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Research Article

5β-Cholanic Acid/Glycol Chitosan Self-Assembled Nanoparticles (5β-CHA/GC-NPs) for Enhancing the Absorption of FDs and Insulin by Rat Intestinal Membranes

Chengyun Yan,[1,4] Jiwei Gu,[2] Yuguang Lv,[3] Weiguo Shi,[3] Zhan Huang,[2] and Ying Liao[1]

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Abstract. The absorption-enhancing effects of glycol chitosan modified by 5β-cholanic acid nanoparticles (5β-CHA/GC-NPs) on a drug with poor absorption in the intestine were studied by the method of *in situ* closed loop. We chose fluorescein isothiocyanate-labeled dextrans (FDs) and insulin as the model drugs. 5β-CHA/GC-NPs loaded to different drugs were prepared by the dialysis method, and the physicochemical characteristics and *in vitro* release profiles of nanoparticles were also estimated. The results showed that 5β-CHA/GC-NPs markedly increased the absorption of insulin and FDs in the jejunum, ileum, and colon. The ratios of absorption for all the drugs in the jejunum were higher than those in the ileum and colon. In addition, the enhancing effect of 5β-CHA/GC-NPs for the absorption of FDs from the jejunum was decreased with increasing molecular weights. In the toxicity test, 5β-CHA/GC-NPs did not significantly increase the release of protein and the activities of LDH, indicating that the nanoparticles did not cause any membrane damage to the intestine. These findings suggested that 5β-CHA/GC-NPs were safe and useful carriers for enhancing the absorption of the drug with poor absorption by intestinal membranes.

KEY WORDS: 5β-cholanic acid/glycol chitosan; self-assembled nanoparticles; intestinal absorption; poorly absorbable drugs.

INTRODUCTION

Oral drug administration is the most convenient and feasible route for the delivery of a drug. However, it still remains a challenge for peptides and proteins to fulfill their curative effect, due to low permeability and high metabolism (1). Among possible strategies, various nanocarriers, including micelles, liposomes, nanoparticles, and copolymer, have been applied in the interest of improvement of the absorption of peptides and proteins by intestinal membranes (2–5).

It was reported that chitosan has been a promising intestinal absorption enhancer for oral peptide delivery, on account of its mucoadhesive and permeability-enhancing properties (6–8); chitosan nanoparticles are an attractive carrier, which have the capacity to prevent peptide and protein drugs from chemical and enzymatic degradation (9,10) and also to reduce the transepithelial electrical resistance of the absorptive epithelial cells (11,12). The low molecular weight chitosan has better water solubility and biological activity compared with a higher degree of polymerization of chitosan (13), and the decreased MW and increased water solubility can enhance the absorption of chitosan molecules by membrane transport or cellular uptake (14).

Glycol chitosan(GC) was obtained through conjugating ethylene glycol branches to low molecular weight chitosan, which has a better aqueous solubility and steric stabilization (15). GC can be easily modified to various derivatives, as a result of the presence of amine and hydroxyl groups. As reported in previous papers, hydrophobically modified glycol chitosan (HGC) nanoparticles could spontaneously form self-aggregates to encapsulate and sustainably release various small molecule drugs and peptides *in vitro* and *in vivo* for various medical applications (16–19). It was a valuable protein-delivery system in the medical field.

In this study, glycol chitosan modified by 5β-cholanic acid nanoparticles (5β-CHA/GC-NPs) were prepared. It was expected that the formulation could exhibit higher resistance to gastric enzymatic degradation, enhanced mucoadhesiveness, and permeability.

We hypothesized that 5β-CHA/GC-NPs could be employed as an effective yet safe polymeric drug carrier to improve insulin delivery across the intestinal mucosal layer thereby enhancing systemic bioavailability.

[1] College of Pharmacy, Guilin Medical University, Guilin, 541004, China.
[2] First Affiliated Hospital of Jiamusi University, Jiamusi, 154000, China.
[3] College of Pharmacy, Jiamusi University, Jiamusi, 154000, China.
[4] To whom correspondence should be addressed. (e–mail: hademan@126.com)

The goal of this research was to evaluate the absorption-enhancing effects of 5β-CHA/GC-NPs on drugs with poor absorption in the intestine. Insulin and fluorescein isothiocyanate-labeled dextrans (FDs) were chosen as the model drugs. These drugs loaded with 5β-CHA/GC-NPs were obtained using a dialysis method. The mean diameter, zeta potential, and drug encapsulation of the nanoparticles and the drug release *in vitro* from 5β-CHA/GC-NPs were also measured.

