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Research Article

Efficient Loading of cisplatin into Superabsorbent Polymer Microsphere in the treatment of hepatocellular carcinoma.

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ABSTRACT

Background: Although Superabsorbent Polymer (SAP) microsphere has been useful in treatment of multiple and large hepatocellular carcinoma, efficient loading procedure of cisplatin onto SAP was not established. Loading efficiency of cisplatin was evaluated from the viewpoint of passive concentration gradient and ionic strength.

Methods: To load cisplatin into SAP particle, 50mg of cisplatin powder was dissolved in three manners of aqueous solutions: (a) 50mg of cisplatin powder plus 35ml of warmed physiological saline solution (0.9%), (b) 5ml of physiological saline solution (0.9%) plus a half of solution (5ml) of the product made from 50mg of cisplatin powder in 10ml of iohexol 350mgI/ml, and (c) 10ml of iohexol 350mgI/ml plus 0.25ml of 10% NaCl solution

Results: Cisplatin dissolved sufficiently in all the three methods. Average amounts of loaded cisplatin into SAP particles were 1.7mg in solution (a), 6.6mg in solution (b), and 11.8mg in solution (c). Since alteration of ionic strength in the method (c) significantly affect the extent of SAP swelling, addition of 0.25ml of 10% NaCl was unquestionably necessary. Release of cisplatin from SAP was evidently gradual, showing 83.1% after 6 hours in static elution experiment and 68.6% after 1 hour in dynamic elution experiment.

Conclusions: Cisplatin-loaded SAP may act as a drug delivery system for treatment of liver cancer, and loading of cisplatin should be consciously performed considering cisplatin concentration and ionic strength. (225words)

Keywords: Superabsorbent polymer microsphere, cisplatin, transcatheter arterial chemoembolization (TACE), hepatocellular carcinoma (HCC)

INTRODUCTION

Since hepatic resection, liver transplantation and percutaneous radiofrequency ablation are applicable in only 30–40% of hepatocellular carcinoma (HCC) patients, transcatheter arterial chemoembolization (TACE) has been recognized as an effective palliative treatment option for patients with intermediately advanced HCC[1,2]. Various procedures and tools for better and more effective TACE have been developed until recently. In the last decade, drug-eluting beads for the transcatheter arterial treatment of HCC have been developed to actively loaded doxorubicin from its solution and to release it in a controlled and prolonged fashion in the tumor's arterial network[3-6]. Among them, Superabsorbent Polymer Microsphere (SAP-MS: HepaSphere, Nippon Kayaku, Tokyo, Japan) became available for TACE in management of HCC, as non-biodegradable, spherical, dry particle made of sodium acrylate and vinyl alcohol copolymer. It has a few standards of particle sizes that can be calibrated in increments of approximately 50µm between 50 and 200µm. SAP-MS swells within several minutes after absorbing fluid, and the swollen particles are elastic and compressible but hold their spherical or oval shape. The microspheres contain anionic groups in their chemical structures which may interact with positively charged parts of doxorubicin or irinotecan by an ion-exchange mechanism, leading to controlled and sustained intra-tumoral drug retention. However, it does not bind with the other chemotherapeutic drugs without positively charged groups, such as cisplatin, mitomycin C, or 5-fluorouracil.

Cisplatin is a relatively potent chemotherapeutic agent for HCC[7-9], and its effect is considered to depend on the concentration and dwelling time in the tumor. Although cisplatin may be one of the ideal chemotherapeutic agents for loading to SAP-MS, its chemical structure does not contain any positively charged part which can bind with the microsphere particles. Moreover, usual cisplatin formulation of pharmaceuticals is a large volume of solution with 100mg of cisplatin in 200ml of water. Although swollen SAP-MS does contain cisplatin in their particles with concentration gradient, only small amount of the diluted anticancer agent can enter the swollen microsphere. A new cisplatin

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Table 1 Amount of loaded cisplatin and particle size in loading method (a), (b), and (c).

