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Investigation of lectin-modified insulin liposomes as carriers for oral administration

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Abstract

The aim of this study was to design and characterize lectin-modified liposomes containing insulin and to evaluate the potential of these modified colloidal carriers for oral administration of peptide and protein drugs. Wheat germ agglutinin (WGA), tomato lectin (TL), or Ulex europaeus agglutinin 1 (UEA1) were conjugated by coupling their amino groups to carbodiimide-activated carboxylic groups of *N*-glutaryl-phosphatidylethanolamine (*N*-glut-PE). Insulin liposomes dispersions were prepared by the reverse-phase evaporation technique and modified with the lectin-*N*-glut-PE conjugates. Lectin-modified liposomes were characterized according to particles size, zeta potential and entrapment efficiency. The hypoglycemic effect indicated by pharmacological bioavailability of insulin liposomes modified with WGA, TL and UEA1 were 21.40, 16.71 and 8.38% in diabetic mice as comparison with abdominal cavity injection of insulin, respectively. After oral administration of the insulin liposomes modified with WGA, TL and UEA1 to rats, the relative pharmacological bioavailabilities were 8.47, 7.29 and 4.85%, the relative bioavailability were 9.12, 7.89 and 5.37% in comparison with subcutaneous injection of insulin, respectively. In the two cases, no remarkable hypoglycemic effects were observed with the conventional insulin liposomes. These results confirmed that lectin-modified liposomes promote the oral absorption of insulin due to the specific-site combination on GI cell membrane.

Keywords: Wheat germ agglutinin; Tomato lectin; Ulex europaeus agglutinin 1; Insulin; Liposomes; Oral administration

1. Introduction

Oral delivery is the preferred route of administration because it offers several advantages over other routes. It is more natural, less invasive, can be self-administered (outside the hospital), and is less expensive (Ahmed et al., 2002). However, oral delivery is generally not an effective method for the delivery of peptides and proteins.

The human gastrointestinal (GI) resists absorption of peptides, proteins and other large molecules until they are broken down into smaller molecules. The

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acidic environment of the stomach combined with an array of enzymes and physical barriers in the intestines either destroy or prevent efficient absorption of nearly all macromolecules. This problem leads to unacceptably low oral bioavailability (Lehr, 2000).

Several approaches to enhance the oral delivery of peptides have been or are currently being pursued. For instance, protective coatings, such as lipids and polymers, have been used to protect peptides during transport through the acidic environment of the stomach (Saffran et al., 1990) and enhance transport across the intestinal wall (Iwanaga et al., 1999). Alternatively, bioadhesive agents are used to enhance contact of the peptide to the intestinal wall (Ponchel and Irache, 1998; Arangoa et al., 2000). Such local delivery to sites allows greater adsorption and stability (Fara et al., 1985; Davis et al., 1988).

To achieve specific delivery of proteins and peptides across the intestinal epithelium after peroral administration receptor-mediated endocytosis can be utilized as a pathway. Recent investigations have focused on drugs conjugated with lectins.

There is evidence from the literature, that some plant lectins can facilitate the transport across cellular barriers (Clark et al., 2000). Lectins are proteins that recognize and bind to sugar complexes attached to proteins and lipids. They do this with very high specificity for the chemical structure of the glycan arrays.

The rationale behind lectin-mediated drug targeting is very simple (Bies et al., 2004). Most cell surface proteins and many lipids in cell membranes are glycosylated and these glycans are binding sites for lectins. The combination of a small number of sugars can produce a vast range of different chemical structures. Different cell types express different glycan arrays and in particular, diseased cells, such as transformed or cancerous cells, often express different glycans compared with their normal counterparts. Therefore, lectins could be used as carrier molecules to target drugs specifically to different cells and tissues. Apart from the concept of using the specificity of protein–sugar interactions for targeting to specific cells only, this kind of receptor-mediated bioadhesion may also be used to convey signals to cells in order to trigger vesicular transport processes into or across polarized epithelial cells. A number of studies have demonstrated the ability of lectins (agglutinins) to bind to intestinal mucosa.

Most cell surface proteins and many lipids in cell membranes of the GI are glycosylated and these glycans are binding sites for lectins. These glycans contain carbohydrate comprising *N*-acetyl-galactosamine, *N*-acetyl-glucosamine, galactose, fucose and sialic acid.

Wheat germ agglutinin (WGA) binds to *N*-acetyl-D-glucosamine and sialic acid exhibiting a molecular weight of 36 kDa. As compared with plant lectins with different carbohydrate specificity, the WGA-binding rate to intestinal cell lines of human origin, human colonocytes and prostate cancer cells was highest (Gabor et al., 1997, 1998, 2001). Moreover, the WGA not only binds to the cell membrane, but it is also taken up into the cytoplasm of enterocyte-like Caco-2 cells (Wirth et al., 1998).

Representing a dietary lectin, WGA is putative non-toxic. Wheat flour contains about 300 mg of WGA per kilogram and wheat germ is consumed either in unprocessed form as muesli or in processed form as bread. The muesli most likely contains the native lectin, bread is supposed to contain rather minimal amounts of intact lectin but the dietary intake of bread is rather high. Another hint towards negligible WGA toxicity is the observation that the viability of neither Caco-2 monolayers nor rats' intestinal epithelial cells was reduced (Ishiguro et al., 1992). On the other hand, daily peroral administration of 42 mg WGA to rats for 10 days provoked antinutritive effects (Pusztai et al., 1993). It should be considered, however, according to a rough calculation this corresponds to a daily intake of 30 g pure WGA in man. To date, a final judgement on toxicity or atoxicity of WGA cannot be given due to lack of studies in vivo. Nevertheless, the amounts of lectins as necessary for glycotargeting of prodrugs or colloidal carrier systems are in the microgram range so that toxic effects should not be provoked (Gabor et al., 2004).

