Mannan-Coated Liposome Delivery of Gadolinium–Diethylenetriaminepentaacetic Acid, a Contrast Agent for Use in Magnetic Resonance Imaging

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Gadolinium–diethylenetriaminepentaacetic acid (Gd-DTPA), a paramagnetic contrast agent for use in magnetic resonance imaging (MRI) was bound to stearylamine and incorporated into the liposomal membranes (Gd-DTPA liposomes). In addition, the Gd-DTPA liposomes were coated with mannan (cholesterol–aminoethylcarbamylmethyl mannan), a polysaccharide, to obtain the mannan-coated liposomes. An *in vitro* MRI study showed that the Gd-DTPA liposomes produced a greater intensity of contrast than did the Gd-DTPA solution with a reduced T_1 relaxation time. Intravenous injection of the Gd-DTPA liposomes containing ^{153}Gd or ^{14}C-DTPA to mice showed an accumulation of Gd-DTPA primarily in the liver and lung. When the mannan-coated liposomes were administered, an increased uptake of Gd-DTPA by these tissues was demonstrated. The mannan-coated liposomes may enhance contrast of the liver in MRI at a lower dose of Gd-DTPA.

Keywords magnetic resonance imaging; Gd-DTPA; liposome; mannan; T_1 relaxation time

Introduction

Magnetic resonance imaging (MRI), a non-invasive diagnostic method, provides information with two major useful characteristics. First, the data it provides on proton (^{1}H) density is as diagnostically useful as that provided by X-ray computed tomography (CT) on electron density. Second, the spin-lattice and spin-spin relaxation times (T_1, T_2 relaxation times), which reflect the physical conditions of the molecules, are diagnostically useful in providing anatomical information of various organs.[1)]

The use of paramagnetic metal ions such as Gd^{3+}, Fe^{3+} and Mn^{2+} has been investigated as a means of improving the quality of the MRI images of the central nervous system and liver.[2)] Of those ions, Gd^{3+} seems one of the best contrast media because its magnetic moment considerably exceeded that of the other metal ions.[3,4)] In contrast, free Gd^{3+} ions are excreted very slowly, leading to an accumulation in the bone and central nervous system, thereby disturbing the living body.[5)] Gadolinium–diethylenetriaminepentaacetic acid (Gd-DTPA) produced by binding Gd^{3+} to the water-soluble chelating agent, diethylenetriaminepentaacetic acid (DTPA), induced a marked reduction in the toxicity of Gd, [6)] since Gd-DTPA in solution is presumed to be excreted rapidly.[7)] In the present study, we prepared small unilamellar vesicle (SUV) liposomes in order to deliver Gd in a chelated form (Gd-DTPA) to the desired sites.[8,9)] We also prepared polysaccharide-coated liposomes to improve their membrane stability and targetability to organs.[10,11)] The distribution of these agents was evaluated *in vitro* and *in vivo*.

Materials and Methods

Materials Gadolinium trichloride ($GdCl_3$) and cholesterol were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Phosphatidylcholine obtained from egg yolk (PC) and stearylamine (SA) were purchased from Wako Pure Chemical Industries Co. (Osaka, Japan). DTPA anhydride and cholesterol–aminoethylcarbamylmethyl–mannan (Chol-AECM-mannan) were purchased from Dojin Chemicals Co. (Kumamoto, Japan). Both ^{153}Gd (37 MBq/mmol) and ^{14}C-DTPA (37 MBq/mmol) were purchased from Amersham International, Ltd. (Buckinghamshire, U. K.).

Preparation of Gd-DTPA–Stearylamine (Gd-DTPA-SA) Gd-DTPA-SA was prepared by the method of Kabalka *et al.*,[9)] with some modifications. SA (71 mg) was melted in distilled water (10 ml) at 90 °C, and mixed with DTPA anhydride (107 mg) to yield DTPA-SA. In addition, $GdCl_3\ 6H_2O$ (111 mg) was added to the mixture to yield Gd-DTPA-SA. The reaction mixture was applied to gel filtration using a Sephadex G-50 column (2.0 × 40 cm) to remove the unreacted compound. The solvent was then removed by means of a rotary evaporator.

Preparation of Gd-DTPA Liposomes The Gd-DTPA liposomes were prepared by the method of Kabalka *et al.*,[9)] with some modifications. Gd-DTPA-SA (250 mg) was dissolved in chloroform (10 ml); PC (100 mg) and cholesterol (50 mg) were then added to the mixture. The solvent was removed by a rotary evaporator under nitrogen gas. Phosphate-buffered saline (pH 7.4, 10 ml) was added to the generated lipid film, and the mixture was shaken for 5 min by a vortex mixer. SUV liposomes were prepared by sonication (Laboratory Supplies Co., Hicksville, New York, U.S.A.) for 30 min. Free Gd and DTPA were removed by gel filtration using a Sephadex G-50 column (2.0 × 40 cm). The mean diameter of liposomes was 310 nm, which was determined by means of a particle counter (Ohtuka Denshi Co., Hirakata, Japan).

