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Small unilamellar liposomes as magnetic resonance contrast agents loaded with paramagnetic Mn-, Gd- and Fe-DTPA–stearate complexes

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Summary

Small unilamellar liposomes were used as carriers for paramagnetic ions to enhance proton relaxation times for magnetic resonance imaging (MRI). The metal ions Fe^{3+}, Gd^{3+} and Mn^{2+} were complexed to liposomes containing various amounts (20–60 mol%) of the lipophilic chelator diethylenetriamine pentaacetic acid (DTPA)–stearate (DTPA-SA). The Mn- and Gd-DTPA-SA liposomes were stable for more than two months and had a mean diameter of 26–36 nm, whereas for the Fe-DTPA-SA liposomes, vesicle sizes up to 2 μm were obtained. Due to their large size the Fe-DTPA-SA liposomes were not further studied. The efficiency of metal complexing averaged at 53 ± 16%, suggesting that the binding of the metal ions is restricted to the outer liposome surface. The complexing capacity was 3.5–11 μmol, corresponding to 0.2–0.6 mg manganese or 0.55–1.7 mg gadolinium per ml liposomes. In vitro release of metal ions was very low, namely 0.4% for gadolinium, 1.8% for manganese and 5% for iron, determined 5 days after complex formation. Pharmacokinetics and organ distribution of radioactive ^{54}Mn and ^{153}Gd-DTPA-SA liposomes (0.03 mM/kg b.wt.) in rats revealed that approximately 35% of the Mn-liposomes were present in the liver after 30–60 minutes with more than 80% eliminated after 24 h. Mn-DTPA-SA was eliminated from the liver by biphasic kinetics with $t_{1/2}(1) = 20$ min and $t_{1/2}(2) = 10$ h. Different results were obtained with Gd-DTPA-SA liposomes with a slower liver absorption over 2–4 h (35–60%) and a slow elimination with $t_{1/2}(2) = 80$ h. Eight days after injection, 17% of the Gd-DTPA-SA could still be detected in the liver. Both complexes are predominantly eliminated through the hepato-biliary route. The very low metal concentrations found in the kidneys suggest that the metal complexes remain stable. Imaging experiments in rats showed that Mn-DTPA-SA liposomes at 0.03 mM/kg b.wt. gave a signal enhancement on T1 weighted images making the liver as bright as fat tissue. Images taken with Gd-DTPA-SA liposomes at the corresponding concentration gave a smaller, but still significant liver signal intensity increase. The DTPA-SA liposomes used as ligands for paramagnetic metals proved to be very efficient signal enhancers of the reticuloendothelial system (RES), mainly for the liver. The Mn-DTPA-SA liposomes in particular, produce a very strong signal enhancement and due to their fast elimination they might represent a viable contrast agent for MR-imaging of the upper abdomen.

Introduction

Magnetic resonance imaging (MRI) has become an important diagnostic tool. However, le-

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sion detection in the upper abdomen and particularly in the liver is still not satisfactory, and ways to improve image quality in this region of the body are currently being sought after (Glazer, 1988). The need for contrast agents in the upper abdomen becomes increasingly apparent with the use of fast imaging sequences which yield a reduction of motion artefacts but possibly with reduced image contrast.

Agents which have the strongest paramagnetic effect on proton T1-relaxation are the metal ions of gadolinium (Gd^{3+}) and manganese (Mn^{2+}), followed by iron (Fe^{3+}) (Gadian et al., 1985; Weinmann et al., 1984). Due to their toxicity, these metals can only be used in the chelated form. To date, complexes of gadolinium with diethylenetriamine pentaacetic acid (Gd-DTPA) as well as very low concentrations of ferrioxamine B have been used in human studies (Carr et al., 1984; Hamm et al., 1987; Manu et al., 1987). The properties of a novel complex of gadolinium, namely gadolinium–tetraazacyclododecane tetraacetic acid (Gd-DOTA), which has a higher complex-forming constant than Gd-DTPA have recently been described (Bousquet et al., 1988).

Contrast enhancing complexes of manganese and iron with aminopolycarboxylates are also in evaluation for clinical applications (Fornasiero et al., 1987).

The fast blood and organ clearance rates of such metal complexes restrict their use to studies of perfusion, of capillary integrity, and of the extracellular space. To prolong intravascular retention, Gd-DTPA coupled to albumin as a carrier molecule has been used as a potential blood pool marker in animal studies (Ogan et al., 1987).

To increase the specificity of the paramagnetic effect for imaging, Gd-DTPA has been linked to monoclonal antibodies specific for human colon adenocarcinoma but a definite proof is still lacking whether specific tumor signal enhancement can be obtained (Curtet et al., 1988).

In a comparable approach, Kornguth et al. (1987) linked Gd^{3+} to DTPA-modified murine monoclonal anti-human T cell antibodies for the detection of intracerebral mononuclear lymphocytes. The intrinsic property of liposomes to target to the reticuloendothelial system (RES) has prompted their use as carriers of contrast agents. Magin et al. (1986) showed that manganese chloride entrapped within the aqueous space of large unilamellar vesicles (LUV) enhanced selectively the intensity of proton NMR signals in the livers of mice. Parasassi et al. (1985) reported comparable effects on T1 and T2 relaxation obtained in animal tissues after i.v. injection of manganese-citrate encapsulated into multilamellar liposomes (MLV). Manganese bound to serum albumin and entrapped in MLV (Navon et al., 1986) and Mn-DTPA in similar liposomes increased manganese accumulation in liver and spleen 2–12-fold, yielding a marked reduction of proton relaxation rates (Caride et al., 1984). However, the entrapped contrast agents leaked from the liposomes due to diffusion and membrane destabilization. To abolish leakage from the carrier vesicles, Kabalka et al. (1987) used DTPA-stearate, a lipophilic derivative of DTPA to anchor the complexing agent within the lipid membranes of MLV. The delivery of gadolinium via such liposomes to liver and spleen increased T1 relaxation markedly.

