The glucose-lowering potential of exenatide orally delivered via a goblet cell-targeting nanoparticles

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ABSTRACT SUMMARY
Exenatide is a synthetic version of exendin-4 which shares several glucoregulatory activities with the mammalian hormone, glucagon-like peptide-1 (GLP-1). In this study, a novel formulation of goblet cell-targeting nanoparticles for oral exenatide delivery was developed. Oral administration of exenatide nanoparticles to the db/db mice could reduce the blood glucose concentration by approximately 40%.

INTRODUCTION
Exendin-4 is an incretin mimetic found in the lizard saliva which is in the new class of antidiabetic agents. In clinical trails, exenatide exhibited glucoregulatory effects such as glucose-dependent stimulation of insulin secretion, suppression of inappropriately elevated glucagon secretion, and slowing of gastric emptying. So far, there isn’t any proper route of administration except subcutaneous (SC) injections which need to be given frequently and could cause pain. It is high time that new administration routes should be investigated.

The oral route is always considered to be the most convenient and comfortable means of drug administration for patients. Oral administration of exenatide would be beneficial to diabetic patients, as it not only alleviates the pain caused by injections, but can also mimic the physiological fate of insulin and may provide a better glucose homeostasis. The major problem faced in delivering a therapeutic peptide through the oral route is poor oral bioavailability due to incomplete and/or erratic absorption through the gastrointestinal tract (GIT), degradation of the drug or drug carriers due to varying pH of the stomach and enzymatic degradation. It has been reported that the use of colloidal carriers such as nanoparticles and nanocapsules can be a promising way to improve the oral bioavailability of peptides and proteins [1]. Chitosan has already been used in the form of nanoparticles and nanocapsules aimed at improving the transmucosal delivery of drugs through different mucosal surfaces [2]. In this study, we used the goblet cell-targeting peptide (CSK) connect to chitosan to prepare the nanoparticle.

EXPERIMENTAL METHODS
The nanoparticles were prepared using ionotropic gelation method. Briefly, exenatide was dissolved in distilled water (2mg/ml) and was added to the CSK peptide modified chitosan (CSK-chitosan) solution, and then, 2.5mg/ml tripolyphosphate (TPP) solution was added dropwisely to CSK-chitosan solution (1mg/ml) at a ratio of 0.15:1 under magnetic stirring. Then the solution was stirred at 500 rpm for 40min, and yielded an opalescent suspension which had obvious Tyndall phenomenon.

Characterization of NPs: the size and zeta potential of the drug loaded nanoparticles were characterized with a Zeta Potential/Particle Sizer (NICOMP™ 380ZLS, NICOMP).

Entrapment efficiency of drug loaded NPs: The amount of encapsulated exenatide was measured by sephadex G50 gel column. Briefly, a 4cm gel column was made by 5ml syringe with a little piece of filter paper blocked the exits and sephadex G50 was pre-swelled with boiled distilled water. The nanoparticles and the free exenatide have the different retention time and the nanoparticles will eluted at a faster speed because of the larger size. The eluted nanoparticles was damaged by adding an amount of acetic acid. The amount of exenatide in the nanoparticles was measured by a reverse-phase high performance liquid chromatography.
Pharmacological and hypoglycemic studies: the db/db mice were chosen as type II diabetes model and administrated with the following formulations: oral administration with physiological Saline (20ml/kg), chitosan NPs (30µg/kg), CSK-chitosan NPs (30µg/kg). Blood samples were collected from the tail veins of the mice prior to drug administration and at different time intervals (1, 2, 4, 6, 8, 10 and 12h) after dosing. And then determine the blood glucose with glucometer (ACCU-CHEK®). The total decrease (D%) of blood glucose were calculated using a modification formula as follow:

\[ D\% = \frac{[AUC(\text{physiological saline}) - AUC(test)]}{AUC(\text{physiological saline})} \times 100 \]

RESULTS AND DISCUSSION

The characteristics of blank and drug loaded NPs were shown in Fig.1. It was shown that both of the NPs made with chitosan and CSK-chitosan were uniform and the size were 120±20nm. The particles were nearly spherical and smooth.

Fig.1.TEM micrograph of the chitosan NPs and CSK-chitosan NPs

The pharmacological effects of chitosan NPs and CSK-chitosan NPs were evaluated on db/db mice after oral administration. As shown in Fig.2, both of chitosan NPs and CSK-chitosan NPs exhibited relatively strong hypoglycemic effects as compared with exenatide solution at 2h and 4h after administration. Moreover, with the modification of CSK peptide, CSK-chitosan NPs showed a better hypoglycemic effect with the blood glucose value reduced to 60% of the original. The chitosan NPs group and the physiological saline group of the mice exhibited stress hyperglycemia symptoms after administration of the drug and this phenomenon didn’t happen in the CSK-chitosan NPs group. Since the CSK-chitosan NPs had fewer irritation to the animals.

Fig.2.Blood glucose levels in db/db mice after administration of chitosan NPs (30µg/kg), CSK-chitosan NPs (30µg/kg) and physiological Saline (20ml/kg)

CONCLUSION

The exenatide nanoparticles which has glucose-lowering potential was successfully made and the hypoglycemic effects were obvious. The NPs were nearly spherical and uniform. CSK-chitosan NPs showed a better hypoglycemic effect with the blood glucose value reduced to 60% of the original.

REFERENCES


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