Furthermore, the effect of 5β-CHA/GC-NPs on the absorption of model drugs in different intestinal regions was evaluated using the method of *in situ* closed loop, and the effect of 5β-CHA/GC-NPs on the intestinal membrane damage will be also examined in rats.

MATERIALS AND METHODS

Materials

Glycol chitosan modified by 5β-cholanic acid (5β-CHA/GC) (MW 168 kDa, DS = 106) was synthesized in our own laboratory (Jiamusi, China). FDs (FD4, FD10, and FD70) were purchased from Macklin Biochemical Inc. (Shanghai, China). Insulin was obtained from Eli Lilly Co. Ltd. (Suzhou, China). LDH CII assay kit, BCA™ Protein Assay Kit, and Insulin-EIA Test Kit were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), Glucose B Test Kit was provided by Pierce Biotechnology Inc. (Wako, Japan). Triton X-100 was obtained from Nacalai Tesque Inc. (Kyoto, Japan). Sodium pentobarbital was provided from Jining Shiye Biotechnology Inc. (Shanghai, China). Other chemicals were of reagent grade.

Preparation of 5β-CHA/GC Nanoparticles

Drugs loaded with 5β-CHA/GC-NPs were obtained in accordance with a previous report with some modifications (20). The 5β-CHA/GC solution was prepared by adding polymer (10 mg) to 2 ml water/methanol (1:1, *v*/*v*), and 1 ml drug–ethanol solution at a concentration (2 mg/ml) was added to the 5β-CHA/GC solution. The solution was on dialysis (molecular cutoff = 10 kDa) for 2 day after a 12-h continuous stirring at 25 °C, the solution was then centrifuged, and the supernatant was filtered by a 0.22-μm micropore filtering film.

Characterization of the Nanoparticles

A particle size analyzer (Nicomp380ZLS, CA, USA) was used to determine the mean diameter and the zeta potential of the nanoparticles. The encapsulation efficiency (EE) and loading capacity (LC) were determined by ultracentrifugation (21). A 20-g sample of drug-loaded nanoparticles was centrifuged (21,000×*g*, 60 min) and washed with water to separate the drugs on the particle surface. Free FDs in the supernatant were examined with a spectrofluorimeter (Shimadzu UV160U, Columbia, USA). The amount of free insulin in the clear supernatant was determined using an enzyme immunoassay method (Insulin-EIA Test Kit, Wako Pure Chemical Industries, Osaka, Japan). The EE and their LC of the nanoparticles were calculated as follows:

$$\%EE = \left[\frac{\text{Total drugs} - \text{Free drugs}}{\text{Total drugs}}\right] \times 100$$

$$\%LC = \left[\frac{\text{Total drugs} - \text{Free drugs}}{\text{Nanoparticles weight}}\right] \times 100$$

Assessment of *In Vitro* Drug Release

Drug release from 5β-CHA/GC-NPs was studied using a modified dissolution method (22). A 1-ml sample of drug-loaded nanoparticle suspension was placed in a dialysis membrane tube. The test tube was suspended in 100 ml PBS (pH 7.4) solution and stirred at 37.4 °C. At given time intervals, 1-ml samples of PBS solution were taken out of the dissolution cells and the drug content was analyzed by the method as described in the experimental section of evaluation of drug encapsulation. The release medium was replaced with fresh buffer to maintain total volume after each withdrawal. The tests were performed in triplicate. The cumulative percentage release rate of drugs from the nanoparticles was calculated.

Effect of 5β-CHA/GC-NPs on the Absorption of Drugs in Rat Intestine

Absorption experiments were performed by the method of *in situ* closed loop (23,24) under the approval of the Animal Ethics Committee at Guilin Medical University (Approval No. IAEC-201706016). For the study, free insulin, insulin loaded with 5β-CHA/GC-NPs, FDs, and FDs loaded with 5β-CHA/GC-NPs were dissolved in PBS buffer (pH 7.4) to yield a final concentration of 20 IU/ml, 20 IU/ml, 2 mg/ml, and 2 mg/ml, respectively. Male Wistar rats (230–250 g) were fasted and anesthetized before the experiment. A closed loop was prepared of the jejunum, ileum, and colon according to a previously described study in detail, and drug solutions (1 ml) were added into the intestinal segment prior to closing. Blood samples (0.5 ml) of each rat were collected periodically to determine the concentration of the drug in the plasma.

Plasma FD concentration was determined with a fluorescence spectrophotometer (Spectrauor Plus, Tecan, Switzerland) at an excitation wavelength of 485 nm and emission wavelength of 535 nm, respectively.

Plasma insulin concentration was estimated using an enzyme immunoassay method (Insulin-EIA Test Kit, Wako Pure Chemical Industries, Osaka, Japan), and the total area under the insulin concentration curve (AUC) from 0 to 6 h was calculated with the sum of successive trapezoids between each data point.

