

Preparation of Gadolinium-Containing Emulsions Stabilized with Phosphatidylcholine–Surfactant Mixtures for Neutron-Capture Therapy

Masahito MIYAMOTO,*^a Kaoru HIRANO,^a Hideki ICHIKAWA,^a Yoshinobu FUKUMORI,^a Yasuyuki AKINE,^b and Koichi TOKUUYE^c

Faculty of Pharmaceutical Sciences, Kobe Gakuin University,^a 518 Arise, Ikawadani-cho, Nishi-ku, Kobe 651–2180, Japan, Institute of Clinical Medicine, University of Tsukuba,^b 1–1–1 Tennoudai, Tsukuba, Ibaraki 305–0006, Japan, and The National Cancer Center Hospital,^c 5–5–1 Tsukiji, Chuo-ku, Tokyo 104–0045, Japan.

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Gadolinium-containing lipid emulsions for neutron-capture therapy were designed to fulfill the following requirements: particle size smaller than 100 nm; gadolinium content as high as possible; surface of the emulsions modified with hydrophilic moieties to provide prolonged circulation in the blood. Emulsions containing soybean oil, water, Gd–diethylenetriaminepentaacetic acid–distearylamine (Gd–DTPA–SA), as an amphiphilic drug, and hydrogenated egg yolk phosphatidylcholine (HEPC), as an emulsifier, in a weight ratio of 7.36 : 92 : 1 : 2 were prepared without co-surfactants by the thin-layer hydration method using a bath-type sonicator. The mean particle size of the emulsions was 280.7 nm. In order to make the droplet size of the emulsions smaller than 100 nm, as well as to modify the emulsion surfaces, a co-surfactant, Tween[®] 80, HCO[®]-60, Pluronic[®] F68, polyoxyethylene alkyl ether (Brij[®]) or polyoxyethylene alkyl ester (Myrj[®]), was introduced into the standard system. Tween 80, HCO-60, Brij 76, 78 and 700 were effective in reducing the particle size to below 100 nm when the co-surfactant weight ratio (CWR), defined as co-surfactant/(HEPC+Gd–DTPA–SA) (w/w), was larger than 0.67; the particle size with Tween 80 and HCO-60 was reduced to 52.7 and 74.7 nm, respectively, at a CWR of 1.0 (w/w). In order to increase the gadolinium content, the weight ratio of Gd–DTPA–SA to HEPC was increased from 1 : 2 of the standard-Gd formulation to 2 : 1 of the high-Gd formulation. The measured particle size of the HCO-60 high-Gd emulsions was 78.7 nm when the CWR was 1.0 (w/w). In this case, the calculated gadolinium content reached 3.0 mg Gd/ml. These results indicate that HCO-60 is an effective co-surfactant not only in terms of particle size reduction but also with respect to gadolinium enrichment.

Key words neutron-capture therapy; lipid emulsion; gadolinium; ultrafine emulsion; co-surfactant; Gd–diethylenetriaminepentaacetic acid–distearylamine

In a previous study,¹⁾ gadolinium-containing reservoir microcapsules for repeated gadolinium neutron-capture therapy (GdNCT) were designed and prepared. Microcapsules around 50 μm in diameter were successfully prepared using the 9 : 9 : 4 poly(ethyl acrylate/methyl methacrylate/2-hydroxyethyl methacrylate) latex system. Although release of water-soluble Gd–diethylenetriaminepentaacetic acid (Gd–DTPA) from the microcapsules was delayed for 3 h, it was difficult to suppress the release over a period of weeks. In contrast, release of Gd–DTPA–distearylamine (Gd–DTPA–SA) from microcapsules could be kept below 1% for longer than 60 d. The content of Gd–DTPA–SA and gadolinium in the microcapsules, whose mass median diameter was as small as 52 μm , was 38.4 and 5.63%, respectively.

Instead of the reservoir microcapsules, it was planned to develop nano-carriers that would deliver gadolinium to tumors through the systemic circulation. Oil-in-water (o/w) emulsions stabilized with emulsifiers such as phospholipids have been attracting much attention as drug carriers because they are biodegradable and biocompatible and, unlike liposomes, they can be prepared on an industrial scale and are relatively stable below 25 °C for long periods.²⁾ One problem with using emulsion particles as drug carriers is how they can leave the vascular space and reach their site of action. The extravascular transfer of particulate carriers largely depends on their size. It is believed that a diameter of approximately 100 nm is the cut-off value for drug carriers able to pass through the discontinuous capillary endothelium of tu-

mors.³⁾ In addition, drug carriers smaller than 100 nm are expected to more easily avoid uptake by the reticulo-endothelial system (RES) and to circulate for longer periods in blood, as estimated from the finding that small unilamellar liposomes of about 70–100 nm are cleared more slowly from the circulation when compared with larger ones of the same composition.⁴⁾

The main object of the present study was to prepare Gd–DTPA–SA-containing emulsions with a reduced particle size, surface properties such that prolonged blood retention was obtained and a high gadolinium content. The effects of the concentration of oil, sonication time, oil type, concentration of glycerol, co-surfactant type and concentration of co-surfactant on the particle size were studied.

