담즙산으로 개질한 키토산의 유전자 전달체로의 응용

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Bile Acids Modified Chitosan Oligosaccharides for Efficient Gene Delivery

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Introduction

Gene therapy has been considered as a powerful treatment to cure acquired or inherited diseases [1]. Owing to the macromolecular and anionic characters of genetic materials, efficient gene therapy requires the carrying materials (gene carriers) that can effectively internalize the genetic materials into somatic cells [2]. Although the limited and less-effective gene transfection efficiency than viral carriers remains unsolved, non-viral gene deliveries also have potentials for clinical applications in consideration of less immunogenic and non-oncogenic characters, unlimited gene size, and safety profiles [3].

Among the several natural and synthetic cationic materials, chitosan has unique advantages such as non-toxicity, biocompativility, and biodegradability for gene carrier [4]. However, the limited and low solubility on water or physiological solutions and low gene transfection efficiency have been located as crucial obstacles for the successful application of chitosan for gene delivery.

In this research, to address these obstacles, two strategies were employed to develop chitosan based gene carriers. To improve solubility, chitosan oligosaccharide with high water solubility and narrow molecular distribution (COS, MW 3000) was prepared and utilized for further modification. To enhance the gene transfection efficiency by facilitating cellular uptake, the COS was chemically modified with hydrophobic bile acids (BAs) with different hydrophobicities (cholic acid, deoxycholic acid, and lithocholic acid). Then the COSBAs were introduced into in vitro gene delivery systems and investigated their potentials for gene carriers.

Experimental methods

The COS with number average molecular weight of 3000 Da was prepared by ultrafiltration techniques with enzymatically depolymerized chitosan lactate (donated from Kittolife co. Korea). The COSBA were prepared by coupling reaction of primary amine group on COS with NHS-activated bile acids (cholic acid, deoxycholic acid, and lithocholic acid; 10 or 20 mole % of glucosamine unit).

The COSBA nanoparticles prepared by diafiltration method were further characterized by dynamic light scattering (DLS). The gene condensation and the condensed gene protection capacities of the COSBAs were investigated by gel-retardation assay and DNase I protection assay, respectively. Finally, COSBA/plasmid DNA complexes were introduced into *in vitro* gene delivery system with

HEK 293 cell line and their gene trasfection efficiencies and cytotoxicities were investigated.

Results and discussion

Due to the amphiphilic properties of the hydrophobized COS, the COSBA can self-associate to form micelle or micelle like self-aggregate in an aqueous environment. The mean diameters of COSBA nanoparticles measured by DLS instrument were in the range of 240 ~ 141 nm. Also, the COSBA series showed typical trends of particle size variation of decreased particle size by increasing hydrophobic contents, as shown in Table 1.

Gene condensation capacities of the COSBAs, investigated by gel retardation assay, revealed that the BA conjugation could slightly enhance gene condensation capacity, owing to the extra hydrophobic interaction between charge complex and hydrophobic BA moieties (Figure 1). Furthermore, the COSBAs more effectively protect the condensed plasmid DNA from endonuclease attack than unmodified COS (data not shown).

Although the COSBAs showed similar trends of nanoparticle formation and gene condensation, the gene transfection efficiencies were dramatically dependent upon kinds of BA and BA contents. In case of hydrophilic BA (cholic acid, 3 hydroxyl groups per molecule), enhanced gene transfection was achieved by increasing conjugation contest. However, deoxycholic acid (2 hydroxyl group) conjugated COSs (COS3DAs) showed the reverse trends. The lithocholic acid (1 hydroxyl group) conjugated COS showed negligible gene transfection independent with the degree of substitution (Figure 2). Dramatic enhancements of gene transfection were achieved by modulation of pH value of transfection medium. In case of COS3CA20 and COS3DA10, the gene transfection efficiencies after 4 h transfection at pH 6.5 were almost compatible with that of Lipofectamine, the one of the most powerful transfection agents.

Not only the high gene transfection efficiency, but the absence of noticeable cytotoxic effects of BA conjugated COS was investigated. As shown in Figure 3, BA conjugated COS showed around 100% relative viability while the PLL and the Lipofectamine showed 80% and 50% relative viability, respectively.

Table 1. Particle sizes of COS-BA nanoparticles.

Raw material[a]	Bile Acids[b]	Samples	d(nm)[c]
COS3	Cholic acid	COS3CA10	181.9±18.4
		COS3CA20	174.3±20.7
	Deoxycholic acid	COS3DA10	240.4±30.8
		COS3DA20	231.0±32.8
	Lithocholic acid	COS3LA10	215.8±27.4
		COS3LA20	141.2±16.9

[a] chitosan oligosaccharide with Mn of 3000 Da
[b] feed ratio per glucosamine unit
[c] mean diameter in water measured by DLS

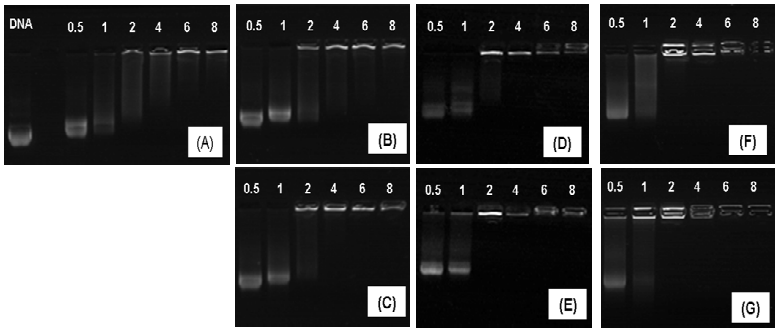

Figure 1. Gel retardation assay of COS3/ (A), COS3CA10/ (B), COS3CA20/ (C), COS3DA10/ (D), COS3DA20/ (E), COS3LA10/ (F), and COS3LA20/ (G) plasmid DNA complexes with various carrier/DNA weight ratios. Numbers indicate the carrier/DNA weight ratio.

