

Preparation of Lecithin Microcapsules by a Dilution Method Using the Wurster Process for Intraarterial Administration in Gadolinium Neutron Capture Therapy

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Received July 30, 1998; accepted October 15, 1998

Lecithin microcapsules containing gadolinium (Gd) were designed and prepared as a dosage form for intraarterial administration to accumulate Gd in tumors in neutron capture therapy. The microcapsules were composed of 1) a lactose core, 2) a layer of distearylamine of gadopentetic acid (Gd-DTPA-SAM) and polyvinylpyrrolidone (PVP) with or without soybean lecithin (SL) and 3) a membrane containing SL, cholesterol, stearic acid and PVP at three different compositions. A dilution method using the Wurster process was developed for small-scale preparation. In spite of using only 2 g of Gd-DTPA-SAM each, three types of microcapsules were obtained with a content of 24.9% as Gd-DTPA-SAM (3.66% as Gd) even at 150% coating level. The swelling type of microcapsules (MC-D1) did not release Gd at all for the entire 120 min of the experiment in a 0.9% saline solution. On the other hand, the rapid-erosion type (MC-D2) and the vesicle-dispersing type (MC-D3) released Gd with a lag time. The percent released depended on the coating level and the SL content in the Gd-fixing layer. A large number of droplet-like particles spouted out, and/or tubular vesicles formed with MC-D2 and MC-D3 in the saline solution. These phenomena implied that the water-insoluble Gd-DTPA-SAM would be entrapped in these particles/vesicles. When MC-D2 and MC-D3 were administered to normal rats *via* the hepatic artery, a Gd-accumulation as high as 70 and 71% of the injected dose was detected in the whole liver 2 h after administration. In addition, biochemical and histological evaluation of the liver after administration indicated that embolization of the microcapsules actually occurred in the blood vessels, and that necrosis induced by ischemia was not serious. These results suggested that administration of these microcapsules might be multiply repeated in order to accumulate the required amount of Gd in tumors.

Key words microcapsule; lecithin; gadolinium neutron capture therapy; intraarterial administration; small-scale preparation; fluidized bed

Neutron capture therapy (NCT) is a promising radiation therapy for treating cancer.¹⁾ In NCT, a nonradioactive compound containing boron (B) or gadolinium (Gd) is accumulated in tumors, and the tumors are irradiated with neutrons. The nuclide in the atom absorbs these neutrons and disintegrates into killing radiation. Several B compounds that accumulate preferentially in tumors have been developed and great therapeutic effects have been reported, especially with borocaptate sodium and *p*-boronophenylalanine against malignant brain tumors and malignant melanoma, respectively.^{1,2)} On the other hand, NCT using Gd (GdNCT) is currently underdeveloped.^{1,3)} GdNCT has the advantages that the thermal neutron capture cross section of Gd is 66 times larger than that of B and that the intracellular presence of Gd is not essential because of the long range of gammas released in the reaction of ¹⁵⁷Gd and neutrons. Currently, there is no Gd compound that selectively accumulates in tumors. Therefore, pharmaceutical techniques will be most helpful to achieve an effective Gd-concentration in tumors.

Soybean lecithin (SL) microcapsules have been developed using the Wurster process by the present authors.⁴⁾ They are composed of a lactose core (75–106 μm), a layer of carbazochrome sodium sulfonate (CCSS, a model drug) fixed with a mixture of SL, cholesterol (CH), stearic acid (SA) and polyvinylpyrrolidone (PVP), and a coat of the mixture. They exhibited short-term delayed and subsequently prolonged release and bioerosion, and these properties are changed flexi-

bly and easily by varying the composition of their membranes.^{4,5)} In a previous study, Gd-containing SL microcapsules were prepared to control Gd-release using three kinds of Gd-compounds with different physico-chemical properties: they were composed of a lactose core subcoated with PVP (75–90 μm), a layer of a Gd-compound and PVP, and a coat of the 5:5:2 (weight ratio) SL-CH-SA mixture containing 42% PVP based on the total weight of SL, CH and SA.⁶⁾ Release of water-soluble Gd-compounds had a short lag time of about 10 min, then a burst followed by sustained release; on the other hand, release of a hydrophobic Gd-compound was almost completely suppressed for the entire 120 min. Therefore, use of the hydrophobic Gd-compound seemed to be favorable, when Gd-release from the fine microcapsules was required to be strongly suppressed.

In this study, Gd-containing lecithin microcapsules as a dosage form for intraarterial administration were designed and prepared to accumulate Gd at a high content in tumors. Taking into consideration of modification of microcapsule formulation for individual patients in preclinical use, a small-scale technique in preparing the microcapsules with a high drug content by the Wurster process will be required. So, a dilution method was developed in this study. Three kinds of Gd-containing microcapsules were prepared by this method, and then, Gd-accumulation in the target organ and the degree of necrosis which would be caused by ischemia were evaluated using normal rat liver after intraarterial administration

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of the microcapsules.

Experimental

Materials Lactose (DMV 200M) was used as a core and a diluent material. SL (reagent grade, about 30% phosphatidylcholine, a minimum of 95% phospholipids) was purchased from Nacalai Tesque, Inc., Kyoto, Japan. The phospholipids contained in SL were described in detail in a previous report.⁷⁾ CH (guaranteed reagent (GP) grade), SA (GP grade), PVP (K-30, M.W.=40000, extra pure reagent grade), methylene chloride and ethanol were used as purchased from Nacalai Tesque, Inc. For the Gd-compound, a hydrophobic distearylamine of gadopentetic acid (Gd-DTPA-SAm), synthesized following Hnatowich *et al.*⁸⁾ and Kabalka *et al.*⁹⁾ was used. Gadopentetate dimeglumine (Gd-DTPA-DM, Magnevist[®], Nihon Schering Co., Ltd., Osaka, Japan) was used as a control. A dextran solution (Dextran 40 lactated Ringer's solution, Low Molecular Dextran L Injection, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) was used as a suspending medium. Glass beads (200 μm , GB-02, Top Co., Ltd., Kyoto, Japan) were used as a diluent in release tests after washing with ethanol and distilled water. Human plasma was supplied by the Japanese Red Cross Society.

Coating A Grow Max (140) spouted bed coater with a draft tube (Fuji Paudal Co., Ltd., Osaka, Japan) was used. A spray nozzle of 0.8 mm diameter and a bag filter with an opening of about 5 μm were employed throughout all experiments.

Preparation of Microcapsules by the Dilution Method Details of cores, compositions of spray solutions and coating conditions are listed in Table 1. Lactose was sieved into 44–53 and 53–63 μm for the diluent and the core, respectively. First, 2 g of Gd-DTPA-SAm (–20 μm) was manually fixed to 1 g of lactose cores using an appropriate amount of PVP with or without SL dissolved in methylene chloride, and then the Gd-fixed cores were prepared to be 75–125 μm prior to coating. The Gd-fixed cores were diluted by 24 g of the lactose of 44–53 μm , and then coated together using the spray solution containing 20 or 40 g of SL, CH, SA and/or PVP by the Wurster process. The Gd-containing microcapsules were collected by sieving. Their coating level is denoted as 75 or 150%, respectively.

