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EFFECTS OF GASTROINTESTINAL ADMINISTRATION OF HUMAN INSULIN AND A HUMAN INSULIN-DEAE-DEXTRAN COMPLEX ENTRAPPED IN DIFFERENT COMPOUND LIPOSOMES ON BLOOD GLUCOSE IN RATS

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ABSTRACT

The effects of gastrointestinal administration (oral, in duodenum and colon) of human insulin and a human insulin-DEAE (diethylaminoethyl) dextran complex entrapped in different compound liposomes in comparing to human insulin alone given subcutaneously on blood glucose level of normal and STZ-diabetic rats were investigated. The liposomes were prepared from a hydrogenated soy lecithin (Epikuron, E 200 H) and by a high pressure homogenization procedure. Samples were lyophilized and reconstituted in 0.067 M phosphate buffer, pH 7.4 before application. The complexed insulin (0.25 and 0.5 IU/kg insulin) showed no diffrences in blood glucose

lowering profiles from the free insulin when both were administered intravenously in normal rats. When given orally, the complex (30 and 60 IU/kg insulin) entrapped in positive liposomes (E 200 H/cholesterol/stearylamine= 7:2:1, in a molar ratio) indicated no effects in STZ rats. However, this complex liposome (6.0 IU/kg insulin) gave a retention effect of blood glucose lowering as % initial level of about 12 % after 5 hours when injected in duodenum and showed two maximum effects of 21 and 22% at 100 and 300 minutes respectively when administered in colon of normal rats. When the free insulin entrapped in positive liposomes was given in duodenum in normal rats, the maximum effect of blood glucose lowering of 10 % was observed at 2 hours (6 IU/kg insulin) and 1 hour (12 IU/kg insulin). For the free insulin (12 IU/kg) entrapped in other liposome systems given in duodenum of normal rats, both negative (E 200 H/cholesterol/dicetyl phosphate=7:2:1, in a molar ratio) and neutral (E 200 H/cholesterol=1:1, in a molar ratio) liposomes indicated the maximum effect of about 30% at 120 minutes. Both cholesterol rich positive (E 200 H/cholesterol/stearylamine=7:7:1, in a molar ratio) and negative (E 200 H/cholesterol/dicetyl phosphate=7:7:1, in a molar ratio) liposomes showed not only a maximum effect of about 20% at 2 hours, but also a retention of glucose lowering of 20% after 7 hours as well. This study suggested that a development of human insulin by complexing with the DEAE-dextran polymer and/or entrapping in liposomes, as a drug delivery system in duodenum and colon, is possible.

INTRODUCTION

For the development of insulin drug delivery system, Schade and his colleagues have done on a totally implantable control release insulin delivery system (1). The infusion systems for insulin have also been developed (2). However, the only advantage of the modified insulin over the unmodified hormone is

the less frequence of the injection. It has long been demonstrated that oral administration of various drugs or medicaments is the most preferable and acceptable route especially for childhood patients (3). Infact, insulin can not be administered orally since it undergoes degradation in gastrointestinal environments. Several attempts have been performed on the development of insulin for oral therapy. These include the finding of new insulin derivatives (4–6), a suitable dosage form (7, 8) and a proper drug carrier which is stable in the gastrointestinal fluids such as the entrapment of the hormone in liposomes (9–19). Insulin together with soybean trypsin inhibitor has been directly injected to rat ileum resulting in a significant drop in blood glucose (7). Bioerodible polymers have been used to deliver insulin and vasopressin via the oral route (8). The drugs were released from the polymers by the reaction of the gut microflora in colon. Both insulin and vasopressin have been demonstrated to be absorbed intact to some extent.