Loading Method	Loaded amount of cisplatin (mg)				Size of Particles	Magnification of Swelling
	N1	N2	N3	Average		
(a)	1.7	1.8	1.7	1.7	377	4.9
(b)	5.7	7.8	6.3	6.6	378	4.8
(c)	11.2	12.5	11.7	11.8	328	4.6

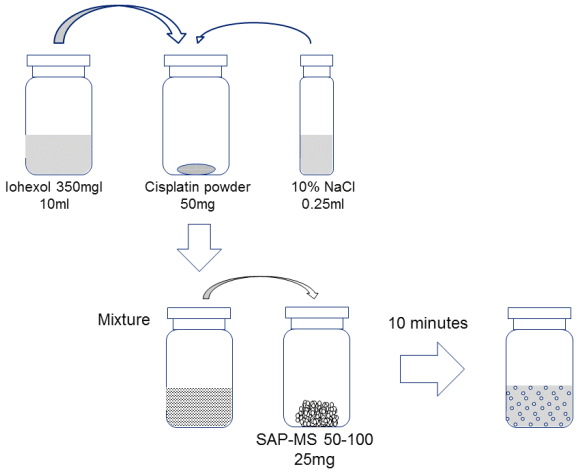

Figure 1 Procedure for loading of cisplatin into SAP microsphere (method (c)). A total of 50mg of cisplatin powder was dissolved in 10ml of iohexol 350mgI, and 0.25ml of 10% NaCl was added in the solution. The 10.25ml of the solution was put in a vial of SAP-MS (25mg), and then the SAP-solution was left at rest at room temperature for 10 minutes

(cis-dichlorodiammineplatinum) powder has been developed for hepatic arterial infusion chemotherapy. This powder is relatively more soluble in saline solution and gives flexibility to adjust the concentration of cisplatin. Maeda et al.[10] reported an in vitro study of this formulation of cisplatin and SAP-MS, suggesting more concentrated cisplatin solution was available using non-ionic iodinated contrast medium. They reported that the particle sizes of SAP and cisplatin elution kinetics were influenced by the type of solvent in which the cisplatin powder was dissolved. The purposes of this study are, therefore, to find appropriate contrast media and ionic strength in the use of cisplatin with SAP-MS, and to confirm the ability of SAP to absorb and elute cisplatin with consideration of the dependence feature of SAP particles on ionic strength of solvent solution.

MATERIALS AND METHODS

Embolic material

SAP-MS (HepaSphere™) is calibrated, spherical, hydrophilic microsphere made from 2 monomers (vinyl acetate and methyl acrylate) that combine to form a copolymer (sodium acrylate alcohol copolymer). Among three standards of SAP-MS available in Japan, we chose the smallest one (50-100μm in dry state size, 25mg/vial) was used in the following experiments. When saline solution or nonionic contrast medium was injected into vacuum-sealed vial of SAP-MS, it absorbs the solution and swells to approximately three to five times its original dry size, according to their ionic strength, within 20 minutes. .

Antitumor agent and its solvents

Although cisplatin is water-soluble chemotherapeutic agent, relatively large amount of solution is usually required to solve the drug completely (100mg cisplatin in 200ml of physiological saline solution, for example). We used cisplatin in powder formulation (IA-Call; Nippon Kayaku, Tokyo, Japan), which is prepared for better solubility in water or other solution (100mg cisplatin in 70ml warmed saline solution). According to Maeda et al.[10] describing that a few nonionic contrast media can dissolve the cisplatin powder with significantly smaller amount of solvents, we used nonionic contrast medium iohexol 350mgI/ml (Omnipaque 350 ™, Daiichi-Sankyo Co. Ltd, Tokyo, Japan) to prepare SAP solution in small volume of solvent.