Particle Size Distribution A sieve analysis was carried out using a row-tap shaker (Iida Seisakusho Co., Ltd., Osaka, Japan).⁵⁾

Drug Release and Content Release tests by the column method using HPLC (Shimadzu LC-3A, Kyoto, Japan) were carried out as reported previously.⁶⁾ Microcapsules were sieved into 106–125 or 125–149 μm , which was the main fraction of single-core microcapsules, and then the microcapsules containing 200 μg as Gd were used in the test. A 0.9% saline solution was used as the test fluid.

The drug content of the microcapsules was determined using the same procedure reported previously.⁶⁾

Polarizing Microscopy An Olympus BX50 polarizing microscope (BXP) was used with a heating stage and a temperature controller (LK-600PM, Linkam, Scurry, UK). Microcapsules (106–125 or 125–149 μm) immersed in the saline solution were observed at 37°C. The microcapsules added in a glass tube containing the saline solution or human plasma and incubated at 37°C under gentle agitation were also observed.

Size Distribution Analysis A laser scattering particle size analyzer (LDSA-2400A, Tonichi Computer Co., Ltd., Japan) was used to measure the size distribution of hydration-induced particles suspending in the saline solution at 37°C under gentle agitation.

Drug Administration Male Wistar rats, 9- or 10-week-old at the time of the experiments, were used. The rats were anesthetized by i.p. injection of pentobarbital sodium (40 mg/kg body weight). Gd-DTPA-DM as a control or microcapsules were administered (340 μg Gd/kg body weight) to the rats by the following surgical procedure. A midline laparotomy was performed and the left gastric artery was exposed. The splenic and the gastroduodenal artery were temporarily ligated by clamps to achieve administration only into a liver; then, dissolved Gd-DTPA-DM or the microcapsules suspended in 1 ml of the dextran solution were administered into the hepatic artery for about 3 min *via* a retrogradely inserted catheter into the left gastric artery. Immediately after administration, the catheter was removed and the left gastric artery was ligated, followed by removing the clamps on the splenic and the gastroduodenal artery in order to restore the circulation. Thereafter, the abdominal wound was closed, except for the rats that were intended to be sacrificed 1 or 2 h after administration. The actual amount of administered Gd was corrected by measuring the Gd remaining in the syringe after administration.

Gd-Accumulation in Liver and Other Organs Gd-DTPA-DM ($n=3$) or microcapsules ($n=9-12$) were administered to the rats. The rats were

sacrificed, and then their livers, hearts, lungs, spleens and kidneys were collected and weighed 120 min after the administration. Each organ was homogenized and freeze-dried; then, a constant amount of the freeze-dried powder was ashed using 3 ml of 6.6N nitric acid. The ashed sample was dissolved in 3 ml of 6.6N nitric acid added again and the Gd-concentration of the obtained solution was measured at 342.247 nm by inductively coupled plasma emission spectrometry (P-5200 ICP system, Hitachi Co., Ltd., Tokyo, Japan) to determine the Gd-accumulation in each organ as a percent of the injected dose (ID).

Histological and Biochemical Evaluation of Liver Blood samples were collected from a carotid artery and livers were removed 1 h and 1, 3 and 7 d after administration of microcapsules ($n=3-6$ at each time). The blood samples were centrifuged to separate serum and the serum samples were kept frozen until analysis. Activities of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and alkaline phosphatase (ALP) in the serum were analyzed according to the UV-Rate method for GOT and GPT and Bessey-Lowry method for ALP using test-kits (test Wako; Wako Pure Chemical Industries, Ltd., Osaka, Japan). Necrosis on the surface of the liver was macroscopically observed. Liver tissue was fixed and dehydrated with 10% formalin and graded alcohols, respectively. Thereafter, they were embedded in paraffin and then sectioned. Staining was performed with hematoxylin-eosin.

Results

Design and Preparation of Microcapsules by the Dilution Method Lecithin microcapsules as a dosage form for intraarterial administration on GdNCT were designed in this study. The important functions that the microcapsules had to possess were: to accumulate Gd with a high content in tumors for a required period; and to degrade or erode in a blood vessel at an appropriate time for multiply repeated administrations. The degradation or erosion would relate to avoidance of serious necrosis induced by ischemia in normal tissue that is adjacent to the tumor.

Two types of microcapsules were designed in this study. The first was microcapsules for accumulating a large amount of Gd in the blood vessels of tumors. In this case, the microcapsules were required to embolize the blood vessels without Gd-release during neutron irradiation and, thereafter, it was favorable for blood flow to be restored by rapid degradation of the microcapsules (delayed-erosion type). The second was microcapsules for dispersing and retaining Gd extensively not only in blood vessels but also in tumor tissue (rapid-erosion type). So, it was required for Gd to be dispersed frequently from the microcapsules, for example, as hydration-induced small particles, following very short-term embolization of the blood vessels.

MC-D1, MC-D2 and MC-D3 were prepared by the dilution method. Schematic diagram of their structures is shown in Fig. 1: Gd-DTPA-SAm was chosen as the Gd-compound. Membrane composition is shown as the weight ratio of SL, CH and SA and the weight percent of PVP based on the total weight of SL, CH and SA. MC-D1 was designed as the delayed-erosion type. In MC-D1, Gd-DTPA-SAm was fixed with PVP and the membrane of the microcapsules was composed of the SL:CH:SA=5:5:2 mixture containing 42% PVP: the weight ratio of SL and PVP used in the fixing will be denoted in parenthesis like MC-D1(0:1) below. Gd-release from these microcapsules was expected to be strongly suppressed.⁶⁾ On the other hand, MC-D2 and MC-D3 were designed as a rapid-erosion type. The membrane of MC-D2(0:1) was composed of the SL:CH:SA=1:0:0 mixture containing 42% PVP. CCSS-containing microcapsules with the same membrane as MC-D2(0:1) eroded rapidly, accompanying the growth of an isotropic oily phase and the rapid

release of CCSS in the previous studies.^{4,5} In MC-D3, a SL-PVP mixture with the weight ratio of 5 : 5, 12 : 5 or 16 : 5 was used as binder in fixing Gd-DTPA-SAm to lactose cores. The addition of SL was expected to induce more frequent dispersion of Gd-DTPA-SAm from microcapsules. The membrane

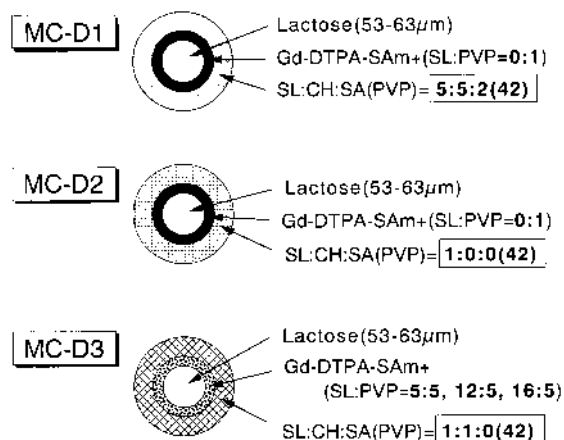


Fig. 1. Schematic Diagram of Microcapsules Prepared by the Dilution Method

of MC-D3 was composed of the SL : CH : SA = 1 : 1 : 0 mixture containing 42% PVP. In this case, dispersion of the membrane as small droplet-like particles was expected, since CCSS-containing microcapsules with the same membrane as MC-D3 showed frequent spouting of small droplets incorporating CCSS from the microcapsules as previously reported.^{4,5}

In spite of the small size of diluents (44–53 μm), the yield of the microcapsules prepared by the dilution method was 81–92% (Table 1). The main size fraction of the microcapsules containing Gd was 90–125 or 106–149 μm at 75% coating level, and 106–149 or 125–177 μm at 150% coating level. Using only 2 g of Gd-DTPA-SAm, microcapsules containing 34.9% and 24.9% of Gd-DTPA-SAm (5.13% and 3.66% as Gd) were obtained at 75% and 150% coating levels, respectively.

