다양한 분자량을 가진 저분자량 수용성 키토산의 응용성

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The application of low molecular water soluble chitosan having various molecular weight

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Introduction

Actual application of genes to human therapy is limited by several problems, including their instability in body fluids, non-specificity to the desired cells, degradation by nucleases, and low transfection efficiency. Gene delivery systems have been investigated in attempt to enhance gene expression and reduce the cytotoxicity. In this point of view, chitosan has also been attractive gene carrier, because of its high positive charges and low toxicity to cells. Chitosan is a natural product, and its low toxicity was well-established[1]. Chitosan is a biodegradable polysaccharide composed of two subunits, D-glucosamin and N-acetyl-D-glucosamine linked together by (1,4)-glycosidic bonds. General lysozymes in the body degrade chitosan in to a common amino sugar, N-acetyl glucosamine, which is incorporated into the synthetic pathway of glycoproteins, and is subsequently excreted as carbon dioxide[2]. Chitosan was first described as a delivery system for plasmids by Mumper et al.[3]. So for, several gene delivery trials have been made with chitosan[4-6]. Oral gene delivery with chitosan-DNA nanoparticles was also tried[7]. However, these trials used relatively high molecular weight chitosan (HMWC, 70K Da) . It was hard for this chitosan to be soluble in water, and it was dissolved in acidic solution. Also water insoluble low molecular chitosan has not been reported. In this study, we prepared low molecular water-soluble chitosan (LMWSC)[8] having various molecular weight (<50K Da) and evaluate the potential as a gene carriers.

LMWSC is highly water soluble and can form complex with plasmids in physiological buffer due to the strong positive charge. The formation of plasmid/LMWSC complex was proved and transfection efficiency in 293T cells was evaluated. The cytotoxicity of LMWSC also was determined by MTT assay. This study showed that LMWSC is one of the candidates for DNA delivery.

Experimental

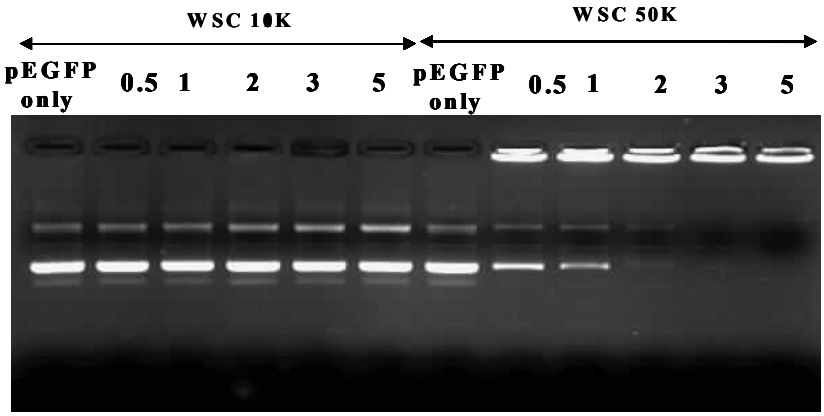
LMWSCs with molecular weight of 11K, 20K, and 50K prepared by previous study (9) were supplied by KITTOLIFE Co., Seoul, Korea. pSV-β-galactosidase plasmid (Promega, Madison, WI) was introduced into *Escherichia coli* strain DH5 (Gibco-BRI, Gaithersburg, MD), and purified by Qiagen Plasmids Maxi Kits (Qiagen, Valencia, CA). For gel retardation assay, plasmid/DNA complexes were electrophoreded on 1% (w/v) agarose gel for 60 minutes at 80 V. DNase I protection assay of the pSV-β-galactosidase/LMWSC complex was performed on a Perkin-Elmer Lambda 19 spectrophotometer. For the transfection studies, 293T cells, a human kidney cell line, were seeded at a density of 2×10^6 cells/dish in 100-mm culture dishes, and incubated for 24 hours before the addition of the plasmid/polymer complex.

Results and Discussion

LMWSCs having various molecular weight were evaluated as a gene carrier due to its strong positive charge with amine group at glucosamine unit. In the previous reports, it was already revealed that chitosan can form complex with plasmid DNA. However, the potential as a gene carrier for water-soluble low molecular weight chitosan was not revealed until now. In this study, we prepared LMWSC with free amine group by novel method and investigated the ability to complex DNA, transfection efficiency, and cytotoxicity etc.

Plasmid/LMWSCs complexes were analyzed in 1.0% agarose gel electrophoresis (Figure 1). The plasmid/LMWSC 20K and 50K were completely retarded above a 1:5, 1:1 weight ratio of plasmid:LMWSC, respectively. However, LMWSC 10K can't form complex with DNA because of low positive charge. The results suggest that LMWSC 20K and 50K were able to condense plasmid DNA into complex.