Tomato lectin (TL) had been shown to have specificity for *N*-acetylglucosamine and derivatives such as its tetramer (Kilpatrick, 1980). It was chosen because it was easy to purify, had been shown to bind to the intestinal mucosa of rats and was relatively resistant to degradation by intestinal enzymes. In addition TL was non-toxic to rats (Kilpatrick et al., 1985) and the fact that raw tomatoes were consumed by millions of people worldwide suggested it was not toxic to humans. Using radiolabelled TL, Naisbett and Woodley showed that it bound strongly to rat intestinal mucosa in vitro, targeting a number of the major glycoproteins

of the intestinal brush border (Naisbett and Woodley, 1994a). As a consequence of binding to mucosal cells, it was also transported across the mucosa in vitro in significantly higher amounts than other macromolecules (Naisbett and Woodley, 1994b).

Ulex europaeus agglutinin 1 (UEA1), a lectin specific for a-L-fucose residues, binds almost exclusively to the apical surface of mouse Peyer's patch M cells in methanol or glutaraldehyde fixed tissues (Clark et al., 1993). Subsequent studies performed on freshly excised tissues or in ligated loops of anaesthetized animals revealed that lectin-binding to living follicle-associated epithelium (FAE) closely resembled that following tissue fixation (Clark et al., 1995; Giannasca et al., 1994; Gebert and Posselt, 1997). Moreover, UEA1 was successfully used to target macromolecules to mouse Peyer's patch M cells in gut loop experiments and to enhance subsequent macromolecule absorption across the intestinal epithelial barrier.

Using ovalbumin as a bystander, mistletoe lectin I strongly stimulated the immune response to the protein. In contrast, WGA and UEA1 exhibited slight adjuvant activity (Lavelle et al., 2001).

Thus the objective of this work is to get evidence whether WGA, TL or UEA1 can enhance the oral delivery of peptides. To follow this approach, model drug insulin had been incorporated in liposomes. Lectins were covalently bound to *N*-glut-phosphatidylethanolamine (*N*-glut-PE) by carbodiimide techniques, then were incubated with insulin liposomes to obtain lectin-modified insulin liposomes. Lectin-modified liposomes were characterized according to particles size, zeta potential and entrapment efficiency. The stabilities were studied in pepsin solution and trypsin solution. The hypoglycemic effect of the lectin-modified insulin liposomes in diabetic mice was compared and the relative bioavailability in rats was evaluated.

2. Materials and methods

2.1. Materials

PE (Sigma); glutaric anhydride (Shanghai Chemical Reagent Co., Ltd., China); 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDC, Fluka); wheat germ agglutinin (WGA, Sigma); tomato lectin (TL, Sigma); Ulex europaeus agglutinin 1 (UEA1, Sigma); 2,4,6-trinitrobenzenesulphonic acid (TNBS, Fluka); soya lecithin (Shanghai Pujiang lecithin Co., Ltd, China); insulin (27.7 Iu/mg, purchased from Xuzhou Biochemical Pharmaceutical Co., Ltd., China); cholate (Shanghai Medical Chemical Reagent Co., Ltd., China); pepsin (1:3000, Shanghai Medical Chemical Reagent Co., Ltd., China); trypsin (≥2500 u/mg, Shanghai Medical Chemical Reagent Co., Ltd., China).

2.2. Preparation of lectin–lecithin conjugates

Lectin was covalently bound to phosphatidylethanolamine (PE) by an appropriate modification of the two-stage carbodiimide method (Irache et al., 1994). In brief, 605 mg PE were dissolved in 60 ml chloroform, then an approx. 10-fold molar excess (940 mg) of glutaric anhydride was added and the mixture incubated at 20 °C for 5 h in the presence of 150 μl pyridine. *N*-glutaryl-phosphatidylethanolamine (*N*-glut-PE) was isolated on a silica gel column with a yield of 90% (Weissig et al., 1986). The carboxylic groups of *N*-glut-PE were activated in PBS by addition of 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDC). After removing the excess carbodiimide reagent, lectins (WGA, TL or UEA1) were then added and coupling was carried out by overnight incubation at 4 °C. The conjugates were isolated on a silica gel column to remove the free lectins. Finally, conjugates were resuspended in PBS containing 5% (w/v) of glycerol and 0.2% (w/v) sodium azide as preservative. All conjugates were stored at 4 °C.

2.3. Determination of the degree of lectin modification

The degree of lectin modification was determined with 2,4,6-trinitro-benzenesulphonic acid (TNBS) assay (Snyder and Sobocinski, 1975). Seven hundred microliters of aqueous solution containing free lectin or *N*-glut-PE modified lectin (all diluted to 0.5 mg of protein/ml) was added to 700 μl of 0.1 M sodium borate buffer (pH 9.2). Three hundred and fifty microliters of TNBS aqueous solution (1.65 mg/ml) was added and the solution was rapidly mixed. After incubation at 40 °C for 45 min, the reaction was stopped by adding 350 μl of 0.1 M NaH_2PO_4 containing 1.5 mM Na_2SO_3

and absorption at 420 nm was determined on a UV/VIS spectrometer (UNICO^{TM} UV-2102PCS).