Liposomes are phospholipid bilayer vesicles which consist of alternating layers of aqueous medium and lipid bilayers (20). By varying the nature and composition of the lipids, different physical properties of particle sizes, surface properties including neutral, positive and negative charges can be conferred on these particles (21). Varying of these physical properties can also affect the location and the rate of metabolism of the liposomes as well as the drugs being entrapped. Recently, an extensive investigation on the use of liposomes as carriers for the introduction of insulin via the oral route has been focussed (9–19). However, some controversial results of insulin entrapped in liposomes administered orally have been reported (9–11, 17, 18). Reduced blood glucose level was observed in mice following the oral administration of insulin entrapped in phosphatidylinositol liposomes (9). An identical reduction of blood glucose level was reported with glucose oxidase entrapped in liposomes of similar composition (10). Nevertheless, little effect was observed when insulin entrapped in liposomes composed of egg lecithin, cholesterol and dicetyl phosphate was administered to diabetic animals (11). It has been

reported that liposomes prepared from synthetic phospholipids imparted greater stability than those made from natural phospholipids (12–15). Insulin entrapped in negatively charged liposomes composed of synthetic phospholipids and dicetyl phosphate (19) or phosphatidic acid (15) have been demonstrated to reduce blood glucose of 40–60% of control in diabetic rats. However, positively charged liposomes prepared from natural phospholipids and stearylamine also indicated a reduction of blood glucose level in acute alloxan diabetic rats (17). But, no reduction of blood glucose in diabetic rats after oral administration of insulin liposomes prepared under acidic conditions was observed (18). It was therefore anticipated that appropriate changes in the type of lipids and the lipid compositions in liposomes would improve the liposome survival in the gut thus allowing the effective transport of liposomes with the encapsulated insulin into the peripheral.

Several reports have indicated that liposomes could be capable in protecting the degradation of insulin by proteolytic digestion and possible facilitating its absorption in the gastrointestinal tract in diabetic animals (9–13, 15–19), but not in normal rats and normal dogs whereas only a small plasma glucose was shown in diabetic dogs (15). Different observations have been obtained in different doses on insulin entrapped in liposomes (15) while plasma immunoreactive insulin level has been shown to be not dose-related (11). Hence, types and the diabetic states of the testing animals as well as the dose of insulin administered should contribute to the hypoglycemic effect of insulin entrapped in liposomes to some extent.

In our laboratory, the characterizations of the human insulin–DEAE dextran complex and the complex entrapped in liposomes have been previously studied (22–24). The complex is anticipated to solve the problem of the leakage of the hormone from the liposomes (21) and the low uptake of liposomes by gastrointestinal epithelial, which is proposed to be probably mediated by endocytosis (25, 26). The large molecular size of the complex may prevent the leakage of the entrapped hormone whereas

the dextran has been demonstrated to be taken up by mamalian cells at higher rates than serum albumin (27-29). In this study, the effects of human insulin as well as a human insulin-DEAE (diethylaminoethyl)-dextran complex entrapped in different liposome compositions administered orally, in duodenum and in colon on blood glucose of diabetic and normal rats were investigated.

MATERIALS

Human insulin (Hoechst AG, D-6230 Frankfurt/M. 80), DEAE-dextran (M.W. 5000,000, Pharmacia GmbH, D-7800 Freiburg), E 200 H (Epikuron 200 H, hydrogenated soy lecithin, Lucas Meyer GmbH & Co., D-2000 Hamburg), cholesterol (Croda GmbH, 4054-Nettetal, 2-Herrenpfad), stearylamine and dicetyl phosphate (Sigma Chemical Company, St. Louis, Mo 63178, USA) were used. A protein assay kit (Sigma Diagnostics, P.O. Box 14508, St. Louis, Mo 63178, USA) and a Glucoquant ^{R}-test kit (Boehringer Mannheim GmbH, D-6700 Mannheim 31) were used. All other chemicals were of reagent grade and used as obtained. For animals, male Wistar rats (Hoe : WISKF) in the weights range of 243±12.9 g (mean±SD) were used.