Experimental

Materials Unless otherwise specified, reagents were used as purchased without any purification. Hydrogenated L- α -phosphatidylcholine from egg yolk (HEPC), soybean oil and Polysorbate 80 (polyoxyethylene (20) sorbitan monooleate, Tween[®] 80) were purchased from Nacal Tesque Inc., Kyoto, Japan. Polyoxyethylene (160) polyoxypropylene (30) glycol (Pluronic[®] F-68) and polyoxyethylene (196) polyoxypropylene (67) glycol (Pluronic[®] F-127) were obtained from Fluka Chemie AG, Buchs, Switzerland and Sigma Chemical Co., St. Louis, MO, U.S.A., respectively. Polyoxyethylene hydrogenated castor oil 60 (Cremophor[®]-HR60, HCO[®]-60) was supplied by BASF Aktiengesellschaft, Ludwigshafen, Germany. Polyoxyethylene (POE) (10) stearyl ether (Brij[®] 76) and POE (20) stearyl ether (Brij[®] 78) were purchased from Fluka Chemie AG. POE (100) stearyl ether (Brij[®] 700), POE (8)-stearate (Myrj[®] 45), POE (40)-stearate (Myrj[®] 52), POE (50)-stearate (Myrj[®] 53) and POE (100)-stearate (Myrj[®] 59) were obtained from Sigma Chemical Co. DTPA and gadolinium chloride (GdCl_3) were purchased from

* To whom correspondence should be addressed.

Nacalai Tesque Inc., and stearylamine (octadecylamine) was purchased from Aldrich Chemical Company, Inc., Milwaukee, WI, U.S.A. Anhydrous chloroform was obtained by adding a quantity of molecular sieve (4A 1/16, Nacalai Tesque Inc.) to chloroform and then allowing it to stand at room temperature for at least 3 d. The distearylamine derivative of Gd-DTPA (Gd-DTPA-SA) was synthesized as described in a previous reports.¹⁾

Preparation of Lipid Emulsions Lipid emulsions were prepared by a thin-film hydration method. The emulsions consisted of soybean oil, water, Gd-DTPA-SA, HEPC and an appropriate co-surfactant. The oil, phospholipid and Gd-DTPA-SA were dissolved in an appropriate amount of chloroform. Co-surfactant, stored as a 75 mg/ml solution in chloroform, was then added to the solution. When the Gd-DTPA-SA content had to be enriched, a little ethanol was added for solubilization. The solution was dried by rotary evaporation, followed by vacuum desiccation for 3–5 h to generate the dry thin-film. The dried film was hydrated using distilled water or aqueous glycerol, and then warmed at 55–60 °C in a thermostated water-bath under N₂. This was followed by swirling/shaking/vortexing to make coarse lipid emulsions. Small lipid emulsions were prepared by 1 h sonication under N₂ with a bath-type sonicator (BRANSON 1210 (BRANSONIC®), Emerson-Japan, Kanagawa, Japan) which was thermostated at 55–60 °C. The sonication was performed by the following procedure: a 3 min-sonication and subsequent 2 min-cooling was repeated for the first 30 min, and then an 8 min-sonication and 2 min-cooling was repeated for the next 30 min.

Particle Size Measurement The droplet size of the emulsions was measured by quasielastic laser light scattering using ZetaPlus equipment with a particle size option, the BI-MAS (BI-8000AT) (Brookhaven Instruments Corp., Ronkonkoma, NY, U.S.A.).

Results and Discussion

Formulation and Operational Factors Gadolinium-containing lipid emulsions for neutron-capture therapy was designed as follows: the particle size of the emulsions had to be smaller than 100 nm; the gadolinium content had to be as high as possible; the surface of the emulsions had to be modified with hydrophilic moieties to allow prolonged circulation in the blood.