Coating of Liposomes with Mannan Chol-AECM-mannan (10 mg) was added to 10 ml of the Gd-DTPA liposome suspension, and the mixture was shaken for 2 h at 15 °C. The mean diameter of the mannan-coated liposomes was 313 nm which was determined by means of the particle counter.

***In Vitro* MRI Study** The Gd-DTPA liposomes at various concentrations were placed in test tubes, and MRI images were taken by the 1.5 *T* MRI system Signa® (General Electric Co., Milwaukee, Wisconsin, U.S.A.). T_1-weighted images were obtained using a repetition time (T_R) of 400 ms and an echo delay time (T_E) of 20 ms by the spin-echo-technique. T_1 and T_2 values of the Gd-DTPA liposomes were obtained by *in vitro* MRI.

***In Vivo* Tissue Distribution of Gd-DTPA Liposomes** Gd-DTPA liposomes containing ^{153}Gd were injected intravenously at a dose of 0.1 mmol/370 kBq/kg to male ddY mice (25—30 g, Clea Japan Inc., Osaka, Japan). The animals were sacrificed at 0.5, 1, 2, 4, 6 or 24 h after dosage, and the radioactivity remaining in the blood (7.8 v/w% of the body weight), liver, lung, spleen and kidney was determined by an Autowell Gamma System ARC-300 (Aloka Co., Mitaka, Japan). The Gd-DTPA liposomes containing ^{14}C-DTPA were similarly administered and the radioactivity was determined by a scintillation counter, Tri-Carb-3385 (Packard Instrument Co., Meriden, CA, U.S.A.).

Results

Effect of Gd-DTPA Liposomes on *in Vitro* MRI Figure 1 shows the effect of the Gd-DTPA liposomes and the Gd-DTPA solution on the T_1 or T_2 value. The inverse of the T_1 value obtained by *in vitro* MRI was proportional to the contrast-enhancing effect of Gd-DTPA. The concentration of Gd-DTPA used clinically is 0.1—1 mM.[7,12)] At this level the liposomes exerted an effect about twice that of the solution. The mannan-coated liposomes exerted a similar

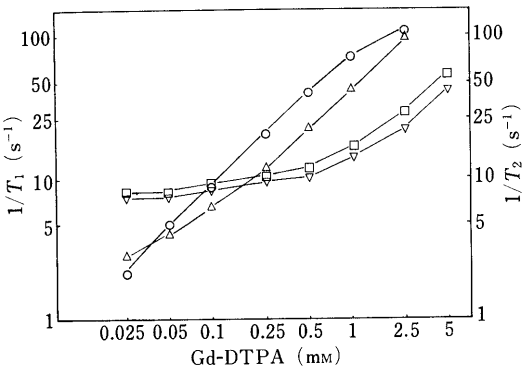

Fig. 1. Effect of Gd-DTPA on T_1- or T_2-Relaxitivity of *in Vitro* MRI

Each point represents the mean T_1 value of five experiments in solution (△) or the non-coated liposomes (○), and the mean T_2 value of five experiments in solution (▽) or the non-coated liposomes (□).

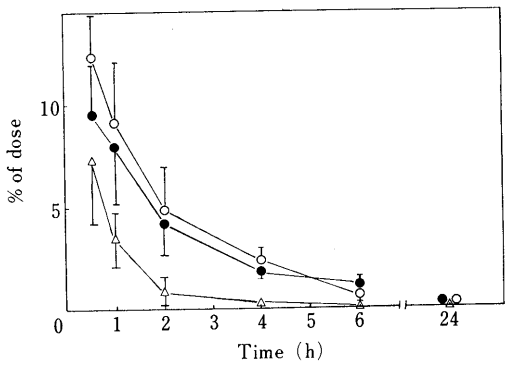

Fig. 2. Blood Concentration Time Courses of ^{153}Gd-DTPA (% of Dose) after Intravenous Injection in Mice

Each point represents the mean±S.E. of 5 experiments after the administration of solution (△), the non-coated liposomes (○) or the mannan-coated liposomes (●).

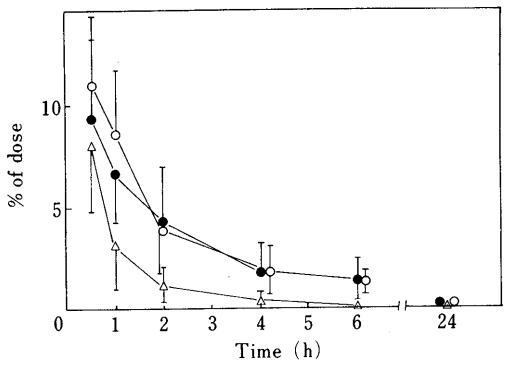

Fig. 3. Blood Concentration Time Courses of Gd-(^{14}C-DTPA) (% of Dose) after Intravenous Injection in Mice

Symbols are the same as those in Fig. 2.

effect to that of the non-coated liposomes (data not shown). The inverse of the T_2 value induced a decrease in the contrast between samples and background when the T_2 value was increased. Neither the liposomes nor the solution induced a change in the T_2 value at concentrations below 1 mM. The mannan-coated liposomes produced results similar to those described above. These *in vitro* results suggest that the contrast-enhancing effect provided by the Gd-DTPA liposomes was about twice that of the Gd-DTPA solution.