METHODS

Preparation of Liposomes

Liposomes were prepared in the first step by the chloroform film method. Liposome systems were neutral liposomes (E 200 H/cholesterol in the molar ratio of 1:1), positive liposomes (E 200 H/cholesterol/stearylamine in the molar ratios of 7:2:1 and 7:7:1) and negative liposomes (E 200 H/cholesterol/dicetyl phosphate in the molar ratios of 7:2:1 and 7:7:1). A total phospholipid mixture weight of 300 mg was dissolved in about 20 ml of

chloroform in a flask. Rotary evaporation of the chloroform at 37° C on a Büchi rotavapor left a thin film on the wall of the flask. An amount of 30.0 ml of human insulin (100 mg) or human insulin (100 mg)-DEAE dextran (2.5 g) complex solution was added to the film which was collapsed into multilamellar liposomes by vortexing for 15 minutes at 53° C with the aid of 0.5 mm glass beads. The resulting liposome dispersion was finally passed through a Gaulin MICRON LAB 40 high pressure homogenizer (Hochdruck Homogenisator, Type MICRON LAB 40, APV Gaulin GmbH, D-2400 Lübeck, FRG) at 80 MPa for 3 cycles in order to obtain a more uniform unilamellar liposome dispersion. The dispersion was further incubated at 53° C for 15 minutes.

The resulting liposomes were separated from the unentrapped human insulin and the DEAE-dextran by centrifugation at 50,000 g, 4° C for 45 minutes on a centrifuge (RC-5 Superspeed Refrigerated Centrifuge, Du Pont de Nemours GmbH, Dieselstrasse 18, D-6350 Nauheim 1). The pellets were washed twice with 7.4 pH, 0.067 M phosphate buffer and recentrifuged. The pellets were collected and redissolved in phosphate buffer. The dispersion was immediately lyophilized by a lyophilizer (Gefriertrocknungsanlage, GTZ, Leybold-Heraeus GmbH & Co. KG, D-5000 Köln) at 0.1 Torr, -30° C for 24-30 hours. The insulin contents in International Unit (IU) per mg of the lyophilized powder were assayed by the Lowry method with protein precipitation using a protein assay kit.

Experimental Procedure

Samples used in animal studies are shown in table 1.

1. Effects of intravenous and oral administration of human insulin-DEAE-dextran complex and the complex entrapped in positive liposomes on blood glucose in normal rats

Clear solutions (0.25 and 0.5 IU/kg) of sample No.1 dissolving in 0.067 M phosphate buffer, pH 7.4 were injected intravenously (2 ml/kg) to 7 normal rats. Suspensions (60 and 30 IU/kg) of sample No. 2 were administered orally (10 ml/kg) to 7

Table 1 Samples Used in the Animal Experiments

Sample No.	Sample Descriptions	Insulin Conc. (IU/mg)
1	Human insulin-DEAE dextran complex powder	1.42
2	Positive liposomes/ complexed human insulin (E 200 H/chol/stea=7:2:1)	0.76
3	Positive liposomes/ human insulin (E 200 H/chol/stea=7:2.1)	2.48
4	Neutral liposomes/ human insulin (E 200 H/chol=1:1)	1.80
5	Negative liposomes/ human insulin (E 200 H/chol/DP=7:2:1)	2.57
6	Cholesterol rich negative liposomes/human insulin (E 200 H/chol/DP=7:7:1)	2.11
7	Cholesterol rich positive liposomes/human insulin (E 200 H/chol/DP=7:7:1)	3.86

Note: chol=cholesterol, stea=stearylamine, DP=dicetyl phosphate

streptozocin (STZ) induced diabetic rats via a nasogastric tube. In both cases, another group of 7 animals was used as positive controls by given subcutaneously of Hoechst human insulin U-40 (0.25 and 0.5 IU/kg for i.v., 15 IU/kg for p.o.)