A water-insoluble Gd-DTPA derivative (Gd-DTPA-SA) was synthesized according to the methods reported by Hnatowich *et al.*^{5a)} and Kabalka *et al.*,^{5b)} and this was used as a gadolinium source. Gd-DTPA-SA has two hydrophobic tails (side-chains) consisting of stearylamine that are connected to the Gd-DTPA moiety *via* an amide linkage. Gd-DTPA-SA can be incorporated into the membrane of liposomal vesicles as a component of the liposomal lamella.^{5b)} In addition, the attachment of two stearyl side-chains to the DTPA should have little effect on its ability to form a complex with the gadolinium ion.^{5b)}

Soybean oil and lecithin have been widely used as a model system for studying emulsions, and are generally used for commercially available fat emulsions such as Intralipid® (Kabi-Pharmacia, Stockholm, Sweden) and Intrafat® (Nihon Pharmaceutical, Co., Ltd., Tokyo, Japan). Thus, soybean oil and lecithin (phosphatidylcholine) were employed as the standard components of the emulsion system studied here. In the standard formulation, better emulsification was obtained with HEPC or hydrogenated lecithin from egg yolk (HEL), whose phospholipid (PL) content was more than 99% and whose phosphatidylcholine (PC) content was approximately 70% (Nacalai Tesque Inc.), rather than with powdered lecithin from soybean (SL), whose PL content was more than 95% and whose PC content was approximately 30% (Nacalai Tesque Inc.). Hence, HEPC was selected as an emulsifier for the standard formulation of Gd-DTPA-SA emulsion. Moreover, considering the chemical stability of lecithin, hydrogenated lecithin, HEPC, may have an advantage.

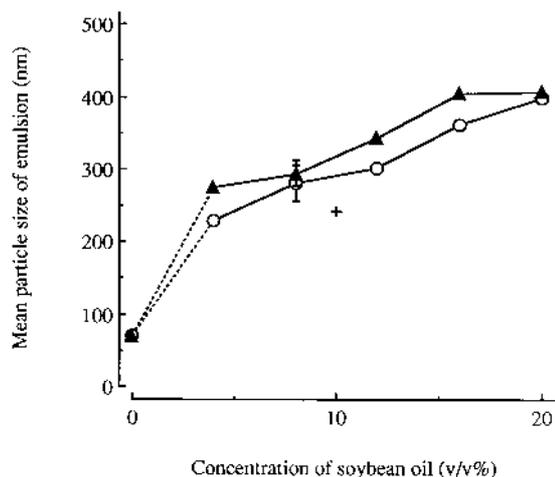


Fig. 1. Effect of Soybean Oil Content on the Mean Particle Size of Gd-DTPA-SA Emulsions

Components: ○, HEPC and Gd-DTPA; ▲, HEPC, Gd-DTPA and glycerol (2.5 w/v%); +, Intrafat®. Formulation: Gd-DTPA-SA, 250 mg; HEPC, 500 mg (the weight ratio of Gd-DTPA-SA : HEPC was 1 : 2). Total volume of soybean oil plus water was 25 ml. Soybean oil concentration: soybean oil/(water+soybean oil), v/v%. Data represent the average ± S.D. of three separate preparations.

Table 1. Formulation of Gd-DTPA-SA emulsion and 10 v/v% Intrafat®

	Gd-DTPA-SA emulsion	Intrafat® ^{a)}
Lecithin ^{b)}		300 mg
HEPC ^{c)}	500 mg	
Gd-DTPA-SA	250 mg	
Soybean oil	2 ml	2.5 g
Glycerol		625 mg
Water	23 ml	Add
Total		25 ml

a) Commercially available lipid emulsion for nutritional purpose. b) From egg yolk. c) L- α -Phosphatidylcholine hydrogenated (from egg yolk).

In a series of preliminary experiments using soybean oil and HEPC, the formulation and preparation conditions were optimized by changing the oil concentration, glycerol content and sonication time. First of all, in order to evaluate the effect of soybean oil concentration on the mean particle size of Gd-DTPA-SA emulsions, a series of samples was prepared with an increasing proportion of soybean oil. The results are shown in Fig. 1. The Gd-DTPA-SA emulsions were prepared at a weight ratio of 1 : 2 Gd-DTPA-SA : HEPC, indicating that about 25 mol% (Gd-DTPA-SA/(Gd-DTPA-SA + HEPC)) of the outer monolayer of the emulsion was shared by Gd-DTPA-SA molecules, because Gd-DTPA-SA is insoluble in the oil. When the Gd-DTPA-SA emulsions were prepared at 20 v/v% (oil/(oil+water)) soybean oil concentration, the mean particle size was 399.3 nm. The particle size fell along with the soybean oil concentration and, finally, the particle size of the 4 v/v% emulsions was as low as 229.3 nm (Fig. 1).

Table 1 compares the formulations of the standard Gd-DTPA-SA emulsion and the commercially available fat emulsion, 10% Intrafat®, which is the same formulation as that of 10% Intralipid®. Although Intrafat® contained 2.5 w/v% glycerol for isotonicity, there was no glycerol in the standard formulation of Gd-DTPA-SA emulsion shown in Table 1.