The stability in the presence of DNase I, endonucleases, is one of the essential parameters of systemic gene delivery. Therefore, DNase I protection assay was carried out (data not shown). As results, The absorbance of naked DNA or



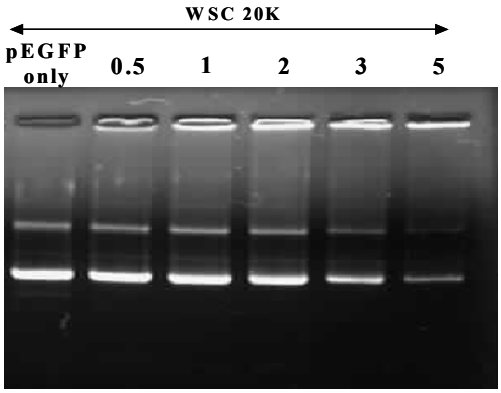


Figure 1. Gel retardation assay

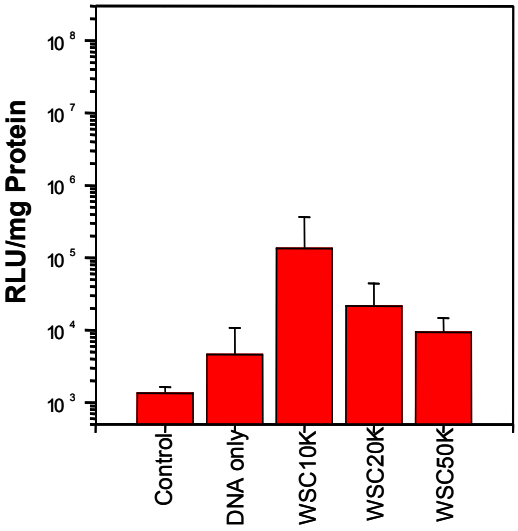


Figure 2. Transfection assay

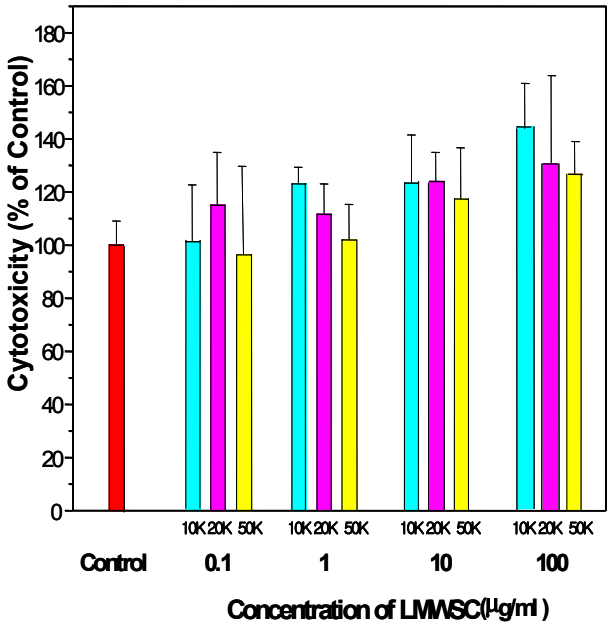


Figure 3. MTT assay

complexes at weight ratio of 1:0.5 increased in the course of DNase I reaction. However, the absorbance of complexes at weight ration of 1:1 or 1:2 were not increased by DNase I digestion. This means that plasmid was protected from DNase I at a ratio of 1:1 or 1:2.

We evaluated whether the LMWSC had an enhanced effect on plasmid delivery into the 293T cells. The efficiency of LMWSC mediated transfection to 293T cells was significantly higher than that of naked DNA. Also, *in vivo* it was revealed that the onset of gene expression using chtosan polyplexes was slower than that of PEI polyplexes, but the gene expression obtained with the chitosan formulation increased over time( ). MTT assay was performed to determine the cytotoxicity of LMWSC. Plasmid/LMWSC for 10K, 20K, and 50K showed negligible cytotoxicity for 293T cells (Figure 3).

Conclusion

To solve the poor solubility of chitosan having free amine group, we prepared LMWSC various molecular weight by novel method.. Generally, chitosan is a natural product and it is well established that the chitosan has low toxicity. In this study, LMWSC 10K, 20K and 50K used to reveal the ability as a gene carrier. As results, used all LMWSC shows negligible cytotoxicity for 293T cells. Also, LMWSCs are highly water-soluble and can effectively form complexes with plasmids in physiological buffer. From MTT assay, it was revealed that LMWSCs protected the DNA efficiently from DNase I. Finally, the efficiency of LMWSC mediated transfection to 293T cells was significantly higher than that of naked DNA.

Therefore, LMWSCs having free amine group will be useful in the development of safe gene carriers.

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