Plasma glucose concentration was examined using the Glucose B Test Kit (Wako, Japan) and the area above the curve (AAC) was calculated by a trapezoidal method. The pharmacological availability (PA%) was calculated from the following equation:

$$PA\% = \left[\frac{AAC(\text{intestine}) \times \text{dose(iv)}}{AAC(\text{iv}) \times \text{dose(intestine)}}\right] \times 100$$

Measurement of Intestinal Membrane Toxicity

To evaluate the intestinal mucosa toxicity of 5β-CHA/GC-NPs, the activity of LDH and the amount of tissue protein were measured in a similar manner as described previously (25,26). The intestinal loop was treated with 5β-CHA/GC nanoparticles (20 mg/ml), Triton X-100 (3%, *v*/*v*), and PBS solution, respectively. The intestinal perfusate was sampled to examine the membrane toxicity. The LDH activity was determined using LDH CII assay kit (Beyotime, China). The tissue protein was measured by BCA™ Protein Assay Kit (Beyotime, China).

Statistical Analyses

All results were expressed as mean value ± SE of three to five animals, and statistical analyses were accomplished using Student's *t* test or one-way ANOVA. A difference of $P < 0.05$ was considered statistically significant.

RESULTS

Physicochemical Characterization of 5β-CHA/GC-NPs

Insulin and FDs loaded with 5β-CHA/GC-NPs with small size and marked positive charge were prepared by the dialysis method. In this study, 5β-CHA/GC and the model drug concentrations were adjusted to a weight ratio of 4:1 to obtain the self-assembled nanoparticles.

Table I summarizes the physicochemical characterization of the model drugs loaded with 5β-CHA/GC-NPs. As shown in Table I, 5β-CHA/GC-NPs have a mean particle size of 202.4–239.4 nm in diameter with narrow size distribution (PdI < 0.08) and a positive value of zeta potential (from 11.3 to 52.2 mV). Results indicated that there was no significant difference in the particle size and zeta potential between FD loading nanoparticles and the plain particles. However, the zeta potential of 5β-CHA/GC nanoparticles was less (11.3 mV) for insulin than that of the plain particles.

Regarding encapsulation efficiency, 5β-CHA/GC-NPs entrapped with insulin displayed a very high insulin association efficiency (87.4%) leading to insulin loading values as high as 24.8%. Table I shows the association efficiency and loading capacity of FDs in 5β-CHA/GC nanoparticles that ranged from 25.6 to 78.3% and from 7.9 to 19.7%, respectively. Results indicated that the association efficiency and loading capacity gradually decrease as the molecular weight of FDs increase.

Assessment of *In Vitro* Drug Release

Figure 1 shows the drug release profiles of 5β-CHA/GC-NPs over a 6-h period in PBS (pH 7.4). As can be seen from Fig. 1, the release curve was characterized by a fast initial burst (30–60% in 30 min), followed by a decelerated drug release for at least 6 h. The extent of release reached approximately 90% at 6 h.

All drugs loaded with 5β-CHA/GC-NPs showed quite similar release patterns. However, a slight difference was found between these release profiles, the release rate of insulin was slightly slow compared with FDs, and the release of FDs was closely related to the molecular weight of the drugs. As the molecular weight of FDs increased, the initial burst accelerated.

Effect of 5β-CHA/GC-NPs on the Absorption of FDs in Rat Intestine

The effect of 5β-CHA/GC-NPs on the absorption of FDs in different intestinal regions is shown in Fig. 2. It was observed that 5β-CHA/GC-NPs significantly increased the absorption of FDs from all the intestinal segments including the jejunum, ileum, and colon.

The AUC values of FD4 and their absorption-enhancing ratio (Ratio) after administration of FD4 solution and FD4–5β-CHA/GC-NP into rat intestine are summarized in Table I. As it could be found in Table II, the AUC value of FD4 was greatly increased in all intestinal loops after administration of FD4–5β-CHA/GC-NP to rats and the ratio value was greater in the jejunum than in the ileum and colon. Also, the enhancement of intestinal absorption of 5β-CHA/GC-NPs was closely related to the molecular weights of encapsulated drugs. It decreased as the molecular weight of FDs increased.