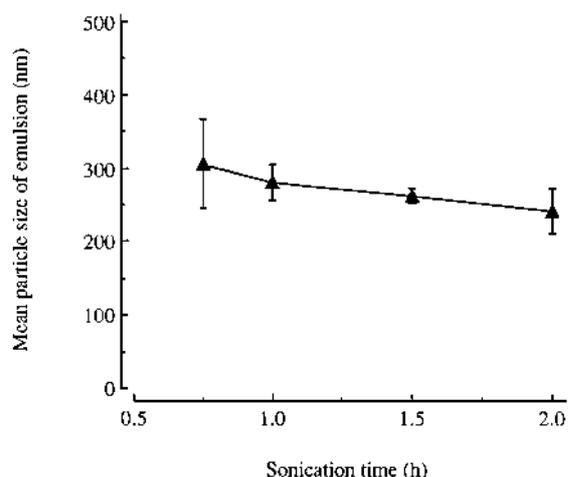


Fig. 2. Effect of Sonication Time on the Mean Particle Size of Gd-DTPA-SA Emulsions

The weight ratio of soybean oil, Gd-DTPA-SA and HEPC was held constant at 7.36:1:2. The concentration of soybean oil in the preparation was held constant at 8 (v/v)%. Data represent the average \pm S.D. of three to five separate preparations.

The isotonic effect of 2.5 w/v% glycerol on the particle size of Gd-DTPA-SA emulsion is also shown in Fig. 1. The mean particle size of isotonic 20 v/v% Gd-DTPA-SA emulsions was 407.1 nm, and that of isotonic 4 v/v% emulsions was 274.5 nm. As shown in Fig. 1, the particle size of standard Gd-DTPA-SA emulsions containing 2.5 w/v% glycerol was very close to that of standard Gd-DTPA-SA emulsions, without glycerol. In fact, the measured mean particle sizes of 10% Intrafat[®], 8 v/v% standard Gd-DTPA-SA emulsion and the corresponding isotonic emulsion were 241.6, 280.7 and 294.3 nm, respectively. This indicates that the addition of 2.5 w/v% glycerol to the formulation of standard Gd-DTPA-SA emulsion did not basically affect the particle size of the emulsions. In addition, the particle size of standard Gd-DTPA-SA emulsions was similar to that of the 10% soybean oil emulsion reported by Illum *et al.*,⁶⁾ the 20% soybean oil emulsion reported by Sakaeda and Hirano,⁷⁾ and the commercial fat emulsion product, 10% Intrafat[®]. This indicates that the preparation procedure for the emulsions described here is in good agreement with other preparation methods which have already been reported by Illum⁶⁾ (ultrasonic probe) and Sakaeda⁷⁾ (microfluidizer system). In order to simplify the system, glycerol was eliminated from the standard formulation, because the addition of glycerol for isotonicity had little effect on the particle size of the emulsions as shown in Fig. 1. Finally, the standard formulation of the Gd-DTPA-SA emulsion was determined as follows: the oil concentration, defined as oil/(oil+water), was 8.0 v/v%; the weight ratio of soybean oil, Gd-DTPA-SA and HEPC was 7.36:1:2 (Table 1). The specific gravity of soybean oil was assumed to be 0.92 (JP XIII).

The effect of the sonication time on the mean particle size is shown in Fig. 2. The particle size was gradually reduced by prolonging the sonication: the particle size of the resulting emulsions fell from 306.1 \pm 59.8 nm at 45 min to 239.9 \pm 36.9 nm at 2 h. Although the effect of the prolonged sonication time on the particle size reduction was not marked, the 45 min-sonication resulted in a poor reproducibility. Since better reproducibility was obtained after 60 min sonication (280.7 \pm 24.6 nm), a sonication time of 60 min was chosen.

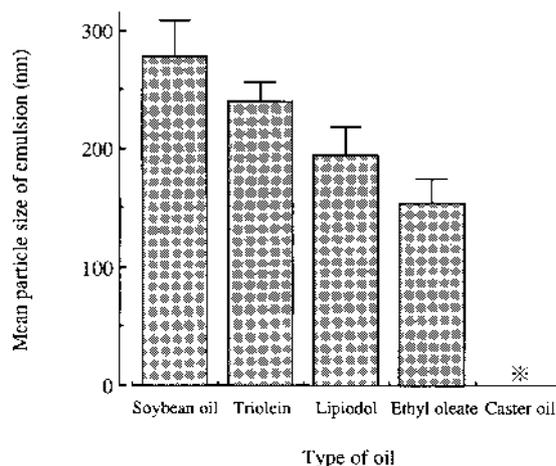


Fig. 3. Effect of Oil Type on the Mean Particle Size of Gd-DTPA-SA Emulsions

The weight ratio of soybean oil, Gd-DTPA-SA and HEPC was held constant at 7.36:1:2. The concentration of soybean oil in the preparation was held constant at 8 (v/v)%. *, emulsion could not be prepared. Data represent the average \pm S.D. of three to five separate preparations.

