Design and Preparation of Gadolinium-Reservoir Microcapsules for Neutron-Capture Therapy by Means of the Wurster Process

Masahito MIYAMOTO,*[,a] Hideki ICHIKAWA,[a] Yoshinobu FUKUMORI,[a] Yasuyuki AKINE,[b] and Koichi TOKUUYE[c]

Faculty of Pharmaceutical Sciences, Kobe Gakuin University,[a] Arise, Ikawadani-cho, Nishi-ku, Kobe 651–21, Japan, Institute of Clinical Medicine, University of Tsukuba,[b] 1–1–1 Tennoudai, Tsukuba, Ibaraki 305, Japan, and The National Cancer Center Hospital,[c] 5–5–1 Tsukiji, Chuo-ku, Tokyo 104, Japan.
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Gadolinium (Gd)-containing microcapsules designed for neutron-capture therapy (NCT) were prepared by a spouted bed coating process. Microcapsules were designed as a Gd-reservoir. They were prepared with the following properties: particle size was smaller than 50 μm, Gd-content was as high as possible, and release of Gd was suppressed as long as possible. Calcium carbonate (20—32 μm) was selected as a seed particle. As a Gd-source, gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA) or a synthesized water-insoluble Gd-DTPA derivative, Gd-DTPA-distearylamide (Gd-DTPA-SA), was layered onto the seed particles. The release-suppressing layer was composed of aqueous acrylic latex of 9:9:4 poly(ethyl acrylate/methyl methacrylate/2-hydroxyethyl methacrylate). In preliminary studies, Gd-DTPA microcapsules with 41—45 μm (mass median diameter) were prepared; they released Gd with a short lag-time and 3 h-prolongation. Complete release suppression was, however, difficult to achieve because of high water-solubility of Gd-DTPA. Hence, a hydrophobic derivative, Gd-DTPA-SA, was next used as a Gd source. Gd-DTPA-SA microcapsules could be prepared with a mass median diameter of 52 μm. Gd-DTPA-SA content of the microcapsules was 38% and release of Gd was suppressed to less than 0.2% over 60 d.

Key words neutron-capture therapy; gadolinium diethylenetriaminepentaacetic acid; microcapsule; coating; spouted bed; poly(ethyl acrylate/methyl methacrylate/2-hydroxyethyl methacrylate)

Neutron-capture therapy (NCT) is based on the nuclear reactions (neutron capture reaction, NCR) that occur when the stable isotopes of a radiosensitizer such as boron-10 and gadolinium-157 absorb low energy non-ionizing radiation of thermal neutrons to yield a lethal radiation. When boron-10 absorbs thermal neutrons, alpha-particles and lithium-7 recoil nuclei are emitted. The energy shared by alpha-particles is restricted since their range is almost the same as a diameter of one cell (about 10 μm).[1] On the other hand, the stable isotope of gadolinium-157 has the highest thermal neutron-capture cross-section among stable nuclides (255000 barn), which is approximately 66-fold greater than that of boron-10 (3833 barn). X-Ray, electron and gamma-rays are emitted in the NCR with gadolinium,[2] and the range of gamma-rays is more than 100 μm, leading to the delivery of a radiation dose to the surrounding tissue which increases the chance of hitting the cells in the tumor.[3] This indicates that the cell inactivation is expected to be induced, even though gadolinium is present in the vicinity of tumor cells.[4] By this remote tumor inactivation, a new radiation therapy is proposed utilizing the benefit of neutron-capture therapy with gadolinium (GdNCT). The therapeutic procedure using gadolinium as a radiosensitizer is as follows: First of all, a sufficient quantity of gadolinium is accumulated or located in the vicinity of tumor cells[4] and remains there. Tumor cells are exposed to the radiation only when the thermal neutron is irradiated to the gadolinium. Neutron irradiation can be continued or repeated until the tumor cell has disappeared.

In a previous study,[5] gadolinium-containing microcapsules for NCT were designed and prepared for *in vivo* experiment with mice to evaluate whether, even if gadolinium existed extracellularly, the cell inactivation could be achieved as a result of NCR with gadolinium. Microcapsules were composed of a lactose core of 53—63 μm, a gadopentetate dimeglumine (Gd-DTPA-DM, Magnevist®) layer, and an ethyl cellulose (EC) and cholesterol (CH) mixture (1 : 1 w/w) for the release-sustaining coating. Although release of Gd-DTPA-DM from the 20% overcoated microcapsules of 75—106 μm was much faster than that of lactose (core material), it was suppressed 2.6% at 10 min and 9.3% at 20 min in an aqueous dextran solution. The Gd-DTPA-DM microcapsules were evaluated as a radiosensitizer for GdNCT *in vivo*.[6] Mice were simultaneously inoculated intraperitoneally with 10^7 Ehrlich ascites tumor cells and microcapsules (gadolinium-containing microcapsules or placebo microcapsules which did not contain gadolinium). The animals were then exposed to thermal neutrons for 12 min. Survival time of the mice given the gadolinium microcapsules and then irradiated by the thermal neutrons was significantly longer than those given placebo microcapsules or control mice which were not irradiated by the neutrons ($p < 0.0001$).[6] These results indicated that radiations induced by the NCR with gadolinium effectively suppressed the growth of ascites tumor cells in mice, even when gadolinium was present in the vicinity of the tumor cells.