Effect of 5β-CHA/GCNPs on the Absorption of Insulin in Rat Intestine

The concentration–time profiles of insulin solution and insulin–5β-CHA/GC-NP administered into the jejunum are shown in Fig. 3. As shown in Fig. 3, it was observed that the level of insulin in plasma was improved significantly when insulin–5β-CHA/GC-NP was administered into the jejunum as compared with the control. Also, the glucose level in the blood was remarkably lowered after the administration of

Table I. Physicochemical Properties of 5β-CHA/GC-NPs ($n = 3$)

Model drugs	Drug/chitosan ratio (*w*/*w*)	Entrapment efficiency (%)	Loading capacity (%)	Zeta potential (mV)	Mean diameter (nm)
Blank	0.0	–	–	52.2 ± 4.6	214.0 ± 17
Insulin	0.2	87.4 ± 4.3	24.8 ± 3.3	11.3 ± 2.9	202.4 ± 9
FD4	0.2	78.3 ± 4.5	19.7 ± 4.1	20.2 ± 4.2	239.4 ± 30
FD10	0.2	34.7 ± 2.3	9.5 ± 0.6	18.0 ± 5.7	233.6 ± 13
FD70	0.2	25.6 ± 1.1	7.9 ± 3.3	16.0 ± 3.3	227.2 ± 21

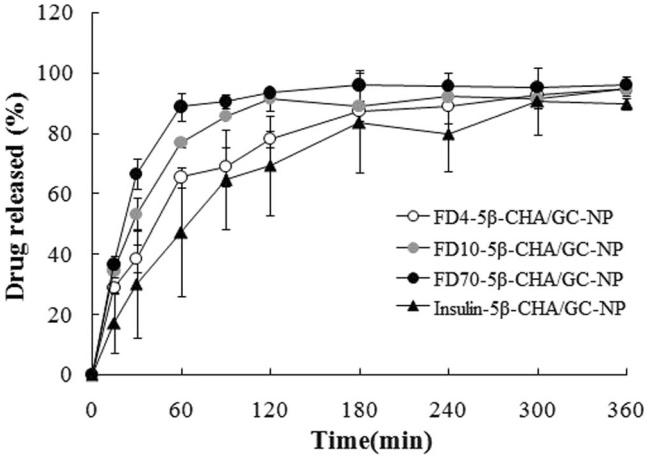

Fig. 1. *In vitro* release profiles of drugs from 5β-CHA/GC-NPs: FD4–5β-CHA/GC-NP (white circle), FD10–5β-CHA/GC-NP (gray circle), FD70–5β-CHA/GC-NP (black circle), and insulin–5β-CHA/GC-NP (black triangle). Data are the mean ± SE ($n = 3$)

insulin–5β-CHA/GC-NP to rats. However, when insulin was administered alone, it did not lead to the drop in blood glucose levels during the experimental period.

Some pharmacokinetic and pharmacodynamic parameters of insulin and insulin–5β-CHA/GC-NP are summarized in Table III. As indicated in Table III, 5β-CHA/GC-NPs significantly enhanced AUC, AAC, bioavailability (BA)%, and PA% of insulin as contrasted with the control, suggesting that 5β-CHA/GC-NPs could increase the absorption of insulin in all regions. The absorption enhancement ratio of insulin in different sites in the order from large to small was as follows: jejunum > ileum > colon.

Mucosal Toxicity of 5β-CHA/GC-NPs

The intestinal membrane toxicity of 5β-CHA/GC-NPs was examined after the jejunal administration of 5β-CHA/GC-NPs. As shown in Fig. 4, a significant increase was seen in the total protein and the activity of LDH for Triton X-100 (3%, *v*/*v*) as a positive control. However, 5β-CHA/GC-NPs (20 mg/ml) did not result in an increase of the toxicity effect in contrast to the control. From the results, it could be