In order to prepare o/w emulsions, some other kinds of oil than soybean oil commonly used have been used as an oil component. The effect of oil type on the particle size of emulsions is illustrated in Fig. 3. The particle size of triolein (Nacalai Tesque)-emulsion (240.3 nm) was almost the same as that of soybean oil-emulsion. Lipiodol Ultra-Fluide[®] (Guerbert Laboratories, France) produced a mean droplet size of 195.2 nm. The particle size with ethyl oleate (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was reduced to 154.4 nm. When castor oil (Nacalai Tesque: 448 mPa·s/25 °C; 262 mPa·s/37 °C^{8a)}) with a higher viscosity was selected as an oil component, no emulsion could be prepared by the procedure used here, while stable emulsions could be prepared by using oils with a lower viscosity such as soybean oil (49 mPa·s/25 °C, 29 mPa·s/37 °C^{8a)}), Lipiodol (37 mPa·s/25 °C, 25 mPa·s/37 °C^{8a)}) and ethyl oleate (4 mPa·s/37 °C^{8b)}). The mean particle size of the emulsions seemed to be closely correlated to the viscosity of the oils: the particle size of the emulsions fell on reducing the viscosity of the oil. This indicates that oil viscosity is an important factor influencing the particle size of the Gd-DTPA-SA emulsions. In spite of this, soybean oil was used in this study because of its widespread application.

Since the oil-in-water (o/w) emulsion exerts a low osmotic pressure, the addition of other materials to achieve a physiological tonicity is required for parenteral administration.²⁾ Although this could be achieved by the addition of electrolytes, in general such electrolytes would adversely affect the emulsion stability. Since glycerol is used in Intralipid[®], the effect of glycerol on the particle size of Gd-DTPA-SA emulsions was investigated (Fig. 4). Intralipid[®] contains 2.5 w/v% glycerol, which does not affect the particle size, as shown in Fig. 1. On the other hand, the emulsion particle size fell on increasing the amount of glycerol added (Fig. 4). When the glycerol concentration was 40 w/v%, the particle size reached 154.1 \pm 17.2 nm. This was probably due to reduced interfacial tension and a similar result was also reported by Shively.⁹⁾

Size-Reduction by Using Co-Surfactants The addition

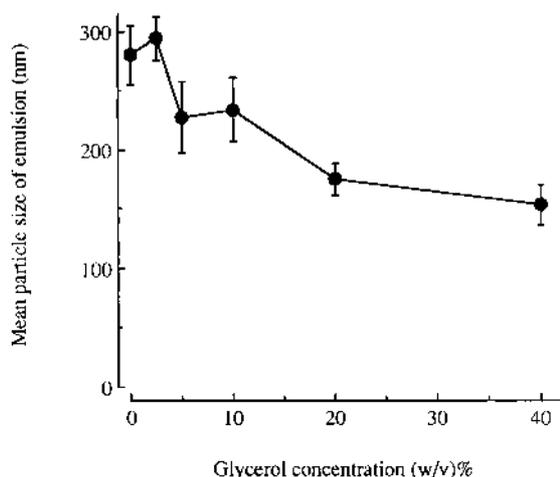


Fig. 4. Effect of Glycerol on the Mean Particle Size of Gd-DTPA-SA Emulsions

The weight ratio of soybean oil, Gd-DTPA-SA and HEPC was held constant at 7.36:1:2. The concentration of soybean oil in the preparation was held constant at 8 (v/v)%. Data represent the average \pm S.D. of three to seven separate preparations.

of emulsifiers (referred to co-surfactants) to the standard formulation of Gd-DTPA-SA emulsion (Table 1) was expected to reduce the particle size of the emulsion. In this study, Tween[®] 80, HCO[®]-60 or polyoxypropylene-polyoxyethylene block copolymers (Pluronic[®] F68) were selected as co-surfactants, since they have been already used as ingredients (emulsifying-agent) in parenteral (intravenous) preparations. The effect of co-surfactant on the mean particle size of the emulsions is shown in Fig. 5. Addition of Pluronic F68 led to a particle size reduction: the particle size was 207.0 nm at a co-surfactant weight ratio (CWR) of 0.5, defined as co-surfactant/(HEPC+Gd-DTPA-SA) (w/w), 184.5 nm at 0.67 (w/w) and 166.1 nm at 1.0 (w/w). However, it was difficult to prepare emulsions smaller than 100 nm using Pluronic F68 as a co-surfactant. The results obtained with Pluronic F127 were similar to those with Pluronic F68 (Fig. 5). These results were probably related to the fact that Pluronic family had a larger HLB value.