The previous Gd-DTPA-DM microcapsules were relatively large in size (mass median diameter; 126 μm (final product)), resulting from remarkable agglomeration in the drug-layering and waterproofing processes due to a high hygroscopicity of Gd-DTPA-DM.[5] However, if microcapsules were to be delivered to the periphery of the liver and other organs, smaller microcapsules would be preferable. In this study, fine gadolinium-containing reservoir-microcapsules for NCT were designed and prepared.

* To whom correspondence should be addressed.

Major problems to be overcome were particle size, gadolinium content and release suppression, and the challenge faced was how to harmonize these incompatible requirements.

Experimental

Materials Unless otherwise specified, reagents were used as purchased without any purification. Calcium carbonate (08 Jyutan, Maruo Calcium Co., Ltd., Hyogo, Japan) sieved into 20—32 μm was used as a core material. Nicotinic acid, polyvinylpyrrolidone (PVP, K30) and the monomers, ethyl acrylate (EA), methyl methacrylate (MMA) and 2-hydroxyethyl methacrylate (HEMA), were purchased from Nacalai Tesque Inc., Kyoto, Japan. Diethylenetriaminepentaacetic acid gadolinium (III) (Gd-DTPA) was purchased from Aldrich Chemical Company Inc., Milwaukee, WIS, U.S.A. and was used as a gadolinium source. DTPA and gadolinium chloride ($GdCl_3$) were purchased from Nacalai Tesque Inc., and stearylamine (octadecylamine) was purchased from Aldrich Chemical Company Inc. Anhydrous chloroform was obtained by adding molecular sieves (4A 1/16) to chloroform and then allowing the mixture to stand at room temperature for at least 3 d.

Preparation of Latex The acrylic polymer latex, poly(EA/MMA/HEMA), was synthesized by an emulsion polymerization technique as previously reported.[7] The molar ratio of EA, MMA and HEMA was 9:9:4 and the total monomer weight was 433 g in each polymerization. Sodium lauryl sulfate (Nacalai Tesque Inc.) and ammonium peroxodisulfate (Nacalai Tesque Inc.) were used as an emulsifier and a reaction initiator, respectively. Prepared latex was dialyzed with a cellulose-tube (1-7/8 Viskase, Sales Corp., Chicago, IL, U.S.A.) against distilled water for 5 d by replacing the water with fresh water every 12 h.

Synthesis of Distearylamide Derivative of Gd-DTPA (Gd-DTPA-SA) DTPA-cyclic-dianhydride was prepared essentially as described by Eckelman *et al.*[8a] Twenty grams (50.8 mmol) of DTPA was suspended in 60 ml pyridine, and then 20.4 g (200 mmol) of acetic anhydride was added to the suspension. The mixture was stirred for 24 h at 65 °C. The product was filtrated, washed repeatedly with acetic anhydride and diethyl ether, and then dried over silica gel under vacuum at room temperature. Distearylamide derivative of DTPA (DTPA-SA) was synthesized according to the method reported by Hnatowich *et al.*[8b] Stearylamine (13.8 g) was dissolved in 800 ml of anhydrous chloroform under N_2 atmosphere. The stearylamine solution was added to DTPA dianhydride (20.0 g) under N_2, and then the resultant suspension was heated to 65 °C, refluxed for 2 d and dried. To hydrolyze the anhydride bonds, the dried product was boiled in distilled water for 3—5 h. The crude DTPA-stearate was purified by recrystallization from hot ethanol. Gadolinium chelation was conducted according to the method reported by Kabalka *et al.*[8c] $GdCl_3$ hexahydrate aqueous solution (4.6 g/46 ml) was added dropwise to a solution of DTPA-SA (10.0 g in 200 ml ethanol) and the resulting solution was refluxed for 30 min. The solution was concentrated to one-third volume and cooled to 0 °C. The resulting solid was collected by vacuum filtration, washed with distilled water, and dried in a desiccator under vacuum.

Coating A Grow Max (140) spouted bed coater with a draft tube (Fuji Paudal Co., Ltd., Osaka, Japan) was used. The pneumatic spray nozzle used had a liquid outlet caliber of 0.8 mm. A laminated bag-filter with about 1 μm opening was set throughout all experiments. The charged weight of calcium carbonate cores was 50 g.

Particle Size Distribution of Microcapsules Sieve analysis was performed using an Alpine 200LS air jet sieve. Stainless steel sieves whose inner diameter was 75 mm and whose height was 20 mm were used for each analysis. Accurately weighed (3 g) microcapsules were loaded. Microcapsules remaining on the sieve were weighed after a 3 min sieving-operation. Sieving was repeated until no weight change was found.

Dissolution Studies Prepared microcapsules were dried under vacuum for 12 h at room temperature. Curing was performed for 12 h in an air steam oven regulated at 80 °C after the microcapsules were mixed with pulverized mannitol powder of 20 or 30% against their weight to prevent particle aggregation. About 240 mg of accurately weighed cured-microcapsules was used for each dissolution test, which was performed on an NTR-VS6P dissolution apparatus (Toyama Sangyo Co., Ltd., Osaka, Japan) according to the Japanese Pharmacopoeia (JP) XIII paddle method. Isotonic phosphate buffer (pH 7.4) was selected as a dissolution fluid. For each experiment, shaft rotation speed was maintained at 200 rpm, temperature was thermostated at 37 °C and the volume of fluid was 500 ml. At the predetermined period, an aliquot of 5 ml was withdrawn through a polyester filter tip (Finefilter F-72, Toyama Sangyo Co., Ltd.), and thereafter an equal volume of fresh dissolution fluid was replaced. Gd-DTPA or Gd-DTPA-SA concentration in the samples was determined by an inductively coupled plasma atomic emission spectrography (ICP-AES) P-5200 ICP system (Hitachi Co., Ltd., Tokyo, Japan) at 355.047 nm.