We dissolve 50mg of cisplatin powder with the following three solvents: (a) 50mg of cisplatin powder plus 35ml of warmed physiological saline solution (0.9%), (b) 5ml of physiological saline solution (0.9%) plus a half of solution (5ml) of the product made from 50mg of cisplatin powder in 10ml of iohexol 350mgI/ml, and (c) 10ml of iohexol 350mgI/ml plus 0.25ml of 10% NaCl solution (Figure 1). The appropriate concentrations of cisplatin were predetermined according to the solubility in each solvent. In the three manners of cisplatin dissolution, one vial (25mg) of SAP-MS was mixed with a total of 10ml of solution from the obtained 35ml, 10ml, and 10.25ml of each solution for 10 minutes.

Measurement of cisplatin and Assessment of cisplatin loading

For each concentration in the solutions, we measured cisplatin concentration at 15 minutes. We collected swollen SAP from each solution with a filter. Filtrated SAP was placed in a 5ml-injector with 5ml of water, and the particles were completely crushed with pumping for 120 seconds using two 5ml-injectors and three-way stopcock. Obtained solution was assayed with an atomic absorption spectrophotometer (Z-2000, Hitachi High Technologies, Tokyo, Japan). In all the three manners of cisplatin loading (a, b, c), supernatant concentration of cisplatin was measured three times, and the averages of cisplatin concentrations were applied to make the drug loading profiles.

Morphology of microspheres after loading cisplatin

Morphological change of SAP-MS was assessed with digital microscope (VHX-1000, KEYENCE Japan, Osaka, Japan) in each loading manner. When sufficient amount of cisplatin was loaded into SAP in certain condition, additional morphological analysis was also performed for the condition controlling the ionic strength.

Assessment of kinetics of cisplatin elution

Static and dynamic kinetics of cisplatin were assessed in each loading patterns. Static measurement of cisplatin

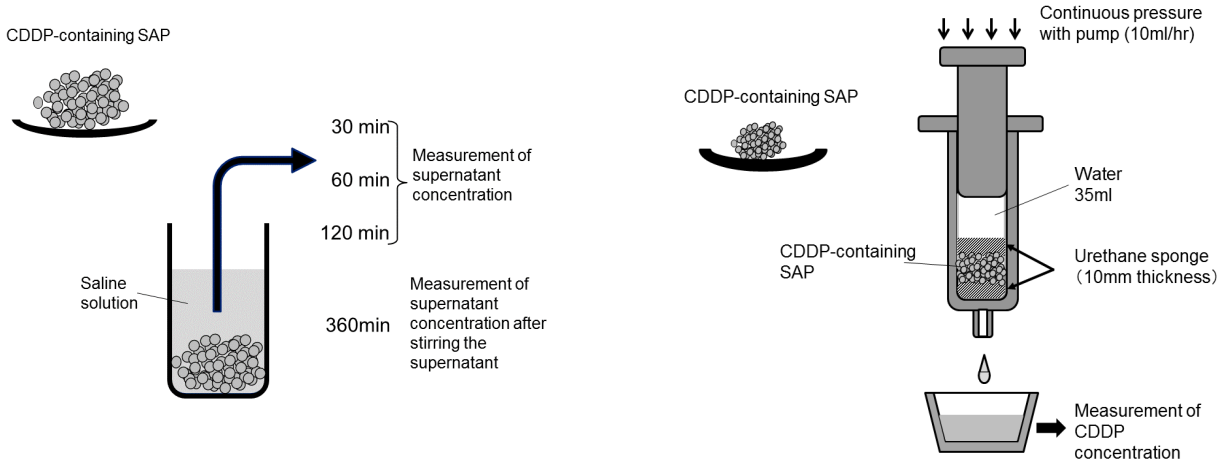

Figure 2 a. Cisplatin elution analysis from SAP particles in static state: Sampling of aliquots from supernatant fluid at 30 minutes, 1 hour, 2 hours, and 6 hours after cisplatin loading. Initial three times were gently performed, and final sampling was performed after stirring the supernatant fluid. b. Cisplatin elution analysis from SAP particles in dynamic state, using an injector with continuous pressure pump (10ml/hr). Sampling of fluid passing through the SAP layer were performed at 5,10, 15, 20, 25, 30, 40, 50, and 60 minutes.