2. Effects of in duodenum administration of human insulin entrapped in positive liposomes on blood glucose of fasting anaesthesized normal rats

After 5 minutes of the induction of the anaesthesization of 7 fasting normal rats by phenobarbital (50 mg/kg, i.p.), a 3-cm long abdominal medium section was performed. A suspension (6.0 IU/kg) of sample Nos. 2 or 3 was injected (2.5 ml/kg) to the duodenum at about 1.5 cm distal from the pylorus. The skin wound was clamped and the animals were irradiated with red light to avoid cooling down of body temperature. The body temperature was controlled from the rectum. Another set of rats was used as controls by giving subcutaneously (2.0 ml/kg) of Hoechst human insulin (0.6 IU/kg) together with the in duodenum administration (2.5 ml/kg) of 0.067 M phosphate buffer. 7 and 3 control animals were used for sample Nos. 2 and 3 respectively.

3. Effects of in colon administration of human insulin-DEAE-dextran complex entrapped in positive liposomes on blood glucose of fasting anaesthesized normal rats

A suspension (6 IU/kg) of sample No. 2 was injected (2.5 ml/kg) into colon of 6 fasting anaesthesized normal rats by the same procedure previously described for the in duodenum administration. Another 6 animals were injected (2.5 ml/kg) in the colon with 0.067 M phosphate buffer together with the subcutaneous (2 ml/kg) administration of the Hoechst human insulin solution (0.6 IU/kg), and were used as positive controls.

4. Effects of in duodenum administration of human insulin entrapped in different compound liposomes on blood glucose of fasting anaesthesized normal rats

Suspensions (12.0 IU/kg) of samples Nos. 4, 5, 6 or 7 were injected (2.5 ml/kg) to duodenum of 7 fasting anaesthesized normal rats by the same procedure previously described. These

animals were also subcutaneously administered (2.5 ml/kg) with 0.067 M phosphate buffer. Another six animals were administered (2.5 ml/kg) both in duodenum and subcutaneously with 0.067 M phosphate buffer, and were used as negative controls. Another set of rats was given in duodenum (2.5 ml/kg) with phosphate buffer and subcutaneously (2.0 ml/kg) with Hoechst human insulin (0.6 IU/kg), and were used as positive controls.

In all cases, the blood samples were drawn from tips of the tails before and at different time intervals following the administration, and were immediately enzymatically analyzed for glucose by a Glucoquant® -test kit. Blood glucose concentrations in mmol/l were obtained and the blood glucose levels as percent of the initial value were then calculated.

RESULTS AND DISCUSSION

Tables 2 and 3 showed the effects of intravenous and oral administration of human insulin-DEAE-dextran complex and the complex entrapped in positive liposomes on blood glucose of normal and STZ diabetic rats respectively. Our previous works have demonstrated that human insulin complexed with a DEAE dextran polymer by the fraction bound of 0.20 was thermally more stable than the uncomplexed insulin (22, 24). From table 2, human insulin-DEAE dextran complex showed the same blood glucose profile with almost the same percent of glucose lowering at the initial until the lasting of the effect, as the uncomplexed human insulin given intravenously. This indicated that the human insulin activity was not disturbed by the complexation and the lyophilization process. However, the complexed human insulin which was mainly formed by an electrostatic interaction appears to be not stable and may dissociate as free insulin and free dextran polymer in the blood circulation. Suzuki et al., have demonstrated a significant difference blood glucose lowering of the insulin-dextran complex in

Table 2 Comparison between the Effects of Intravenous Administration of the Complexed and the Uncomplexed Human Insulin on Blood Glucose in Fasting Normal Rats