2.4. Preparation of lectin-modified liposomes

The liposomes were prepared by the reverse-phase evaporation technique. Soya lecithin and cholesterol were dissolved in 10 ml ethyl ether. Insulin was dissolved in 1 ml 0.01 M hydrochloric acid and diluted to 3 ml with PBS buffer (pH 7.0). Then the insulin solution and the ethyl ether solution were mixed and to form emulsion by sonication (Sonic Purger CQ250, Academy of Shanghai Shipping Electric Instrument). The emulsion was rotary-evaporated for 2 h, then was hydrated with 3 ml of the conjugates solution obtained as described in the last section. PBS was used instead when preparing lectin-free liposomes.

2.5. Physicochemical characterization of liposomes

The morphology of the particles was examined by transmission electron microscopy (TEM) (JEM-1200EX, Japanese Electric). The average diameter, polydispersity index and *Z*-potential of the liposomes were determined by the laser light scattering technique (Zetasizer 3000SH, Malvern Instruments Ltd.).

2.6. In vitro aggregation assay for modified liposomes

The carbohydrate binding activities and specificities of the immobilized lectins were examined in vitro using an aggregation assay (Chen et al., 1996). The substrate for WGA, TL or UEA1 (see Fig. 2 legend) was dissolved in PBS buffer with different concentrations and 200 μl was added to dispersions of lectin-modified liposomes (lipid concentration adjusted to 1 mg/ml). The total volume was 3.0 ml. The mixtures were vortexed and incubated at 25 °C for 20 min and then transferred to the UV/VIS spectrometer. Liposomes aggregation was followed by the turbidity increase (A_{450}) of the dispersions. Carbohydrate specificity of the immobilized lectins was tested by incubating the lectin-modified liposomes with 200 μmol of the inhibitors (see Fig. 2 legend) before the substrates were added.

2.7. The drug entrapment efficiency

The dispersions of lectin-modified liposomes were separated by a Sephedex-G50 column (20 cm × 2.5 cm), washed by PBS buffer (pH 7.0) at a flow rate of 0.5 ml/min. The liposomes entrapping insulin and the free insulin were collected. The lipid membranes of the liposomes were destroyed using 10 mg/ml sodium cholate, then the lipids were extracted using chloroform, the drug content in the supernatant was determined by an HPLC method at 214 nm using an Inertsil ODS-3 column (4.6 mm × 25 cm, GL Sciences Inc. Japanese) with a mobile phase consisting of 0.025 mol/l NaH_2PO_4–0.05 mol/l Na_2SO_4–hydrocyanic ether (36:36:28), pH was modified to 2.0 with H_3PO_4 at a flow rate of 1 ml/min (Zhang et al., 2001). The drug entrapment efficiency were calculated from the following equation:

$$\text{Drug entrapment efficiency} = \frac{\text{analyzed weight of drug entrapped in liposomes} \times 100}{\text{analyzed weight of drug entrapped in liposomes} + \text{analyzed weight of free insulin}} \quad (1)$$

2.8. Stability studies

Stability studies were carried out in pepsin solution (0.05 mg/ml, Tris–HCl buffer, pH = 2) and trypsin solution (0.36 mg/ml, phosphate buffer, pH = 7.4) (Wu et al., 2003). 1.5 ml different proteolytic enzymes solution were added 1.5 ml different insulin liposomes dispersions or insulin solution, then incubated at 37 °C right after the addition. Two hundred microliters samples were withdrawn at each time interval and the pepsin digestion reaction was stopped by adding 100 μl 0.05 mol/l NaOH, the trypsin digestion reaction was stopped by adding 100 μl 0.1 mol/l HCl. The insulin concentrations in the samples were determined by the HPLC method as described above.

2.9. The hypoglycemic effect of the lectin-modified insulin liposomes in diabetic mice

Alloxan-induced diabetic mice (20–25 g, the blood glucose levels ≥10.08 mmol/l, from the medical animal

test center of Shandong University) were housed under normal conditions with free access to food and water. Six groups of six mice each were used to study the in vivo uptake of lectin-modified liposomes or lectin-free liposomes. Mice from groups one to four were gavaged with 0.5 ml WGA modified liposomes, TL modified liposomes, UEA1 modified liposomes or lectin-free liposomes (insulin 350 Iu/kg). The fifth group was given PBS only. This group was used as a control for the experiment. The sixth group was injected abdominal cavity of insulin (insulin 50 Iu/kg). Food was restored immediately after administration. Forty microliters blood was withdrawn from the ophthalmic choroid vein using capillary at each time interval and was then centrifuged (3000 rpm for 15 min, 80-2, Shanghai Surgery Instruments, China). Twenty microliters serum was separated to determine the blood glucose using the glucose oxidase method (glucose determination agent box, Shanghai Rongsheng Biological technology Co., Ltd., China) (Wu et al., 2003). Their relative pharmacological bioavailability (PA) was calculated by the area over the hypoglycemic curve (AOC) versus time profile after administration (Scott-Moncrieff et al., 1994; Radwan and Aboul-Enein, 2002).

