A Study of Embolizing Materials for Chemo-embolization Therapy of Hepatocellular Carcinoma: Embolic Effect of Cisplatin Albumin Microspheres Using Chitin and Chitosan in Dogs, and Changes of Cisplatin Content in Blood and Tissue

Yutaka NISHIOKA,*[,a] Shojiro KYOTANI,[a] Masashi OKAMURA,[a] Masako MIYAZAKI,[b] Yoshiya SAKAMOTO,[b] Masanori MORITA,[b] Kazuichi OKAZAKI,[b] Saburo OHNISHI,[b] Yasutake YAMAMOTO[b] and Kenichi ITO[c]

Department of Pharmacy, Kochi Medical School Hospital,[a] The First Department of Internal Medicine[b] and Vice President, Kochi Medical School,[c] Kohasu, Okoh-cho, Nankoku, Kochi 783, Japan. *Received May 13, 1991*

Hepatic artery of dogs was embolized with cisplatin (CDDP) albumin microspheres containing chitin and chitosan to investigate the *in vivo* CDDP release kinetics from CDDP albumin microspheres, the CDDP cumulative characteristics in the liver, and the influence of microsphere administration on hepatic tissue. Results showed that changes in blood CDDP content were dependent on CDDP albumin microsphere type and that release kinetics were better sustained when chitin was added to the microspheres or when the microspheres were treated with chitosan. In particular, the administration of CDDP in the chitin-containing CDDP chitosan albumin microspheres showed a blood CDDP content of approximately 0.26 μg Pt/ml 14 d after administration. The administration of chitin-containing or chitosan treated CDDP microspheres showed a CDDP content in the hepatic tissue of 0.14 to 0.23 μg Pt/g 28 d after administration. They also showed better control of CDDP release than those without chitin or chitosan treatment. No CDDP influence on hepatic tissue was observed. We conclude that, even *in vivo*, chitin and chitosan are effective embolic materials.

Keywords cisplatin; chitin; chitosan; albumin microsphere

In recent years, hepatic arterial chemo-embolization as an effective hepatocellurar carcinoma therapy[1-3] has attracted considerable attention. In a previous report,[4,5] cisplatin (CDDP) albumin microspheres, chitin-containing CDDP albumin microspheres, and chitin-containing CDDP chitosan microspheres were investigated as to *in vitro* CDDP release kinetics. It was determined that CDDP release kinetics were controlled better when chitin was added or when CDDP was treated with chitosan. In this paper, we report on mongrel dog hepatic artery embolization with various CDDP albumin microspheres, and we researched microsphere CDDP release kinetics *in vivo*, as well as CDDP content in hepatic tissue and the influence of CDDP albumin microspheres on hepatic tissue.

Experimental

Reagents CDDP powder was kindly supplied by Nippon Kayaku Co., human serum albumin by the Green Cross Co., CDDP injection by Nippon Kayaku Co., chitin and chitosan (70% of deacetylation) by Nakarai Tesque Co., Ltd., pentobarbital sodium injection by Dainippon Pharmaceutical Co., Ltd., and iopamidol (61.24%) by Nippon Schering K. K. All other reagents employed were commercial special-grade products.

Preparation of CDDP Albumin Microspheres, Chitin-Containing CDDP Albumin Microspheres, and Chitosan Treated Chitin-Containing CDDP Albumin Microspheres CDDP powder and chitin (1.5% concentration) were mixed to a fine powder with a mortar and pestle. Then, 2 ml of albumin solution was added and mixed well. This solution was added to toluene–chloroform mixed with ethylcellulose and emulsified according to the method of preparation for w/o emulsion, and hardened with glutaraldehyde. The product was washed with acetone, air-dried at 50 °C for 2 h. To obtain chitosan treated chitin-containing albumin microspheres, chitin-containing albumin microspheres were stirred for a set time in an acetic acid solution of chitosan (1.5%) and washed, and then air-dried at 50 °C for 2 h. These microspheres were sieved into grades (74—177 μm) and sterilized by dry heat at 135 °C for 2 h.

Administration of Microspheres *in Vivo* Thirty-six dogs (body weight 12—15 kg each) were divided into four equal groups. CDDP albumin microspheres, chitin-containing CDDP albumin microspheres, or chitosan treated chitin-containing CDDP albumin microspheres were administered through each dog's hepatic artery. The control was administered CDDP *via* the hepatic artery. To measure CDDP content, 2 ml of blood were taken from a foreleg vein at 1, 2, 3, 4, 6, 24 h and 2, 3, 7, 14, 21, 28 d after administration. One mg of CDDP per 1 kg body weight was administered. Solutions were administered during laparotomy under sodium pentobarbital anesthesia. CDDP was measured by atomic absorption spectrophotometry. Blood chemistries were performed with vision analyzer for T-bilirubin, glutamic oxaloacetic transaminase (GOT), blood urea nitrogen (BUN) and, creatinine. To confirm the presence of embolus, hepatic arteriogram with iopamidol was performed before and after administration.