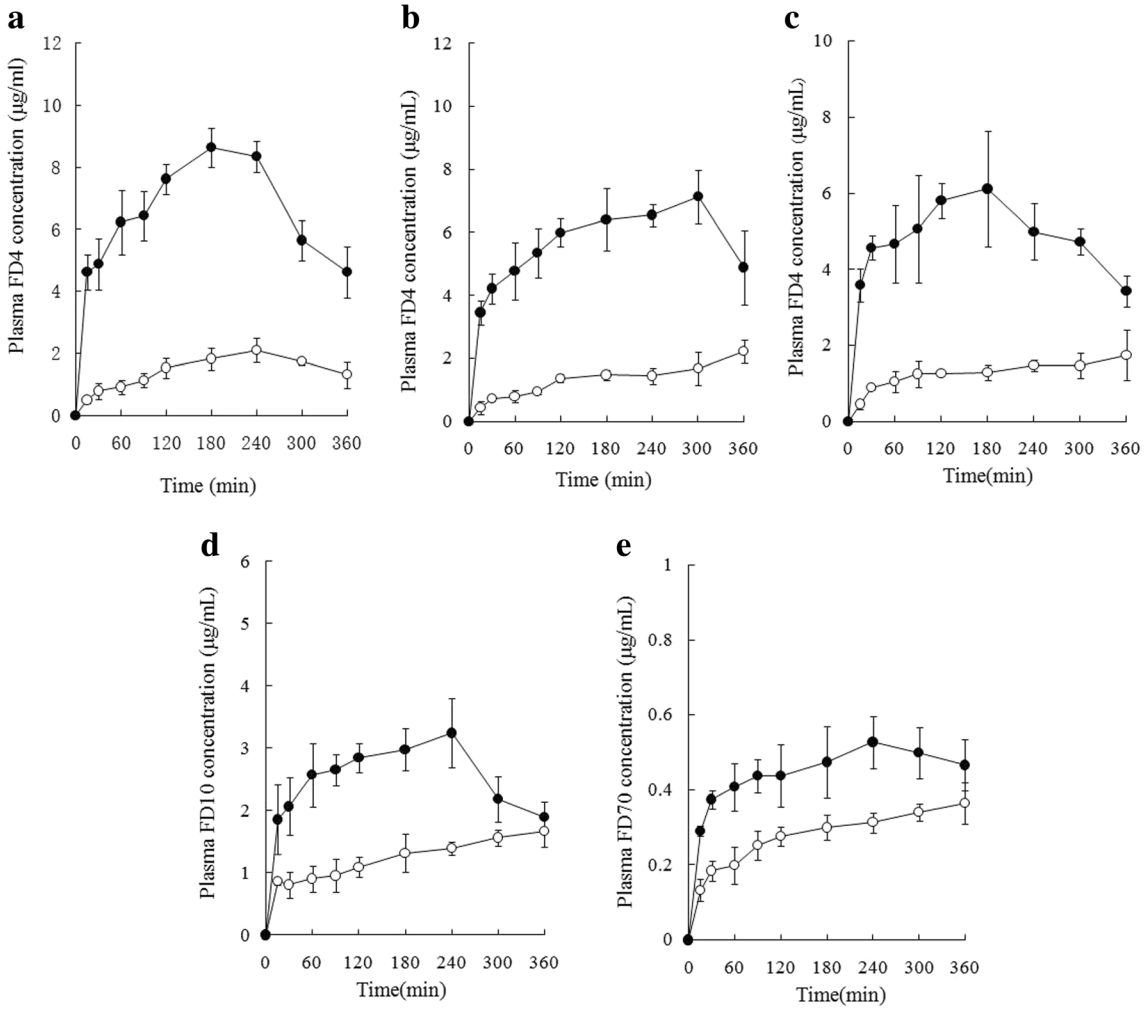

Fig. 2. Plasma concentrations of FDs after administration of FD solution and FDs–5β-CHA/**GC-**NP into rat intestines: **a** FD4 in the jejunum, **b** FD4 in the ileum, **c** FD4 in the colon, **d** FD10 in the jejunum, and **e** FD70 in the jejunum. Data are the mean ± SE of three to five determinations. Key: control (white circle); FDs–5β-CHA/GC-NP (black circle)

Table II. Pharmacokinetic Parameters of FDs After Administration of FD Solution and FDs–5β-CHA/GC-NP to Different Intestinal Regions

	Jejunum		Ileum		Colon	
	AUC_{0-360} (μg min/ml)	Ratio	AUC_{0-360} (μg min/ml)	Ratio	AUC_{0-360} (μg min/ml)	Ratio
FD4						
Control	533.6 ± 98.9	1.0	481.2 ± 63.5	1.0	459.7 ± 75.2	1.0
FD4–5β-CHA/GC-NP	2396.2 ± 235.6**	4.5	2073.6 ± 213.6**	4.3	1763.0 ± 141.4**	3.8
FD10						
Control	443.4 ± 58.5	1.0	448.2 ± 84.5	1.0	426.5 ± 61.3	1.0
FD10–5β-CHA/GC-NP	920.8 ± 142.4**	2.1	818.0 ± 140.3**	1.8	696.2 ± 121.6**	1.6
FD70						
Control	100.3 ± 11.6	1.0	108.3 ± 14.0	1.0	87.6 ± 10.6	1.0
FD70–5β-CHA/GC-NP	161.8 ± 9.8**	1.6	162.7 ± 22.1**	1.5	123.9 ± 15.2**	1.4

Data are the mean ± SE of three to five determinations
**$p < 0.01$, *$p < 0.05$

concluded that the 5β-CHA/GC-NPs were less toxic, which did not lead to any membrane damage to the jejunum.