Time(mins)	Blood Glucose Content as % of Initial Level (mean ± SEM), n = 7			
	Uncomplexed (IU/kg)		Complexed (IU/kg)	
	0.25	0.5	0.25	0.5
0	100.00±2.13	100.00±1.93	100.00±2.31	100.00±2.20
30	82.42±3.19	64.67±2.64	80.78±2.85	67.58±1.47
60	96.63±3.73	82.60±4.57	110.68±6.05	88.46±2.01
120	99.47±1.42	105.27±2.99	102.31±3.38	109.71±1.65
180	107.64±2.84	108.44±2.64	106.94±1.60	108.24±3.48
240	107.99±1.78	107.38±1.58	105.16±2.31	110.26±2.01
300	107.10±1.95	101.58±0.35	94.84±2.49	106.59±2.75

Table 3 Comparison between the Effects of Oral (p.o.) and Subcutaneous (s.c.) Administration of the Complexed Human Insulin Entrapped in Positive Liposomes and the Non-Entrapped Human Insulin on Blood Glucose in STZ-Diabetic and Normal Rats Respectively

Time(hrs)	Blood Glucose Contents as % of Initial Level (mean±SEM), n = 7		
	Human Insulin	Complexed Insulin Liposomes	
	15 IU/kg, s.c.	30 IU/kg, p.o.	60 IU/kg, p.o.
0	100.00±4.67	100.00±2.61	100.00±5.02
1	38.31±4.95	96.46±1.81	95.63±2.58
2	19.63±2.80	96.46±3.76	97.82±1.00
3	31.78±5.14	95.58±4.03	102.18±3.93
4	68.69±10.79	102.65±4.42	117.03±5.90
5	86.45±10.98	95.14±1.42	101.31±4.50
6	98.13±13.79	104.89±3.78	106.55±2.36

comparing to the uncomplexed hormone, when both were administered intravenously into alloxan-diabetic rats (5). But, dextrans 40 and 70 have been used instead in their study and the complex was formed by a chemical covalent bonding.

The insulin complex entrapped in positive liposomes showed no effects when given orally in STZ rats whereas the free human insulin administered subcutaneously in 2 and 4 folds of lesser insulin doses than the complex liposomes, gave the maximum blood glucose lowering effect of about 80% at 2 hours in normal rats (Table 3). The positive liposome system has indicated the highest percent of entrapment of insulin in liposomes in our previous study (23), however, the system appears to be not effective when administered orally. This result seems to agree with the previous study of the oral administration of ^{14}C-PEG-4000, ^{3}H-hydrocortisone and ^{14}C-salicylic acid entrapped in L-alpha-phosphatidyl choline/cholesterol or egg lecithin/cholesterol multilamellar and unilamellar liposomes (30). Our study indicated that the complex and/or the liposomes may be not stable in the gastrointestinal environments. Moreover, lyophilization may also affect the stability of our liposome systems. An intact liposome may not obtain after reconstitution of the lyophilized liposome powder. However, intact structures of the reconstituted lyophilized liposomes prepared from dipalmitoyl phosphatidylcholine have been previously demonstrated by a SEM study (31). Inaddition, positive charges on the surfaces of our liposome systems are probably neutralized by or interacted with the acidic ions in the stomach thereby destroying the liposomal structures before endocytosis and/or absorption of the liposomes together with the entrapped hormone could occur.

Nevertheless, when this complex liposome system was administered in duodenum (Table 4) and in colon (Table 5), some effects on the blood glucose of normal rats were observed. Although the complex positive liposomes given in duodenum in the dose of ten times higher than the free insulin administered subcutaneously showed no effect up to 4 hours whereas the free

Table 4 Comparison between the Effects of in Duodenum (i.d.) and Subcutaneous (s.c.) Administration of the Complexed Human Insulin Entrapped in Positive Liposomes and the Non-Entrapped Human Insulin Respectively on Blood Glucose in Anaesthesized Fasting Normal Rats

