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COMMUNICATION

Erythrocytes as Oral Delivery Systems for Human Insulin

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ABSTRACT

The use of different forms of human red blood cells as oral carrier systems for human insulin in vivo was the subject of this investigation. Male Wistar rats were made diabetic by a single intraperitoneal injection of streptozocin (100 mg/kg). Three days after the injection, rats were found diabetic as evidenced by elevated fasted blood glucose concentration (200 mg/dl or higher). Rats received orally one of the following (100 U, 2 ml): an insulin solution, a ghosts–insulin suspension, a vesicles–insulin suspension, a liposomes–ghosts–insulin suspension, or a liposomes–vesicles–insulin suspension. Free carrier suspensions or sodium chloride solution (0.9%) were also given orally as controls. Blood glucose concentration was determined just before administration and at 1, 2, 3, 4, 5, 6, and 7 hr post administration. The results show that all treatment groups, except liposomes–ghosts–insulin, were significantly different statistically from their respective controls (i.e., the free carriers).

INTRODUCTION

Human insulin (Hins) has almost replaced all other sources of insulin in the current management of type I diabetes. The search for an alternative route of administration other than the parenteral route has been the subject of several publications (1–3).

We have shown (4) that human insulin can bind to erythrocyte membrane. The resulting complexes from this binding were tested intraduodenally and buccally (5–7). We have shown that intraduodenal administration of these complexes in male Wistar diabetic rats resulted in lowering blood glucose concentration significantly (5,7). Similarly, the buccal administration of Hins free or complexed resulted in a decrease in blood glucose level in female Wistar diabetic rats (6). Recently, we have shown a very high correlation between the effect obtained from the intraduodenal administration and the amount of Hins diffused through Caco-2 differentiated cells in culture (8).

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In this study, we present our findings of the oral administration of Hins free or complexed to erythrocyte carrier systems.

METHODOLOGY

Materials

Human insulin (Humulin® R, Eli Lilly) was purchased from NC Mutual, NC. Red blood cells were obtained from the American Red Cross, NC. Streptozocin and all other chemicals were from Sigma Chemical Company (St. Louis, MO).

Methods

Preparation of Dosage Forms

1. Erythrocyte-ghosts: This suspension was prepared according to the method described previously (4). Briefly, human red blood cells were washed first with an isotonic phosphate buffer solution and then hemolyzed and washed several times with a hypotonic phosphate buffer solution. The resulting suspension was stored in the refrigerator and used within 7 days.
2. Erythrocyte-vesicles: This preparation was according to the method described previously (4). Briefly, 5 ml of ghosts suspension was ultra sonicated for 3 min. The resulting suspension was stored in the refrigerator and used within 7 days.
3. Liposomes-ghosts and liposomes-vesicles: These two suspensions were made according to the method described previously (4). Briefly, a lipid film (cholesterol and phosphatidylcholine) was coated on the internal surface of a round-bottom flask. Ghosts or vesicles suspensions were diluted with an equal volume of a swelling solution and then added to the dry lipid film. The flash was gently shaken for 1 hr at room temperature. The resulting mixture was centrifuged after a 2-hr standing period at room temperature. The supernatant was separated, and to the sediment 5 ml of the swelling solution was added to make 6 ml of final preparation.
4. Preparation of carriers-insulin suspension: 1 ml of a carrier was mixed with 1 ml of Hins solution (100 U). The resulting mixture was incubated at 37°C for 24 hr (4). All carriers-insulin suspensions were prepared fresh before the day of administration.

Animals and Treatment

Male Wistar rats (Charles River Laboratory, Wilmington, MA) with an average weight of 300 g were used. Rats received an intraperitoneal injection of streptozocin (100 mg/kg) dissolved in a citrate buffer (0.1 M). Diabetic state was determined by testing the fasting blood glucose concentration 3 days following the streptozocin injection. The overnight (about 16 hr) fasting blood glucose concentration was (mean $\pm$ SD, number of observations) 238.6 $\pm$ 51.5, 61. Diabetic rats received a carrier-insulin suspension, a carrier without Hins, or sodium chloride solution (0.9%), orally (2 ml). Rats were anesthetized with sodium pentobarbital solution 65 mg/ml (initial dose = 80 mg/kg, i.p.; hourly maintenance doses = 20 mg/kg, i.m.). Blood glucose concentration was determined from tail blood using a glucometer (One Touch II, Lifescan Inc., Milpitas, CA). Blood samples were collected just before administration and at 1, 2, 3, 4, 5, 6, and 7 hr post administration. Rats were sacrificed by carbon dioxide gas inhalation.

Statistical Analysis

Groups were compared using an ANOVA repeated measures technique (9). A p value of 0.05 or less was considered statistically significant.