2.10. The relative bioavailability studies in rats

SD rats (250 ± 25 g, from the medical animal test center of Shandong University) were housed under normal conditions with free access to food and water. Six groups of six rats each were used to study the in vivo uptake of lectin-modified liposomes or lectin-free liposomes. Rats from groups one to four were gavaged with 0.5 ml WGA modified liposomes, TL modified liposomes, UEA1 modified liposomes or lectin-free liposomes (insulin 50 Iu/kg). The fifth group was given PBS only. This group was used as a control for the experiment. The sixth group were injected subcutaneous of insulin (insulin 2 Iu/kg). After the rats were anaesthetized with ether inhalation, 0.5 ml blood was withdrawn from the sub-raclaviculares vein at each time interval and was then centrifuged (5000 rpm for 15 min, 80-2, Shanghai Surgery Instruments, China). Twenty microliters serum was separated to determine the blood glucose using the glucose oxidase method. Their relative pharmacological bioavailability (PA) was calculated by the area over the hypoglycemic curve (AOC) versus time profile after administration. One hundred microliters serum was separated to determine the insulin concentrations using the radioactive immunity analysis (RIA, Insulin radioactive immunity analysis box, Navy radioactive immunity technology center, China; GC-911γ-ray radioactive immunity analysis instrument, Keda Zhongjia Technology Co., Ltd., China), the relative bioavailability was calculated by area the curve of serum insulin concentration versus time profile after administration, the vales of endogenous rat insulin level were subtracted from the observed data.

3. Results and discussion

3.1. Preparation of lectin–lecithin conjugates

The lectins attachment to *N*-glut-PE was assessed as a function of the carbodimide reagent concentration and the reaction time (Ezpeleta et al., 1996). Ligand binding increased with the carbodiimide concentration. In this case, a plateau could be reached at a reagent concentration of about lectin: *N*-glut-PE = 1:10 (mol/mol). Similarly, lectins binding increased with the carbodiimide activation time. Moreover, an equilibrium was reached after incubation for at least 6 h.

3.2. Preparation of lectin-modified liposomes

Lectins are natural components of the daily diet. They bind to particular sugar structures specifically with affinities similar to those of monoclonal antibodies. In contrast to antibodies, lectins are generally stable in the gastrointestinal tract (Gabor et al., 2002). When the liposomes are modified with lectins, the lectin–sugar specific interactions may allow the differentiation among intestinal epithelial cells and therefore facilitate targeted delivery of the liposomes. Lectins are non-membrane hydrophilic molecules. They can be incorporated into the membrane by modifying them with a hydrophobic anchor, *N*-glut-PE. During liposome formation, increased lipophilicity will force the hydrophobic tail region to partition into the membrane phase and expose the lectins to the water phase (Chen et al., 1996). The changes of the morphology and the increases of the average diameter of the liposomes after the modification were testimonies of the immobilized lectins on the liposomes surfaces.

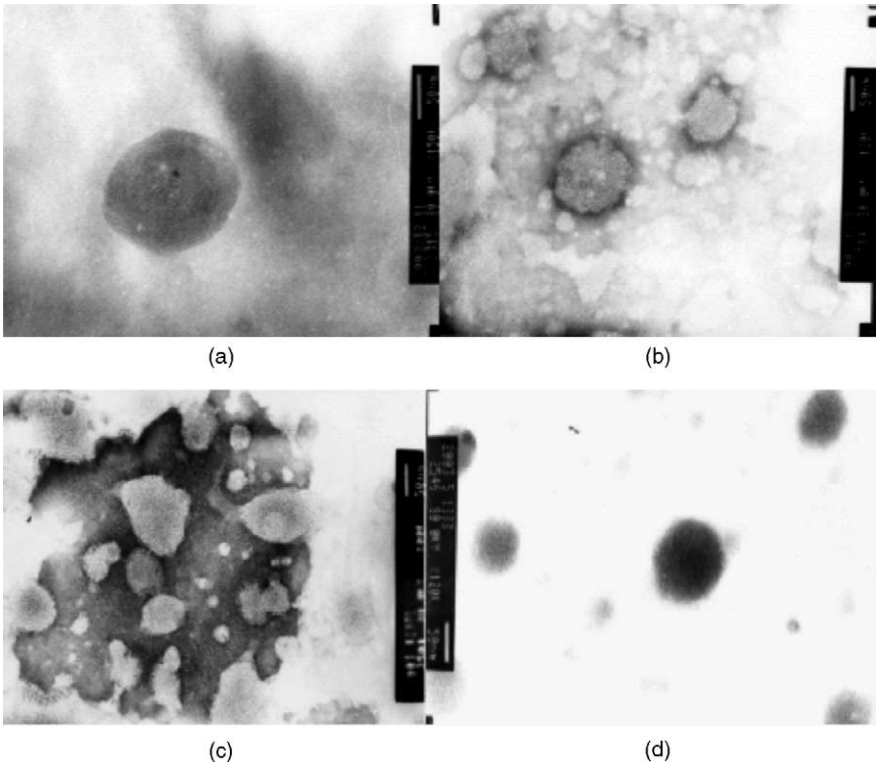

Fig. 1. Transmission electron photomicrograms of insulin liposomes: (a) conventional liposomes, (b) WGA modified liposomes, (c) TL modified liposomes, (d) UEA1 modified liposomes.

