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INTRADUODENAL ADMINISTRATION OF BIOCARRIER-INSULIN SYSTEMS

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ABSTRACT

Successful oral administration of insulin for systemic therapeutic effects has long been a major pharmaceutical challenge. Intraduodenal administration of insulin to rats, free or in a form of carrier-insulin, was the subject of this study. Several erythrocyte-membrane carrier systems (ghosts, vesicles, liposomes-ghosts, and liposomes-vesicles) were tested. Insulin (100 U) was incubated with each of the carriers at 37°C for 24 hr. The carrier-insulin system, insulin solution, sodium chloride solution, or carrier-free insulin was then introduced into the duodenum of anesthetized male Wistar diabetic rats. Blood samples were collected from the tail at different time intervals and then analyzed for glucose content using an o-toluidine method. There was no significant difference in blood glucose concentrations among the control groups. However, ghosts-insulin, vesicles-insulin, and liposomes-vesicles-insulin all showed a statistically significant difference in lowering blood glucose levels when compared to control groups. Liposomes-ghosts-insulin did not show any significant difference from its control group. The results indicate

that liposomes-vesicles-insulin was the most efficient in delivering insulin into the circulation in its pharmacologically active form of any other carrier tested. The findings of this study may be of significance in the search for a suitable oral carrier for insulin or perhaps other proteinaceous substances.

INTRODUCTION

The current treatment of type I diabetes involves administering insulin by injection, due to destruction of the drug by digestive enzymes when taken orally. However, repeated administration of the drug by injection results in discomfort to the patient. In addition, this way of administration does not correspond well to the normal passage of insulin in the body (i.e., from the pancreas to the circulation via the hepatic portal vein through the liver).[1] A more desired approach is to give the drug orally. Liposomes[2,3,4,5,6,7,8,9] and nanocapsules[10] have been suggested as drug carriers for insulin for oral administration. The type of liposomes used in these studies was found to have an influence on the amount of insulin bound. Multilamellar liposomes were found to bind insulin to a much lesser extent than unilamellar liposomes.[11,12] However, when multilamellar liposomes were in the form of liposomes-incorporating-ghosts, a significant entrapment (70-80%) of insulin occurred.[11] Polyalkylcyanoacrylate nanocapsules,[10] with an average size of 220 nm, were used to entrap insulin with a 54.9% rate of entrapment. These nanocapsules were shown to have a sustained hypoglycemic effect for 20 days after an oral dose of 50 U/kg nanocapsules-insulin.

Insulin has a strong affinity for binding to certain membrane lipids.[1,2] Such lipids include the negatively charged phosphatidylserine,[1] which is a normal component

of most biological membranes including erythrocytes. Although intact erythrocytes,[13,14] erythrocyte-ghosts,[11] and liposomes-incorporating-ghosts[11] have been suggested as carrier systems for insulin, the use of these membranes as carriers for orally administered insulin has not been assessed.

In this study we report the findings of using liposomes-incorporating-ghosts or vesicles, erythrocyte-ghosts, and erythrocyte-vesicles as biological carriers for insulin when administered intraduodenally to Wistar rats.

MATERIALS AND METHODS

Materials

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

Animals and Treatments

A total of 95 male Wistar rats weighing on average 462.3 ± 46.6 g (mean ± S.D.) were used in this study. Food and water was provided ad libitum. Rats were made diabetic with a single intravenous injection of streptozocin (65 mg/kg).[10] The presence of diabetes mellitus was diagnosed by gross observation (polyuria, polydipsia, and weight loss) and by measuring the blood glucose concentration (324.62 ± 15.29 mg/dl, mean ± S.D.). After the diabetic state was established, animals were fasted overnight prior to experimentation (water was

provided ad libitum). On the day of the experiment, rats were placed in an infants incubator (Isolette, Air-Shields, Inc., Hatboro, Pennsylvania) to control body temperature. Rats (7-14/dosage form studied) were anesthetized with pentobarbital sodium (80 mg/kg, i.p.). A midline incision was made to expose the duodenum and 2 ml of the drug (insulin-carrier or free insulin), at a concentration of 50 U/ml, was introduced into the duodenum followed by 1 ml of saline. To determine the effect of the carrier systems on blood glucose concentration, control rats received either physiological saline or one of the carrier suspensions (without insulin) intraduodenally. Blood glucose concentration was determined from tail blood just prior to administration (baseline reading) and at 0.5, 1, 2, 3, 4, and 5 hr post administration. At the end of the 5 hr period, rats were sacrificed by exsanguination.