RESULTS AND DISCUSSION

The initial blood glucose concentration is listed in Table 1. The average initial blood glucose concentration was over twofold higher than that for a normal male Wistar rat. Changes in blood glucose concentrations from the baseline level are presented in Figs. 1–3 for all treatment groups. The administration of Hins solution resulted in a statistically significant decrease in blood glucose level as compared with the normal saline group (p = 0.002) (Fig. 1). The administration of erythrocyte-ghosts-insulin [Fig. 2(a)] or erythrocyte-vesicles-insulin [Fig. 3(a)] also resulted in a statistically significant lowering of blood glucose in contrast to their respective control groups (p = 0.001 for ghosts; p = 0.016 for vesicles.) No difference in response between

Table 1

Initial Blood Glucose Concentration in Overnight Fasting Male Wistar Rats

Group	Blood Glucose Concentration (mg/dl) Mean ± SD; Number of Rats)
Insulin solution	254.5 ± 50.0 (6)
Ghosts-insulin	230.2 ± 27.7 (6)
Ghosts	211.3 ± 19.6 (6)
Vesicles-insulin	246.0 ± 53.3 (6)
Vesicles	219.3 ± 30.6 (6)
Liposomes-ghosts-insulin	213.2 ± 28.3 (5)
Liposomes-ghosts	238.5 ± 34.7 (8)
Liposomes-vesicles-insulin	279.2 ± 122.8 (6)
Liposomes-vesicles	263.2 ± 19.2 (6)
Sodium chloride solution (0.9%)	226.7 ± 36.0 (6)

liposomes-ghosts-insulin and the free-carrier liposomes-ghosts control group was found ($p = 0.665$) [Fig. 2(B)]. Similar results for the carrier liposomes-ghosts were found after intraduodenal administration (5,7). A statistically significant difference in blood glucose changes between liposomes-vesicles-insulin and the free-carrier liposomes-vesicles existed ($p = 0.049$) [Fig. 3(B)]. The decrease in blood glucose level seen over the 7 hr period for the control group may be related to a residual effect of insulin in this diabetic rat model. A significant residual concentration in diabetic rats, induced by streptozocin injection, was reported in previous work (10,11). Therefore, it is important that an appropriate control group be used in studies using the streptozocin rat model.

Table 2 shows the average difference (mg/dl) of blood glucose concentration between the treatment groups and their corresponding controls over the 7 hr