TNBS reacts with the free amino groups in proteins and gives absorption at 420 nm. Since the *N*-glut-PE conjugation also takes place on the amino groups in the lectins, the TNBS assay is used to quantify the reduction in the number of amino groups during lectin conjugation. The relative decrease in A_{420} measured for the same lectin before and after the conjugation reaction can be used to calculate the extent of lectin conjugation. A typical degree of modification obtained using this assay was between 40 and 70%. All insulin-liposomes were of spherical or ellipsoidal shape (Fig. 1). The average diameter and *Z*-potential of the liposomes were found to be slightly increasing after the modification with lectins (Table 1).

Table 1
Physicochemical characterization of liposomes

liposomes	Average diameter (nm)	Polydispersity index	*Z*-potential (mV)
Conventional	166.2 ± 16.02	0.416	−2.10
WGA modified	191.0 ± 13.62	0.448	+5.78
TL modified	194.1 ± 21.95	0.453	+8.70
UEA1 modified	189.7 ± 10.67	0.462	+3.40

3.3. In vitro aggregation assay

During the modification, structural changes were introduced in the lectins. It is crucial to verify lectin binding activity as well as carbohydrate specificity after the modification processes. This is done using the in vitro aggregation assay. When the substrates (molecules that contain multiple copies of the specific carbohydrate residues recognized by lectins) are added into the lectin-modified liposomes dispersions, interaction between the carbohydrate residues and the surface-immobilized lectins should lead to 'bridge' formations among the liposomes, if the lectins remain biologically active. This results in liposome aggregation and can be estimated by following the increase in the turbidity

of liposome dispersions. When inhibitors (small sugar molecules containing a single copy of the carbohydrate residues) are incubated with the lectin-modified liposomes first, they should compete with the substrates for lectin binding, if the lectins still recognize the same carbohydrate residues. As a result, the bridges can no longer form and aggregation should disappear. This assay can therefore provide insight as to whether the immobilized lectins still retain their carbohydrate binding activities as well as their sugar specificities.

Turbidity changes of the liposomes dispersions in the presence of their corresponding substrates are plotted in Fig. 2. When substrates were added to lectin-modified liposomes, the turbidity of the suspensions increased with increasing substrate concentration, indicating increasing aggregation of the lectin-modified liposomes (Fig. 2a–c). This establishes that the lectins maintained their binding activity after immobilization. In the presence of the inhibitors, however, virtually no change in turbidity was observed even when the same substrates were added (Fig. 2a and c). This indicates that the carbohydrate specificity of these lectins remained unaltered after immobilization. In this case, the inhibitors for TL were $(GlcNAc)_3$ or $(GlcNAc)_4$ (Hussain et al., 1997), which we did not obtain, so the sugar specificity assay for TL could not be conducted.

3.4. The drug entrapment efficiency

Under the chromatographic conditions used, insulin presented a major peak at 12 min. Concerning the validation procedure of the technique, the standard curve was linear for the range from 2 to 100 μg/ml ($r = 0.9999$). The drug entrapment efficiency was

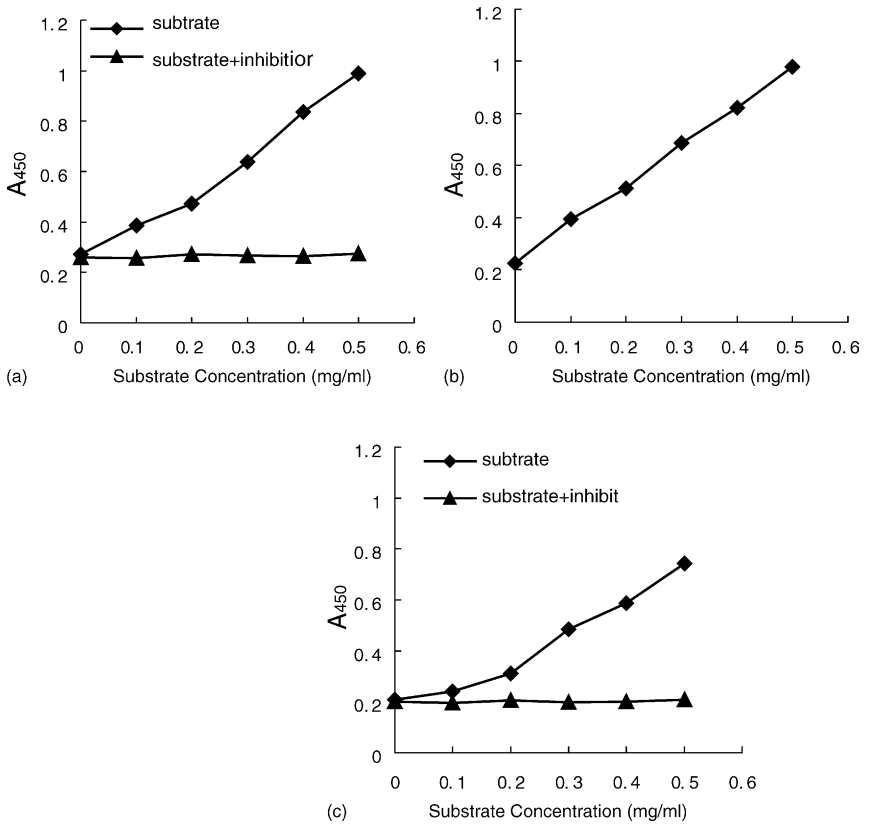

Fig. 2. In vitro aggregation of lectin-liposomes in the presence of their substrate: (a) glycophorine (Sigma) for WGA; (b) glycophorine (Sigma) for TL; (c) 2′-fucosyl-lactosamine BAS conjugate (Sigma) for UEA1; or in the presence of their substrates as well as inhibitors: (a) *N*-acetylglucosamine (Shanghai Boao Biological technology Co., Ltd., China) for WGA; (c) L-fucose (Shanghai Boao Biological technology Co., Ltd., China) for UEA1.