Determination of Drug Content To determine the gadolinium content, microcapsules were completely pulverized with a mortar and pestle made of agate. The pulverized Gd-DTPA-containing microcapsules, weighing about 30.0 mg, were dispersed in 30 ml phosphate buffer (pH 7.4). The dispersion was sonicated for 15 min and then centrifuged (Kubota KN-30F) at 3000 rpm for 10 min. The concentration of Gd-DTPA in the supernatant was determined with ICP-AES, and this was also used to estimate the value of 100% release in dissolution tests. In a Gd-DTPA-SA-containing microcapsule, about 15.0 mg of pulverized microcapsules was dispersed in a glass tube with 5 ml of 1 N HCl, the glass tube was completely sealed and then heated at 110 °C for 12 h. The resulting solution was filtered and appropriately diluted with distilled water; then, the concentration of Gd-DTPA-SA was determined by ICP-AES.

Scanning Electron Microscopy (SEM) Samples of microcapsules were attached to a stage *via* double-sided tape and sputter-coated with gold. Scanning electron microscopy was performed using a JEOL JSM-5300SL (JEOL Ltd., Tokyo, Japan).

Results

Particle Design of Microcapsules (MC) Characteristics required for reservoir-microcapsules at this stage of the study were as follows: 1) Release of gadolinium from microcapsules (MC) is suppressed as long as possible to make repeated NCT possible; 2) the microcapsules are smaller than 50 μm to assure their easy suspending in the dissolution fluid and passing through a syringe needle and catheter; 3) microcapsules do not necessarily have to be biodegradable; 4) content of gadolinium must be as high as possible. Gadolinium-containing microcapsules designed for evaluating coating performance and release properties are illustrated in Fig. 1. Calcium carbonate and aqueous polymeric latex of 9:9:4 poly(EA/MMA/HEMA) were selected as seed-particles and coating material for the release-suppressing layer, respectively. Gd-DTPA or Gd-DTPA-SA was used as a gadolinium source. Prepared microcapsules were covered with pulverized mannitol powder to prevent the particle aggregation in curing.

Details of the cores, the composition of spray solution and the coating conditions and performances are listed in Table 1. Gd-DTPA and nicotinic acid were directly fixed to the core particles using polyvinylpyrrolidone (PVP) as a binder. Ten grams of Gd-DTPA and 5 g of nicotinic acid were dissolved in 84 ml of distilled water; 1 g of PVP and 250 ml of methanol were then added to the solution to make a Gd-DTPA-containing spray solution. In Gd-

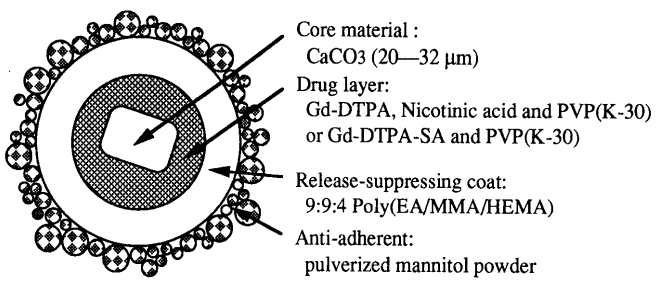

Fig. 1. Schematic Diagram of Gadolinium-Containing Microcapsule

Table 1. Formulations of Spray Solution, Operating Conditions and Coating Performances for Preparation of Gd-Containing Microcapsules

	Drug-layering		Release-suppression	
	Gd-DTPA MC	Gd-DTPA-SA MC	Gd-DTPA MC	Gd-DTPA-SA MC
Core: $CaCO_3$ (20—32 μm) (g)	50	50		
Gd-DTPA (g)	10			
Gd-DTPA-SA (g)		10		
Nicotinic acid (g)	5			
PVP (K-30) (g)	1	1		
Polymer (g)[a]			50	50
Methanol–water (3 : 1)	add			
Ethanol–CH_2Cl_2 (1 : 1)		add		
Water			add	add
Total (ml)	334	250	333	333
Product (g)/core (g)[b]	1.32	1.22	2.32	2.22
Compound content (Theoretical) (%)	15.2	16.3	8.6	9.0
(Measured) (%)	14.3	13.6	6.6	6.8
Gd content (Theoretical) (%)	4.5	2.4	2.5	1.3
(Measured) (%)	4.1	2.0	1.9	1.0
Inlet air temperature (°C)	65	40	45	45
Outlet air temperature (°C)	27—28	22	27	26
Inlet air flow rate (m^3/min)	0.05	0.06	0.05—0.08	0.06
Liquid flow rate (ml/min)	3	3.4	2.4—3.3	3.0—3.3
Spray air pressure (atm)	1.8—2.0	1.8	2.2—2.6	1.9—2.4
Yield (%)			92	95
Mass median diameter (μm)			45	41

a) 9 : 9 : 4 poly(EA/MMA/HEMA). *b*) This ratio was against $CaCO_3$ core (50 g).