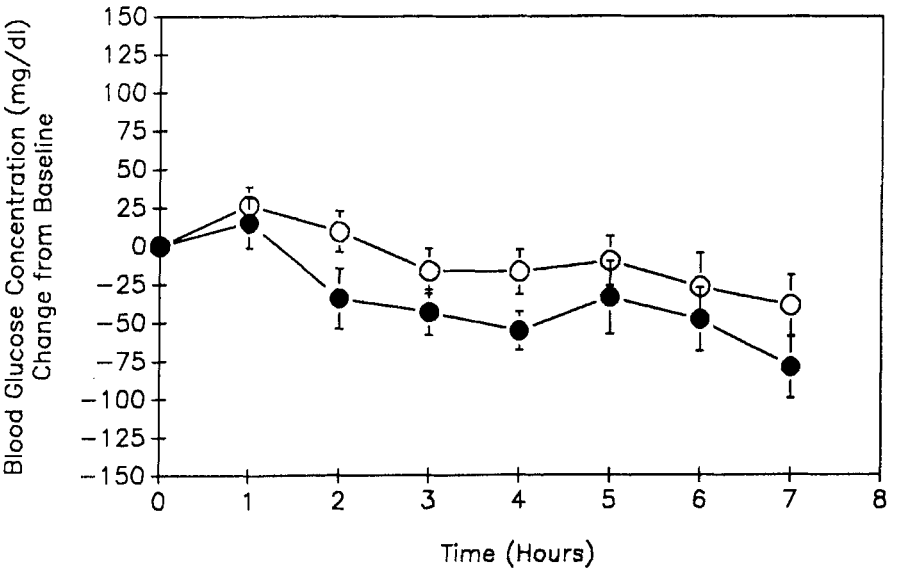
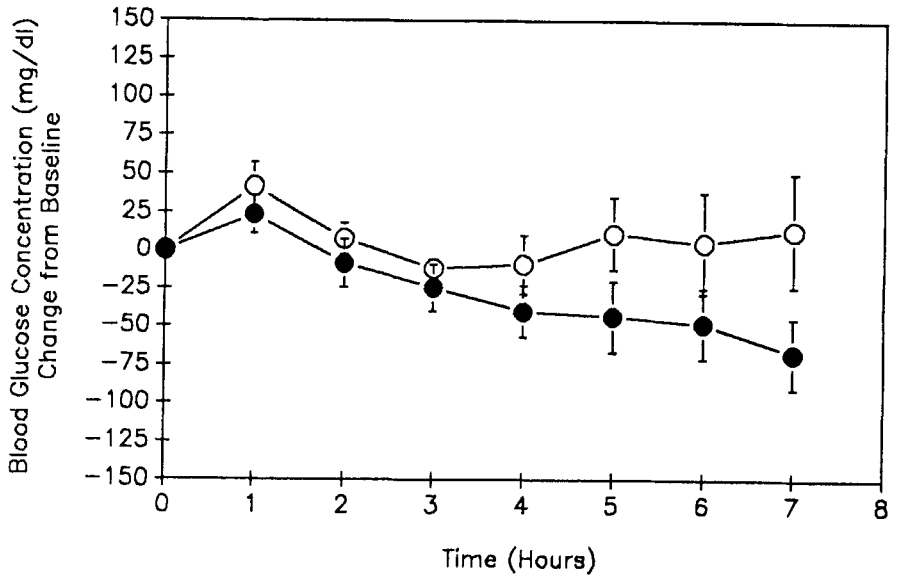
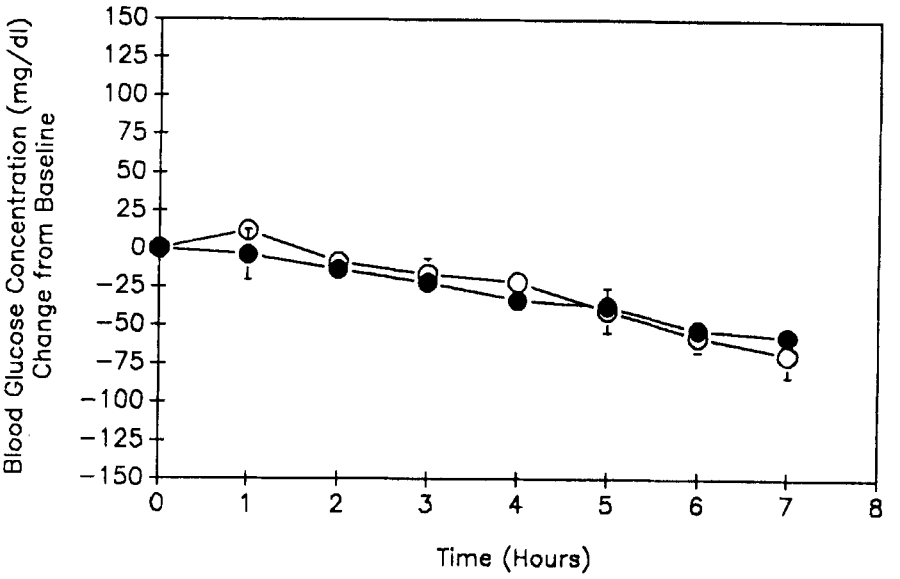


Figure 1. Change in blood glucose concentration (mg/dl) vs. time (h). (○), sodium chloride solution (0.9%) and (●) human insulin solution (100 U). Data points are mean ± SE of 6 observations.

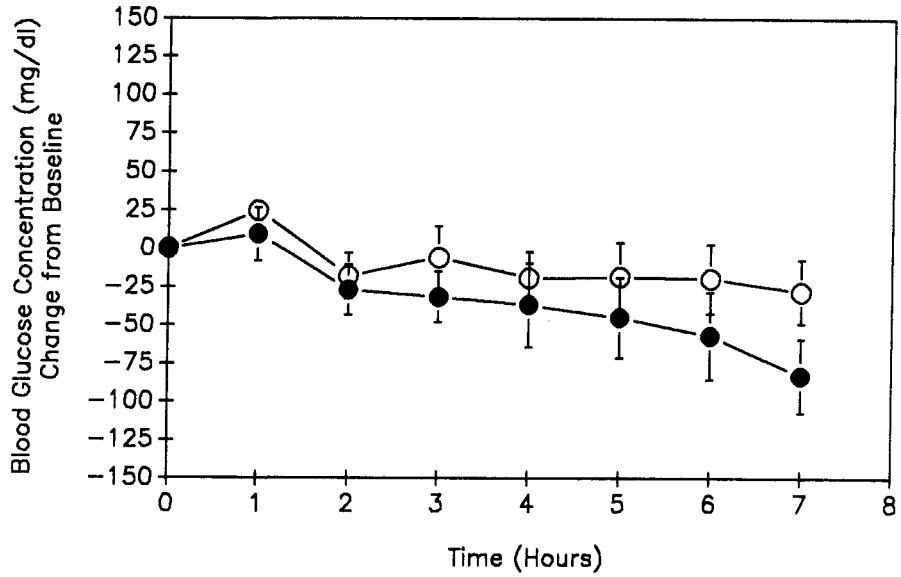


(a)