Table 2
Entrapment efficiencies of insulin-liposomes

Liposomes	Entrapment efficiency (%) ($n = 3$)
Conventional liposomes	30.30 ± 2.77
WGA modified liposomes	69.33 ± 4.54
TL modified liposomes	82.50 ± 5.57
UEA1 modified liposomes	39.55 ± 7.28

increased when modified with WGA and TL, slightly increased when modified with UEA1. The insulin liposomes modified with TL showed higher entrapment efficiency (82.50%) as compared with the other lectin-modified liposomes (Table 2). The modifications were performed at neutral pH. Under these conditions, insulin (p*I* 5.0) was negative electricity while WGA (p*I* 7.0) and TL (p*I* 10.0) were positive electricity. The electricity attraction between insulin molecules and WGA molecules or TL molecules maybe the reasons for the increased drug entrapment efficiencies. UEA1 (p*I* 6.5) was negative electricity under the modification conditions, no notable increase was observed in drug entrapment efficiency.

3.5. Stability studies

Lectin-modified liposomes could protect insulin from peptic and tryptic digestion. Fig. 3 showed the remaining ratio of insulin after incubation of insulin-liposomes modified by three kind of lectins in pepsin solution, the remaining ratio of insulin after incubation in trypsin solution was shown in Fig. 4. In all lectin-modified liposomes, the protective action of TL modified liposomes was strongest. In addition, no protective action was observed by the conventional liposomes.

Lectins are generally stable in the gastrointestinal tract (Gabor et al., 2002). In vitro, after preincubation of WGA and TL with abnormal high amounts of pepsin, trypsin, pancreatin, and elastase, no degradation products were observed and the cell-binding characteristics were fully retained (Gabor et al., 1997). The modified liposomes were coated by lectins and lectins are stable with the enzymes, so the stabilities of the modified liposomes were improved.

3.6. The hypoglycemic effects of the lectin-modified insulin liposomes in diabetic mice

The insulin liposomes modified with WGA showed a better hypoglycemic effect as compared with the other modified liposomes. The minimum blood glucose was 35.39% of the initial blood glucose induced by WGA modified liposomes. The blood glucose reducing effect lasted for 12 h after oral administration to diabetic mice. The relative pharmacological bioavailability of

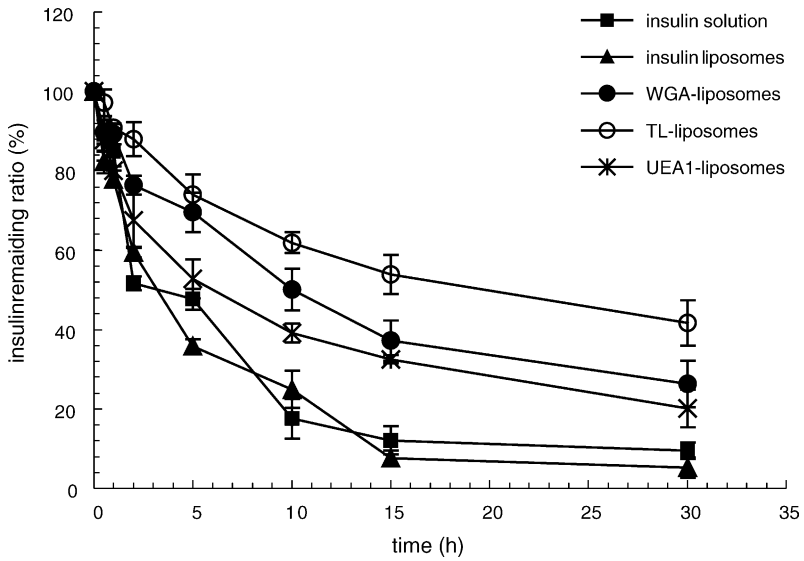

Fig. 3. Remaining ratio of insulin after incubation of insulin solution, insulin liposomes, and insulin-liposomes modified by three kind of lectins in pepsin solution (the mean ± S.D., $n = 3$).