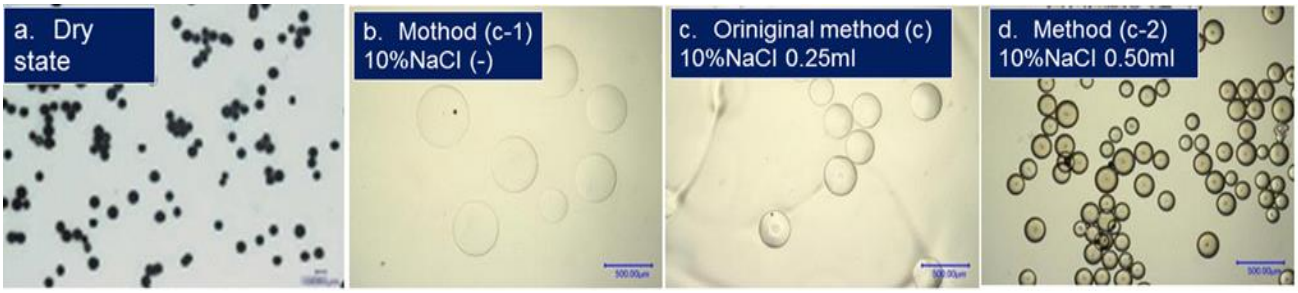

Figure 3 Microscopic analysis of morphology of SAP microspheres. a. Dry state before absorption of fluid. b. Microspheres generated with method c-1. Microspheres became over-swollen. c. Microspheres generated with method c. Microspheres became appropriately swollen with addition of 0.25ml of NaCl. d. Microspheres generated with method c-2. Microspheres showed small and insufficient swelling.

concentration was performed as intermittent sampling of supernatant from cisplatin containing SAP-MS solution. Sampling was performed at 30 minutes, 1 hour, 2 hours, and 6 hours after mixing of cisplatin solution and SAP-MS. Although initial 3 times of samplings were performed gently from the middle of supernatant, final time of sampling was performed from the middle of supernatant after stirring it (Figure 2a).

Dynamic kinetics of cisplatin elution was assessed in a 50ml-disposable injection syringe (Figure 2b). Cisplatin-containing SAP was placed between two layers (each 10mm) of urethane sponges. Thirty-five milliliter of water was also put over the urethane layers. Plunger of the injection syringe was continuously pressed with a rate of 10ml per hour. Pressed drips of the fluid were collected in a container, and concentration of cisplatin was intermittently measured for them: 0min, 5, 10,15,20,35,30,40,50, and 60 min.

RESULTS

Cisplatin solubility

Fifty milligrams of cisplatin powder was sufficiently dissolved in all the solutions described as the three manners: (a) 50mg of cisplatin powder plus 35ml of warmed physiological saline solution (0.9%), (b) 5ml of physiological saline solution (0.9%) plus a half of solution (5ml) of the product made from 50mg of cisplatin powder in 10ml of iohexol 350mgI/ml, and (c) 10ml of iohexol 350mgI/ml plus 0.25ml of 10% NaCl solution.

Cisplatin loading assessed by crushed SAP particles

Ten milliliters of the solution was collected from the three solutions, and was injected into containers of 25mg of SAP-MS in dry state. After 15 minutes, microscopic measurement of the size of SAP (50-100μm in dry state) showed a significant increase with approximately four to five times of original dry state. Median sizes of a total of 100 SAP particles were 377μm in solution (a), 378μm in solution (b), and 328μm in solution (c), respectively.