DTPA-SA, 10 g of the derivative was dissolved in 250 ml of ethanol-dichloromethane (CH_2Cl_2) (1 : 1 v/v); then, 1 g of PVP was also dissolved in the solution. Nicotinic acid was essential for the fixing of Gd-DTPA, since it effectively reduced particle agglomeration. Next, 9 : 9 : 4 poly(EA/MMA/HEMA) latex was coated onto the gadolinium-fixed particles. This release-suppressing layer was coated up to the 100% level based on the weight of calcium carbonate cores. Yield of the final product reached more than 90% (Table 1: Gd-DTPA MC, 92%; Gd-DTPA-SA MC, 95%). A steady circulation of particles whose diameters were larger than 20 μm could previously be achieved under the usual conditions.[9b] In this study, however, the particle size of core materials (calcium carbonate (20—32 μm), 50 g) was unusually small.[5,9b] Hence, the inlet air flow rate was adjusted to a very low level, 0.05—0.08 m^3/min. Spray air pressure was set to 1.8 atm in gadolinium-fixing and, thereafter, increased to 2.6 atm in the coating of the release-suppressing layer. Both the inlet air flow rate and the spray pressure were gradually increased with increase in particle size during the coating. Inlet air temperature (45 °C) was adjusted to be slightly lower than the softening temperature of the 9 : 9 : 4 polymer (46 °C).[10]

Figure 2 shows the particle size distribution of microcapsules prepared with Gd-DTPA and Gd-DTPA-SA. As seen in the figure, microcapsules with narrow particle size distribution were prepared. Mass median diameter of Gd-DTPA MCs was 45 μm and that of Gd-DTPA-SA was 41 μm. When the microcapsules were observed under a microscope, particles larger than 63 μm were agglomerates composed of only two or three-core particles. Therefore, the degree of agglomerations (fractions larger than 63 μm) was found to be only 3—5%.

Dissolution of Gadolinium In order to evaluate the release of gadolinium from microcapsules, a dissolution test was performed according to the JP XIII paddle method in phosphate buffer (pH 7.4). Figure 3 shows the dissolution profiles of gadolinium from microcapsules. A clearly delayed release profile was observed for Gd-DTPA MCs (Fig. 3). After a lag-time of about 30 min, the release rate of Gd-DTPA was increased and the release was completed at about 3 h. To elucidate this fast Gd-DTPA release, change of the microcapsule morphology was observed microscopically after immersing in the dissolution fluid maintained at 37 °C. Microcapsules were swollen and enlarged in size, and burst of the

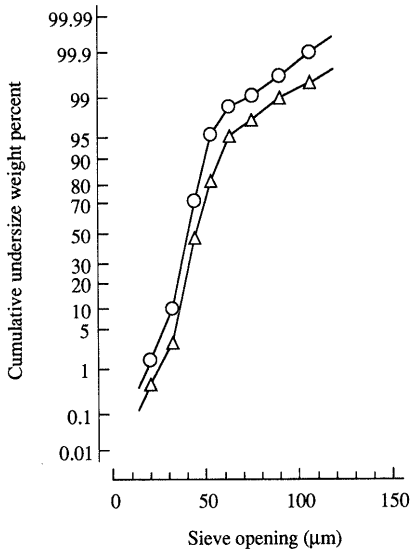

Fig. 2. Cumulative Undersize Distribution of Gadolinium-Containing Microcapsules

Microcapsules: △, Gd-DTPA MC; ○, Gd-DTPA-SA MC. Coating applied: 100%.

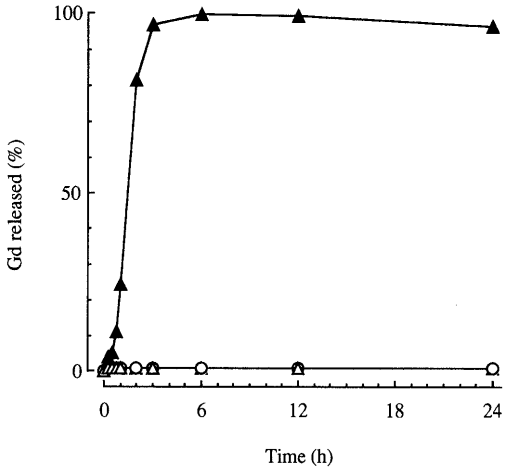

Fig. 3. Release of Gadolinium from Gd-DTPA or Gd-DTPA-SA Microcapsules

All microcapsules were cured at 80 °C for 12 h after being mixed with 20% pulverized mannitol powder. Microcapsules, coating level: ▲, Gd-DTPA MC, 100%; ○, Gd-DTPA-SA MC, 25%; △, Gd-DTPA-SA MC, 100%. Dissolution test: JP XIII paddle method. Dissolution fluid: isotonic phosphate buffer (pH 7.4). Volume of dissolution fluid: 500 ml. Dissolution temperature: 37 °C.

Table 2. Formulations of Spray Solution, Operating Conditions and Coating Performances for Preparation of NCT-SA Microcapsules

	Drug-layering	Release-suppression
Core: $CaCO_3$ (20—32 μm) (g)	40	36[a]
Gd-DTPA-SA (g)	43	
PVP (K-30) (g)	4.3	
Polymer (g)[b]		7.5
Ethanol–CH_2Cl_2 (1 : 1)	add	
Water		add
Total (ml)	1000	75
Product (g)/core (g)[c]	2.18	2.64
Compound content		
(Theoretical) (%)	49.3	40.8
(Measured) (%)	43.2	38.4
Gd content (Theoretical) (%)	7.23	5.99
(Measured) (%)	6.33	5.63
Inlet air temperature (°C)	40	45
Outlet air temperature (°C)	21—25	21
Inlet air flow rate (m^3/min)	0.05	0.06
Liquid flow rate (ml/min)	3.5—4.7	2.7
Spray air pressure (atm)	1.8	2.2
Yield (%)	82.7	97.7
Mass median diameter (μm)		52
Coarse fraction (>63 μm) (%)		5.5

a) Gd-DTPA-SA-fixed particles. *b*) 9:9:4 poly(EA/MMA/HEMA). *c*) This ratio was against initial $CaCO_3$ cores.

release-suppressing membrane was observed 1—3 h after immersion (data not shown). This was in good accordance with the *in vitro* release profile shown in Fig. 3. On the other hand, release of water-insoluble Gd-DTPA-SA from microcapsules (coating applied, 100%) was strongly suppressed as expected (Fig. 3). No release of Gd-DTPA-SA as particles was also observed. The percentage of released Gd-DTPA-SA was only 0.6% during 24 h. This extremely low release was maintained even at the coating level of 25% (based on core weight); percentage of Gd-DTPA-SA released from the microcapsules could be kept at only 0.2% for 40 d (data not shown).