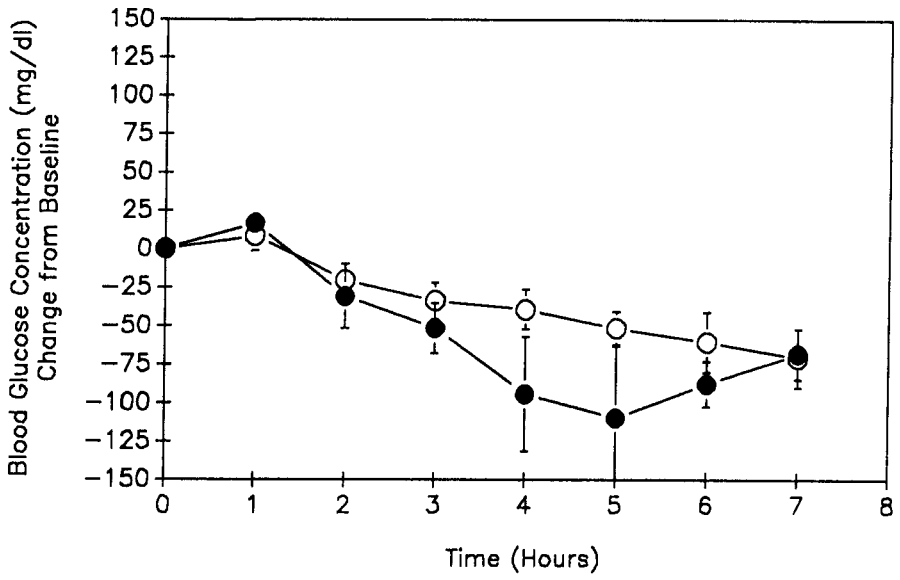


(b)

Figure 2. Change in blood glucose concentration (mg/dl) vs. time (hr). (a) erythrocyte-ghosts (○) vs. erythrocyte-ghosts-insulin (100 U) (●) and (b) liposomes-ghosts (○) vs. liposomes-ghosts-insulin (100 U) (●). Data points are mean ± SE of 5–8 observations.



(a)



(b)

Figure 3. Change in blood glucose concentration (mg/dl) vs. time (h). (a) erythrocyte-vesicles (○) vs. erythrocyte-vesicles-insulin (100 U) (●) and (b) liposomes-vesicles (○) vs. liposomes-vesicles-insulin (100 U) (●). Data points are mean ± SE of 6 observations.

Table 2

The Average Difference Between the Treatment Groups and Their Corresponding Controls

Group	Difference in mg/dl (Control and Treatment)
Sodium chloride solution/insulin solution	28.14
Ghosts/ghosts-insulin	39.09
Vesicles/vesicles-insulin	27.59
Liposomes-ghosts/liposomes-ghosts-insulin	1.38
Liposomes-vesicles/liposomes-vesicles-insulin	25.45

period. The highest average difference was seen with the erythrocyte-ghosts suspension followed by insulin solution, erythrocyte-vesicles, liposomes-vesicles, and finally liposomes-ghosts.

CONCLUSION

The oral administration of several erythrocytes carriers of human insulin in diabetic rats resulted in a statistically significant hypoglycemic effect. The erythrocyte-ghosts carrier showed the highest hypoglycemic response.

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REFERENCES

1. S. Lee and J. J. Sciarra, J. Pharm. Sci., 65, 567 (1976).
2. A. E. Pontiroli, M. Alberetto, A. Secchi, G. Dossi, and G. Pozza, Br. Med. J., 284, 303 (1982).
3. N. Das, M. K. Basu, and M. K. Das, Biochem. Intern., 16, 983 (1988).
4. A. Al-Achi and R. Greenwood, Drug Dev. Ind. Pharm., 19(6), 673 (1993).
5. A. Al-Achi and R. Greenwood, Drug Dev. Ind. Pharm., 19(11), 1303 (1993).
6. A. Al-Achi and R. Greenwood, Res. Comm. Chem. Pathol. Pharmacol., 82(3), 297 (1993).
7. A. Al-Achi and R. Greenwood, Drug Dev. Ind. Pharm., 20(14), 2333 (1994).
8. R. Greenwood and A. Al-Achi, Drug Dev. Ind. Pharm., 23(2), 221 (1997).
9. S. Bolton, *Pharmaceutical Statistics: Practical and Clinical Applications*, Marcel Dekker, New York, 1990.
10. P. D. Winocour, M. Lopes-Virella, M. Laimins, and J. A. Colwell, J. Lab. Clin. Med., 106, 319 (1985).
11. H. Xiang and J. H. McNeill, Biochem. Biophys. Res. Comm., 187(2), 703 (1992).