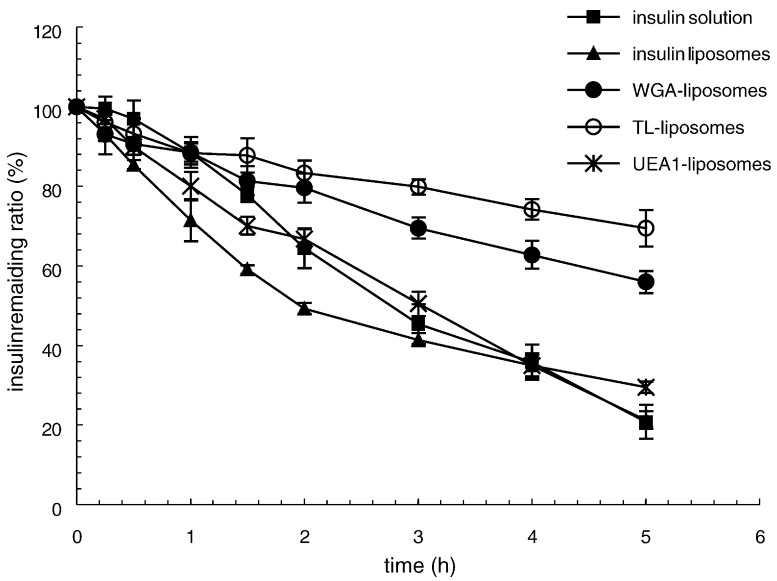

Fig. 4. Remaining ratio of insulin after incubation of insulin solution, insulin liposomes, and insulin-liposomes modified by three kind of lectins in trypsin solution (the mean ± S.D., $n = 3$).

insulin liposomes modified with WGA, TL and UEA1 were 21.40, 15.28 and 8.92% in diabetic mice, respectively. No remarkable hypoglycemic effects were observed with the conventional insulin liposomes (Fig. 5).

3.7. The relative bioavailability studies in rats

After oral administration of the insulin liposomes modified with WGA, TL and UEA1 to rats, the blood glucose level obviously decreased. The relative

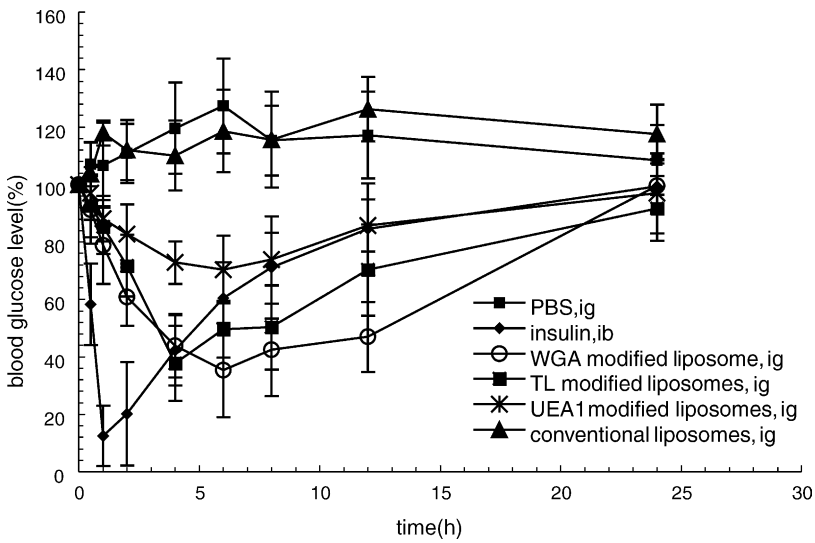

Fig. 5. The blood glucose changed after oral administrated some insulin preparations in diabetic mice (means ± S.D.).

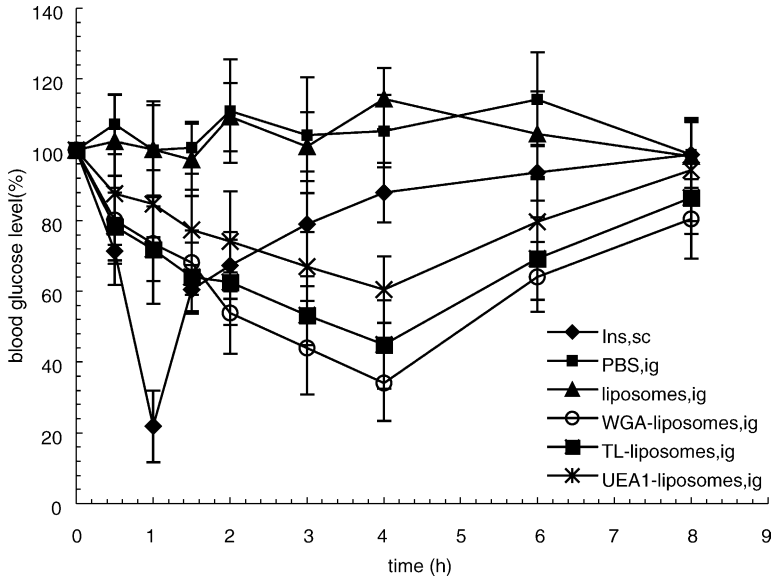

Fig. 6. The change of serum glucose levels (%) after oral administration of some insulin preparations in rats (means ± S.D.).

pharmacological bioavailabilities were 8.47, 7.29 and 4.85% while no significant hypoglycemic effects were observed with the conventional insulin liposomes (Fig. 6). Fig. 7 shows the serum insulin concentrations changing after oral administration of some insulin preparations in rats. The experimental data were assessed by Pkanalyst computer program and found to best fit the one-compartment open model. The relative