Preparation of Gd-DTPA-SA-Containing Microcapsules for NCT (NCT-SA MC) Based on the above preliminary experiments, highly Gd-DTPA-SA-containing microcapsules were designed and prepared for practical use. The amount of 9:9:4 poly(EA/MMA/HEMA) was designed so that thickness of the release-suppressing layer would be the same as that of 25% coated Gd-DTPA-SA microcapsules prepared by the method in Table 1. Here, this polymer layer eventually acted as a protective barrier against erosion of the Gd-DTPA-SA layer from microcapsules. Gadolinium content of microcapsules would be designed to be not less than that of the previous Gd-DTPA-DM microcapsules.[5]

Table 2 shows formulations, operating conditions and coating performances for preparation of the microcapsules. Mass median diameter of prepared microcapsules was 52 μm (Table 2). When the microcapsules were observed by microscope, particles larger than 63 μm consisted of agglomerates of two or three core-particles. The degree of agglomerations defined as the fractions larger than 63 μm was only 5.5%. The gadolinium content in the microcapsules was measured to be 5.63%; that is, Gd-DTPA-SA content (drug content) was 38.4% (Table 2).

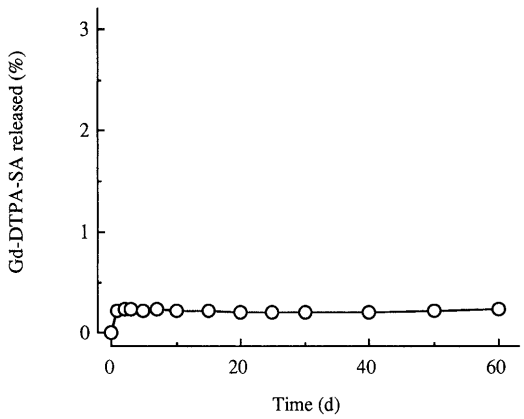

Fig. 4. Release of Gd-DTPA-SA from NCT-SA Microcapsules

Microcapsules were cured at 80 °C for 12 h, after being mixed with 30% pulverized mannitol powder. Size fraction (μm): 32—44. Dissolution test: JP XIII paddle method. Dissolution fluid: isotonic phosphate buffer (pH 7.4). Volume of dissolution fluid: 500 ml. Dissolution temperature: 37 °C.

A dissolution test was carried out in phosphate buffer (pH 7.4) for 60 d, and the results are shown in Fig. 4. To avoid aggregation, pulverized mannitol powder (30% based on the weight of the microcapsules) was mixed with microcapsules before heating. Although the weight of gadolinium layered on the cores was 5.35 times larger than that of the preliminary microcapsules (Tables 1, 2), dissolution of Gd-DTPA-SA from the microcapsules was maintained at a very low level; about 0.2% during 60 d. Figure 5 shows photographs of calcium carbonate cores (Fig. 5a)) and the microcapsules (Fig. 5b) and c)). The final product was well layered with mannitol powder as an anti-adherent (Fig. 5c)).

Discussion

Atoms of rare-earth elements do not form stable, covalent bonds with organic molecules; therefore, the gadolinium ion has to be detoxified by complexation. Gadolinium is known to form stable chelate with DTPA;

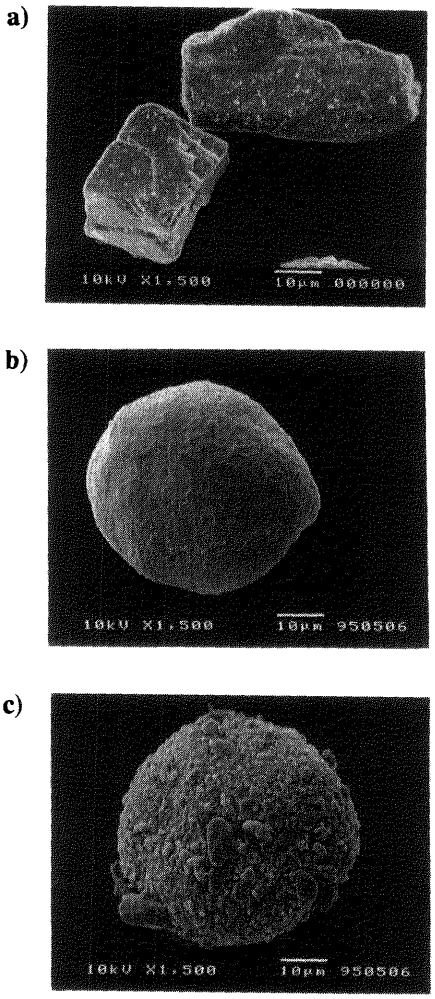

Fig. 5. Typical Photographs of NCT-SA Microcapsules in the 32—44 μm Fraction

a) Calcium carbonate core, b) uncured microcapsule, c) cured microcapsule after being mixed with 30% pulverized mannitol powder.

the formation constant (log k) for Gd-DTPA is 22—23.[11a] The stable Gd-DTPA complex has an LD_{50} of 10 mmol/kg in rat after intravenous injection, while the LD_{50} of gadolinium chloride was only 0.3 mmol/kg.[11b] This means that Gd-DTPA can be administered at an unusually high dose compared with usual chemotherapy. If localized or accumulated in a tumor, Gd-DTPA would eventually be activated to kill the tumor cells by localized neutron-irradiation.