Preparation of Erythrocyte-Ghosts (EG)

Erythrocyte-ghosts were prepared as previously described.[15] Briefly, a volume of human red blood cells was washed with isotonic buffer solution and then the cells were hemolyzed and washed several times with a hypotonic buffer solution. The resulting EG suspension was stored at 4°C. The average yield of ghosts suspension produced by this method has been shown to be 4×10^6 ghosts/ml.[15]

Preparation of Erythrocyte-Vesicles (EV)

Erythrocyte-vesicles, vesicular fragments of the erythrocyte membrane (diameter approximately 0.2 μm), were prepared using a method previously described.[15] Five ml of EG suspension were sonicated using a sonic dismembrator set at an energy level of 50.

Preparation of Liposomes-Incorporating-Ghosts or Vesicles (LEG and LEV)

These two dosage forms were prepared by a modification of a method previously described.[11] Lipids (containing 0.375 g of L-α-phosphatidyl-choline [type XI-E from fresh egg yolk] and 0.05 g cholesterol) were dissolved in 50 ml chloroform, then evaporated to dryness under vacuum. Then, 2.5 ml of a swelling solution (0.0725 M of each of NaCl, KCl, and CsCl) and 2.5 ml of either the EG or the EV suspensions, were added to the dry lipids. The mixture was shaken gently for one hour at room temperature then allowed to stand overnight at 4°C. The mixture was centrifuged at 23,500 x g for 20 min and the supernatant was discarded. Five ml of the swelling solution was added to the sediment, and the mixture was gently vortexed. The final preparation was stored in the refrigerator until use.

Incubation of Insulin with the Carriers

Human insulin was incubated with the carriers at 37°C for 24 hr. One ml of insulin solution (100 U/ml) was mixed with one ml of the various carrier suspensions. Following incubation, the mixture was centrifuged at 23,500 x g for 20 min. The amount of insulin associated with one ml of either one of the carrier systems under the above conditions are shown in Table 1. All dosage forms were prepared fresh on the day of the experiment.

Blood Glucose Level Assay

Blood samples (0.2 ml) taken from the tail were mixed in test tubes with 0.5 mg of sodium fluoride and 0.4 mg of potassium oxalate. Then, 1.8 ml of a 3 %

TABLE 1
Amount of Insulin (U) Associated with One ml of the Carrier.

Carrier	Amount of Insulin Associated (U) Average ± S.D. (n)[a]
Erythrocyte-Ghosts	56.12 ± 12.56 (11)
Erythrocyte-Vesicles	32.50 ± 15.06 (6)
Liposomes-Ghosts	45.44 ± 6.10 (6)
Liposomes-Vesicles	26.63 ± 7.55 (6)

a. Number of Observations.

trichloroacetic acid solution were added to each of the blood samples. The samples were centrifuged for 10 min at 2,000 r.p.m. One ml of the supernatant was mixed with 5 ml of o-toluidine reagent and the whole mixture was placed in boiling water for 10 min to allow a blue-green color to develop. The mixture was cooled in tap water for 3 min. The absorbance of the cooled samples was determined spectrophotometrically at 630 nm within 30 min of cooling.

Data Analysis

Changes in blood glucose concentrations associated with the various treatment regimens were compared using a repeated measures technique (split-plot method).[16] Significantly greater reductions produced by carrier-insulin treatments than by saline, or insulin or carrier alone was considered to be evidence of carrier efficacy.

RESULTS AND DISCUSSION

The present study tests the effect of different erythrocyte-membrane carriers on the absorption of human

TABLE 2
Initial Blood Glucose Concentrations (Baseline Concentrations) for All Treatment Groups.

Treatment Group	Glucose Concentration (mg/dl)[a]
Sodium Chloride Solution	325.49 ± 70.11(14)[b]
Human Insulin Solution	319.95 ± 52.08(11)
Erythrocyte-Ghosts	326.19 ± 37.23(8)
Erythrocyte-Ghosts-Insulin	329.38 ± 63.71(10)
Erythrocyte-Vesicles	331.28 ± 61.74(8)
Erythrocyte-Vesicles-Insulin	337.68 ± 55.74(10)
Liposomes-Ghosts	341.79 ± 71.00(8)
Liposomes-Ghosts-Insulin	317.87 ± 48.43(10)
Liposomes-Vesicles	286.29 ± 50.99(7)
Liposomes-Vesicles-Insulin	330.30 ± 42.97(10)

a. Average ± S.D.
b. Number of Rats in the Treatment Group.

insulin from the intestine of rats. Blood glucose concentrations were measured before and at different time intervals following the administration of insulin intraduodenally. Table 2 shows the initial blood glucose concentrations for the various treatment groups. All the control groups showed an increase in blood glucose concentrations over the 5-hour period following administration (Fig. 1). This increase may be due to stress imposed on animals during and after the surgery.

