Release Characteristics of Cisplatin Chitosan Microspheres and Effect of Containing Chitin

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To increase cisplatin (CDDP) content, to suppress burst effect during the initial phase of drug release, and to improve the capacity of the system for sustained release, we prepared various types of CDDP chitosan microspheres incorporating chitin and investigated the content of CDDP and its *in vitro* release kinetics from these microspheres. The results of this study showed that the CDDP content increased with increasing chitosan concentration and that the incorporation of chitin in the carrier matrix produced a more pronounced increase in drug content.

The addition of chitin also led to inhibition of the initial burst effect. The rate of CDDP release reduced with increasing concentration of chitosan: that is, the 50% CDDP release time was about 0.5 h with the microspheres prepared with 1.0% of chitosan and about 4.5 h with those prepared with 5.0% of chitosan, indicating about nine-fold prolongation.

The addition of chitin further resulted in retardation of the rate of CDDP release. Meanwhile, our chitosan microspheres were shown to undergo enzymatic degradation by lysozymes.

Keywords cisplatin; chitosan microsphere; chitosan; chitin

In recent years, hepatic arterial chemo-embolization has been widely used as an effective therapeutic modality for hepatocellular carcinoma, and, to produce higher therapeutic efficacy, various embolizing materials and injection methods have been extensively investigated.[1-3] Macromolecular substances such as albumin, starch, and polylactic acid are candidates for useful embolizing materials.[4-7] In previous studies,[8,9] we prepared cisplatin (CDDP) microspheres using albumin as the carrier matrix and investigated the CDDP content and release kinetics from this system.

Chitin and chitosan, biodegradable macromolecules, contributed to the increase in CDDP content and to better control of drug release. Albumin microspheres, however, have some drawbacks, including difficulty in loading a greater amount of CDDP in the matrix and poor control of the burst effect during the initial phase of drug release. To overcome these shortcomings, we prepared CDDP chitosan microspheres using chitin and chitosan which possess immunoadjuvant and antitumor activities.[10] In the present study, the internal and surface morphologies of the microspheres, CDDP content, *in vitro* kinetics of drug release, and enzymatic degradation of the microspheres by lysozymes were investigated.

Experimental

Reagents CDDP powder was kindly supplied by Nippon Kayaku Co. In addition, the following agents were employed: chitosan (degree of deacetylation, 70%), chitin, and soya bean oil (Nakarai Tesque Co., Ltd.), and lysozyme (Sigma Co., Ltd.). All other reagents employed were commercial special-grade products.

Preparation of CDDP Chitosan Microspheres and Chitin-Containing CDDP Chitosan Microspheres To 2 ml of an acetic acid solution (5%) of chitosan, 100 mg CDDP powder was added and mixed well. To this mixture 20 ml of bean oil was added. This was emulsified according to the method of preparation for w/o emulsion and, hardened with glutaraldehyde. The product was washed with methanol, air-dried at 50 °C for 2 h, and then was sieved into grades (74—177 μm). Chitin-containing CDDP chitosan microspheres were prepared in the same way with CDDP and various concentrations of chitin dispersed in an acetic acid solution (5%) of chitosan.

CDDP Content According to the method described in the previous report,[8] weighed quantity of each type of microsphere was added to 5 ml of normal saline and homogenized, and the CDDP content in the chitosan microspheres was measured by an atomic absorption spectrophotometer (Hitachi Z-9000).

Observation of CDDP Dispersion by an Electron Microscope Equipped with an Energy Dispersive X-Ray Microprobe CDDP dispersion in each type of chitosan microsphere was examined with an electron microscope equipped with an energy dispersive X-ray microprobe (Jeol JEM-1200EX, Link 860-500J).

CDDP Release Test The test was conducted as described in the previous report.[8] Normal saline (100 ml) as a release solution was placed in a release cell, which was immersed in a thermostated tank maintained at 37 °C. A cell (nitrate cellulose membrane, pore size 3 μm) containing a suitable amount of the sample was immersed in this tank. The contents of the cell were stirred (50 rpm) with a stirring rod, and aliquots of the solution were serially taken. The CDDP content in the release solution was measured by atomic absorption spectrophotometry.

Effect of Lysozyme on Microspheres Lysozyme was added to normal saline at a concentration of 1.5×10^{-2} mg/ml. The CDDP released from the microspheres into the solution was measured by atomic absorption spectrophotometry, and changes in the form of the microspheres were examined by microscopy.

Results

CDDP Content and The Effect of Chitosan and Chitin Concentration The effects of chitosan and chitin concentrations on the CDDP content and yield are shown in Table I.