Gd-Release from Microcapsules Release profiles of MC-D1, MC-D2 and MC-D3 in the saline solution at 37 °C by the column method are shown in Fig. 2. Gd was not released from MC-D1(0 : 1) coated at either 75% and 150% levels (MC-D1(0 : 1–150)) for the entire 120 min (Fig. 2A). On the other hand, a small initial burst of Gd-release was observed with MC-D2(0 : 1) (Fig. 2A). With an increase in

Table 1. Microcapsules Prepared by the Dilution Method and the Characteristics of the Products

		MC-D1(0 : 1)		MC-D2(0 : 1)		MC-D3(5 : 5)		MC-D3(12 : 5)		MC-D3(16 : 5)	
		(75)	(150)	(75)	(150)	(75)	(150)	(75)	(150)	(75)	(150)
Drug fixing:											
Core: Lactose	(g)	1		1		1		1		1	
(53–63 μm)											
Drug: Gd-DTPA-SAm	(g)	2		2		2		2		2	
Binder: PVP K-30	(g)	0.76		0.50		0.30		0.30		0.25	
SL	(g)					0.30		0.72		0.80	
Solution: CH ₂ Cl ₂	(ml)	25		13		7.5		6		5	
Yield (g)	75–125 μm	2.76		2.74		2.88		3.03		2.71	
Coating: ^{a)}											
Diluent: Lactose	(g)	24		24		24		24		24	
(44–53 μm)											
Spray solution:											
SL	(g)	5.89	11.80	14.00	28.00	6.72	13.40	6.76	13.50	6.68	13.40
CH	(g)	5.89	11.80			6.72	13.40	6.76	13.50	6.68	13.40
SA	(g)	2.36	4.71								
PVP K-30	(g)	5.89	11.80	5.82	11.70	6.72	13.40	6.76	13.50	6.68	13.40
Ethanol-CH ₂ Cl ₂		added		added		added		added		added	
Total	(ml)	401	801	401	801	401	801	405	810	401	801
Total yield (%)		—	86	—	83	—	81	—	90	—	92
Yield of MC (g)	90–106 μm	0.02	—	0.26	—	0.21	(0.91)	0.11	(1.07)	0.23	—
	106–125 μm	0.17	(1.09)	0.26	(1.09)	0.16	(0.72)	0.09	0.53	0.15	0.90
	125–149 μm	0.25	1.90	0.19	1.46	0.05	0.50	0.01	0.22	0.07	0.31
	149–177 μm	0.07	0.55	0.12	0.47	—	(0.12)	—	—	0.03	(0.07)
	177 μm—	0.02	—	0.02	—	—	—	—	—	—	0.05
Gd content (%)	90–106 μm	3.28	—	3.10	—	4.06	(2.18)	2.78	(1.32)	2.42	—
	106–125 μm	3.96	(2.12)	3.82	(1.92)	5.13	3.03	3.33	2.35	3.47	2.28
	125–149 μm	4.30	2.76	4.00	2.61	4.14	3.66	— ^{b)}	2.71	— ^{b)}	2.94
	149–177 μm	— ^{b)}	3.35	— ^{b)}	2.59	—	(2.02)	—	—	— ^{b)}	(1.78)
	177 μm—	— ^{b)}	—	— ^{b)}	—	—	—	—	—	—	— ^{b)}
Gd-compound content (%)	90–106 μm	22.4	—	21.1	—	27.7	(14.8)	19.0	(9.0)	16.5	—
	106–125 μm	27.0	(14.4)	26.0	(13.1)	34.9	20.6	22.7	16.1	23.6	15.6
	125–149 μm	29.3	18.8	27.3	17.8	28.2	24.9	— ^{b)}	18.5	— ^{b)}	20.0
	149–177 μm	— ^{b)}	22.8	— ^{b)}	17.6	—	(13.8)	—	—	— ^{b)}	(12.1)
	177 μm—	— ^{b)}	—	— ^{b)}	—	—	—	—	—	—	— ^{b)}

a) Operating conditions: inlet air temperature, 27–38 °C; outlet air temperature, 20–24 °C; inlet air flow rate, 0.07–0.22 m³/min; spray liquid flow rate, 2.6–3.5 ml/min; spray air pressure, 2.1–2.4 atm; nozzle diameter, 0.8 mm. b) Not measured. The fraction where contamination of diluents into Gd-containing particles were observed are shown in parenthesis.

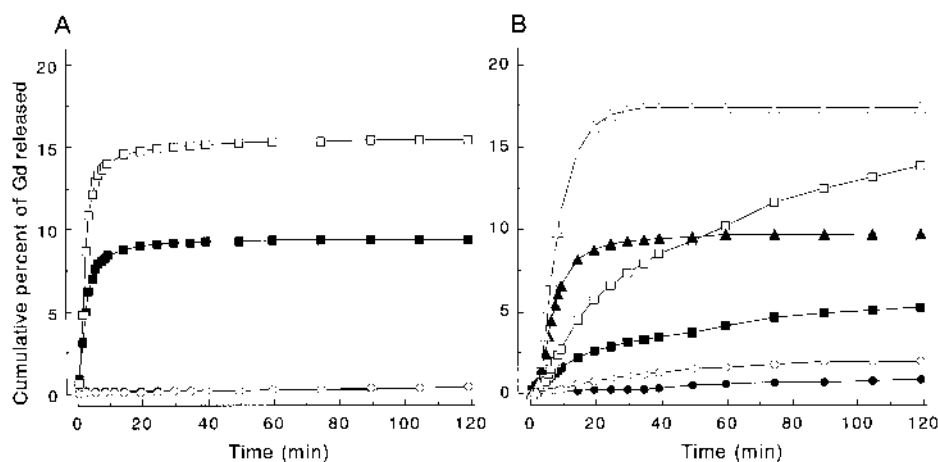


Fig. 2. Cumulative Percent of Gd Released from Microcapsules in Saline Solution at 37°C

A) Microcapsule: ●, ○, MC-D1(0:1); ■, □, MC-D2(0:1). B) Microcapsule: ●, ○, MC-D3(5:5); ■, □, MC-D3(12:5); ▲, △, MC-D3(16:5). Closed, 75% coating; open, 150% coating. Size fraction of microcapsules used in release tests: MC-D1(0:1) and MC-D2(0:1), 125–149 μm ; MC-D3, 106–125 μm .

coating level, the amount of Gd released initially was increased but thereafter no more Gd-release was observed. In Fig. 2B, Gd-release from MC-D3 is shown. An increase in both the SL content in the Gd-fixing layer and the coating level promoted Gd-release.