In the cases of Tween 80 and HCO-60, a marked reduction in particle size was observed. The particle size of Tween 80 emulsions, 175.2 nm at a CWR of 0.5 (w/w) fell dramatically to 63.9 nm at a CWR of 0.67 (w/w) and 52.7 nm at a CWR of 1.0 (w/w). When HCO-60 was selected as a co-surfactant, the particle size fell in the same manner, reaching 74.7 nm at a CWR of 1.0 (w/w). Emulsions with a mean particle size smaller than 100 nm and a translucent appearance could be prepared by using Tween 80 or HCO-60 as a co-surfactant (Fig. 5). Their droplet size did not significantly change when stored over a month at room temperature (date not shown). The ultrafine Gd-DTPA-SA emulsions could be prepared at the above high co-surfactant concentration in this study. In general, microemulsions require a high amount of surfactant, in the range 6–8 (w/w)%; in contrast, 2–3 (w/w)% surfactant is required for conventional emulsions.¹⁰ In the present study, the co-surfactant concentration was only 1.9% at a CWR of 0.67 (w/w) and 2.8% even at a CWR of 1.0 (w/w). The amount of co-surfactant for the Gd-DTPA-SA emulsions was almost the same as that for conventional emulsions.

Surface Modification with Polyoxyethylene Stearyl

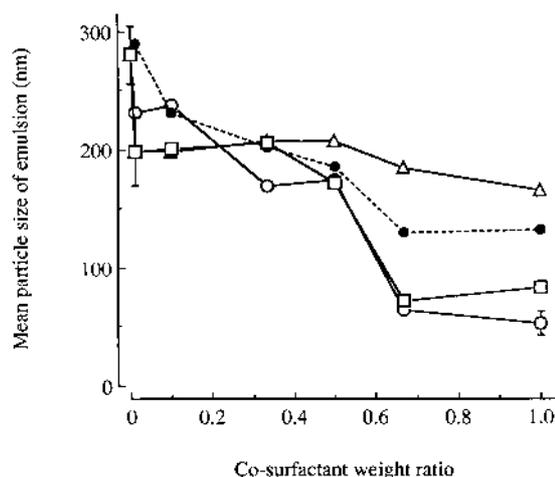


Fig. 5. Effect of Co-Surfactant on the Mean Particle Size of Gd-DTPA-SA Emulsions

Co-surfactant: \circ , Tween 80; \square , HCO-60; \triangle , Pluronic F68; \bullet , Pluronic F127. CWR was defined as co-surfactant/(HEPC+Gd-DTPA-SA). The weight ratio of soybean oil, Gd-DTPA-SA and HEPC was held constant at 7.36:1:2. The concentration of soybean oil in the preparation was held constant at 8 (v/v)%. Data represent the average \pm S.D. of three to five separate preparations.

Ethers and Esters In general, the conventional nano-carriers are readily taken up by the reticulo-endothelial system (RES), so that it is necessary to develop a carrier-system that avoids this. In the case of liposomes, prolonged blood circulation could be achieved by coating the liposome surface with ganglioside GM1 or amphipathic poly(ethylene glycol) (PEG).¹¹ Long-circulating nano-carriers can slowly accumulate by so-called 'passive targeting' at pathological sites with a 'leaky' vasculature such as tumors and inflamed areas, improving or enhancing drug delivery to those areas.¹² In order to build up the steric hindrance on the emulsion surface, Brij and Myrjs, non-ionic surfactants, were selected as co-surfactants (Figs. 6 and 7). Of course, Tween 80, HCO-60 and Pluronic F68 would be also expected to prolong the blood circulation time of the resultant emulsions, because they have PEG-like structures. The effect of various Brij members on the mean particle size of the emulsions is shown in Fig. 6. When the number of polyoxyethylene (POE)-units of Brij was less than 10, no stable emulsion could be prepared because of gelation or immediate separation (data not shown). As shown in Fig. 6, at a low CWR level such as 0.1–0.5 (w/w), the Brij family was more effective in reducing the particle size than Tween 80 and HCO-60. The particle size reduction-effect was not strongly dependent on the number of POE-units, although the droplet size with Brij 78 ($C_{18}POE_{20}$) emulsions was a little smaller than that with other Brij (Brij 76; POE-10 or Brij 700; POE-100). With every Brij shown in Fig. 6, emulsions with particle sizes smaller than 100 nm could be prepared when the CWR was greater than 0.5 (w/w). The results with the unsaturated Brij family, which contains a double-bond (Brij 90 series; POE (*m*)-oleyl ether ($C_{18-1}POE_m$)), were almost the same as those with the corresponding saturated Brij family (data not shown).

