Oligoarginine-modified Biodegradable Nanoparticles: A Novel Delivery Carrier for Oral Administration of Insulin

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ABSTRACT SUMMARY

Cell-penetrating peptides-functionalized biodegradable nanoparticles were devised as a carrier for oral delivery of insulin. With poly(arginine)₈ (R8) modification, the nanoparticles were endowed with improved cellular transportation ability and enhanced insulin absorption via intestinal administration. Compared to L-form R8, its D-form enantiomer exhibited superior absorption both in vitro and in vivo.

INTRODUCTION

Incorporating bioactive macromolecules into nanoparticles could protect them from enzymatic degradation in the harsh environment of the gastrointestinal tract. However, plain nanoparticles remain unsatisfactory in their transduction efficiency. Thus, a more efficient strategy is imperative to facilitate the oral absorption of therapeutic macromolecules.

Cell-penetrating peptides (CPPs) are a class of transporters with a significant capability for membrane translocation, and they have been employed for the delivery of a wide variety of cargoes. Oral administration of macromolecules with the aid of CPPs is restricted to covalent linkage or electrostatic interaction between the cargo and CPPs.

In this study, we devised an approach using CPP-modified biodegradable nanoparticles to enhance the intestinal absorption of insulin (INS). Poly(lactide-co-glycolide acid) (PLGA) nanoparticles were covalently decorated with poly(arginine), enantiomers (L-R8 and D-R8) on their surface via polyethylene glycol (PEG) bridges. The R8-modified nanoparticles (R8-NPs) have the potential to deliver a wide variety of biomolecules, irrespective of the presence or absence of physicochemical interactions, and will not affect the bioactivity of the encapsulated macromolecules. The membrane-penetrating and absorption-enhancing capabilities of the R8-modified nanoparticles were evaluated. In addition, the effects of peptide conformation on ileal INS absorption were also discussed.

EXPERIMENTAL METHODS

A double emulsion and solvent evaporation process was used to produce plain PEG-PLGA nanoparticles loaded with coumarin-6 (C6-NPs) or INS (INS-NPs), respectively. L-R8 and D-R8 were separately modified on the nanoparticle surface via a maleimide-mediated covalent conjugation, which was confirmed by nuclear magnetic resonance, Fourier transform infrared spectroscopy and X-ray photoelectron spectroscopy. The morphology, mean diameter and zeta potential of the nanoparticles were also determined.

In the uptake experiments, after cultured in DMEM medium for 2 weeks, the Caco-2 cell monolayers were incubated with C6-NP or L-/D-R8-C6-NP (3% R8-modification, by mole) at 37°C for 1 h, and were visualized using a Leica DMI 4000B fluorescent microscope. To evaluate the transportation, Caco-2 cells were grown in Millipore transwell plates for 3 weeks, and the intactness of the monolayer was monitored by trans-epithelial electrical resistance. C6-NP or L-/D-R8-C6-NP were added on the apical side. The concentrations of C6 in basal chambers were measured using a Hitachi F-1000 fluorescence spectrophotometer.

Ileal loop absorption studies were performed with anesthetized rats. Dispersions of 1 mL INS-NP or L-/D-R8-INS-NP (10 IU INS/kg body weight, 50% R8-modification, by mole) were separately administered into a 10 cm ileal loop made from the pretreated segment. The relative bioavailabilities of the enterally administered nanoparticles were calculated in comparison with the subcutaneous injection of 0.5 mL INS solution to rats at a dose of 1 IU/kg body weight. A 0.5 mL aliquot of blood was collected from the tail tip at predetermined time intervals. The INS concentrations in plasma were determined by radioimmunoassay, and the blood glucose levels were determined by the glucose oxidase method.