DISCUSSION

It was reported that 5β-cholanic acid-modified glycol chitosan spontaneously formed nano-sized self-aggregates consisting of a hydrophobic inner core and a hydrophilic outer shell, and various anticancer drugs could easily be entrapped in the hydrophobic inner cores (25). The goal of this research was to assess the absorption-enhancing effect of 5β-CHA/GC-NPs on the drugs with poor absorption in the intestine. In this work, insulin and FDs loaded with 5β-CHA/GC-NPs were obtained by the dialysis method. As observed from Table III, 5β-CHA/GC-NPs exhibited a mean particle size in the range from 202.4 to 239.4 nm and positive zeta potential values (from 11.3 to 52.2 mV). Further, their PdI values were all below 0.09. These results indicated that the self-assembled nanoparticles were spontaneously formed, which have a narrow size distribution. The physicochemical properties were in good agreement with previous studies (18,19,27,28). It was found that insulin–5β-CHA/GC-NPs have a surface charge of 11.3 ± 2.9 mV and an average size of 202.4 ± 9 nm, respectively. For the plain particles, the surface charge and the average size were 52.2 ± 4.6 mV and 214.0 ± 17 nm, respectively. The insulin-encapsulated 5β-CHA/GC-NP showed a smaller size compared to those of plain particles. The reduction of insulin–5β-CHA/GC-NP in diameter and surface charge could be due to the ionic interaction between negatively charged insulin and positively charged 5β-CHA/GC-NPs.

The physicochemical properties of nanoparticulates are an important factor in promoting the transmucosal absorption of macromolecular drugs. The uptake by intestinal cells and

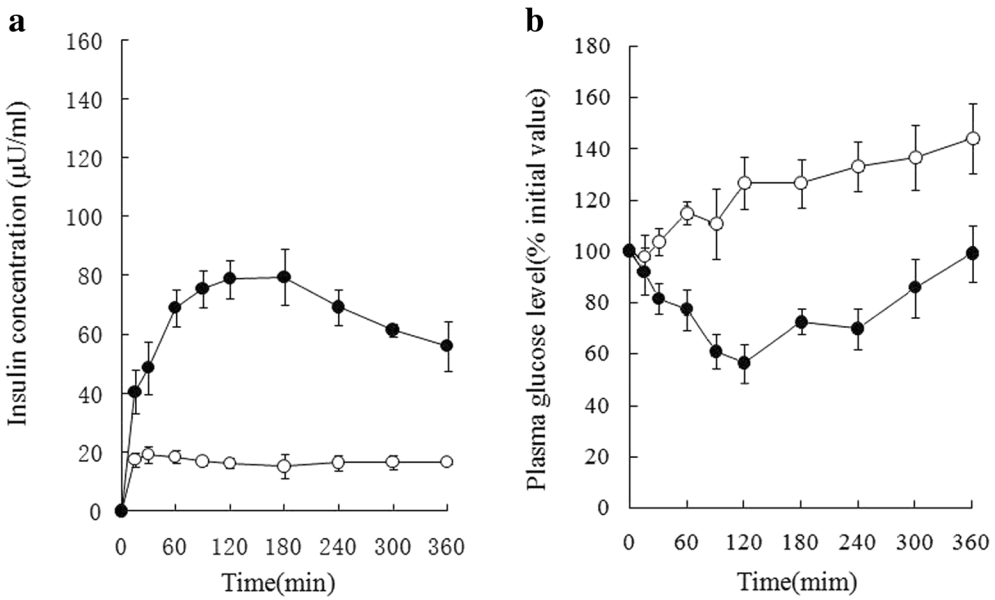

Fig. 3. Plasma concentrations of insulin and glucose after administration of insulin solution and insulin–5β-CHA/GC-NP to the jejunum: **a** insulin and **b** glucose. Data are the mean ± SE of three to five determinations. Key: control (white circle); insulin–5β-CHA/GC-NP (black circle)

Table III. Pharmacokinetic Parameters of Insulin After Intestinal Administration of Insulin Solution and Insulin–5β-CHA/GC-NP to Rats

	$AUC_{(0–360)}$ (μU min/ml)	BA (%)	AAC (%glu.reduc.min)	PA (%)
Jejunum				
Control	5899.2 ± 524.0	14.7 ± 1.3	0.0 ± 0.0	0.0
Insulin–5β-CHA/GC-NP	23,926.5 ± 2166.4**	59.8 ± 5.4	8654.9 ± 1881.6	36.1 ± 7.8
Ileum				
Control	5328.2 ± 493.2	13.3 ± 1.2	0.0 ± 0.0	0.0
Insulin–5β-CHA/GC-NP	19,867.4 ± 1459.6**	49.7 ± 3.6	8050.7 ± 1070.6	33.5 ± 4.5
Colon				
Control	5164.0 ± 437.4	12.9 ± 1.1	0.0 ± 0.0	0.0
Insulin–5β-CHA/GC-NP	15,711.3 ± 1816.2**	39.3 ± 4.5	7780.2 ± 1266.4	32.4 ± 5.3