Time(mins)	Blood Glucose Contents as % of Initial Level, (Mean±SEM), n = 7	
	Human Insulin 0.6 IU/kg, s.c.	Complexed Insulin Liposomes 6.0 IU/kg, i.d.
0	100.00±3.21	100.00±3.39
60	79.41±4.55	101.83±3.13
80	70.32±2.67	99.48±3.66
100	76.20±4.01	96.08±3.39
120	73.53±5.35	99.22±2.09
180	87.97±5.35	99.22±4.18
240	90.37±4.01	99.74±4.18
300	96.52±6.68	87.99±7.57

Table 5 Comparison between the Effects of in Colon (i.c.) and Subcutaneous (s.c.) Administration of the Complexed Human Insulin Entrapped in Positive Liposomes and the Non-Entrapped Human Insulin Respectively on Blood Glucose in Anaesthesized Fasting Normal Rats

Time(mins)	Blood Glucose Contents as % of Initial Level (mean±SEM), n = 6	
	Phosphate Buffer,2.5 ml /kg,i.c. and Human Insulin 0.6 IU/kg,s.c.	Complex Liposomes, 6 IU/kg,i.c. and Phosphate Buffer, 2 ml/kg,s.c.
0	100.00±8.77	100.00±2.91
60	70.38±4.74	87.92±2.01
80	62.09±5.69	81.66±1.57
100	60.43±5.92	79.19±2.24
120	63.98±5.45	80.54±1.54
180	73.46±4.98	82.77±1.12
240	72.27±4.50	84.79±2.91
300	76.07±3.55	76.73±2.91

insulin gave the maximum blood glucose lowering effect of about 30% after 80 minutes, the complex liposome showed a retention effect in decreasing of blood glucose of about 12% after 5 hours (Table 4). Perhaps, some of the remaining intact reconstituted liposomes and/or the DEAE dextran polymer may be able to protect the hormone from degradation in the duodenum to some extent. Importantly, this result indicated that the complexed insulin entrapped in liposomes can be absorbed in the duodenum. Interestingly, when this complex liposome was given in colon, the two maximum glucose decreasing effects of about 21% and 22% were found at 100 and 300 minutes respectively (Table 5). Similarly, the liposomes and/or the polymer may have some influences on the stability of the hormone in colon. In this case, a retention effect of glucose lowering of insulin was also observed after 5 hours. Hence, the complexed insulin entrapped in positive liposomes appears to be more stable and better absorbed in the colon than in the duodenum. However, the maximum glucose lowering effect of this complex liposome given in colon was only 50% of the free human insulin administered subcutaneously with insulin doses of ten times lesser than the complex liposomes.

Tables 6 and 7 showed the comparison between the uncomplexed human insulin entrapped in different compound liposomes administered in duodenum and the non-entrapped insulin given subcutaneously in normal rats. For the free insulin entrapped in positive liposomes, only the marginal values of the maximum glucose lowering effect of about 10% was observed at 2 hours for 6 IU/kg and at 1 hour for 12 IU/kg (Table 6). In contrary to the complex liposomes, no retention of glucose lowering effects were observed after 5 hours. Hence, the dextran polymer seems to contribute a more protection effect for the hormone than the liposomes in duodenum.

In comparing the blood glucose lowering effects of human insulin entrapped in other different liposome compositions given in duodenum, human insulin alone administered subcutaneously and a 0.067 M phosphate buffer solution given in duodenum of

Table 6 Comparison between the Effects of in Duodenum (i.d.) and Subcutaneous (s.c.) Administration of the Entrapped and the Non-Entrapped Human Insulin in Positive Liposomes on Blood Glucose in Anaesthesized Fasting Normal Rats

