Preparation and Evaluation of Albumin Microspheres and Microcapsules Containing Cisplatin

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Albumin microspheres and microcapsules containing cisplatin (CDDP) were prepared and tested as chemotherapeutic agents for the treatment of hepatocellular carcinoma. CDDP albumin microspheres were prepared by hardening with glutaric aldehyde in accordance with the method to prepare W/O emulsion. On the other hand, microcapsules were prepared by formation of a coacervate by the phase isolation method. CDDP albumin microspheres and microcapsules thus prepared were sieved and sterilized by dry heat at 135 °C for 4 h prior to use. The content and release of CDDP were determined. The CDDP contents for albumin microspheres and microcapsules were found to be 9.2% and 33.3%, respectively. Release of CDDP *in vitro* was found to be significantly different between the two formulations. CDDP release *in vivo* was also investigated by injecting albumin microspheres and microcapsules into the hepatic artery of adult dogs. The blood CDDP concentrations after injection of both formulations were lower than those noted after injection of CDDP injectable solution, indicating that CDDP might be accumulated in the liver at a higher concentration and that use of the two formulations might result in alleviation of CDDP side effects.

Keywords cisplatin; albumin microsphere; microcapsule; chemo-embolization; cisplatin release

Attention has been focused on chemo-embolization as an effective therapeutic modality for hepatocellular carcinoma in recent years. Various materials have been tried as embolizing materials, such as albumin, starch microspheres and ethyl cellulose microcapsules. The carcinostatic agents mitomycin C and adriamycin can be used concurrently with chemo-embolization. More recently, it has been reported that cisplatin (CDDP) is effective for the treatment of hepatocellular carcinoma.[1-6] However, no report has yet appeared on the use of CDDP albumin microspheres in chemo-embolization for the treatment of hepatocellular carcinoma. Thus, we prepared CDDP albumin microspheres and compared their properties with those of CDDP microcapsules.

Experimental

Reagents CDDP powder was kindly supplied by Nippon Kayaku Co. In addition, the following agents were employed; CDDP injectable solution (Nippon Kayaku Co.), human serum albumin (The Green Cross Co.), ethylene vinyl acetate (containing 28% vinyl acetate) (Toyo Soda Inc.), polyisobutylene (average M. W. 1300000) (Aldrich Co.), ethyl cellulose (Nacalai Chemical Co.) and HCO-60 (Nikko Chemical Co.). All other reagents employed were commercial special-grade products.

Preparation of CDDP Albumin Microspheres Albumin microspheres were prepared according to the method of Longo *et al.*[7] Suitable amounts of 25% human serum albumin solution and CDDP were taken into an agate mortar, mixed well and used as a dispersing solution. This dispersing solution was added to a toluene–chloroform mixture dissolved in ethyl cellulose, emulsified according to the method of preparation for W/O emulsion, and hardened with glutaric aldehyde. The product was washed with acetone, air-dried at 50 °C for 2 h, then sieved into grades and sterilized by dry heat at 135 °C for 4 h.

Preparation of CDDP Microcapsules CDDP microcapsules were prepared by the phase isolation method in which cyclohexane solution (heated to 82 °C) containing ethyl cellulose, ethylene vinyl acetate and polyisobutylene were mixed, and then stirred with a CDDP suspension in cyclohexane. Microcapsules thus prepared were washed with *n*-hexane containing 1% HCO-60 as well as with *n*-hexane, followed by air drying at 50 °C for 2 h, and then the product was sieved into grades and sterilized by dry heat at 135 °C for 4 h.

CDDP Release *in Vitro* 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. Into this was immersed a cell (nitrate cellulose membrane 3 μm) containing a suitable amount of the sample. The contents of the cell were stirred (50 rpm) with a stirring rod, and measured aliquots of the release solution were serially taken. The CDDP content in the release solution was measured by atomic absorption spectrophotometry.

CDDP Release *in Vivo* Nine mongrel adult dogs were divided into 3 groups and injected with CDDP injectable solution, CDDP albumin microspheres or CDDP microcapsules. The dosage was set at 1 mg/kg body weight in terms of CDDP. The abdomen was opened under nembutal anesthesia, the drug was injected into the hepatic artery, and blood was serially withdrawn from the anterior limb vein for measurement of blood CDDP concentrations. CDDP was quantitatively assayed by atomic absorption spectrophotometry. In the case of CDDP albumin microspheres and microcapsules, samples with granule diameters of 74 to 177 μm were suspended in normal saline prior to use.

Results

CDDP Content CDDP content were found to be 9.2% for albumin microspheres and 33.3% for microcapsules. The recovery rates were 71% for albumin microspheres and 60% for microcapsules.