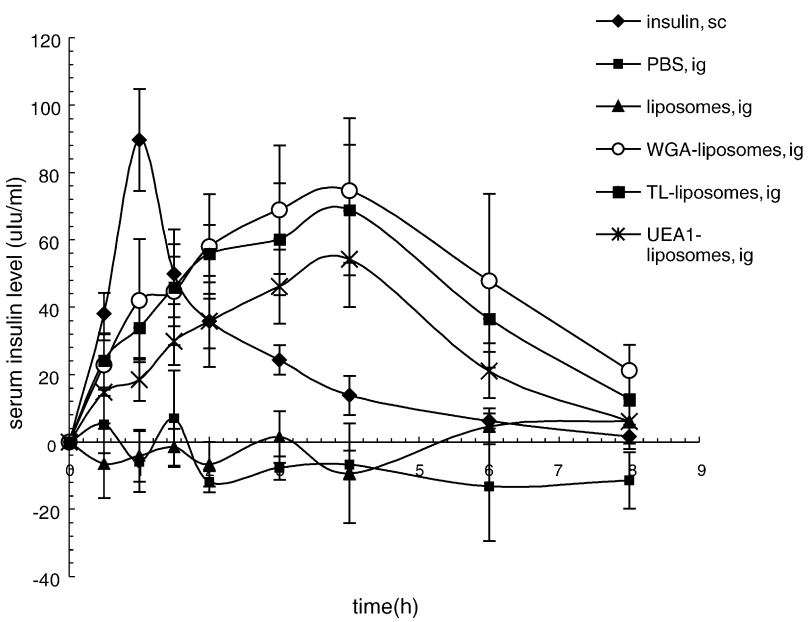

Fig. 7. Serum insulin concentration after oral administration of some insulin preparations in rats (means ± S.D.) (μIu/ml).

bioavailability calculated by area the curve of serum insulin concentration versus time profile were 9.12, 7.89 and 5.37% in comparison with subcutaneous injection of insulin, respectively. There was a linear relationship between blood glucose level and serum insulin concentration.

After oral administration of the lectin-modified insulin liposomes to diabetic mice or to rats, the blood glucose level obviously decreased. And there was a linear relationship between blood glucose level and serum insulin concentration. These results suggested that lectin-modified liposomes could enhance intestinal absorption of insulin. Lectin is a specific ligand which shows an affinity for a receptor located into the GI cavity, can be grafted on the surface of drug carrier and mediate an adhesive interaction between the carrier and the biological surface. For oral delivery, lectins can be good tools for this purpose due to their relatively good resistance to acidic pH and enzymatic degradation and the ubiquitous presence of binding sites along the GI tract. WGA is affinity to sialic acids, recognizes glycoconjugates present on both M cells and regular intestinal absorptive cells, liposomes modified with WGA were beneficial to absorption of insulin across the intestinal epithelium and to uptake by Peyer's patches (Chen et al., 1996; Pusztai et al., 1993). TL recognizes glycoconjugates present on the regular intestinal absorptive cells, liposomes modified with TL would bind to the epithelial cells and enhance the absorption of insulin across the intestinal epithelium (Carreno-Gomez et al., 1999). UEA1 recognizes glycoconjugates present on M cells, liposomes modified with UEA1 would bind to M cells and enhance the uptake of insulin by Peyer's patches (Clark et al., 1995; Chen et al., 1996). As the specialized 'sampling' cells, M cells are the primary phagocytotic cell which can take up the liposomes (Ponchel and Irache, 1998). Liposomes modified with UEA1 would be taken up more effectively by M cells (Jepson et al., 1996, 2004). The relative pharmacological bioavailability of the UEA1 modified insulin liposomes were 8.92% in diabetic mice, 4.85% in rats while no significant hypoglycemic effects were observed with the conventional insulin liposomes, respectively. The amounts of the regular intestinal absorptive cells are enormous while the amounts of M cell is relatively rare (Kraehenbuhl and Neutra, 2000). When the insulin liposomes modified with TL, the absorption of insulin was enhanced through the receptor-mediated endo/transcytosis (Haas and Lehr, 2002; Gabor et al., 2004). The relative pharmacological bioavailability of the TL modified insulin liposomes were 15.28% in diabetic mice, 7.29% in rats, higher than the hypoglycemic effects induced by the liposomes modified with UEA1. WGA bind to all epithelial cells with equal specificity, the relative pharmacological bioavailability of the modified insulin liposomes were 21.40% in diabetic mice, 8.47% in rats, higher than the hypoglycemic effects induced by the liposomes modified with TL. The receptor-mediated endo/transcytosis was observed in the absorption process of WGA modified drug carriers (Faivre et al., 2003; Mo and Lim, 2004). The insulin liposomes modified with WGA showed a better hypoglycemic effect as compared with the other modified liposomes.

The potential of protein–sugar interaction has been appreciated of drug delivery and targeting than bioadhesion in the GI tract (Lehr, 2004). As some of the lectins were also taken up into the cells, lectins were proposed to enhance intestinal drug transfer. As presented here, the WGA modified liposomes is a powerful delivery system for transporting peptide and protein drugs. The relative bioavailability calculated by area the curve of serum insulin concentration versus time profile were 9.12% in comparison with subcutaneous injection of insulin. This is still an unacceptably low oral bioavailability. Maybe using the protease inhibitors at the same time would increase the bioavailability (Ahmed et al., 2002). Future scenarios may include additional reservoirs, permitting incorporation of permeation enhancers and enzyme inhibitors to increase the effectiveness of the drug. The characterization lectin-modified liposomes and the comparison of the three lectins is the first step in the successful development of microdevices that will play an important roll in a new generation of drug delivery.

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