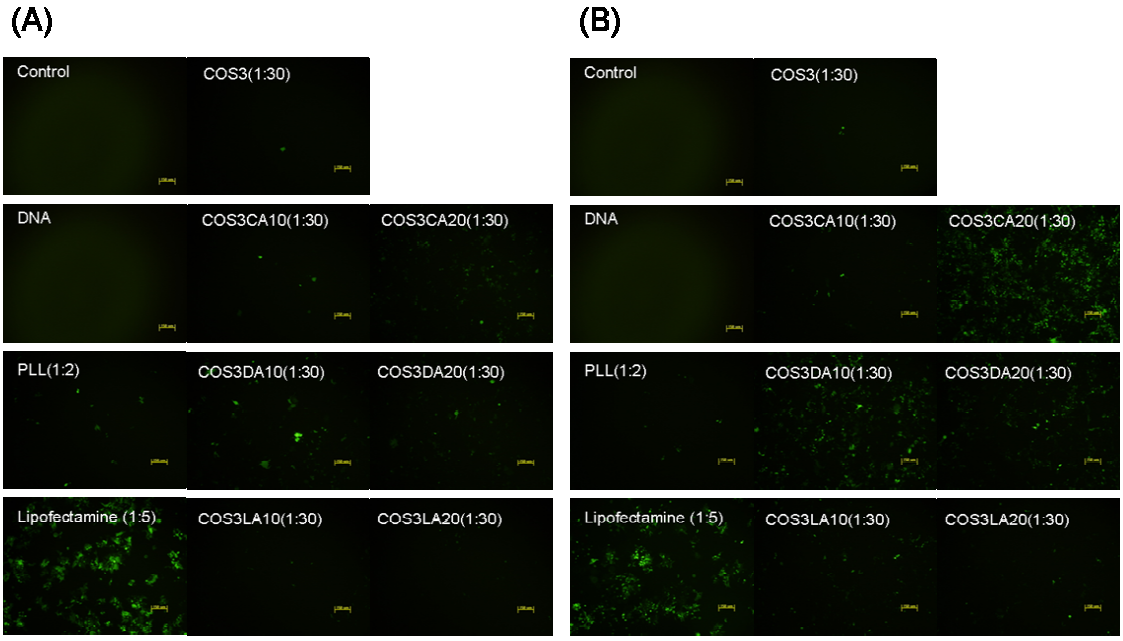

Figure 2. Fluorescence microscope images of GFP gene transfected HEK 293 cells after transfection for 48h at pH 7.4 (A), and pH 6.5 (B).

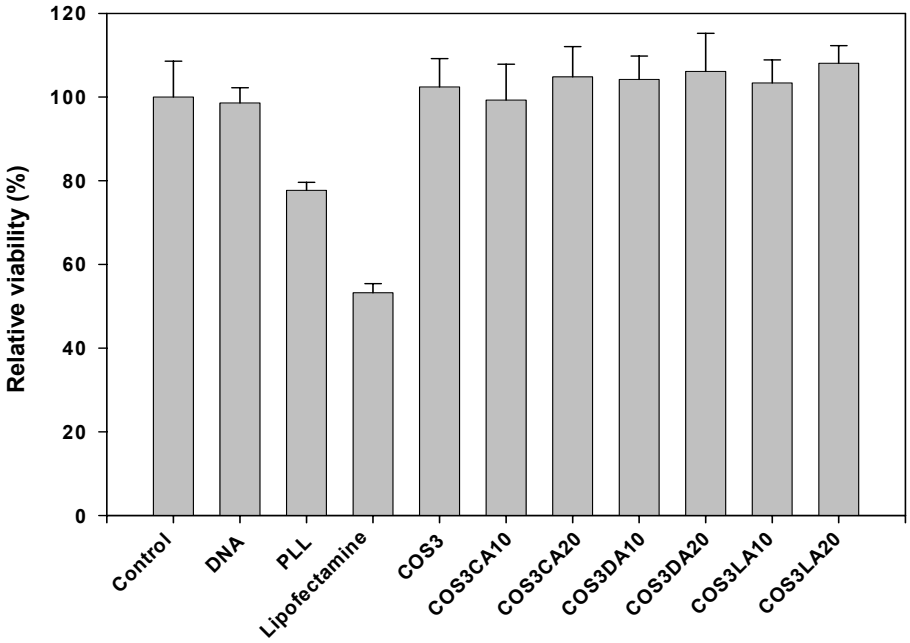

Figure 3. Cytotoxicity of COSBAs with negative control groups of PLL and Lipofectamine.

Conclusion

In this study, we demonstrated the efficacy of COSBAs on gene delivery system. The COSBAs showed great ability to form complexes with DNA and showed enhanced physicochemical properties compared with unmodified chitosans. Some of the COSBAs also showed superior gene transfer efficiency than unmodified chitosans and positive control goup of PLL and Lipofectamine. Therefore, bile acid-modified chitosan oligosaccharide can be considered for efficient gene condensing materials for gene delivery systems.

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