-Ghosts Without Insulin Administered Intraduodenally. Data Points are Mean ± S.E.

concentration. When the data were corrected for the respective control, there was no significant difference between the carrier-insulin systems at all doses (i.e., 200 U/kg, 400 U/kg, and 600 U/kg) and insulin solution (600 U/kg). This indicates that the carrier systems at a dose of 200 U/kg were as efficient in delivering insulin to the systemic circulation as an insulin solution given in a dose of 600 U/kg. This glucogenic effect of the carrier systems was reported also for a microemulsion-insulin delivery system injected i.p. in mice (25). In that study, there was

a steady decrease in blood glucose level over a one hour period after insulin injection, whereas insulin in a microemulsion resulted in an increase in blood glucose levels.

CONCLUSION

This study has demonstrated that human insulin is absorbed from rat intestine following intraduodenal administration. Insulin absorption from a solution of insulin administered intraduodenally, as evidenced by a decrease in glucose concentrations, was enhanced by increasing the volume of the dose and/or increasing the total dose of insulin given. This absorption process was pharmacologically significant after administering a dose of 600 U/kg in streptozocin-induced diabetic male Wistar rats. Because of the glucogenic effect of the carrier used, the effect of insulin was masked when carrier-insulin was administered, though the efficacy of delivering insulin by carriers was as good as insulin solution.

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