Lithocholic acid-Modifed Exendin-4 Loaded Glycol Chitosan Nanoparticle (HGC) for Efficient Protein Drug Delivery System

Sohee Son[1], Su Young Chae[1], Kwangmeyung Kim[2], Ick Chan Kwon[2], and Kang Choon Lee[1]

[1]College of Pharmacy, SungKyunKwan University, Suwon 440-746, Korea
[2]Biomedical Research Center, Korea Institute of Science and Technology, Seoul 136-791, Korea
kclee@skku.edu

Statement of Purpose:

The insulinotropic peptide of exendin-4 (Ex-4, GLP-1 analogue) has excellent therapeutic potentials in type 2 diabetes [1, 2]. However, the Ex-4 still does not free from fast glomerular filtration, and frequent injections (twice a day) are required for proper glycemic control. Therefore long-acting GLP-1 agonists have been widely investigated for incretin-based type 2 diabetic therapies [3].

To develop both long acting and enzymatic resistance Ex-4 based drug delivery systems, the long-acting lithocholic acid (LA)-conjugated Ex-4 derivative was physically entrapped into the hydrophobically modified glycol chitosan nanoparticles (HGC). Then, therapeutic potentials and efficacies of the novel drug delivery system were investigated *in vitro* and *in vivo*.

Methods:

Synthesis and purification of the LA-Ex-4

The LA modified Ex-4 were synthesized by coupling reaction of NHS-activated LA with epsilon amine groups in Ex-4. After reaction, the positional isomers of Lys^{12}, Lys^{27}, and Lys^{12,27}-LA modified Ex-4s were separated by RP-HPLC. Among them, Lys^{27}-LA-Ex-4 (LAM1-Ex4) was further studied for drug delivery application.

Preparation of LA-Ex-4 loaded HGC nanoparticles

The LA-Ex4 was loaded into HGC nanoparticles by using dialysis method .

Characterization of LA-Ex-4 loaded HGC nanoparticles

To evaluate the LA-Ex-4 loaded HGC nanoparticles, drug contents were measured by RP-HPLC, and the particle sizes were analyzed by dynamic light scattering.

Stability of LA-Ex-4 loaded HGC nanoparticles

To investigate the enzymatic stability, LA-Ex4 and LA-Ex4 loaded HGC nanoparticles were incubated with 2mM trypsin for 0, 1, 3, 5, 10, 20 min. After incubation, the quantitative analysis of LA-Ex4 was performed by RP-HPLC.

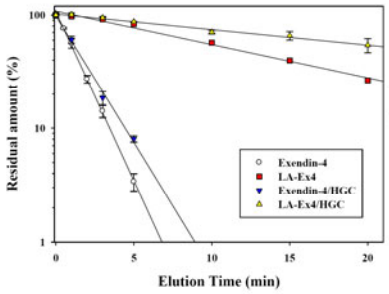
Results:

The LAEx-4 was successfully loaded into a HGC nanoparticle as shown in Table 1 (above 63% loading efficiency) and the LA-Ex4 loaded nanoparticles showed narrow size distrubition (about 270 nm).

Samples	Feed ratio (%, Drug/HGC)	Drug contents (%)	Loading efficiency (%)	Particle Size (nm)
HGC	-	-	-	361.4±52.9
HGC-Ex4	5.0	2.5	63.4	387.9±58.5
	10.0	6.0	75.2	360.9 ±60.6
HGC-LAEx4	5.0	3.7	77.6	283.7±42.0
	10.0	6.0	66.0	261.5±36.4

Table 1. loading efficiencies and particle size analysis of LA-Ex-4 loaded HGC nanoparticles.

The LA-Ex4 loaded HGC nanoparticles also showed different proteolytic stability. In a drug comparison, a LA-Ex4 showed about10 times increased degradation half - life than that of Ex-4, and the stability was further improved in LA-Ex4 loaded HGC nanoparticle (Figure 1).



Samples	$t_{1/2}$ (min)
Exendin-4	1.017±0.06
LA-Ex4	10.280±1.18
Exendin-4/HGC	1.356±0.03
LA-Ex4/HGC	22.408±4.17

Figure 1. Enzymatic stability of LA-Ex4 loaded HGC nanoparticle.

Conclusions:

The present study demonstrated the synthesis of LA-Ex-4s and its application for protein drug delivery system. The LA-Ex4 was successfully loaded into HGC nanoparticles and the drug loaded nanoparticles showed narrow size distribution presumably due to hydrophobic characteristics of LA-Ex4. Finally, the elevated enzymatic stability revealed that the LA-Ex4 loaded HGC nanoparticles have excellent potentials as a long-acting protein drug delivery system.

References

1. Deacon CF. *Diabetes* 2004:53: 2181- 2189
2. Gehmann HC, et al. *Endocrinology* 1992:130:159- 166
3. Rolin B, et al. *Am J Physiol Endocrinol Metab.* 2002: 283:E745