저분자량 수용성 키토산 나노입자와 레티노익산 복합제의 제조와 특성

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Preparation and Characterization of Low Molecular Water-Soluble Chitosan nanoparticles formed Complexes with All-Trans Retinoic Acid

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Introduction

Chitosan, one of the most plentiful biomass in nature, is a natural polymer derived from chitin by deacetylation and composed of (1,4)-linked 2-amino-2-deoxy-β-D-glucan. Since chitosan is already known as biocompatible, biodegradable, and non-toxic material, it is an interesting biomaterial for its drug carrying ability and ease of modification.[1] Furthermore, chitosan has been reported to enhance drug delivery across the nasal or mucosal layer without damage.[2,3] Despite its superiority as a biomaterial, chitosan is not fully soluble in water but soluble in acidic solution. Aqueous solubility of chitosan has been known to limit its application only in acidic solution to bioactive agents such as gene delivery carriers, peptide carriers, and drug carriers. Recently, wedeveloped water-soluble chitosan (WSC) with low molecular weight and free-amine group.[4,5] Since WSC is readily soluble in neutral aqueous solution, it has remarkable advantages such as ease of modification, usefulness as a gene or peptide drug carriers, and drug carriers.[6]

All-trans retinoic acid (atRA)is effective in the treatment of epithelial and hematological malignancies such as breast cancer, head and neck cancer, ovarian adenocarcinoma, and acute promyelocytic leukemia (APL).[7-9] However, some side effects such as retinoid acute resistance, hypertriglyceridermia, mucocutaneous dryness, and headache were reported in spite of its pronounced effects,[10-12] and they limit the clinical applications of atRA. The rapid decrease of half life of atRA with continuous oral administration or intravenous injection was reported by several authors.[13-14] Furthermore, very low aqueous solubility (0.1 μM at pH 7.3), photolability, and local-irritating reactions of atRA also limits its clinical application.[15,16] Various kinds of atRA formulation have been reported to overcome the side effects and solubility problem of atRA. Giordano et al.[17] reported that atRA-loaded microspheres are

effective in reducing the incidence of tractional retinal detachment by a sustained release of atRA. It was reported that liposome-encapsulated atRA showed higher serum tretinoin concentrations and maintained them longer rather than oral atRA, resulting that liposomal-atRA was found to be effective in diagnosingacute promyelocytic leukemia (APL).[18] Solid lipid nanoparticles are useful formulation in solving the poor aqueous solubility of atRA and able to use it by intravenous injection.[19] Ezpeleta et al.[20]reported that atRA was encapsulated into gliadin nanoparticles.

An alternative approach would be to make the complex between atRA and positively charged carrier. Thünemannand Beyermann[21] reported that polyethyleneimine (PEI) complexes with retinoic acid form nanoparticles for controlled release of atRA. They showed that a nanoparticulate complex between atRA and PEI has sizes ranging from 170 to 580 nm. In other reports,[22] they reported the presence of complexes between poly(ethylene oxide)-b-poly(L-lysine) and RA and formed core-shell type micelles.

For this study, we have studiedchitosan nanoparticles encapsulating atRA. WSC with free amine group has advantages for preparation of nanoparticles due to their aqueous solubility and chitosan nanoparticle could be formed polyelectrolyte complexes between free-amine group of WSC and carboxyl group of atRA. Formation of nanoparticles and their physicochemical properties were investigated using various analytical equipments ; TEM, DLS, and fourier-transform infrared (FT-IR) spectroscopy

Experimental

WSC with M.W. of 3,000 (3K), 5,000~10,000 (5K), 21,000 (21K), 50,000 (50K), 100,000 (100K) was kindly supplied by Kitto Life Co. Ltd. Korea. All-trans retinoic acid (atRA), acetone, and dimethylformamide (DMF) was purchased from Sigma Co. USA. WSC was dissolved in 10 ml of deionized water (0.5 %, w/w). To this solution, atRA dissolved in 1 ml of DMF was dropped with sonicaton using bar type sonicator and sonicated for 30 s (3 s X 10) at 40 W. After that, this solution introduced into dialysis tube (molecular weight cutoff (MWCO) : 2,000 g/mol) and then dialyzed against 1 L X 6 for 12 h. After that, dialyzed solution was used for analysis or lyophlilized. The particle size was monitored by dynamic light scattering method using electrophoretic light scattering spectrophotometer (ELS-8000, OTSUKA Electronics, Japan) at a fixedangle of 90^{o} and at room temperature.