Gd-DTPA is freely soluble in water as its salt with sodium and/or with *N*-methylglucamine (*i.e.* meglumine).[11c] In a previous study,[5] dimeglumine salt of Gd-DTPA (Gd-DTPA-DM, Magnevist®) was used as a gadolinium source, since it is clinically used as an magnetic resonance imaging (MRI) contrast agent. The high water-solubility and rapid excretion of Gd-DTPA-DM make it an ideal contrast agent for MRI, but this high solubility also make it difficult to control its release when it is introduced in microcapsules. The strongly hygroscopic property of Gd-DTPA-DM led to an enlarged size of the microcapsules due to enhanced agglomeration, and also to rapid release of Gd-DTPA-DM.[5] Hence, Gd-DTPA was chosen as compound with more potential as a gadolinium source, since the solubility of this commercially

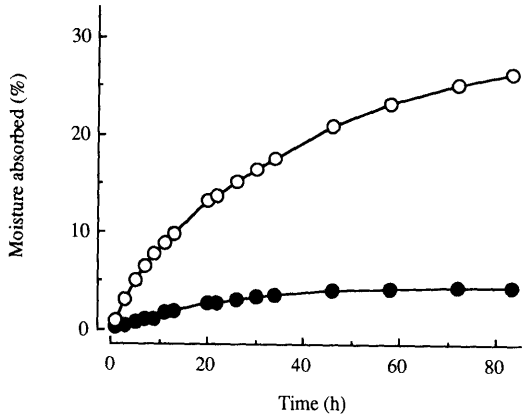

Fig. 6. Time Course of Moisture Absorption of Gd-DTPA-DM and Gd-DTPA

Temperature: 37 °C. Relative humidity: 71.5%. Gadolinium compound: ○, Gd-DTPA-DM; ●, Gd-DTPA.

available reagent was expected to be lower than that of its dimeglumine salt.

To confirm the difference in hygroscopicity, moisture absorption of Gd-DTPA-DM and Gd-DTPA was determined at 37 °C and 71.5% relative humidity (Fig. 6). Gd-DTPA-DM powder was obtained by freeze-drying the commercially available solution (Magnevist®). Gd-DTPA was clearly less hygroscopic than its dimeglumine salt: the percentage of moisture absorbed during 83 h was 26.2% with Gd-DTPA-DM, but was only 4.3% with Gd-DTPA. Moisture absorbency of Gd-DTPA-DM was 6—7 times higher than that of Gd-DTPA at all time points. This indicated that Gd-DTPA was more favorable than Gd-DTPA-DM at least for improving both coating operation and release kinetics.

A water-insoluble Gd-DTPA derivative (Gd-DTPA-SA) was synthesized according to the methods reported by Hnatowich *et al.*[8b] and Kabalka *et al.*[8c] and was also used as a gadolinium source. Gd-DTPA-SA had two hydrophobic tails (side-chain) consisting of stearylamine. These tails were connected to Gd-DTPA moiety *via* amide linkage. The attachment of two hydrophobic side-chains to the DTPA should have little effect on its ability to make a complex with the gadolinium ion.[8c]

In the Wurster process the upward forces acting on the particles are primarily affected by the velocity of the fluidizing air, while the downward forces are mainly dependent on gravity. Hence, heavier particles are more steadily circulated.[12] In the previous study,[5] lactose was used as a core material. Its low specific gravity (1.54), however, seemed unsuitable for the coating with fine particles. Consequently, calcium carbonate was chosen as a core material, since its high specific gravity (2.93) made it favorable for fine particle operation.

When Gd-DTPA was layered on the core-particles, a nicotinic acid was essential. Addition of nicotinic acid to the Gd-DTPA layer did not enrich gadolinium in microcapsules, but it could not be removed from the formulation because it was effective in reducing agglomeration by hindering the homogeneous film-formation, possibly due to its incompatibility with Gd-DTPA and PVP.[13] Gd-DTPA, nicotinic acid and PVP were

added at a weight ratio of 10:5:1 (Table 1). Organic solvent systems have advantages in the coating operation, for example, in reducing the operating time. In addition, when coating is to be operated using water-soluble and/or hygroscopic compounds, these systems make it possible to reduce the agglomeration effectively. Thus, Gd-DTPA- and nicotinic acid-containing organic solvent-based spray solution was prepared for the layering process (Table 1). Gd-DTPA was soluble in water, but only slightly soluble or insoluble in alcohol (methanol or ethanol). The organic-solvent-based spray solution was designed so that water would be a co-solvent of organic solvent. Gd-DTPA and nicotinic acid was dissolved in distilled water, and methanol was poured into the aqueous solution; PVP was then added to the solution. A clear solution was obtained until the methanol to water ratio reached 3:1. However, the Gd-DTPA spray-solution could not be obtained when a methanol-water (3:1 v/v mixture) was directly added to the Gd-DTPA and nicotinic acid.