RESULTS AND DISCUSSION

The nanoparticles were generally spherical with a uniform size about 200 nm (Fig. 1), on which surface R8 was successfully immobilized. As summarized in Table 1, surface decoration by R8 did not result in obvious changes in the physical properties of the nanoparticles, except for their zeta potentials.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Particle size (nm)</th>
<th>Polydispersity index</th>
<th>Zeta potential (mV)</th>
</tr>
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<tbody>
<tr>
<td>INS-NP</td>
<td>189.6±13.3</td>
<td>0.169±0.07</td>
<td>-34.0 ± 7.39</td>
</tr>
<tr>
<td>L-R8-INS-NP</td>
<td>196.8±16.4</td>
<td>0.188±0.03</td>
<td>14.7 ± 4.85</td>
</tr>
<tr>
<td>D-R8-INS-NP</td>
<td>200.1±15.6</td>
<td>0.200±0.01</td>
<td>15.3 ± 5.43</td>
</tr>
</tbody>
</table>

Table 1. Effects of surface modification by R8 on the characteristics of PEG-PLGA nanoparticles loaded with INS (mean ± S.D., n=3).
Fig. 2. Micrographs of the uptake of C6-NP (A), L-R8-C6-NP (B) and D-R8-C6-NP (C) by Caco-2 cells. The concentration of C6 in all nanoparticle suspensions was 100 ng/mL.

The fluorescent images in Fig. 2 show that R8-C6-NPs were internalized more efficiently by Caco-2 cells than unmodified C6-NP. As illustrated in Fig. 3, the cellular transportation percentage of 3% R8-modified nanoparticles was 2.95- (for L-R8) or 3.23- (for D-R8) times higher at 4 h than that of the unmodified nanoparticle at a low concentration (Fig. 3A), which was 1.31- (for L-R8) or 1.89- (for D-R8) times higher at a high concentration (Fig. 3B). Modification of the nanoparticles by R8 resulted in a significant improvement of transportation across the Caco-2 cell monolayer at the low concentration. The difference in the ability to promote transportation between L-R8 and D-R8 became more explicit with the increased concentration of the nanoparticles.

Fig. 3. Time course of apical-to-basolateral transportation of the nanoparticles across the transwell-grown Caco-2 cell monolayer at (A) low concentration (C6 200 ng/mL) and (B) high concentration (C6 1000 ng/mL).

In rats, more ileal absorption of insulin (Fig. 4A) correlated well with the lower blood glucose level (Fig. 4B). Minor insulin absorption and hypoglycemic effects were observed following the administration of unmodified INS-NP. In contrast, the absorption of insulin increased significantly after administered R8-INS-NP, and the resultant hypoglycemic effects became obvious at the same time. As shown in Fig. 4, insulin absorption increased more remarkable in the D-R8-INS-NP group than in the L-R8-INS-NP group. The relative bioavailabilities of insulin for INS-NP, L-R8-INS-NP and D-R8-INS-NP were 3.13%, 10.18%, 13.91%, and the pharmacological bioavailabilities were 3.02%, 7.56%, 11.49%, respectively.

The superiority of modifying the nanoparticles with oligoarginine was confirmed by the results of pharmacokinetic and pharmacodynamic studies. Compared to the unmodified nanoparticles, intestinal insulin absorption was substantially enhanced by the R8-modification (improved by 3.2-times for L-R8 and 4.4 times for D-R8), and more pronounced hypoglycemic effects were also observed (lowered by 2.5-times for L-R8 and 3.8-times for D-R8). The D-form R8 was more powerful in promoting insulin absorption than its L-form enantiomer. This finding is consistent with the results in cellular transportation study, and could be attributed to the different sensitivities of the two peptide conformations to enzymic degradation.

Fig. 4. Plasma insulin (A) and blood glucose (B) concentration profiles following in situ administration of 10 IU/kg INS-loaded nanoparticles into the ileal segments of rats; a 1 IU/kg INS solution administered via subcutaneous injection served as the control. Each data point represents the mean ± S.D. (n=4). *p<0.05 and **p<0.01 compared with the unmodified INS-NP.

CONCLUSION
Surface modification of biodegradable nanoparticles with polyarginine, especially with the D-form enantiomer, showed remarkable advancement in promoting the intestinal absorption of insulin. This delivery system is also promising for the delivery of a wide variety of bioactive macromolecules by oral administration.

REFERENCES

ACKNOWLEDGMENTS
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