CDDP Release *in Vitro* Figure 1 shows that CDDP was released from albumin microspheres and microcapsules in significantly different patterns. In the case of albumin microspheres, more over the release pattern depended on the granule diameter.

CDDP Release *in Vivo* Figure 2 shows changes in blood

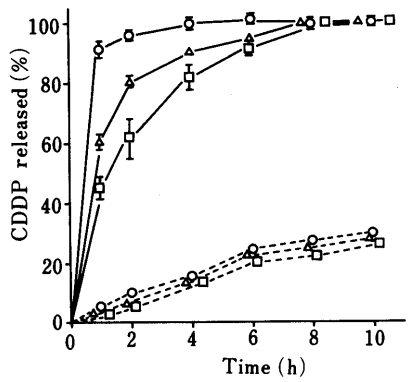

Fig. 1. Effect of Particle Size on the CDDP Release Profiles from Albumin Microspheres and Microcapsules

Particle size: ○, 20—37 μm; △, 37—74 μm; □, 74—177 μm. ——, albumin microspheres; ----, microcapsules.

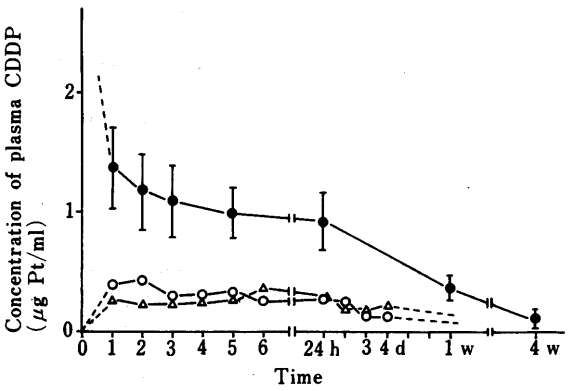

Fig. 2. Time Course of Plasma CDDP after Arterial Injection of CDDP Albumin Microspheres and Microcapsules

○, CDDP albumin microspheres; △, CDDP microcapsules; ●, CDDP injection solution.

CDDP concentration following administration of CDDP injectable solution, albumin microspheres and microcapsules in adult dogs. In the case of CDDP injectable solution, the blood CDDP concentration at 1 h was 1.65 μg Pt/ml, which subsequently declined gradually. At 4 weeks after administration, blood CDDP was still detectable, although the concentration was low (0.15 μg Pt/ml). In the case of albumin microspheres, the blood CDDP concentration peaked at 1 to 2 h, ranging from 0.41 to 0.42 μg Pt/ml, which was about one-fourth of that after CDDP injectable solution. The level fell below 0.1 μg Pt/ml within 1 week after administration. In the case of microcapsules, no clear peak of blood CDDP concentration was observed; the mean blood CDDP concentrations ranged from 0.22 to 0.28 μg Pt/ml during the first 6 h. As was the case with albumin microspheres, the level fell below 0.1 μg Pt/ml within 1 week after administration. An examination for possible effects on the liver and kidney following administration of albumin microspheres and microcapsules revealed that glutamate pyruvate transaminase and alkaline phosphatase activities were elevated until the 3 rd day after administration but declined thereafter, returning to the baseline at 2 to 3 weeks. No definite change was observed in creatinine.

Discussion

One-shot intra-arterial injection or continuous intra-arterial infusion has been used as an effective therapeutic method for the treatment of hepatocellular carcinoma. In recent years, transcatheter artery embolization (TAE) therapy has become widely used. In an attempt to enhance its therapeutic effect, TAE therapy has recently been combined with chemotherapy and clinically applied in the form of chemo-embolization or intra-arterial chemotherapy using lipiodol as a carrier. In order to examine the slow release of CDDP from the embolizing material as well as the embolizing effect on the feeding artery, we prepared albumin microspheres and conducted an experimental study to determine CDDP content and release *in vitro* and *in vivo*. A significant difference was seen in CDDP release *in vitro* between CDDP albumin microspheres and microcapsules. With respect to CDDP release *in vivo*, however, no difference was observed between the two drugs. This may be explained by the morphological differences of the two formulations, which probably resulted in differences in the embolization status of the artery. Administration of albumin microspheres and microcapsules resulted in lower blood CDDP concentrations, suggesting that most of the CDDP released from the two formulations was accumulated in the liver. Furthermore, the lower blood CDDP concentrations would be expected to be beneficial in reducing CDDP side effects. In view of these findings, we believe that CDDP albumin microspheres would be useful for chemo-embolization, and that albumin as a drug carrier would be better than microcapsules in the terms of biodegradability and biocompatibility. It may be possible to increase the content and slow-release capacity of CDDP by the use of other biodegradable macromolecules such as chitin and chitosan.

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