Loaded cisplatin was directly measured for smashed and solution-state of collected SAP-MS particles. Average amounts of loaded cisplatin were 1.7mg in solution (a), 6.6mg in solution (b), and 11.8mg in solution (c). Each amount of loaded cisplatin was directly correlated with surrounding supernatant concentration of cisplatin, considering the extent of the SAP swelling (Table 1).

Morphology of SAP-MS according to ionic strength of solution

Microscopic examination of various conditions of swollen SAP particles was compared with original dry

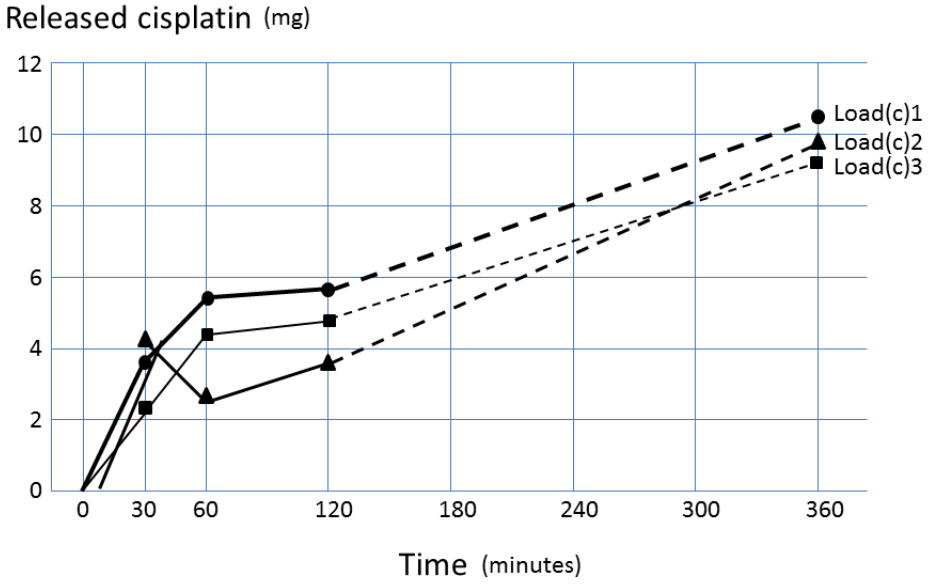

Figure 4 Cisplatin elution experiment in static state. Cisplatin gradually eluted to surrounding fluid with an average rate of 83.1% per 6 hours.