Erosion of Microcapsules Erosion of microcapsules immersed in the saline solution was observed at 37°C under a polarizing microscope (Fig. 3a, b, e, f, i, j). The membrane of MC-D1(0:1—150) was swollen, and droplet-like particles sometimes spouted out from cracks on the surface which was caused by the swelling (Fig. 3a, b). Even at 120 min, the core remained opaque (Fig. 3b). The membrane of MC-D2(0:1) coated at 150% level (MC-D2(0:1—150)) was highly hydrated and became an isotropic oily phase (Fig. 3e, f). Their Gd-fixed cores gradually became small and some of them disappeared during the observation. When MC-D3(16:5) coated at 150% level (MC-D3(16:5—150)) was observed, many small droplet-like particles spouted out initially (Fig. 3i). Then, many anisotropic tubular vesicles appeared on their membrane (Fig. 3j). The droplet-like particles and tubular vesicles were more significantly observed with both the higher coating levels and the higher contents of SL in the drug-fixing layer of MC-D3.

When microcapsules were incubated in the saline solution under gentle agitation at 37°C, a greater number of small droplet-like particles with MC-D2(0:1—150) and of anisotropic vesicles with various forms, for example, tubular, spherical, oval and dumbbell-like, with MC-D3(16:5—150) were dispersed in the fluid (Fig. 3g, k). The size distributions of such secondarily induced particles in the fluid after 120 min incubation are shown in Fig. 4. The mean diameters were 14 and 11 μm with MC-D2(0:1—150) (Fig. 4A) and MC-D3(16:5—150) (Fig. 4B), respectively. The large isotropic oily phase as seen in Fig. 3g is not included in the distribution due to their deposit.

Microcapsules were similarly observed in human plasma at 37°C under gentle agitation (Fig. 3d, h, l). Small, anisotropic vesicles were generated around the swollen membrane of MC-D1(0:1—150) (Fig. 3d), different from the observation in the saline solution (Fig. 3b, c). On the other hand, the behavior of MC-D2(0:1—150) in human plasma

(Fig. 3h) was basically similar to that in the saline solution (Fig. 3g), but the hydrated phase seemed to be more finely dispersed. With MC-D3(16:5—150), the tubular vesicles greatly grew in length (Fig. 3l): some of them had a length of about 300 μm in human plasma. A number of anisotropic vesicles were also dispersed in the fluid.

Gd-Accumulation in Liver and Other Organs Gd-accumulation in the whole liver of normal rats 2 h after hepatic arterial administration was evaluated using MC-D1(0:1—150), MC-D2(0:1—150) and MC-D3(16:5—150). Gd-DTPA-DM dissolved in dextran solution was also administered as a control. Gd-concentrations in the lungs, heart, spleen and kidneys, which can be attributable to leakage of Gd into the hepatic vein, were also investigated. When Gd-DTPA-DM was administered, less than 1% ID was detected in the liver (Fig. 5). However, significantly higher Gd-accumulations were detected in the liver with the microcapsules: 52% ID for MC-D1(0:1—150), 71% ID for MC-D2(0:1—150), and 70% ID for MC-D3(16:5—150). On the other hand, less than 1% ID was detected in the heart and spleen, and a slightly higher Gd-accumulation, about 2% ID, in the lungs with all three microcapsules. In the kidneys, 4% ID was detected with MC-D1(0:1—150), while the Gd level was very low with the other microcapsules.

Biochemical Evaluation of Liver Functions Biochemical evaluation of liver functions after hepatic arterial administration of microcapsules was performed in order to study the degree of damage induced by ischemia (Fig. 6). Activities of GPT, GOT and ALP in serum were measured after administration of MC-D2(0:1—150) and MC-D3(16:5—150) in this study. Only 1 h after administration, great increases in the activities of GPT and GOT were observed in both MC-D2(0:1—150) (Fig. 6a, b) and MC-D3(16:5—150) groups (Fig. 6d, e). The high activities continued 1 d after administration. Then, 3 d after administration, the activities in both groups decreased to almost the same level as those in a control. No meaningful change in ALP was found until 7 d after administration in both groups (Fig. 6c, f). There was no significant difference in each activity between two microcapsule-administered groups.

Observation of Liver Necrosis The surface of the liv-

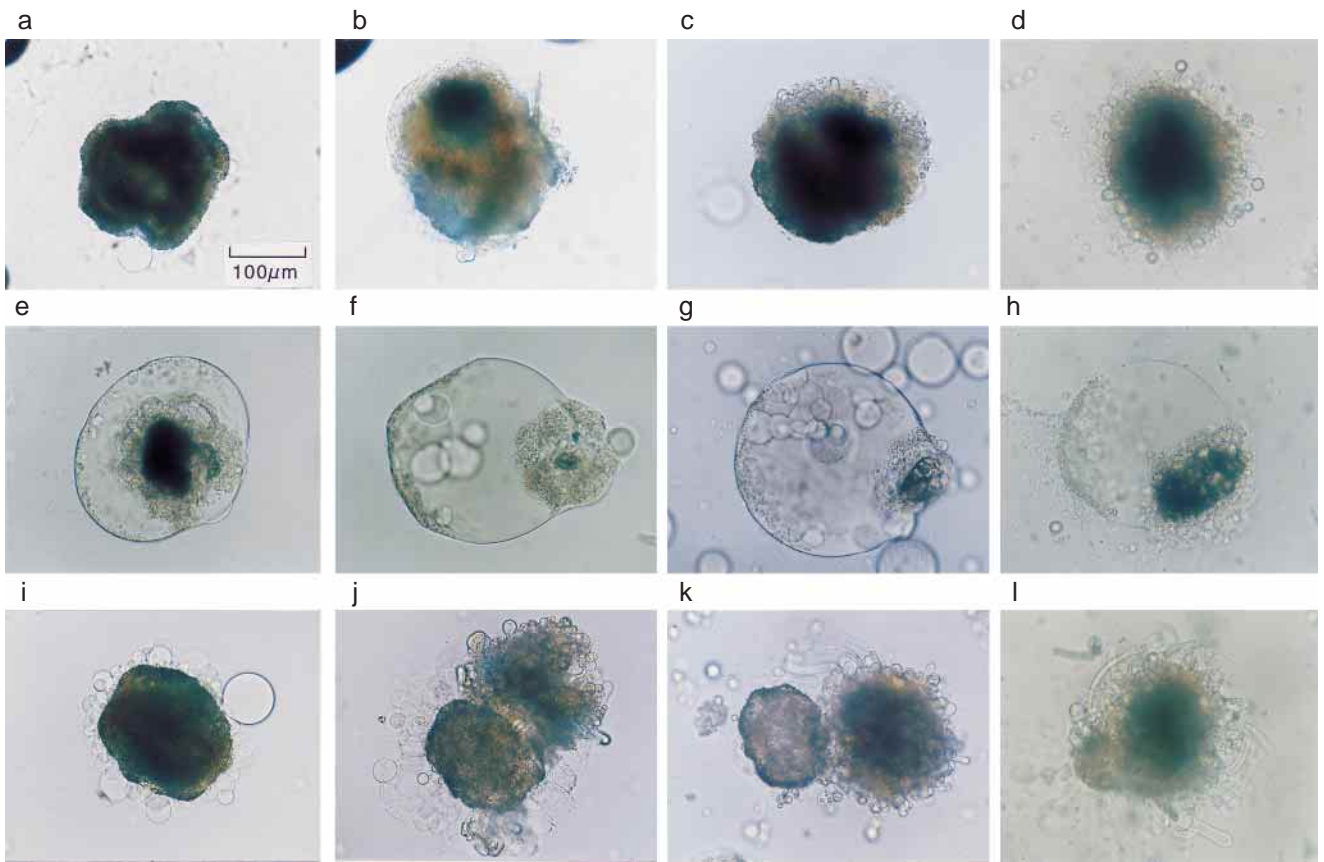


Fig. 3. Photomicrographs of Microcapsules in the Saline Solution or Human Plasma at 37 °C