In the fine powder coating operation, segregation of particles in the coating chamber was a serious problem. Even a slight segregation gradually broadened the particle size distribution. In fact, Wesdyk *et al.* reported that larger and heavier beads within a batch of fine and coarse beads coated by the Wurster method received a thicker film.[12] They displayed a significantly lower rate of dissolution compared to smaller and lighter beads. As also reported,[9a] smaller particles tended to retard from particles steadily circulating in the coating chamber due to their adhesion to the wall by electrostatic charge or van der Waals force and, consequently, they exhibited a faster release. To evaluate the steady particle-circulation, the measured microcapsule size was compared to the calculated particle size. The densities of calcium carbonate and 9:9:4 poly-(EA/MMA/HEMA) were 2.93 and 1.25 g/cm^3, respectively. The density of the gadolinium-containing layer was assumed to be the same as that of 9:9:4 polymeric latex (1.25 g/cm^3). Thus, the calculated mean particle diameter of microcapsules prepared with Gd-DTPA and Gd-DTPA-SA was 42.7 and 41.7 μm, respectively. These were in good agreement with the measured sizes (Gd-DTPA MCs, 45 μm; Gd-DTPA-SA MCs, 41 μm). These results indicated that segregation or loss of circulating particles was very minor during the coating operation and that coating efficiency was high.

The organic solvent system of EC-CH mixture (1:1 w/w) was previously applied to coating.[5] In the present study, however, the aqueous colloidal dispersion of poly(EA/MMA/HEMA) (polymeric latex) was selected as a coating material because its performance had been well established.[7,14,15] Agglomeration was suppressed effectively using the latex system, when the monomer composition was appropriately selected. To suppress agglomeration, the coating operation had to be adjusted so as not to allow completion of the film-formation; usually, the inlet air temperature had to be lower than the softening temperature of latex polymer. When microcapsules were prepared with the aqueous latex systems, the fraction of agglomeration was less than 1% for lactose cores larger than 44 μm; even for 32—44 μm cores, it was only 3.0%.[15b] Such a low agglomeration tendency

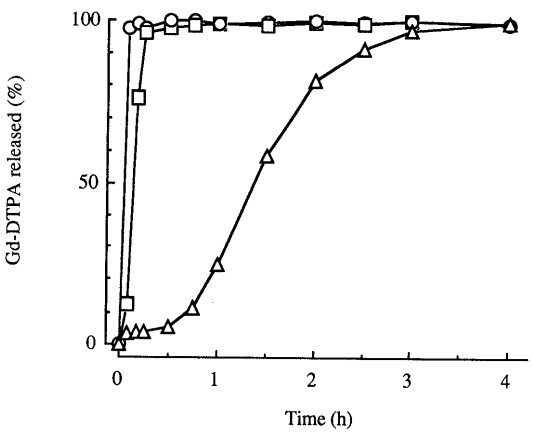

Fig. 7. Effect of Coating Level on the Release of Gadolinium from Gd-DTPA Microcapsules

Coating level: ○, 25%; □, 50%, △, 100%. Dissolution test: JP XIII paddle method. Dissolution fluid: isotonic phosphate buffer (pH 7.4). Volume of dissolution fluid: 500 ml. Dissolution temperature: 37 °C.

observed in the latex systems was difficult to achieve in the solution systems. In the latex systems, coating efficiency was sufficiently high in spite of the poor film-formability; therefore, a subsequent curing process made it possible to complete the film-formation. According to the monomer composition, release kinetics could change dramatically.[7,10] The 9:9:4 poly(EA/MMA/HEMA) latex was selected for coating material in the present study, because it had the lowest content of hydrophilic HEMA among the polymers previously synthesized; it was also the most hydrophobic among the polymers having a softening temperature above 40 °C, that was the lowest temperature at which the inlet air temperature could be stably controlled during operation.[7]

Figure 7 shows the effect of coating level on the dissolution of Gd-DTPA from microcapsules. Release of Gd-DTPA was prolonged as the coating level increased, but it was too fast for 25% and 50% coated microcapsules to be used for a practical release-suppressing pharmaceutical. This result indicated that the release-suppressing layer of 9:9:4 poly(EA/MMA/HEMA) could not act as a permeation barrier when subjected to only a 25 or 50% coating level. On the other hand, a clearly delayed release profile was obtained when the release-suppressing coating was applied at the 100% level against core particles (Fig. 7). After about 30 min lag-time, the subsequent release rate of Gd-DTPA was increased, and all Gd-DTPA was released in a 3 h period in the dissolution test. This coating suppressed the release more than the previous one.[5]

A change in morphology was observed microscopically during microcapsules being immersed in the dissolution fluid maintained at 37 °C. When the latex membrane (9:9:4) was directly put onto lactose-cores in a previous study, the microcapsules were swollen and enlarged in size, but there was no bursting of the membrane.[10] Gd-DTPA MCs were similarly swollen and enlarged in size, but bursting of the release-sustaining membrane was observed 1—3 h after their immersion in the dissolution fluid. Difference in the dissolution profile was at least partially attributable to the film thickness. Theoretically, the membrane thickness of 100% coated Gd-DTPA MCs was calculated as only 5.7 μm under the assumptions

described above. The membrane thickness of the 9:9:4 latex-coated lactose microcapsules, in contrast, was calculated to be 9.1 μm,[10] which was 1.6 times thicker than that of the present Gd-DTPA MCs. Probably such a thin film as 5.7 μm could not satisfactorily act as a permeation barrier of highly water-soluble Gd-DTPA.