TABLE I. Effect of Chitin and Chitosan on CDDP Content and Yield

Concentration of chitosan (%)	Chitin added (%)	Content of CDDP (μg Pt/mg)	Yield (%)[a]
1.0	0	82.43	70.3
	1.0	101.25	84.5
	1.5	132.84	89.6
2.0	0	94.53	78.4
	1.0	123.80	86.2
	1.5	150.32	93.6
3.0	0	108.52	80.1
	1.0	143.26	89.8
	1.5	162.41	94.8
5.0	0	121.32	82.5
	1.0	152.64	93.1
	1.5	179.82	96.9

($n=5$)

a) Recovered weight of microspheres per total weight of CDDP, chitin and chitosan employed.

The CDDP content was greatly influenced by the content-ratios of chitosan and chitin. The CDDP content increased by about 1.5-fold to 121.32 μg Pt/mg when the concentration of chitosan was increased from 1.0% to 5.0%. The addition of chitin led to further increases in the CDDP content: at a constant chitosan concentration of 1.0%, the CDDP content was 82.43 μg Pt/mg with the formulations containing chitin at 1.0% and 1.5%, respectively. The CDDP yield increased markedly with the addition of chitosan and chitin at increasing concentrations.

Observation of CDDP Dispersion in the Microspheres by X-Ray Microprobe Photograph The CDDP dispersion patterns in the chitosan microspheres are shown in Fig. 1 (chitosan was used at a concentration of 2.0%). (A) is the surface appearance and (B) the cross sections of the microspheres. On the microsphere surface, the CDDP dispersion was inhomogeneous with spots of highly concentrated CDDP.

Inside the carrier matrix, meanwhile, CDDP was ascertained to be uniformly dispersed.

CDDP Release from Chitosan Microspheres The CDDP release profiles from chitosan microspheres are shown in Fig. 2. The time required for 50% release was markedly prolonged from about 0.4 h to about 4.5 h when the chitosan concentration was increased from 1.0% to 5.0%. The CDDP release rate was greatly modified by the chitosan concentration.

CDDP Release from Chitosan Microspheres Containing Chitin The CDDP release profiles from chitosan microspheres at various chitin concentrations are shown in Fig. 3. The higher the chitin concentration, the slower the CDDP release rate: at the constant chitosan concentration of 2.0%, the time required for 50% release was about 1.0 h in the absence of chitin, but was markedly prolonged up to about 2.6 and 5.5 h at 1.0% and 1.5% of chitin, respectively. The results obtained with other formulations containing different concentrations of chitosan were consistent with

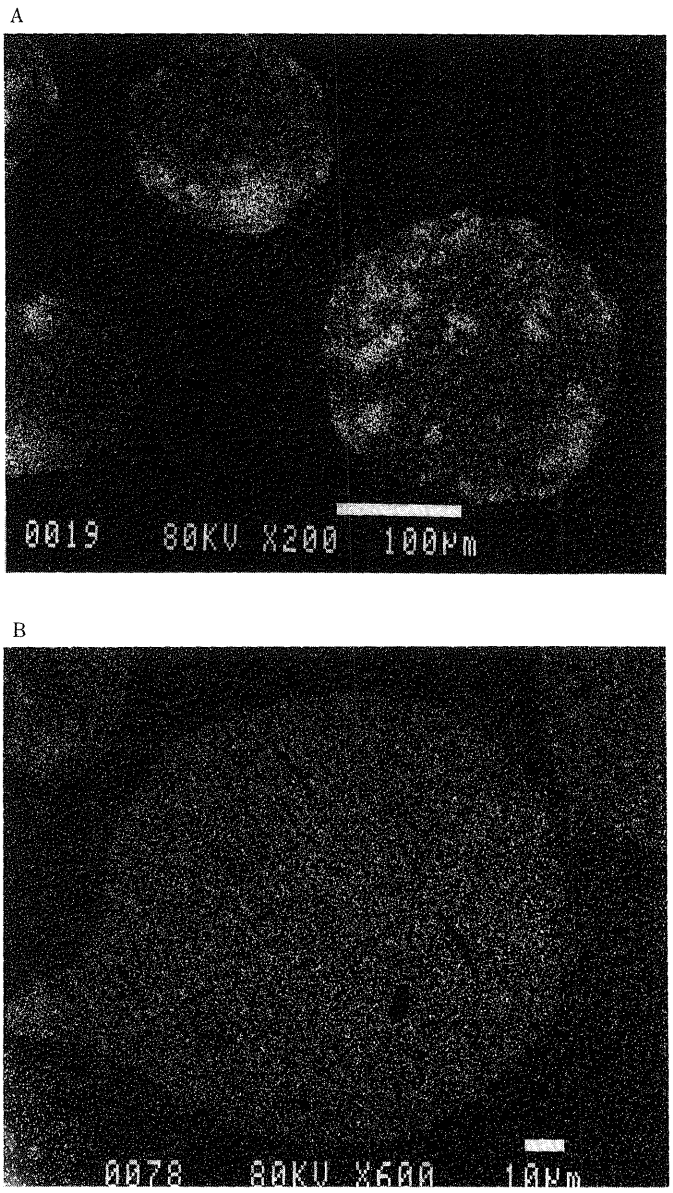

Fig. 1. X-Ray Microprobe Photographs of CDDP Chitosan Microspheres

(A) surface, (B) cross section.