Changes in blood glucose levels from the baseline are presented in Figure 1 for all the treatment groups. EG, EV, and LEV showed a significant decrease in the glucose concentrations following the administration of the carrier-insulin complex (p values < 0.003, 0.009, and 0.0001, respectively). This decrease in concentration was sustained for a period of 4 hours. The magnitude of this decrease was about 50 mg/dl. In the case of LEG, there was no difference in blood glucose levels as compared to control.

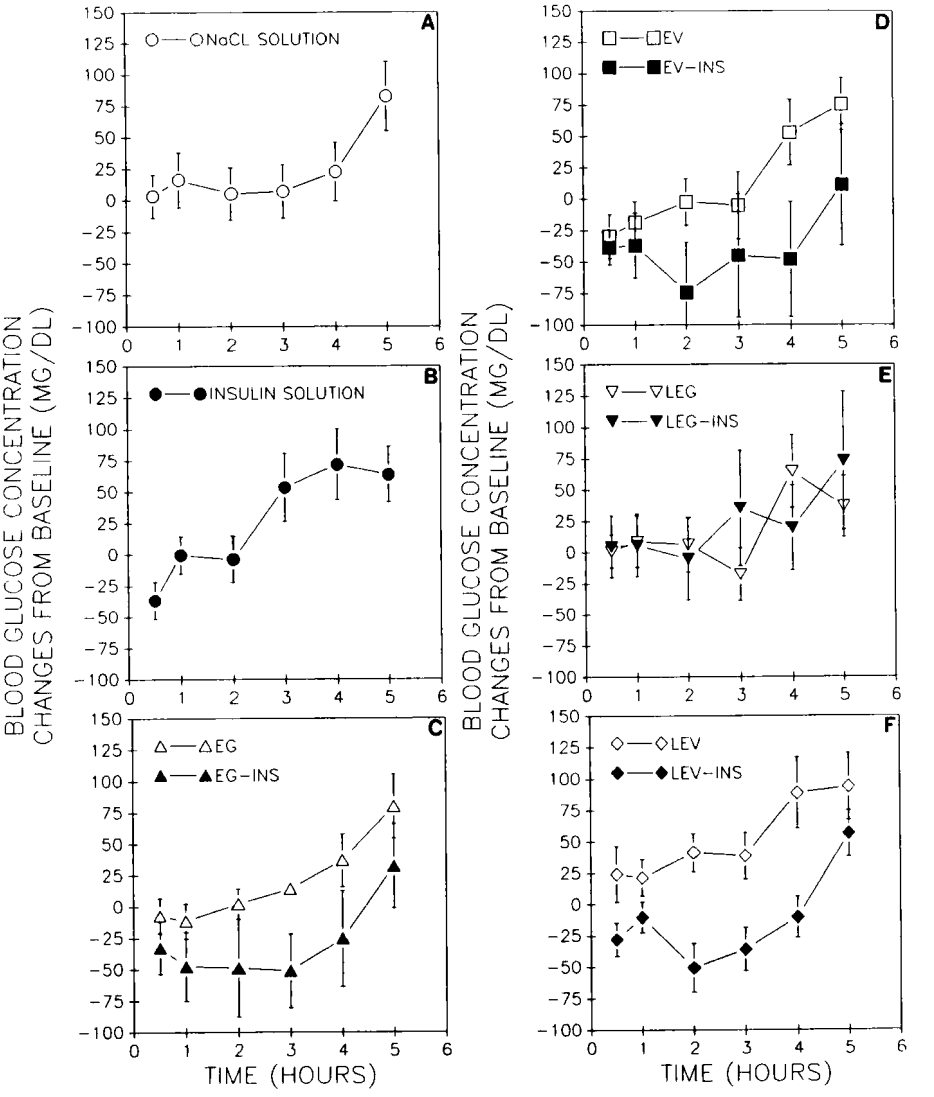

Fig. 1. Changes in blood glucose concentrations (mg/dl) from baseline (i.e., the initial blood glucose levels.) A) ○—○ Sodium chloride solution (0.9 %); B) ●—● Insulin solution (100 U); C) △—△ EG and ▲—▲ EG-Insulin; D) □—□ EV and ■—■ EV-Insulin; E) ▽—▽ LEG and ▼—▼ LEG-Insulin; and F) ◇—◇ LEV and ◆—◆ LEV-Insulin. Data points are mean ± S.E.