Microcapsule: a—d, MC-D1(0 : 1—150); e—h, MC-D2(0 : 1—150); i—l, MC-D3(16 : 5—150). Condition: a, b, e, f, i, j, immersed; c, d, g, h, k, l, agitated. Medium: a—c, e—g, i—k, saline solution; d, h, l, human plasma. Time: a, e, i, 3 min; b—d, f—h, j—l, 120 min. Size fraction of microcapsules used: MC-D1(0 : 1—150) and MC-D2(0 : 1—150), 125—149 μm ; MC-D3(16 : 5—150), 106—125 μm .

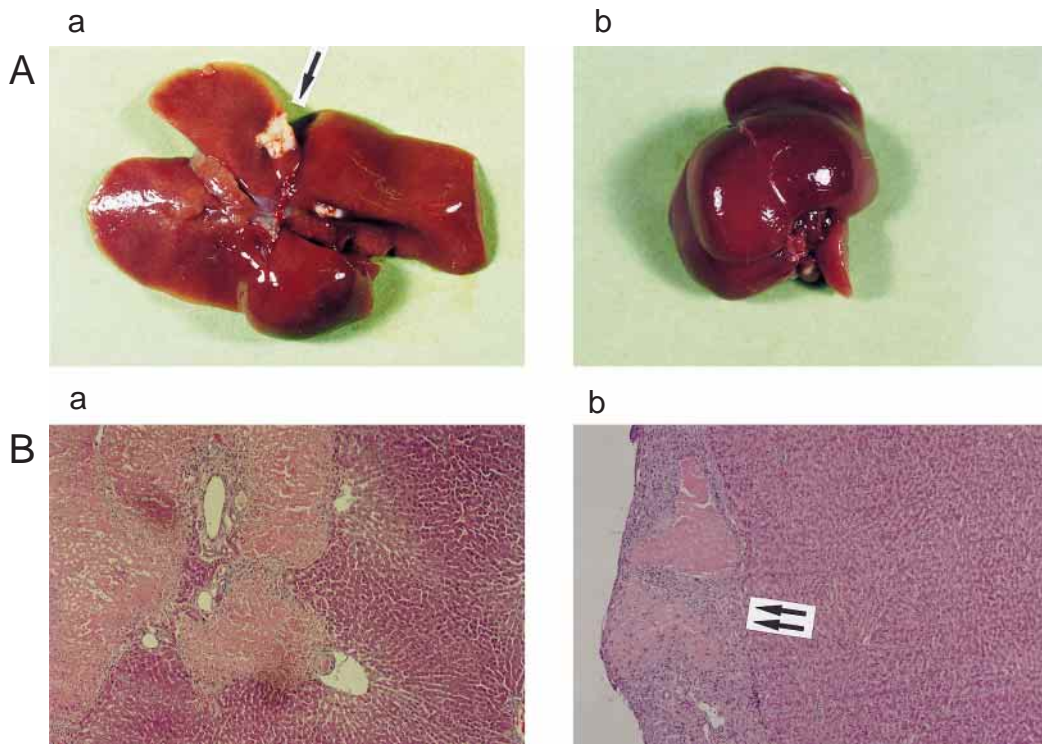


Fig. 7. Macroscopic (A) and Histological (B) Observations of Liver after Hepatic Arterial Administration of MC-D2(0 : 1—150) to Rats

Size fraction of administered microcapsules, 125—149 μm . Administration dose is 340 μg Gd/kg body weight. a, 3 d after administration; b, 7 d. Necrosis was observed on peripheral part 3 d after administration (at arrow). A large number of fibroblasts were surrounding the necrosis 7 d after administration (at arrows).

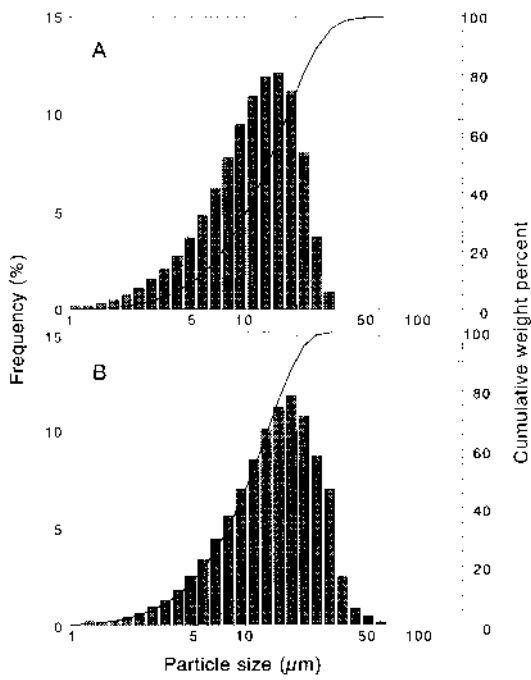


Fig. 4. Size Distribution of Hydration-Induced Particles Dispersed in the Saline Solution 120 min after Incubation at 37°C with (A) MC-D2(0:1—150) and (B) MC-D3(16:5—150)

Size fraction of microcapsules used: MC-D2(0:1—150), 125—149 μm; MC-D3(16:5—150), 106—125 μm.