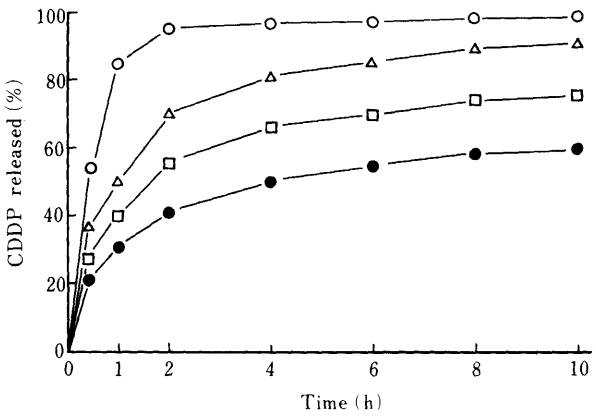

Fig. 2. Release of CDDP from Chitosan Microspheres Using Different Concentrations of Chitosan

Concentration of chitosan: ○, 1.0%; △, 2.0%; □, 3.0%; ●, 5.0%.

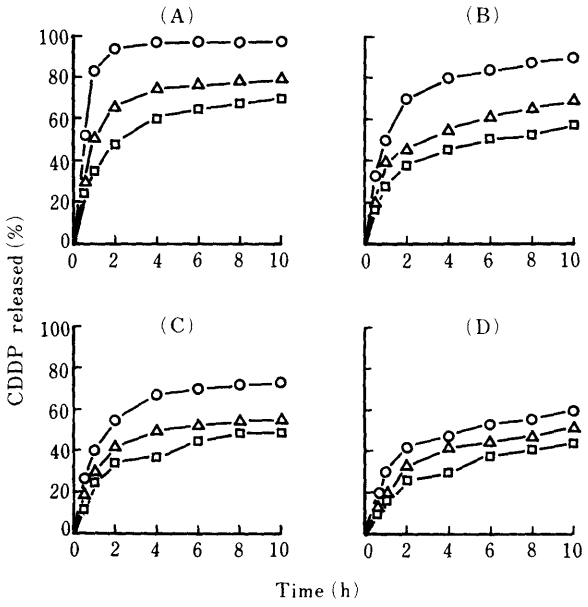

Fig. 3. Release of CDDP from Chitosan Microspheres Using Different Concentrations of Chitin

Concentration of chitin: ○, 0%; △, 1.0%; □, 1.5%. (A) 1.0% chitosan, (B) 2.0% chitosan, (C) 3.0% chitosan, (D) 5.0% chitosan.

TABLE II. Effect of Lysozyme on the CDDP Release from CDDP Chitosan Microspheres

Concentration of chitosan (%)	Chitin added (%)	Time of 50% release (h)	
		Physiological saline solution	Physiological saline solution containing lysozyme
1.0	0	0.4	0.3
	1.0	0.8	0.6
	1.5	2.3	1.6
2.0	0	1.0	0.4
	1.0	2.6	1.7
	1.5	5.5	2.8
3.0	0	1.7	0.5
	1.0	5.1	3.4
	1.5	8.5	3.7
5.0	0	4.5	0.9
	1.0	8.0	6.3
	1.5	11.2	7.8

the above findings.

Effects of Lysozymes on CDDP Release The effects of lysozymes on the CDDP release from chitosan microspheres and chitin-containing chitosan microspheres are shown in Table II. The addition of lysozymes to the dissolution medium enhanced the CDDP release rate and shortened the time for 50% release. The effect of lysozymes, however, was variable, particularly depending on the concentration of chitin, and the time for 50% release was markedly prolonged with increasing chitin concentration. Changes in the form of the microspheres owing to decomposition by lysozyme were observed.

Discussion

CDDP chitosan microspheres were prepared using two biodegradable macromolecules, chitosan and chitin, and the morphology, CDDP content, *in vitro* release of CDDP, and biodegradability of these microspheres were investigated. The CDDP content increased with the increasing concentration of chitosan and chitin added. This is thought to be explained by the intense ionic interaction between the negatively charged groups in the chitosan and chitin molecules and the cationic substances such as CDDP. The rate of CDDP release was retarded by chitosan and chitin added at increasing concentrations. The burst effect occurring during the initial phase of drug release was also inhibited by the addition of chitin. A possible explanation of these effects could be: an increase in the chitosan concentration led to the enhanced solidification of chitosan, *i.e.*, formation of a chitosan aldehyde bond, and thereby increased the integrity of microspheres; then CDDP capacity of the matrix was increased when chitin was added. The microspheres should show no immunogenicity *in vivo* because of their susceptibility to lysozyme. These findings as well as the immunoadjuvant and antitumor activities of chitosan and chitin suggest that chitosan microspheres containing chitin are useful as the embolizing material for chemo-embolization of hepatocellular carcinomas.

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