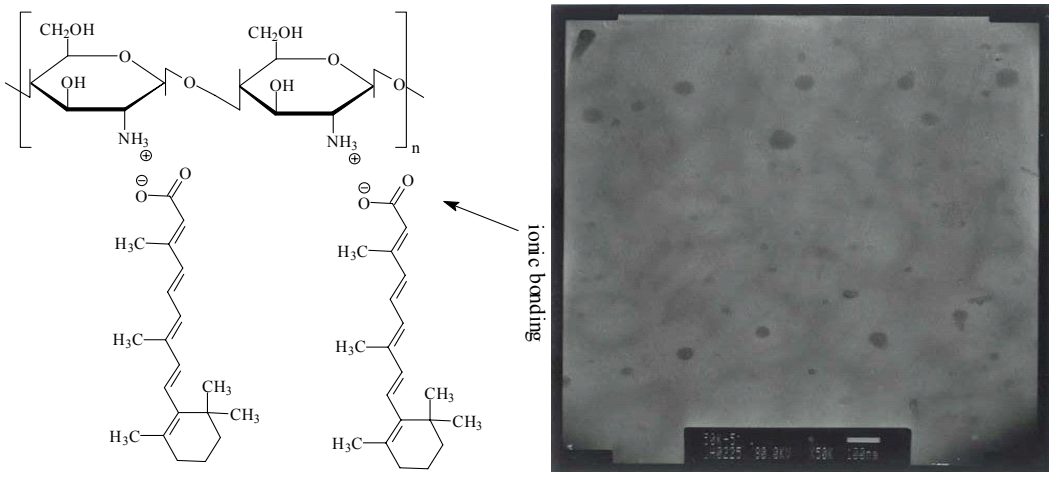
To transmission electron microscope(TEM) images, a drop of nanoparticles suspension was placed on a carbon film coated on a copper grid for TEM. The specimen on the copper grid was not stained. Observation was done at 80 kV in a JEOL JEM-2000 FX Ⅱ.

To measure drug contents and loading efficiency, volume of dialyzed chitosan-atRA aqueous solution was adjusted to 20 ml and 0.1 ml of this solution was diluted with 9.9 ml of acetone. Drug concentration was measured at 365 nm withUV spectrophotometer (UV-1601, Shimadzu Co., Japan). Chitosan aqueous solution (0.5 %, w/w) without atRA was used for blank test.

Result and discussion

To form polyelectrolyte complex with WSC and atRA, various M.W. of WSC were used and almost M.W. of WSC showed ability to form nanoparticulate polyelectrolyte complexes. Particle sizes of chitosan-drug polyelectrolyte complexes were 50 - 800 nm according to the

M.W. of chitosan and drug amount. Particle size of polyelectrolyte complex of chitosan and drug was not proportional to the increased M.W. of chitosan. Since particle size of chitosan polyelectrolyte complexes showed below 300 nm with 3K - 50 K M.W. of WSC, polyelectrolyte complex nanoparticles are acceptible for intravenous injection or oral administration. Furthermore, loading efficiency was over 60 % at all of the formulation.



(a) (b)

Figure 1. Scheme of nanoparticle formation based on polyelectrolytes complexes of chitosan and atRA (a) and TEM photo of polyelectrolyte complex nanoparticles of atRA and WSC (b). Bar = 100 nm.

Chitosan M.W.	Chitosan/atRA feeding ratio (mg/mg)	Drug contents (%, w/w)	Loading efficiency (%, w/w)	Particle size (nm)		Zeta potential (mV)	
				Wt conv.	No conv.		
3K	50/2	3.2	82.3	133.6±67.1	94.8±27.1	34.66	Nanoparticle
	50/5	8.5	93.4	191.0±58.2	158.6±36.2	26.44	Nanoparticle
	50/10	12.1	69.1	146.0±128.1	130.8±9.5	38.17	Precipitate
5K-10K	50/2	3.5	89.3	236.7±105.8	103.2±60.5	28.9	Nanoparticle
	50/5	8.2	89.0	191.0±103.0	118.9±41.1	28.99	Nanoparticle
	50/10	12.5	71.7	181.4±94.2	129.7±35.4	33.98	Precipitate
21K	50/2	3.0	78.5	158.3±58.0	124.0±31.4	47.11	Nanoparticle
	50/5	9.1	99.6	176.7±101.1	155.1±30.1	47.96	Nanoparticle
	50/10	12.7	73.0	236.0±88.9	107.3±71.6	48.3	Nanoparticle
50K	50/2	3.0	76.8	296.4±69.2	261.4±49.9	71.94	Nanoparticle
	50/5	6.9	73.8	289.6±60.9	260.7±46.2	71.76	Nanoparticle
	50/10	12.3	70.2	360.1±95.2	333.0±75.8	70.23	Nanoparticle

Table 1. Characterization of atRA-encapsulated chitosan nanoparticles

Conclusions

Nanoparticulate polyelectrolyte complexes between chitosan and drug were successively formed through ultrasonication and particle size was ranged about 50 - 800 nm. Morphology

of chitosan polyion complex nanoparticles observed with transmission electron microscope (TEM) was spherical shapes. Loading efficiency of drug into chitosan polyelectrolyte complexes was over 40 % (w/w). This drug loading efficiency was higher than other types of drug carriers such as liposomes, conventional nanoparticles, and lipid nanoparticles.

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