Tissue Distribution of Gd-DTPA Liposomes Figure 2 shows the blood level of ^{153}Gd in mice throughout the 24 h after intravenous administration of the Gd-DTPA liposomes or the Gd-DTPA solution containing ^{153}Gd. One h

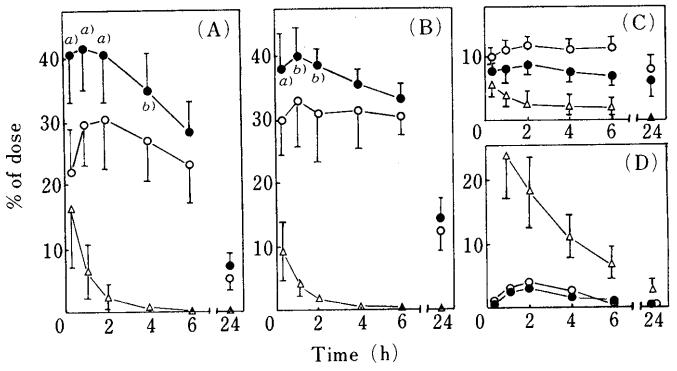

Fig. 4. Tissue Distribution Time Courses of ^{153}Gd-DTPA (% of Dose) after Intravenous Injection in Mice

(A) liver, (B) lung, (C) spleen, (D) kidney. Symbols are the same as those in Fig. 2. *a*) Significantly different from the non-coated liposomes. $p<0.01$. *b*) Significantly different from the non-coated liposomes. $p<0.05$.

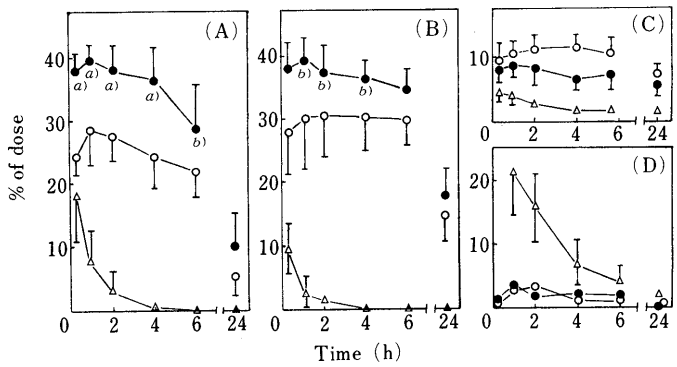

Fig. 5. Tissue Distribution Time Courses of Gd-(^{14}C-DTPA) (% of Dose) after Intravenous Injection in Mice

(A) liver, (B) lung, (C) spleen, (D) kidney. Symbols are the same as those in Fig. 2. *a*) Significantly different from the non-coated liposomes. $p<0.01$. *b*) Significantly different from the non-coated liposomes. $p<0.05$.

after administration of the solution, the blood level of ^{153}Gd was 3.5% of the dose, indicating a rapid disappearance of Gd-DTPA chelates from circulation. In contrast, after the administration of the non-coated liposomes and the mannan-coated liposomes, the blood levels of ^{153}Gd were 9% and 8% of the dose, respectively.

Figure 3 shows the blood level of ^{14}C-DTPA in mice after administration of the liposome preparations or the solution containing ^{14}C-DTPA. The liposomes containing ^{14}C-DTPA and ^{153}Gd produced similar blood levels.

Figure 4 shows the level of ^{153}Gd in the liver, lung, spleen and kidney of mice after intravenous administration. The retention of ^{153}Gd was prolonged in the liver and lung in the case of liposome preparation, while ^{153}Gd rapidly disappeared from these organs in the case of solution. The mannan-coated liposomes showed a greater uptake as compared with the non-coated liposomes.

Figure 5 shows the level of ^{14}C-DTPA in each tissue after injection of the liposomes or the solution. The results were similar to those seen in Fig. 4. Considering that the chelate coefficient of Gd and DTPA is very large (10^{22}),[7] the levels observed over 24 h suggest that Gd did not separate from DTPA.

Discussion

We succeeded in delivering Gd-DTPA in the liposomal

membrane to the liver and lung, and demonstrated the potential of these agents in MRI. *In vitro* MRI studies revealed that the T_1 values of two liposome preparations were shorter than that of the solution, and the effect of liposomes on enhancing the contrast was twice that of the solution (Fig. 1). The range within which Gd-DTPA is transferred as an entire molecule has been increased by the binding of Gd-DTPA to apparent macromolecules such as liposomes, leading to an increase in the frequency of Gd-DTPA that approaches the protons.[13] It is therefore considered that a liposome preparation may provide an enhanced MRI contrast.

In vivo tissue distribution also revealed an accumulation of liposomes in the reticuloendothelial systems (RES) such as the liver (Figs. 4 and 5). The liposomes coated with mannan induced a significant increase in the uptake of Gd-DTPA due to mannan recognition by the organs.[10,14] These results suggest that administration of the mannan-coated liposomes may enhance the MRI contrast of the liver at a decreased dose of Gd-DTPA.

References and Notes

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