Measurement of CDDP Content in the Hepatic Tissue and Histpathological Discussion Mongrel dogs were killed 1, 7 and 28 d after administration of microspheres and the livers were extirpated. 0.5 g of each extirpated liver and 3 ml of concentrated nitric acid was thermolyzed at 80 °C for 5 h. After each sample was air cooled, 5 ml of 40% sodium hydroxide and 2 ml of 25% sodium bicarbonate were added for a pH between 7 and 9. Then, 300 mg of sodium diethyldithiocarbamic acid, a Pt chelating agent, was added and heated at 80 °C for 1 h. After air-cooling, 3 ml of chloroform was added and shaken to extract CDDP. The procedure was repeated three times. The chloroform layer was evaporated and dried using an evaporator and the residue was dissolved with 0.5 ml of methanol. CDDP was measured by atomic absorption spectrophotomertry. The extirpated livers were fixed with formalin and paraffin slices were prepared and HE dyed in the standard manner, and sections were analyzed histopathologically.

Results

Confirmation of Embolus by Hepatic Arteriogram Figure 1 shows hepatic arteriogram before and after CDDP albumin microsphere embolization. After administration, the hepatic artery was large and serpentine, vasodilated, or showed multiple artery branching.

CDDP Release *in Vivo* Figure 2 shows the change of blood concentration of CDDP. Albumin microspheres showed the highest CDDP at approximately 0.4 μg Pt/ml 1 to 2 h after administration. After peaking it gradually decreased and was below the limit of measurement within a week. Chitin-containing albumin microspheres showed the highest CDDP at approximately 0.6 μg Pt/ml after 1 h. It was below the limit of measurement within 2 weeks (w). Chitosan treated chitin-containing albumin microspheres showed the highest CDDP at approximately 0.5 μg Pt/ml 1 or 2 h after administration similar to albumin microspheres administration. On the other hand, release was sustained and CDDP content 14 d after administration was still approximately 0.26 μg Pt/ml. With CDDP injection, the content was approximately 1.4 μg Pt/ml 1 h after administration and showed a gradual decrease after 4 w to

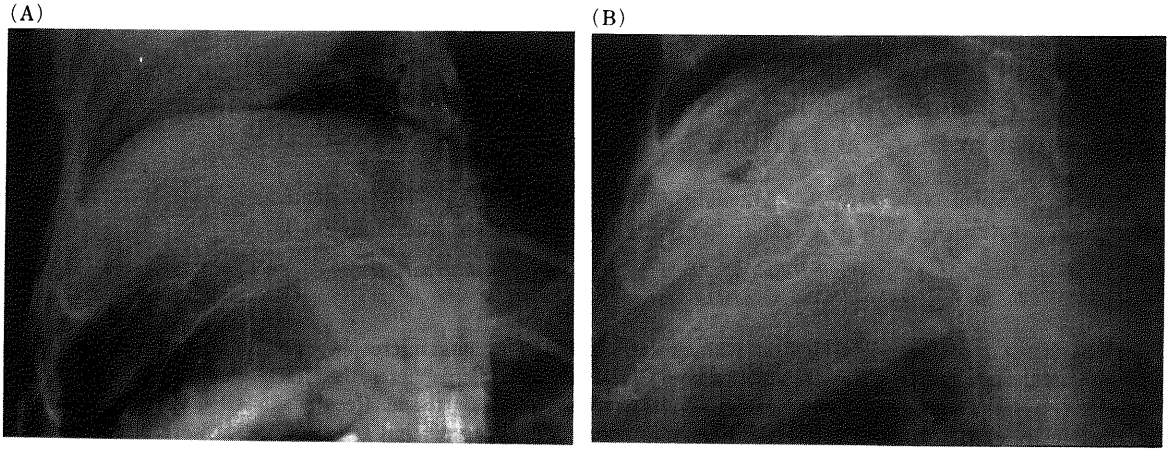

Fig. 1. Confirmation of Embolus by Hepatic Arteriogram

(A) Before CDDP albumin microsphere embolization. (B) After CDDP albumin microsphere embolization.