Time(mins)	Blood Glucose Contents as % of Initial Level (mean±SEM)		
	Human Insulin 0.6 IU/kg,s.c. n = 3	Human Insulin Liposomes i.d., n = 7	
		6.0 IU/kg	12.0 IU/kg
0	100.00±5.80	100.00±2.64	100.00±6.87
60	75.99±11.08	92.55±1.92	90.52±14.22
80	75.46±10.03	93.75±2.64	100.24±3.32
100	76.25±11.35	94.71±4.09	95.97±3.32
120	73.88±6.07	90.38±2.16	95.50±2.61
180	85.75±7.39	93.27±2.40	99.53±2.84
240	96.57±7.92	93.99±5.05	96.92±3.08
300	99.74±5.28	93.27±3.85	96.45±2.61

normal rats were used as positive and negative controls respectively (Table 7). The insulin entrapped in neutral (Sample No. 4) and negative (Sample No. 5) liposomes showed a blood glucose lowering profile the same as the positive control. Both systems gave the maximum glucose decreasing effect of about 30% whereas the positive control showed 34% at 120 minutes. However, doses of the hormone given in both cases were 20 folds more than that used in positive controls. The neutral and negative liposome systems appear to be better delivery systems for the human insulin than the positive liposomes in duodenum. The neutral liposomes contain higher amount of cholesterol than the positive liposomes while the negative liposomes have an opposite charge in comparing to the

Table 7 Comparison between the Effects of in Duodenum (i.d.) and Subcutaneous (s.c.) Administration of the Entrapped Human Insulin in Different Compound Liposomes and the Non-Entrapped Human Insulin Respectively on Blood Glucose in Anaesthesized Fasting Normal Rats

Time(mins)	Blood Glucose Contents as % of Initial Level (mean±SEM)		
	Positive Control n = 7	Negative Control n = 12	Sample No. 4 12 IU/kg,i.d. n = 11
0	100.00±6.27	100.00±4.06	100.00±3.71
60	74.67±7.05	94.03±2.15	87.62±6.93
80	68.41±5.74	87.35±1.67	79.95±5.20
100	66.84±6.27	83.77±1.43	75.99±4.95
120	66.31±5.22	84.01±2.15	71.78±4.29
180	72.32±5.48	85.68±1.91	75.50±4.70
240	84.07±2.61	85.92±2.39	84.65±3.22
300	84.33±3.13	84.01±2.39	88.11±2.48
360	85.38±3.39	84.73±2.39	88.37±2.72
420	85.12±3.66	85.44±3.58	90.10±2.97

Table 7 (continued)

Time(mins)	Blood Glucose Contents as % of Initial Level (mean±SEM)		
	Smaple No. 5 12 IU/kg,i.d. n = 12	Sample No. 6 12 IU/kg,i.d. n = 12	Sample No. 7 12 IU/kg,i.d. n = 12
0	100.00±4.94	100.00±3.71	100.00±6.37
60	85.45±7.27	93.27±2.55	91.98±2.12
80	74.03±7.53	92.74±2.91	87.50±1.89
100	74.29±8.31	83.99±2.32	82.08±1.89
120	71.17±7.53	80.05±3.02	81.37±2.59
180	72.73±7.27	81.90±3.71	82.08±4.01
240	80.01±7.01	87.47±2.78	95.28±6.60
300	81.56±6.49	83.76±2.55	87.50±3.07
360	84.42±4.68	81.44±2.78	78.77±4.25
420	89.35±2.86	79.35±3.71	80.19±3.07

positive liposomes. Negative charges on the liposome surfaces may have less chances to be attacked by ions in duodenal environments than the positive liposome systems.

For cholesterol rich negative (Sample 6) and positive (Sample 7) liposome systems, a maximum blood glucose lowering effect from the initial of about 20 % was found in both systems after 2 and 7 hours. Obviously, a retention of the effect was also observed in these systems after 7 hours . With the presence of higher amount of cholesterol in liposomes, liposomes may be more stable and resistant to degradation by the duodenal secretions thus staying intact longer in the duodenum. Some effects of cholesterol on our liposome systems have been previously discussed (22, 23).

In summary, this investigation suggested that an application of the human insulin complexed with the DEAE-dextran polymer and/or entrapped in liposomes as drug delivery systems in duodenum and colon is possible. However, further development and modification of the insulin and/or the liposomes are still required.

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