Preparation and Characterization of Nanocomplexes Based on Lithocholic acid-Modified Exendin-4 and Glycol Chitosan Bearing β-Cyclodextrin.

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Purpose

Exendin-4, a naturally occurring dipeptidyl peptidase-IV resistant GLP-1 analog, has emerged as a promising therapeutics for type 2 diabetics. However, the therapeutic efficacy of exendin-4 is hampered by its short biological half-life, thus requiring twice a day injections in the clinical settings. Although several methods, including PEGylation, have been developed to overcome this critical issue, the long-acting formulation of exendin-4 still remains an intriguing challenge. In recent years, supramolecular systems have gained significant attention for delivery of biotherapeutics. For example, lithocholic acid-modified exendin-4 (LAM_1-Ex4) remarkably increased duration of action, presumably due to the nanoparticlar formation and interactions with albumin. Based on the strong binding of lithocholic acid with β-cyclodextrin(β-CD), we envisaged that design of supramolecular nanoparticular systems based on β-CD could further prolong the duration of action. To realize our concept, in the present study we have synthesized glycol chitosan bearing β-CD(β-CDGC) conjugate and investigated their complexation behavior with LAM_1-Ex4.

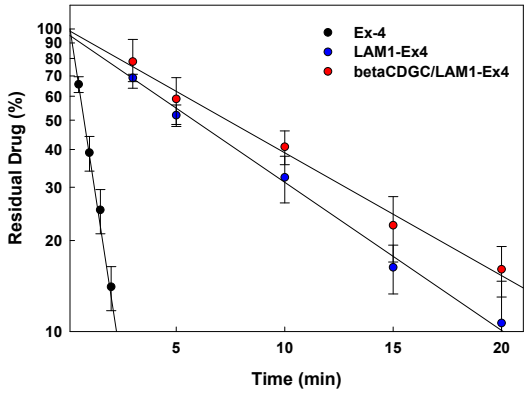
Methods

β-CD bearing glycol chitosan(β-CDGC) was prepared by the reaction with mono functionalized cyclodextrin and LAM_1-Ex4 (Lys^{27}-LA-Ex4) were obtained by reverse-phase HPLC separation. LAM_1 was physically encapsulated into β–CDGC nanoparticles by dialysis method. The physicochemical characteristics of the LAM_1-Ex4 loaded β-CDGC conjugate(LAM_1/β-CDGC) were examined by using transmission electron microscope (TEM), dynamic light scattering (DLS). The stability of LAM_1-Ex4 and LAM1/β-CDGC was studied in the presence of Trypsin at 37℃. Further, db/db mice were administered a single subcutaneous(S.C.) injection of LAM_1/β-CDGC nanocomplexes and blood glucose levels were monitored using a glucometer and tail-tip blood samples..

Results

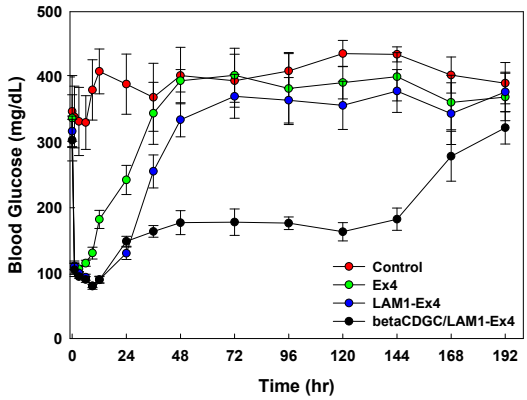
The size of the LAM_1-GC, LAM_1/αCDGC and LAM_1/β-CDGC nanocomplexes was found to be 655, 461 and 325 nm, respectively. This indicates that particle size of LAM_1/β-CDGC nanocomplexes can decreases by strong interaction between lithocholic acid and β-Cyclodextrin. The half-life of LAM_1/β-CDGC was increases 20% to 7.35min compared with LAM_1-Ex4.

Figure1. The stability of Exendin-4, LAM_1-Ex-4 and LAM_1/β-CDGC in presence of trypsin at 37℃.



Futher, the results obtained from phamacodynamics study indicated that the glucose lowering effect of LAM_1/β-CDGC nanocomplexes continued for a week *in vivo*. Calculated glucose AUC value also revealed that LAM_1/β-CDGC had greater antidiabetic effects than exendin-4.

Figure2. Pharmacodynamics characterizations of Exendin-4, LAM_1-Ex4 and LAM_1/β-CDGC after an S.C. injection (100nmol/kg, n=6).



Conclusions

We prepared nanocomplex system based on lithocholic acid-modified exendin-4 and glycol chitosan bearing β-cyclodextrin. This nanocomplex system might have a potential as long-acting therapeutics for type 2 diabetics.

References:

1) J Controlled Release 142 (2010) 206-213
2) J. Med. Chem. 2009, 52, 6889-6896
3) Biomaterials 31 (2010) 4121-4128
4) Tetrahedron Letter, Vol. 38, pp. 6171-6172, 1997