Whether or not this decrease in glucose concentration is therapeutically significant is to be seen. It is an indication, however, that some of the insulin was absorbed from the intestine in its pharmacologically active form.

The sustained effect seen with EG, EV, and LEV indicates that the release profile of insulin from the dosage forms which occurred over the 4-hour period was in a uniform manner. EV resemble polyalkylcyanoacrylate nanocapsules[10] in their size (200 nm).[15] It was suggested that nanocapsules can penetrate the intestinal membrane barrier and then accumulate in the liver.[10] EV may have acted in the same manner to produce a hypoglycemic effect. For EG and LEV the mechanism of transporting insulin could be either by endocytosis, fusion between the carrier and the plasma membrane where subsequently insulin is released inside the intestinal cells, and/or by surface adsorption of carriers on the plasma membrane of intestinal cells and releasing insulin to the intestinal cells.[17] It is not clear from the data obtained in this study why LEG could not produce any hypoglycemic effect. Relatively speaking, LEG are larger in size than any other carrier tested which might hinder endocytosis or possibly other processes. Further work is needed to explain this finding.

The changes in blood glucose concentrations observed between the free carrier groups and their corresponding carrier-insulin groups are shown in Figure 2 (i.e., changes observed between EG and EG-INS, EV and EV-INS, and LEV and LEV-INS). Fitting these data to the following equation[18] (this equation has been used to quantitate the relationship between drug response and time):

$$R = R_0 - (m\ K/2.303)\ t \qquad (1)$$

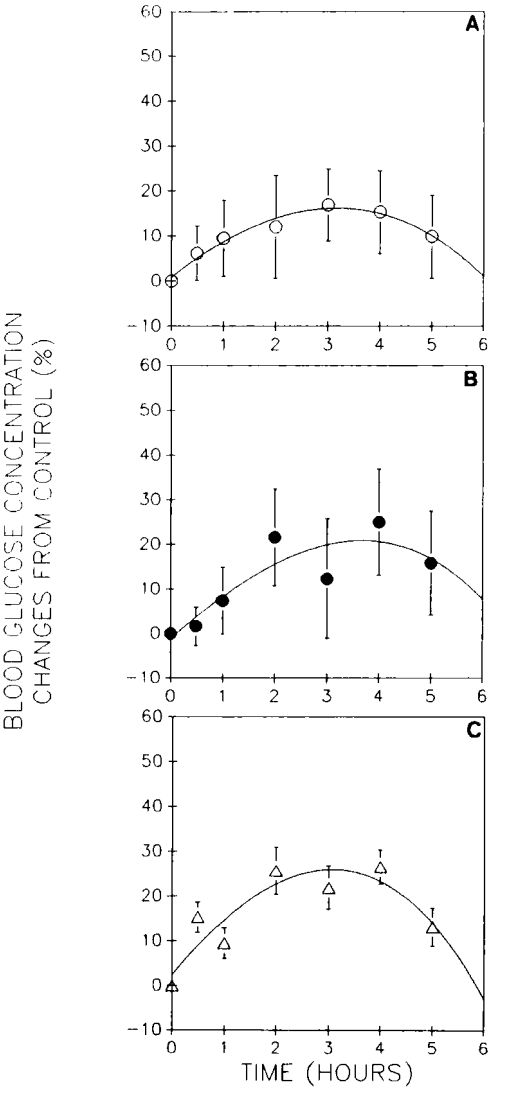

Fig. 2. Changes in blood glucose concentrations (expressed as percent) from control groups. A) ○—○ EG-Insulin; B) ●—● EV-Insulin; and C) △—△ LEV-Insulin. Data points are mean ± S.E.

where, **R** is the response (i.e., decrease in blood glucose concentrations),
R_0 is the maximum response,
m is the slope of the response versus log C (i.e., insulin plasma concentration),
K is the apparent first order rate constant, and
t is the time.