Furthermore, migration of the dissolved Gd-DTPA or nicotinic acid into the film layer during the coating process would occur, leading to the fast release. Yang and Ghebre-Sellassie reported that in aqueous coating systems significant migration occurred during the coating process.[16] They also reported that the pellets with a sealcoat between the drug layer and the release-sustaining coat released the drug at slower rates than those without sealcoat. This indicated that migration of water-soluble substances into the film layer was suppressed in the presence of sealcoat.

Although a challenge in this study was how to harmonize the incompatible requirements of release-suppression and small particle size, the release was only slightly delayed when the water-soluble Gd-DTPA was used for sufficiently small microcapsules; of course, these Gd-DTPA MCs can be applied depending on the purpose of treatment. For a device to completely suppress, therefore, Gd-DTPA-SA was selected as a gadolinium source. The preliminary experiments described above demonstrated at least that the 9:9:4 poly(EA/MMA/HEMA) latex achieved a very low degree of agglomeration even in the coating of 20—32 μm particles of calcium carbonate; the particle size distribution of prepared microcapsules was very narrow (Fig. 2) and the degree of generated agglomeration (fractions larger than 63 μm) was only 3—5%. However, the ability of this latex to suppress release of water-soluble Gd-DTPA was inadequate. Since incorporation of a large amount of Gd-compound in microcapsules smaller than 50 μm was required for NCT-SA microcapsules, and simple membrane-thickening for suppressing release would obviously lead to enlargement of particle size, water-insoluble Gd-DTPA-SA was used which offered release-suppression, small particle size and high gadolinium content.

Release of Gd-DTPA-SA from the microcapsules was suppressed to less than 1% for longer than 40 d, as expected, since Gd-DTPA-SA was insoluble in water. This indicated that the Gd-DTPA-SA layer was completely protected from erosion by the existing release-suppressing layer. The amount of 9:9:4 poly(EA/MMA/HEMA) was designed so that the thickness of the release-suppressing layer of highly gadolinium-loaded microcapsules (NCT-SA MC) would become the same thickness as that of 25% coated Gd-DTPA-SA MCs (Table 1, Fig. 3). The thickness of the release-suppressing layer for the 25% coated Gd-DTPA-SA MCs in the preliminary experiments (Table 1) was 1.9 μm using the assumption described above. Hence, thickness of the release-suppressing layer of the NCT-SA MCs was designed to become 2.0 μm. Microcapsules were designed to have a gadolinium content of not less than the Gd-DTPA-DM MCs previously prepared[5]: Gd-DTPA-DM and gadolinium contents in the previous Gd-DTPA-DM MCs were 26.5 and 4.44%, respectively. The microcapsules were successfully prepared as designed (Table 2), and had a mass median diameter of 52 μm. The coarse fractions larger than 63 μm (agglomerates) accounted for only 5.5%, while Gd-DTPA-SA and gadolinium contents were 38.4 and 5.63%, respectively. Release of Gd-DTPA-SA from microcapsules was also suppressed to less than 1% for longer than 60 d (Fig. 4).

Released or leached Gd-DTPA-SA would be accumulated into the body, since amide linkage of this derivative could not be expected to degrade to amines and Gd-DTPA residues in the body. Slow elimination from the body would be a potentially serious problem. In fact, when metal–DTPA-SA complex was incorporated into the liposome membrane and injected, this lipid-soluble metal–DTPA complex was very stable in the animal.[17] The marker (metal–DTPA-SA complex) is very slowly eliminated from the mice, and the calculated half-life of such elimination is approximately 22 d. Kabalka *et al.* reported the biodegradable Gd-DTPA derivatives,[8c] ester and thioester derivatives of Gd-DTPA. These derivatives might be beneficial as gadolinium sources to introduce in the microcapsules. It is clear that when these biodegradable derivatives are used in the future, microcapsules can be prepared similarly.

Intra-arterial particulate radiation therapy for liver tumors has been performed in humans, most recently using yttrium-90.[18] Yttrium-90 glass microspheres for brachytherapy were prepared with a 15—30 μm diameter (TheraSpheres™).[18a] Intra-arterial radiation therapy using beta-emitting microspheres consists of injecting the spheres in a suitable suspension medium through a catheter into an artery leading to the tumor. Typical microsphere diameters of 15—30 μm cause their entrapment in the tumor capillary bed, where the short-ranged, highly ionizing beta emissions deliver a large local radiation dose with little irradiation to neighboring organs. The injection of TheraSpheres into the hepatic artery of dogs was well tolerated.[18b] It produced clinically silent changes within the liver. These reports suggested that the present NCT-SA MCs might be applied to GdNCT to further investigate its effectiveness *in vivo*.

Conclusion

A new cancer treatment proposed in this paper takes advantage of the remote tumor inactivating property of GdNCT. Gadolinium-containing reservoir microcapsules for NCT were designed and prepared. Three major problems of particle size, gadolinium content and release suppression must be overcome. Microcapsules around 50 μm in diameter (41—52 μm, mass median diameter) were successfully prepared using the 9:9:4 poly(EA/MMA/HEMA) latex system. The resultant microcapsules had a very narrow size distribution. A clearly delayed release profile was obtained with Gd-DTPA MCs. Release of water-soluble Gd-DTPA from the microcapsules was delayed for 3 h; however, it was difficult to suppress the release over weeks. In contrast, release of Gd-DTPA-SA from microcapsules was suppressed to less than 1% for longer than 60 d. The content of Gd-DTPA-SA and gadolinium in the microcapsules whose mass median diameter was as fine as 52 μm was 38.4 and 5.63%,

respectively.

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