The effect of various Myrjs members on the mean particle size of the emulsions is shown in Fig. 7. The Myrj family had a rather poor particle size reduction-effect compared with the Brij family. The mean particle size of the Myrj 53

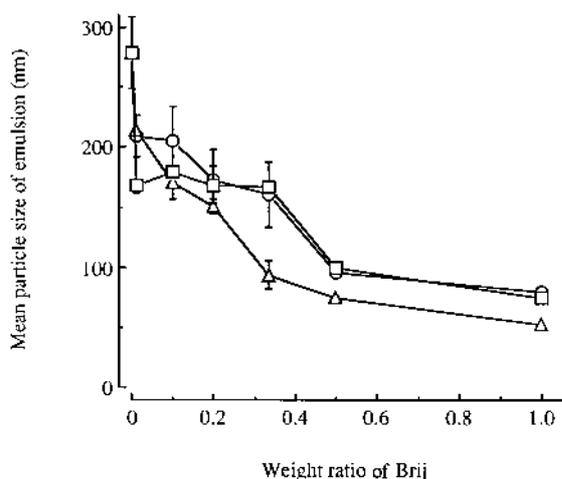


Fig. 6. Effect of Various Brij's on the Mean Particle Size of Gd-DTPA-SA Emulsions

Brij is a polyoxyethylene stearyl ether ($C_{18}POE_n$). Co-surfactant: \circ , Brij 76 ($C_{18}POE_{10}$); \triangle , Brij 78 ($C_{18}POE_{20}$); \square , Brij 700 ($C_{18}POE_{100}$). The weight ratio of Brij was defined as Brij/(HEPC+Gd-DTPA-SA). The weight ratio of soybean oil, Gd-DTPA-SA and HEPC was held constant at 7.36:1:2. The concentration of soybean oil in the preparation was held constant at 8 (v/v)%. Data represent the average \pm S.D. of three separate preparations.

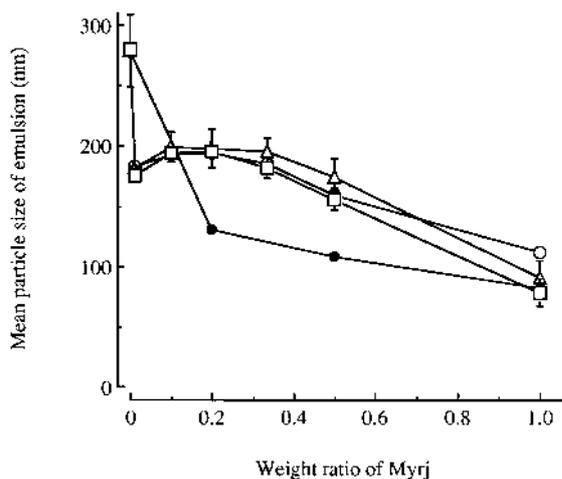


Fig. 7. Effect of Various Myrj's on the Mean Particle Size of Gd-DTPA-SA Emulsions

Myrj is a polyoxyethylene stearyl ester ($C_{18}POE_n$). Co-surfactant: \bullet , Myrj 45 ($C_{18}POE_8$); \circ , Myrj 52 ($C_{18}POE_{40}$); \triangle , Myrj 53 ($C_{18}POE_{50}$); \square , Myrj 59 ($C_{18}POE_{100}$). The weight ratio of Myrj was defined as Myrj/(HEPC+Gd-DTPA-SA). The weight ratio of soybean oil, Gd-DTPA-SA and HEPC was held constant at 7.36:1:2. The concentration of soybean oil in the preparation was held constant at 8 (v/v)%. Data represent the average \pm S.D. of three separate preparations.

($C_{18}POE_{50}$) emulsion was 173.8 nm at a CWR of 0.5 (w/w) and 90.3 nm at a CWR of 1.0 (w/w). In addition, the results with Myrj 59 ($C_{18}POE_{100}$), having a larger POE chain, were almost the same as those with Myrj 53: the particle size was 155.0 nm at a CWR of 0.5 (w/w) and was 78.5 nm at a CWR of 1.0 (w/w). In the case of Myrj 45 ($C_{18}POE_8$), having a shorter POE chain (Fig. 7), it was difficult to prepare stable emulsions, because separation occurred within a few days of their preparation (data not shown).

When the weight ratio of soybean oil:HEPC:Gd-DTPA-SA:co-surfactant was 7.36:2:1:3 (CWR, 1.0 w/w) and the molecular weight (M.W.) of the HEPC was 762.1, calculated assuming that HEPC was an equimolar mixture of dipalmitoyl-phosphatidylcholine (DPPC, M.W.: 734.05) and dis-