Data are the mean ± SE of three to five determinations
$^{**}p < 0.01$, $^{*}p < 0.05$

the permeability of nanoparticles were influenced by particle size and surface properties (21). It was reported that the smaller size nanoparticles more efficiently penetrated to the mucous layer (29), and the positively charged nanoparticles could lead to greater mucoadhesion to the mucus layer (30). As we have demonstrated above, it can be deduced that 5β-CHA/GC-NPs had an excellent mucoadhesive property and permeability to the mucous layer, because of their smaller size and positive charge.

In the present study, the model drugs loaded with 5β-CHA/GC-NPs with small size and marked positive charge were prepared as shown in Table I. The EE and LC of insulin–5β-CHA/GC-NP were 87.4 and 24.8%, respectively. 5β-CHA/GC-NPs displayed an excellent capacity for the association of insulin, due to electrostatic interactions between the peptide drugs and nanoparticles (31). The results corresponded to those of preliminary studies reported (32). However, the FD loading capacities were much lower, and this was possibly attributed to its hydrophilic nature and macromolecule. Table I shows the EE and LC of FDs in 5β-CHA/GC-NPs that ranged from 25.6 to 78.3% and from 7.9 to 19.7%. The results indicated that the association efficiency and loading capacity gradually decrease as the molecular weight of FDs increases. These small, uniformly sized particles with marked positive charge obtained by us were suitable for the delivery of poorly absorbable drugs across the intestinal mucosa.

The drugs' *in vitro* release behavior from 5β-CHA/GC-NPs are shown in Fig. 1. All drugs loaded with 5β-CHA/GC-NPs exhibited quite similar release patterns, which were composed of a fast burst release and then a slow release. These release profiles conferred an increase in release rates compared to those of the previous studies (18,19,27). It can be due to the fact that these hydrophilic macromolecule and protein molecules were loosely bound to 5β-CHA/GC-NPs. The initial fast release was because of swelling of the polymer, creating pores, or diffusion of the drug from the surface of the 5β-CHA/GC-NPs (33). Then, the sustained release of drugs from the nanoparticles was maintained for 6 h continuously though drug diffusion from the inner core or the degradation of 5β-CHA/GC-NPs (34).

A slight difference was found between these release profiles, and the release rate of insulin was slightly slow compared with FDs, and it was possibly because insulin can

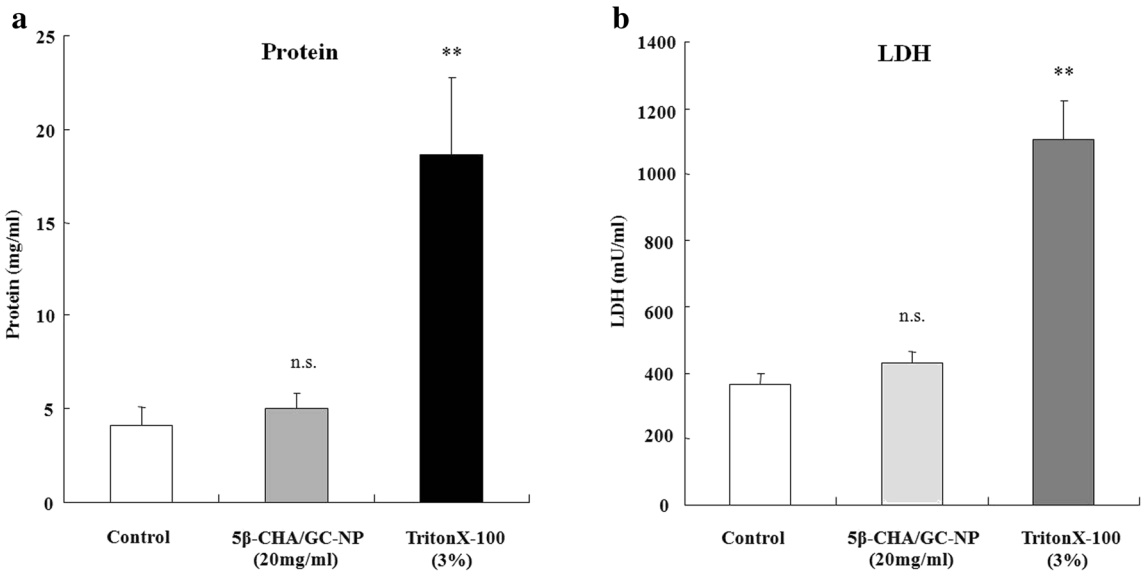

Fig. 4. The total protein (**a**) and the activity of LDH (**b**) at 6 h after the jejunal administration of 5β-CHA/GC-NPs. Data are the mean ± SE of three to five determinations, $^{**}p < 0.01$, $^{*}p < 0.05$

be incorporated into the hydrophobic inner core of the self-assembled 5β-CHA/GC-NP by hydrophobic and ionic interactions leading to form a more compact nanoparticle (19).