The value of m and R_0 can be estimated from the fitted line using the calculated data points at 3, 4, and 5 h where the effect curve begins to decline.

The value of K was determined in a study done to define the biologic activity and pharmacokinetics of human insulin in male Wistar rats.[19] The calculated K value was 0.093 hr^{-1}. Table 3 presents the predicted value of m and R_0 for the different groups, assuming the calculated K value as shown above.

The larger the value of the slope m, the larger the change in blood glucose concentration for a given change in dose. This indicates that LEV is the most effective carrier in delivering insulin in its active form to circulation. This observation is also reflected in the

TABLE 3
Predicted Value of the Slope of the Response Versus log C Curve (m) and the Maximum Response (R_0).

Treatment Group	m[a]	R_0[a]
Erythrocyte-Ghosts-INS	75.29(26.85)[b]	16.76(1.39)
Erythrocyte-Vesicles-INS	38.28(32.70)	20.70(1.70)
Liposomes-Vesicles-INS	265.54(19.37)	34.95(1.00)
Liposomes-Ghosts-INS[c]	-	-

a. Statistically Significant Difference ($p < 0.0001$).
b. Values in Parentheses Represent Standard Error of the Estimate.
c. Not Calculated (No Significant Difference Between LEG and LEG-INS.)

maximum response R_0, where LEV-INS produced a two-fold greater response than EG-INS.

CONCLUSION

The ability of erythrocyte-membrane-insulin carrier systems to produce a hypoglycemic effect after intraduodenal administration in rats warrants further investigation in formulating oral dosage forms using these carriers. Information gained from such studies may be of importance for the delivery of any proteinaceous drugs to be given orally.

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REFERENCES

1. D.S. Schade and R.P. Eaton. N. Engl. J. Med., 312, 1120 (1985).
2. H.M. Patel and B.E. Ryman. FEBS Lett., 62, 60 (1976).
3. G. Gregoriadis, G. Dapergolas, and E.D. Neerunjun. Biochem. Soc. Trans., 4, 256 (1976).
4. H.M. Patel and B.E. Ryman. Biochem. Soc. Trans., 5, 1739 (1977).
5. H.M. Patel, N.G.L. Harding, F. Logue, C. Kesson, A.C. MacCuish, J.C. MacKenzie, B.E. Ryman, and I. Scobie. Biochem. Soc. Trans., 6, 784 (1978).

6. C. Weingarten, A. Moufti, J.F. Desjeux, T.T. Luong, G. Durand, J.P. Devissaguet, and F. Puisieux. Life Sci., 28, 2747 (1981).
7. J.F. Arrieta-Molero, K. Aleck, M.K. Sinha, C.M. Brownscheidle, L.J. Shapiro, and M.A. Sperling. Hormone Res., 16, 249 (1982).
8. H.M. Patel, R.W. Stevenson, J.A. Parsons, and B.E. Ryman. Biochim. Biophys. Acta, 716, 188 (1982).
9. N. Das, M.K. Basu, and M.K. Das. Biochem. Intern., 16, 983 (1988).
10. C. Damge, C. Michel, M. Aprahamian, and P. Couvreur. Diabetes, 37, 246 (1988).
11. C.P.J. Caygill and W.D. Stein. Life Sci., 8, 809 (1969).
12. J.H. Wiessner and K.J. Hwang. Biochim. Biophys. Acta, 689, 490 (1982).
13. Y. Ito, T. Ogiso, M. Iwaki, and M. Kitaike. J. Pharmacobio-Dyn., 12, 193 (1989).
14. E. Pitt, C.M. Johnson, and D.A. Lewis. Biochem. Pharmacol., 32, 3359 (1983).
15. A. Al-Achi and M. Boroujerdi. Drug Dev. Ind. Pharm., 16, 1325 (1990).
16. S. Bolton, "Pharmaceutical Statistics: Practical and Clinical Applications," Marcel Dekker, New York, 1990.
17. M. Schneider, in "Drug Targeting," P. Buri and A. Gumma, eds, Elsevier Science Publishers, Amsterdam, 1985, p. 119.
18. M. Gibaldi and D. Perrier, "Pharmacokinetics," Marcel Dekker, New York, 1982.
19. P.A. Halban, M. Berger, A. Gjinovci, and A.E. Renold. Diabetes Care, 4, 238 (1981).