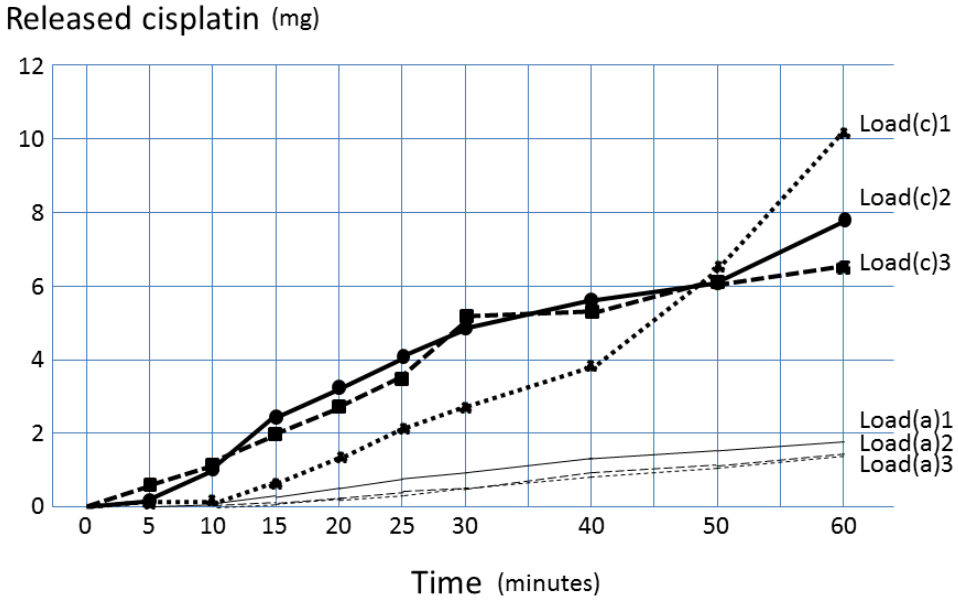

Figure 5 Cisplatin elution experiment in dynamic state. Cisplatin gradually eluted to passing fluid with an average rate of 68.6% per 60minutes in method c.

particle (Figure 3a). Since SAP contained the most amount of cisplatin (11.8mg) in dissolution method (original c) (Figure 3c), we performed additional two conditions with different ionic strength. We dissolve 50mg of cisplatin in 10ml of iohexol 350mgI/ml only (c-1) (Figure 3b), and in 10ml of iohexol 350mgI/ml plus 0.50ml of 10% NaCl solution (c-2) (Figure 3d). The former solution was much lower ionic strength than original dissolution method (c), and the latter was higher ionic strength. Morphologically, smaller and insufficiently swollen particles were observed in the method (c-1), and larger and over-inflated particles were found in the method (c-2). The latter over-swollen SAP particle was fragile and easily destructed with small mechanical pressure.

Cisplatin elution kinetics

To elucidate how slowly the loaded cisplatin diffuse to surrounding tissue or fluid, a few aliquots of supernatant solution were assessed for cisplatin concentration in static condition. When 11.8mg of cisplatin was loaded with the method of dissolution (c), average amounts of eluted cisplatin were 3.7mg after 30minutes, 4.1mg after 1hr, 4.9mg after 2hrs and 9.8mg after 6hrs, respectively (Figure 4a). The loaded cisplatin was slowly released from SAP to surrounding fluid slowly with a rate of 83.1% per 6 hours.

The elution rate of loaded cisplatin from SAP was also assessed with dynamic experiment using injection syringe under slow fluid flow (Figure 4b). Serial samples of eluted fluid from SAP with 11.8mg of loaded cisplatin (dissolution method (c)) showed that the average amounts of cisplatin were 0.2mg after 5 min., 0.8mg after 10min.,

1.7mg after 15min., 2.5mg after 20min., 3.2mg after 25min., 3.9mg after 30min., 5.0mg after 40min., 6.2mg after 50min., and 8.1mg after 60min., respectively. A total of 68.6% of loaded cisplatin was released and diffuse from SAP-MS after 60 min. in the study. When serial sampling was performed in the dissolution method (a) with a loaded cisplatin of 1.7mg, the average amounts of released cisplatin were 0mg after 5 min., 0mg after 10min., 0.2mg after 15min., 0.3mg after 20min., 0.5mg after 25min., 0.6mg after 30min., 1.0mg after 40min., 1.3mg after 50min., and 1.5mg after 60min., respectively. Eighty-eight percent of loaded cisplatin gradually diffused to surrounding tissue after 60minutes.

DISCUSSION

TACE is widely recommended for intermediately-advanced HCC, multiple nodules and/or a large tumor difficult to resect, according to any guidelines from the Japan Society of Hepatology[11], American Association of Study of Liver Disease[12] and European Association of Study of Liver Disease[13].

Aside from the property of calibrated, targeted, and more complete occlusion of peripheral tumor vessels, drug eluting beads were recently developed to deliver drugs directly to tumor tissue to obtain sustained release of antitumor agents, and thereby improve the clinical outcomes of patients with intermediate stage of HCC with less systemic toxicity. Of the drug eluting bead, SAP particles can absorb fluids and swell within several minutes. SAP has a negative charge in its structure, and it can ionically attract positively charged antitumor agent, doxorubicin HCl reversibly. SAP-MS also mechanically absorb aqueous-based solutions whether or not they are positively charged: SAP shows reservoir effect of anticancer drug when it swells, according the outer concentration of drugs.