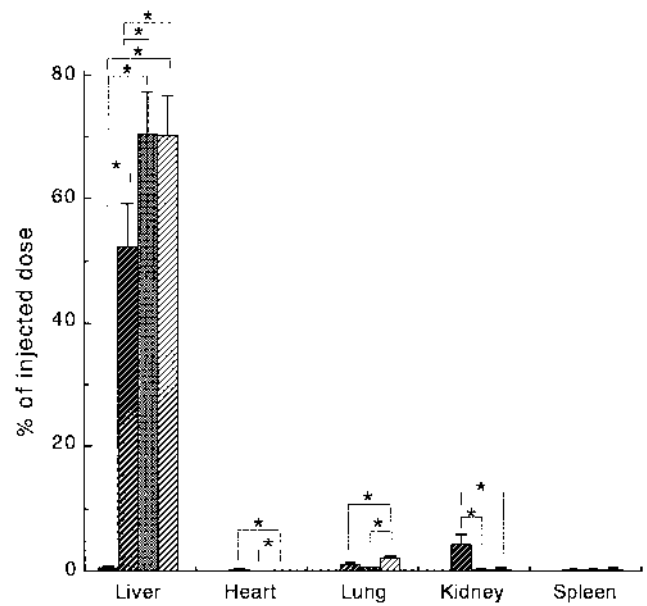


Fig. 5. Accumulation of Gd in Various Organs 2h after Hepatic Arterial Administration of Gd-Solution or Microcapsules to Rats

■, Gd-solution ($n=3$); ▨, MC-D1(0:1—150) ($n=9$); □, MC-D2(0:1—150) ($n=9$); ▩, MC-D3(16:5—150) ($n=12$). Size fraction of administered microcapsules: MC-D1(0:1—150) and MC-D2(0:1—150), 125—149 μm; MC-D3(16:5—150), 106—125 μm. Administration dose is 340 μg Gd/kg body weight. Values are mean percent of administered Gd±S.E. of the mean. * $p<0.1$ compared with each group. The dextran solution was used as a suspending medium. Gd-DTPA-DM dissolved in the dextran solution was used as Gd-solution; accumulation of Gd was measured only for liver.

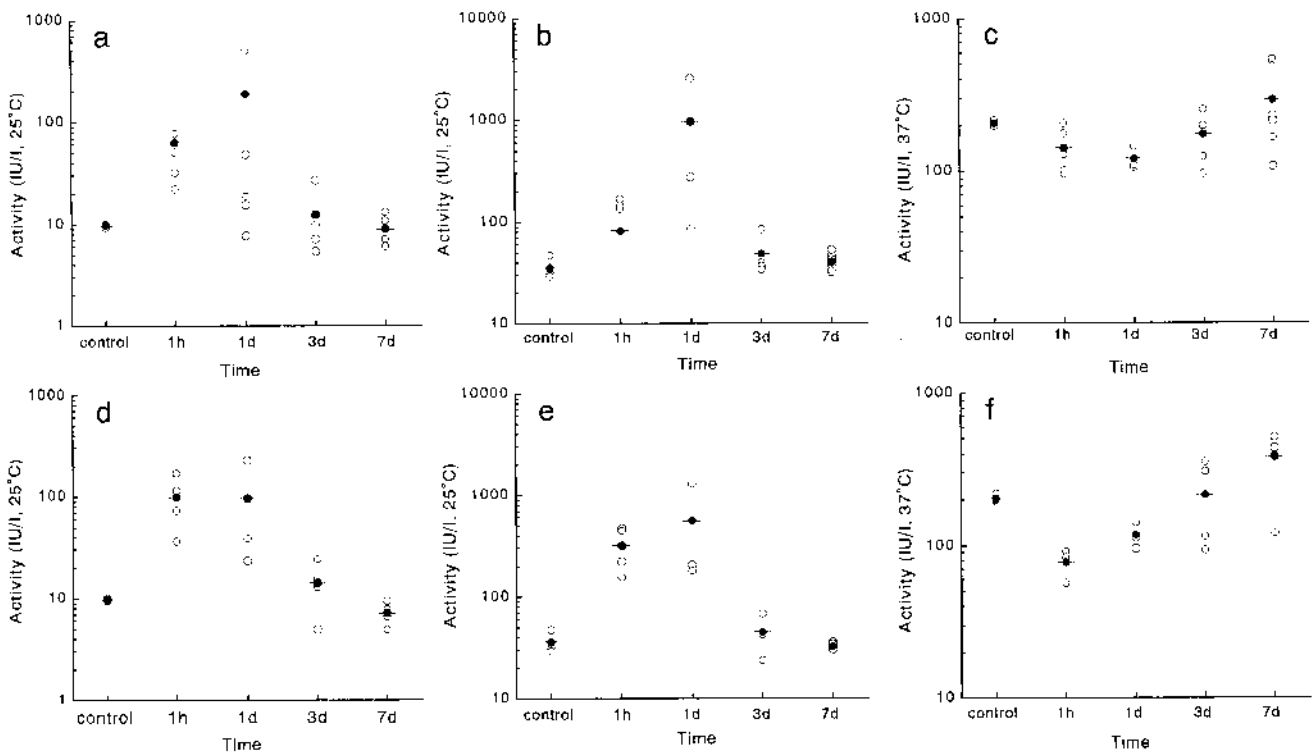


Fig. 6. Change of GPT, GOT and ALP Activities in Serum after Hepatic Arterial Administration of Microcapsules to Rats

Microcapsule: a, b, c, MC-D2(0:1—150); d, e, f, MC-D3(16:5—150). Activity: a, d, GPT; b, e, GOT; c, f, ALP. Symbol: open, individual value; closed, mean. Size fraction of administered microcapsules: MC-D2(0:1—150), 125—149 μm; MC-D3(16:5—150), 106—125 μm. Administration dose is 340 μg Gd/kg body weight. ($n=3-6$).

er was macroscopically observed (Fig. 7A). When MC-D2(0:1—150) was administered, no change was observed 1 h after administration. One day after administration, however, small, dark-red degenerations were sporadically and peripherally found; then, white, small degenerations were observed only on a peripheral part 3 d after administration (Fig. 7A a). Thereafter, they were found only rarely 7 d after administration (Fig. 7A b). When MC-D3(16:5—150) was administered, the change was similar to that with MC-D2(0:1—150) (data not shown).

Histological evaluation of liver tissue was also performed after administration of the microcapsules. With MC-D2(0:1—150), partial necrosis, stained pink, was observed on the peripheral part of the liver 3 d after administration, as shown in Fig. 7B a. Then, a large number of fibroblasts were observed to surround the necrosis 7 d after administration, as shown by the arrows in Fig. 7B b. This indicated that 7 d after administration, the necrosis was in the process of repair.

Discussion

In GdNCT, a high accumulation of Gd in tumors is required for high therapeutic effects. The optimal ^{157}Gd -concentration in tumors was predicted to be around 100 ppm^{3c)} or 50 to 200 $\mu\text{g/g}$ tumor.^{3b)} This value is much larger than the concentration required for antitumor drugs which have been clinically used so far; for example, IC_{50} s of cisplatin, bleomycin and 5-fluorouracil against Ehrlich tumor cells are 0.035, 5.9 and 0.072 $\mu\text{g/ml}$, respectively, at 24 h of exposed time.¹⁰⁾ However, since chelated Gd itself has very low toxicity, whose LD_{50} in rats after i.v. injection is 10 mmol/kg if it is not the Gd^{3+} ion form,¹¹⁾ it is possible to administer a considerably higher amount of the chelated Gd-compound. In GdNCT study, Gd-DTPA-DM solution has been most commonly used as the Gd-compound, because of its clinical use as a contrast medium for magnetic resonance imaging and its high stability.^{3a,c)} The rapid clearance of Gd-DTPA-DM from tissue, however, makes it less effective for GdNCT.

In order to accumulate the extremely high amount of Gd in tumors for the appropriate period, SL microcapsules containing Gd were designed and prepared as a dosage form for intraarterial administration on GdNCT. Their characteristics such as short-term delayed and subsequently prolonged release, bioerodibility and other widely variable properties⁵⁾ seemed to make SL microcapsules suitable as the dosage form for GdNCT. A dilution method was developed to prepare microcapsules with such a variety of properties and a high drug content in a small-scale by the Wurster process.