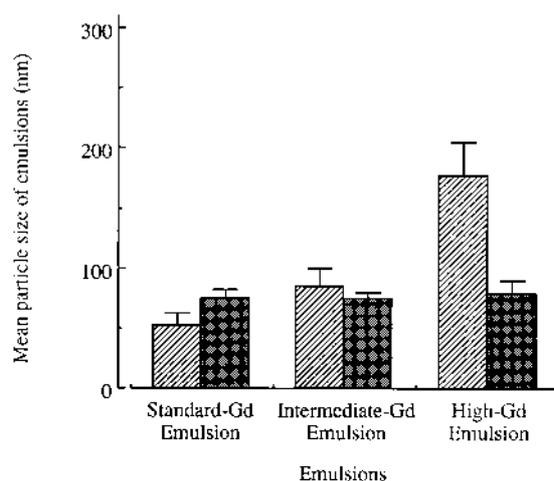


Fig. 8. Effect of Weight Ratio of Gd-DTPA-SA against HEPC on the Mean Particle Size of Gd-DTPA-SA Emulsions

Symbols: \square , emulsion with Tween 80; \blacksquare , emulsion with HCO-60. The weight ratio of soybean oil, Gd-DTPA-SA and HEPC: standard-Gd emulsion was 7.36:1:2; intermediate-Gd emulsion was 7.36:1.5:1.5; high-Gd emulsion was 7.36:2:1. Total weight of Gd-DTPA-SA plus HEPC was kept constant and the CWR was held constant at 1.0 (w/w). The concentration of soybean oil in the preparation was also held constant at 8 (v/v)%. Data represent the average \pm S.D. of three to five separate preparations.

tearoyl-phosphatidylcholine (DSPC, M.W.: 790.16), the molar percentage of Myrj 53 in the surface monolayer of the emulsion was about 25.3 mol% (co-surfactant/(co-surfactant+HEPC+Gd-DTPA-SA)). In the case of POE100 co-surfactant (Brij 700 and Myrj 59), the corresponding figure was 15.2 mol%. In addition, the co-surfactant (Myrj 53) weight ratio relative to HEPC (co-surfactant/(co-surfactant+HEPC)) was 19.6 mol%. In general, sterically stabilized liposomes contain about 5–10 mol% PEG or ganglioside GM1.^{11e,13} The amount of co-surfactant in the Gd-DTPA-SA emulsions was rather greater than that in the sterically stabilized liposomes.

Increase in Gadolinium Content The effect of the weight ratio of Gd-DTPA-SA:HEPC on the particle size of the emulsion is shown in Fig. 8. Here, Tween 80 or HCO-60 was used as a co-surfactant at a CWR of 1.0 (w/w). In the case of Tween 80 emulsions, when the weight ratio of Gd-DTPA-SA:HEPC was increased from 1:2 of the standard-Gd formulation to 1:1 of the intermediate-Gd formulation, the emulsions had relatively similar particle sizes, 52.7 and 85.1 nm, respectively; the theoretical gadolinium content increased from 1.5 mg Gd/ml to 2.2 mg Gd/ml. Any further increase in the weight ratio of Gd-DTPA-SA:HEPC, unfortunately, resulted in an increase in particle size: the particle size of high-Gd emulsions at a weight ratio of 2:1 (Gd-DTPA-SA:HEPC) was 177.4 nm.

In contrast, when HCO-60 was used as a co-surfactant, the particle size of the emulsions remained at 70–80 nm for all the weight ratios of Gd-DTPA-SA:HEPC studied here. In this case, the particle size of the high-Gd emulsions was 78.7 nm and the theoretical gadolinium content reached 3.0 mg Gd/ml. In addition, when HCO-60 was used as a co-surfactant, the resulting emulsions had a diameter of about 100 nm even at a CWR of 0.67 (w/w) (data not shown).

Conclusion

Gadolinium-containing lipid emulsions were designed and

prepared for neutron-capture therapy. The Gd-DTPA-SA emulsions were prepared by a thin-layer hydration method using a bath-type sonicator. The formulation of the standard Gd-DTPA-SA emulsion was soybean oil, water, Gd-DTPA-SA and HEPC at a weight ratio of 7.36:92:1:2, and the measured mean particle size of the standard emulsions was 280.7 nm. Ultrafine emulsions, whose particle size was less than 100 nm and whose appearances were translucent like microemulsions, were successfully prepared by adding emulsifiers (co-surfactant) to the standard Gd-DTPA-SA emulsion. In particular, Tween 80 and HCO-60 were effective in reducing the particle size: when the CWR defined as co-surfactant/(HEPC+Gd-DTPA-SA) (w/w) was greater than 0.67, the particle size was less than 100 nm. At a CWR of 1.0 (w/w), the particle size of Tween 80 and HCO-60 emulsions was 52.7 and 74.7 nm, respectively. When the weight ratio of Gd-DTPA-SA:HEPC was increased from 1:2 of the standard-Gd formulation to 2:1 of the high-Gd formulation, HCO-60 was most effective in keeping the particle size small: the particle size of HCO-60 high-Gd emulsions was 78.7 nm when the CWR was 1.0 (w/w). These results indicate that HCO-60 is an effective co-surfactant not only in terms of particle size reduction but also with respect to gadolinium enrichment.

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