For the use of antitumor agents without protonated groups in their structure, we should use concentrated drugs in a small amount of solution, and we should consider the ionic strength of the solution. As was well known that SAP cannot be used with ionic contrast medium, or doxorubicin in pure water, appropriate swelling of SAP particles greatly depend on ionic strength of dissolving solution. SAP does not swell sufficiently when ionic activity of solution was high, and it explosively over-swells unable to occlude tumor vessels when ionic activity of solution was low. In the use of non-charged anticancer drug, therefore, SAP-MS should be carefully managed, considering both use of concentrated drug in solution and adjustment of ionic strength.

Cisplatin has been used in the treatment of HCC for more than 20years, and its antitumor effect was considered more potent than anthracyclines when used as intra-arterial chemotherapy or TACE[7-9].Therefore, cisplatin in the form of drug-eluting microspheres may be more effective as it will prolong the drug release in high concentration. Powder formulation of cisplatin (IA-Call™, Nippon Kayaku, Tokyo, Japan) was recently introduced in Japan, which has been used after dissolution in saline solution to yield a higher concentration cisplatin (1.4mg/ml) than previous one (0.5mg/ml). This powder is reported as highly soluble in nonionic contrast medium with the maximum concentration of 6.6mg/ml in iohexol 300mgI/ml[10]. Since cisplatin does not ionically bind with SAP particle, we tried to load as much amount of cisplatin as possible, using concentrated cisplatin with adjustment of ionic strength of dissolving solution. Comparing the other manners of cisplatin solution, the formula of 50mg of cisplatin powder in 10ml of iohexol 350mgI plus 0.25ml of 10% NaCl, attained the most amount and highest concentration of cisplatin in 25mg of SAP-MS particles. One vial (25mg) of SAP-MS contained a total of 11.8mg of cisplatin as an expanded reservoir without ionic binding, which was higher amount of cisplatin reported by Maeda[14]. Our *in vitro* experiment demonstrated the highest cisplatin loadability.

Since cisplatin was not considered bound strongly to SAP particles, elution kinetics of cisplatin from dissolved solution was also assessed in two manners of experiments. In the first experiment of sampling of aliquots from supernatant in static state, cisplatin slowly diffused from SAP to surrounding fluid part with a rate of 83.1% per 6 hours. In the second dynamic experiment of serial sampling of fluid passing through cisplatin-loaded SAP, cisplatin also eluted slowly with a rate of 68.6% per hour. Although cisplatin cannot strongly bind ionically with SAP, it was likely elute much more slowly than mere arterial injection.

Our results will enable the interventional radiologist to load more amount of cisplatin into SAP-MS and to inject high amounts of platinum agent directly into the tumor warranting a sustained high-dose delivery over time while embolizing the tumor feeding arteries. There is no standard method for the choosing of drug in TACE, and actual comparisons of treatment effect are not sufficient among cisplatin, epirubicin, mitomycin, and miriplatin. In the TACE treatment of HCC in Japan, anthracyclines are commonly used at the first time of TACE together with lipiodol. When antitumor effect of repeated TACE was insufficient and suboptimal in those cases with HCC, we usually expect chemotherapeutic activity of platinum agents, including cisplatin. Since DC Bead™ (Biocompatibles Co. Ltd. UK) is considered to have a high affinity for anthracyclines but is very difficult to contain or load cisplatin, SAP-MS should be utilized for the patients who are regarded as candidates for platinum agents. .

The optimal combination of the particle size and dispersed contrast medium was not elucidated. Exact releasing properties of cisplatin from SAP, and ideal releasing time also remained to be examined. In the future, we should develop more convenient and efficient procedures for loading higher doses of platinum agents, and research ideal use of cisplatin and SAP.

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