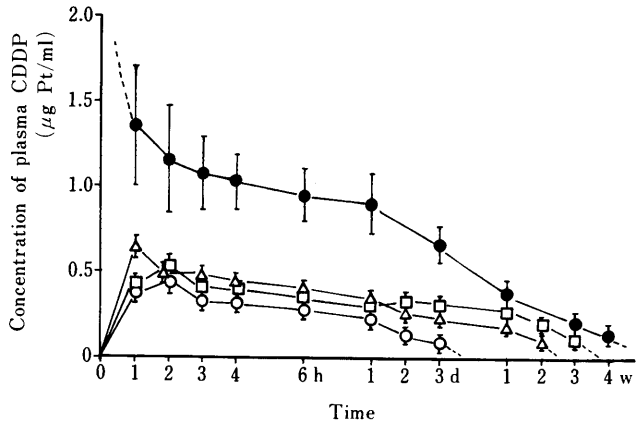

Fig. 2. Time Course of Plasma CDDP after Arterial Injection of CDDP Albumin Microspheres or CDDP

Each point represents the mean ± S.D. ($n=3$). ○, albumin microsphere; △, chitin-containing albumin microsphere; □, chitosan treated chitin-containing albumin microsphere; ●, CDDP.

0.3 μg Pt/ml.

Platinic Content in Hepatic Tissue Table I lists platinic content in hepatic tissue. Platinic content in hepatic tissue differed among albumin microspheres, chitin-containing albumin microspheres and chitin-containing chitosan treated albumin microspheres.

Biochemical and Histpathological Examinations Biochemical analysis in blood after the administration of various CDDP microspheres showed minor changes in total bilirubin, GOT, BUN, and creatinine but all parameters were within normal limits. Histopathological examination 7 d after the administration of various CDDP microspheres showed no histological alterations.

Discussion

Chitin and chitosan are harmless macromolecules with favorable characteristics such as good biological compatibility and degradation ability *in vivo* and are attracting attention as new biopolymer materials. In this study, to control CDDP release kinetics, various CDDP albumin microspheres were prepared incorporating chitin or chitosan and their adaptability as embolic materials *in vivo* for hepatic arterial chemo-embolization therapy were investigated. As a result, when chitin was added to the microspheres or when the micropheres were treated with

TABLE I. The Assay of CDDP in Dog Liver

Days	CDDP concentration (μg Pt/ml)			
	Microsphere			CDDP solution
	A	B	C	
1	1.23±0.12	0.23±0.05	0.14±0.03	1.51±0.36
7	0.57±0.08	1.48±0.17	0.64±0.10	0.85±0.23
28	0.05±0.02	0.14±0.04	0.23±0.06	0.11±0.08

The data represent the mean ± S.D. of three dogs. A was albumin microsphere, B was chitin-containing albumin microsphere and C was chitosan-treated chitin-containing albumin microsphere.

chitosan, changes in blood platinic content as well as platinic content in hepatic tissue were better sustained. We presume that chitin or chitosan combines with anion macromolocules such as chondroitin sulfate *in vivo* and control degradation of microspheres by enzymes *in vivo*. As a result, CDDP release kinetics were sustained. Since it is reported that chitosan is likely to form a complex with heavy metal ions,[6] we presume that platinic ion combines with chitosan amino group to form a coordination compound, which controls CDDP release kinetics.

CDDP kinetics were better sustained when chitin was added or chitosan treated *in vivo*. Moreover, biochemical analysis and histopathological examination confirmed their safety. Together with carcinostatic activity[7] and the suppressive effect of cartinomatous metastasis[8] of chitin or chitosan, chitin and chitosan are expected to greatly improve the cartinostatic effect in hepatocellular cartinoma.

References

1) T. Kato, R. Nemoto, H. Mori and I. Kumagai, *Cancer*, **46**, 14 (1980).
2) Y. Okamoto, A. Konno, K. Tagawa, T. Kato, Y. Tamakawa and Y. Amano, *Br. J. Cancer*, **53**, 369 (1986).
3) N. Willmoto, *Cancer Treat Rev.*, **14**, 143 (1987).
4) Y. Nishioka, S. Kyotani, M. Okamura, Y. Mori, M. Miyazaki, K. Okazaki, S. Ohnishi, Y. Yamamoto and K. Ito, *Chem. Pharm. Bull.*, **37**, 1399 (1989).
5) Y. Nishioka, S. Kyotani, H. Masui, M. Okamura, Y. Mori, M. Miyazaki, K. Okazaki, S. Ohnishi, Y. Yamamoto and K. Ito, *Chem. Pharm. Bull.*, **37**, 3074 (1989).
6) A. Domard, *Int. J. Biol. Macromol.*, **9**, 98 (1987).
7) J. Iida, T. Une, C. Ishihara, K. Nishimura, S. Tokura, N. Mizukoshi and I. Azuma, *Vaccine*, **4**, 151 (1987).
8) J. Murata, I. Saiki, S. Nishimura, N. Nishi, S. Tokura and I. Azuma, *Jpn. J. Cancer Res.*, **80**, 866 (1989).