It has been generally believed that peptide drugs hardly were absorbed across the intestinal mucosa, owing to their low permeability and high metabolism. However, a remarkable decrease in blood glucose, especially in the jejunum, was displayed after 30 min following administration of the insulin–5β-CHA/GC-NP into different intestinal loops, while no decrease was examined in the control (Table III and Fig. 3). In these tests, the decrease in blood glucose level lasted for at least 6 h. This prolonged hypoglycemic effect of 5β-CHA/GC-NPs might be attributed to its physicochemical properties. The results indicated that insulin released from 5β-CHA/GC-NPs cannot be degraded by enzymes in the process of intestinal absorption (12,35).

In an additional test, FD–5β-CHA/GC-NPs, with the control, were added into different intestinal loops to prove the effect of 5β-CHA/GC-NPs on the absorption of macromolecule drugs in rat intestine. It could be seen from the concentration–time curves of FD4 in Fig. 2a–c that the high concentrations of FD4 lasted a long time in plasma after administration of FD4–5β-CHA/GC-NP into different intestinal loops, which confirmed the prolonged effect of 5β-CHA/GC-NPs in the intestine, compared to the FD4 solution. It is plausible that 5β-CHA/GC-NPs increased the affinity to adhere to the intestinal mucosa, allowing FD4 to be absorbed more easily *via* the rat intestine. The same result was obtained in the jejunum when using FD10 and FD70 loaded with 5β-CHA/GC-NPs (Fig. 2d, e).These results suggested that 5β-CHA/GC-NPs as a drug carrier could lead to retention of the drug lasting for a long time at the mucus layer. Meanwhile, the entrapped peptide drug might be avoided from various degradation before they are absorbed. Overall, the adsorption studies demonstrated that 5β-CHA/GC-NPs had an outstanding enhancement effect for increasing the absorption of insulin and FDs in the rat intestine, due to their bioadhesive potential and penetration.

It was a usual method that the mucosal toxicity of 5β-CHA/GC-NPs was analyzed by examining the protein and LDH released from the intestinal mucosa (36). As shown in Fig. 4, a significant increase in the total protein and the activity of LDH was caused by the positive control of Triton X-100 (3%, *v/v*). However, 5β-CHA/GC-NPs (at the largest dose of 20 mg/ml of administration) did not enhance the toxicity effect in contrast to the control. These findings suggested that 5β-CHA/GC-NPs (20 mg/ml) did not lead to any membrane damage to the jejunum, which was less toxic and safe.

CONCLUSION

The goal of this research was to assess the absorption-enhancing effect of 5β-CHA/GC-NPs on drugs with poor absorption in the intestine by the method of *in situ* closed loop. In this work, insulin and FDs loaded with 5β-CHA/GC-NPs were obtained by the dialysis method. The results indicated that 5β-CHA/GC-NPs exhibited a mean particle size in the range from 202.4 to 239.4 nm with narrow size distribution and positive zeta potential values (from 11.3 to 52.2 mV), having EE and LC values that ranged from 25.6 to 87.4% and from 7.9 to 24.8%, respectively. *In vitro* release data showed that the release profile was composed of a fast burst release and then a slow release, and the extent of release reached approximately 90% at 6 h. The results of intestinal absorption showed that 5β-CHA/GC-NPs markedly increased the absorption of insulin and FDs in the jejunum, ileum, and colon. The ratios of absorption for all the drugs in the jejunum were higher than those in the ileum and colon. In addition, the enhancing effect of 5β-CHA/GC-NPs for the absorption of FDs from the jejunum was decreased with increasing molecular weights. In the toxicity test, 5β-CHA/GC-NPs did not significantly increase the release of protein and the activities of LDH, indicating that the nanoparticles did not cause any membrane damage to the intestine.

The research suggested that 5β-CHA/GC-NPs with small size and marked positive charge were useful carriers for poorly absorbable drug delivery, which can effectively enhance the absorption of peptides and macromolecules by intestinal membranes without any mucosal toxicity to the intestinal tissues. 5β-CHA/GC-NPs could be used as an effective yet safe drug carrier for the improvement of the intestinal absorption of a poorly absorbable drug.

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