The success of the dilution method is related to the binding strength of the membrane material.¹²⁾ In Fig. 8, the size distributions of microcapsules which were prepared with the membrane composed of the SL:CH:SA=5:5:2 mixture containing 42% PVP and the cores different in size are shown, compared with those using the latex of 6:12:8 poly(ethyl acrylate (EA)/methyl methacrylate (MMA)/2-hydroxyethyl methacrylate (HEMA)) as a membrane material, which showed an extremely low degree of agglomeration in the previous study.¹³⁾ The microcapsules contained no drug and were coated at 40% level against the core weight. The fraction of agglomerates which was estimated from a broken point on each distribution curve was only 5% for 44—53 μm

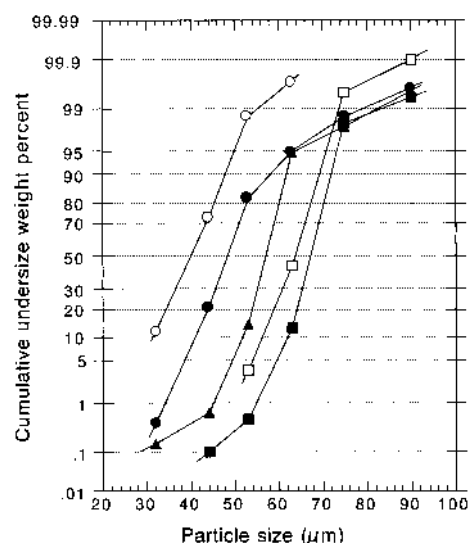


Fig. 8. Cumulative Undersize Distributions of SL Microcapsules with Various Core Size and Microcapsules Using the Latex of 6:12:8 Poly-(EA/MMA/HEMA) as a Membrane

Membrane materials: open, 6:12:8 poly (EA/MMA/HEMA); closed, SL:CH:SA=5:5:2 containing 42% PVP. Core size: ●, ○, 32—44 μm ; ▲, 44—53 μm ; ■, □, 53—63 μm .

of cores in the SL microcapsules, while 10% of microcapsules could not escape from agglomeration when such small cores as 32—44 μm were used. So, drug-fixed particles of 75—125 μm were diluted with the finer lactose particles of 44—53 μm to operate at the charged weight of about 25 g (Table 1): the efficient coating of fine cores (32—44 μm) using the Wurster process in such a small-scale as 25 g has already been established by the present authors.¹⁴⁾ The yield of the products was 81—92% (Table 1), indicating that the coating was successfully performed with a low degree of spray-drying and of loss of the core particles. Since the drug-fixed particles were to be separated from the finer diluent by sieving, it was important for each particle to be coated as a single-core particle without agglomeration. In fact, there was only a negligible contamination in the main fractions of Gd-containing particles.

Kabalka *et al.* reported that metal-DTPA-SAm complex was stable, and no transfer to serum proteins (such as albumin) and/or no metabolism to small-molecular weight compounds (such as metal-DTPA) was detected, when In-DTPA-SAm incorporated in liposomes was incubated in human serum at 37 °C up to 24 h.¹⁵⁾ Therefore, Gd would be retained in MC-D1(0:1) and released from MC-D2(0:1) and MC-D3 as a form of Gd-DTPA-SAm.

The swelling of membrane (Fig. 3b, c) with MC-D1(0:1—150) prepared by the dilution method was similar to that with the microcapsules prepared previously by the normal method, which had the same microcapsule construction except the composition of Gd-containing layer and the coating level.⁶⁾ With intact Gd-fixed cores which were used for MC-D1(0:1) and MC-D2(0:1), Gd-release was not detected at all, similar to that from MC-D1(0:1); the cores kept their shapes for a long time in the saline solution under microscope (data not shown), whereas the lactose core would immediately dissolve. These results suggested that Gd-DTPA-SAm could not be released as a form detectable in the column dissolution method used here, when its interaction

with membrane did not exist.

On the other hand, a small amount of burst-release was initially observed with MC-D2(0:1), in spite of using the same drug-fixed cores as MC-D1(0:1) (Fig. 2A). Interestingly, this initial burst increased with the coating level. The membrane of MC-D2(0:1) more highly hydrated in the saline solution (Fig. 3f, g), whose hydration was not different essentially from that of CCSS-containing microcapsules composed of the same membrane materials^{4,5}: the Gd-fixed core in MC-D2(0:1) gradually became small and disappeared at 120 min on microscopic observation (Fig. 3f, g), different from that in MC-D1(0:1) (Fig. 3b, c). These results suggested that a part of water-insoluble Gd-DTPA-SAM might interact with the highly hydrated membrane to be transformed into a dispersion which could be released from the column. The formation of droplet-like particles might be related to the transformation: although they were a few μm to several tens μm under gentle agitation (Fig. 4), they could be reduced in their size and passed through the 2- μm filter in the column by water pressure. A large amount of Gd might be in the isotropic oily phase or in large droplet-like particles remaining in the column, resulting in only 9.4% and 15.5% of Gd-release with MC-D2(0:1) (Fig. 2A).

In the case of MC-D3, SL was added to the Gd-fixing layer with the purpose of dispersing Gd from microcapsules more frequently, taking account that the dispersion of highly hydrated SL might contribute to the release of Gd-DTPA-SAM. As expected, Gd-release from MC-D3 was increased with not only the coating level but also SL content in the Gd-fixing layer, maintaining a small lag time (Fig. 2B). These results indicated that Gd-release could be enhanced by highly-hydratable SL phase. The initial spouting of droplet-like particles with MC-D3(16:5—150) came from the property which the membrane materials themselves originally had (Fig. 3i).^{4,5} Interestingly, a large number of tubular vesicles, however, appeared on particles only with MC-D3 thereafter (Fig. 3j, k), but not with the CCSS-containing microcapsules. The membrane seemed to be restructured into the vesicles incorporating Gd-DTPA-SAM in the process of hydration; then they dispersed as small anisotropic vesicles. The amount of highly-hydratable SL phase, which would not incorporate a sufficient amount of both CH and SA,⁵ in the Gd-fixing layer and the coat would be related to the release of Gd-DTPA-SAM from the microcapsules.

¹¹¹In-DTPA-SAM is commonly used as a marker of liposome biodistribution study due to its mimic phospholipids structure and its *in vivo* stability.¹⁶ This comes from the fact that In-DTPA-SAM incorporated into liposomes behaves together with lipids. This supported that there was possibility for Gd-DTPA-SAM to be entrapped into SL phases in both the Gd-containing layer and the membrane, and dispersed as the droplet-like particles and anisotropic vesicles in MC-D2(0:1) and MC-D3.

If such small vesicles and particles as several tens to 100 nm in size containing Gd-DTPA-SAM are spontaneously spouted from the microcapsules, they will easily permeate into tumor tissue. There are many studies on microemulsion and other self-emulsifying systems using surfactants, oils, cosurfactants and water.¹⁷ In such systems, fine emulsions with 5—140 nm particles were formed under only gentle agitation. Trotta *et al.* reported microemulsion systems contain-

ing lecithin as a surfactant with a diameter smaller than 60 nm.^{17b,c} On the other hand, spherical unilamellar vesicles were spontaneously formed in the system with lecithin-water-geraniol, a branched biological alcohol.¹⁸ Depending on composition, the lecithin formed a lyotropic liquid-crystal multilamellar phase, spherical unilamellar vesicles, or entangled multilamellar tubular vesicles with a width from tens nm to 1 μm and a length of nano order to 100 μm at 25 °C without agitation. Therefore, secondarily induced particles with a few μm or nano order size were likely to be formed from the microcapsules using lecithin by modifying membrane composition or introducing additives like a cosurfactant.

In order to evaluate Gd-accumulation in the organ into which microcapsules were administered *via* its feeding artery, the liver of the rat was chosen as a model. Since the aim of this study was just to know how much Gd accumulated in the organ after administration, the whole liver of the normal rat was targeted here. It was a little surprising that Gd-accumulations in the liver were higher with MC-D2(0:1—150) and MC-D3(16:5—150) than that with MC-D1(0:1—150) (Fig. 5), because Gd was not released at all from MC-D1(0:1—150) but from MC-D2(0:1—150) and MC-D3(16:5—150) on release test (Fig. 2). Two possible explanations may account for the low Gd-accumulation with MC-D1(0:1—150). First, unexpected rapid erosion of microcapsules and/or fast Gd-release occurred in the artery, resulting in leakage of Gd to the hepatic vein with less retention in the liver, whereas the microcapsules only slightly eroded in human plasma by 120 min on microscopic observation (Fig. 3d). Second, the microcapsules were refluxed into the gastroduodenal or the celiac artery during or just after administration owing to both strong embolization in blood vessels of the liver and pressure induced by injection. On the other hand, it was suggested with MC-D2(0:1—150) and MC-D3(16:5—150) that the rapid-erosion of microcapsules and the formation of droplet-like particles and tubular vesicles (Fig. 3e—l) might be related to the high Gd-accumulation. Microcapsules with the membrane eroded and hydrated would easily move into superior vessels, resulting in minimizing the reflux.

It was reported that Gd-DTPA-SAM was predominantly eliminated by the hepato-biliary route; as a result, 16% of Gd was detected in the collected feces over 5 d after *i.v.* administration of the liposomes.¹⁹ This would make a suggestion that at least a part of about 30% ID which was not detected in collected organs (the liver, heart, lungs, kidneys and spleen) 2 h after administration of MC-D2(0:1—150) and MC-D3(16:5—150) might be eliminated by the hepato-biliary route.

Considering *in vitro* and *in vivo* stability and accumulation for a period exceeding 11 d of Gd-DTPA-SAM with the liposomes reported by Kabalka *et al.*,¹⁵ it would be hard for Gd-DTPA-SAM, which not only remained in the microcapsules, but was also released from them, to be metabolized and to be excreted. This would be an advantage for achieving a high concentration of Gd in tumors for a sufficiently long period. On the other hand, retention of the metal in a body for an unnecessarily excessive period might be a problem. It was reported that Gd-DTPA distearylester and distearylthiolester were more labile than Gd-DTPA-SAM, because they showed

both their transfer to serum albumin and their metabolism in a time-dependent manner.¹⁵⁾ Even if these Gd compounds are to be used, the required properties of microcapsules would be easily attained, for example, by varying membrane composition.

Biological and histological evaluations of the liver using MC-D2(0:1—150) and MC-D3(16:5—150) without irradiation gave some important information on *in vivo* behavior of the microcapsules in blood vessels after intraarterial administration (Figs. 6, 7). The increase in activities of GPT and GOT in serum (Fig. 6) and the necrosis of the liver (Fig. 7a) demonstrated that embolization in the hepatic artery was actually induced by the microcapsules and/or the secondarily induced-particles. Additionally, a decrease in the activities induced (Fig. 6) and the appearance of a large number of fibroblasts (Fig. 7B b) indicated that the tissue with damage caused by the embolization was in the process of reparation 7 d after administration. This suggested that the damage caused to the normal tissue adjacent to tumors by ischemia would not be serious and, if necessary, multiply repeated administration of the microcapsules can be carried out at an appropriate interval in order to store an extremely large amount of Gd in tumors.²⁰⁾

Superselective catheterization has been developed for angiography and has been also applied for treatment.²¹⁾ Park *et al.* reported superselective transcatheter arterial embolization with ethanol and iodized oil to treat hepatocellular carcinoma, where a catheter was inserted into a feeding vessel up to the third-order branch of a hepatic artery.^{21b)} When MC-D2(0:1—150) or MC-D3(16:5—150) are selectively delivered into a feeding artery of the tumor using this technique, 70% ID should be accumulated in the tumor for at least 2 h.

Since distribution of thermal neutron fluence rates is exponentially decreased as the depth increased in water phantom,²²⁾ NCT seemed to be effective only for superficial tumors. Actually, most of the clinical successes on NCT were found in the treatment of melanoma and malignant glioma where the skull of a patient was opened during irradiation. Therefore, there are few attempts for tumors which do not superficially exist in organs on NCT.²³⁾ Application of NCT to the tumor, for instance, in the liver is, however, long-expected, since NCT is free from serious side effects frequently encountered in cancer chemotherapy.

NCT has been rapidly developing in preclinical research. A small number of pharmaceutical studies to deliver B- or Gd-compounds selectively, to increase the accumulation and to prolong residence time were mostly performed on liposomes²⁴⁾ and low density lipoproteins,²⁵⁾ and rarely on microcapsules^{6,26)} and microspheres.²⁷⁾ This study on lecithin microcapsules also demonstrated that utilizing pharmaceutical techniques to control the behavior of the compound will contribute to the development and the success of NCT.

Conclusion

Lecithin microcapsules were applied as a dosage form for intraarterial administration on GdNCT in order to accumulate a large amount of Gd in tumors. The dilution method developed in this study was a promising method to prepare microcapsules with a high content of a drug on a small scale using the Wurster process.

Release of Gd-DTPA-SAm varied depending on the prop-

erties of the microcapsule membrane, and increased depending on the amount of highly-hydratable SL phases in the Gd-fixing layer and the coat. It was suggested that Gd-DTPA-SAm was released being incorporated in droplet-like particles and tubular vesicles with MC-D2(0:1) and/or MC-D3.

After hepatic arterial administration of MC-D2(0:1—150) and MC-D3(16:5—150) into normal rats, a Gd-accumulation as high as 70% ID was detected in the whole liver 2 h after administration. It was suggested that formation of the particles and the vesicles and erodibility of the microcapsules might contribute to the high accumulation. In addition, necrosis induced by ischemia after administration was not serious.

Acknowledgments We are indebted to Shinkichi Hatae, from Mishima Institute for Dermatological Research, for his contribution to the histological evaluation. This work was supported in part by a Grant-in-Aid for Cancer Research (6—15) from The Japanese Ministry of Health and Welfare, Grants-in-Aid for Scientific Research (B) (08457598) and (C) (09672204) and for International Scientific Research (University-to-University Cooperative Research) (10045080) from The Japanese Ministry of Education, Science, Sports and Culture and a Grant-in-Aid for Health Science from Kobe Gakuin University.

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