Even though superparamagnetic ferrite has been introduced as RES specific contrast agent, it produces signal loss of normal liver parenchyma, which will not yield optimal contrast to lesions such as metastases on T1 weighted images (Saini et al., 1987). Agents which enhance T1-relaxation may thus be advantageous, particularly in high field MRI.

In our approach, we used the concept of stable incorporation of the complexing agent within the liposome membrane. In this work we present the physicochemical characteristics, in vivo pharmacokinetic and MR imaging data of small unilamellar liposomes (SUV) containing Gd-, Mn- and Fe-DTPA-stearate as contrast enhancers for the RES.

Materials and Methods

Materials

Diethylenetriamine pentaacetic acid (DTPA), octadecylamine (stearylamine; SA) and cholesterol (USP, from lanolin) were from Fluka AG, Buchs, Switzerland. Cholesterol was recrystallized twice

from methanol before use. Soy phosphatidylcholine (SPC, Epikuron 200) was from Lukas Meyer, Hamburg, F.R.G.

Sodium-cholate, DL-α-tocopherol, manganese chloride ($MnCl_2 \cdot 4H_2O$), iron chloride ($FeCl_3 \cdot 6H_2O$) as well as buffer salts and organic solvents, all of analytical grade were from Merck AG, Darmstadt, F.R.G. Gadolinium chloride ($GdCl_3$, 99.9%) was from Ventron, Karlsruhe, F.R.G.

The radioactively labeled salts $^{153}GdCl_3$ (18.5–185 MBq/mg Gd), $^{54}MnCl_2$ (> 3.7 MBq/μg Mn), and $^{59}FeCl_3$ (110–740 MBq/mg Fe) were from Amersham Int., Amersham, U.K. The capillary dialyzer used for liposome preparation was a type ST-12 disposable haemodialysis cartridge from Travenol, Deerfield, IL, U.S.A.

Synthesis of DTPA-stearate

DTPA-stearate was essentially prepared as described for fatty acid derivatives of EDTA by Eckelman et al. (1975). DTPA anhydride (**I**) was obtained by suspending 20 g (50.8 mmol) of DTPA in 60 ml pyridine followed by the addition of 20.4 g (200 mmol) acetic anhydride. The mixture was stirred for 24 h at 65 °C. The product was filtered and washed with cold acetic anhydride and diethylether and dried over silicagel.

Stearylamine, 13.5 g (50 mmol) was dissolved in 500 ml chloroform and 20 g (55 mmol, 1.1 molar excess) of **I** was added and the mixture heated to 60 °C under reflux during 3 days. To hydrolyze the anhydride bonds, the dry product was boiled in acidified water for 5 h and left standing at 25 °C for 4 days.

The washed and dried DTPA-stearate (DTPA-SA) was recrystallized from boiling ethanol. Infrared spectra confirmed the presence of the amide bond as well as the quantitative cleavage of the anhydride linkages.

Preparation of DTPA-SA liposomes

Unilamellar bilayer liposomes containing various amounts of DTPA-SA (20–60 mol% referred to SPC) were prepared as follows: 3 g SPC, 0.3 g cholesterol, 0.5–1.5 g DTPA-SA and 0.02 g α-tocopherol were dissolved together with corresponding amounts of sodium-cholate in ethanol/methanol/chloroform (2 : 2 : 1 v/v).

The molar ratio of total lipids, including the complexing agent, to detergent was 0.7. After removing the organic solvents by rotatory evaporation at 40 °C, the dry lipid/detergent mixture was solubilized with 100 ml of 67 mM phosphate buffer (PB) to give micellar solutions of 30 mg SPC and 5–15 mg DTPA-SA/ml.

The micelles were then dialyzed according to the method described by Schwendener (1986). Briefly, sodium-cholate was removed from 100 ml micelle solution by dialysis through a Travenol ST-12 haemodialysis cartridge during 2–4 h with 10 liters of PB as dialysis fluid.

The liposomes were filtered through 0.2 μm sterile filters (Nalgene disposable filters, Nalge, Rochester, NY, U.S.A.) and stored at 4 °C.

Formation of metal complexes with DTPA-SA liposomes

Complexation of Gd^{3+}, Mn^{2+} and Fe^{3+} was carried out as follows: to 20 ml of DTPA-SA (40 mol%) liposomes, which were adjusted to pH 6 with 1 N HCl, an aqueous solution of $GdCl_3$ (1 ml containing a 1.1 M excess of Gd^{3+}) was slowly added under stirring and control of the pH which was not allowed to drop below 5. When addition was completed the pH was readjusted to 7.4 with 1 N NaOH. Likewise, Mn^{2+} was complexed to the DTPA-SA liposomes, whereas complex formation with Fe^{3+} was effectuated at a pH of 2.5 with subsequent neutralization. Excess of occasionally formed metal hydroxides were removed by filtration or short centrifugation at 2000 rpm for 5 min.

To obtain labeled liposomes, trace amounts of $^{153}GdCl_3$, $Mn^{54}Cl_2$ or $^{59}FeCl_3$ were added to the metal salt solutions before complexation.

Complete removal of uncomplexed metal ions was ensured by elution of the liposomes through columns of Sephadex G 25m or BioRad P-6 GD (15 × 3 cm) with PB.

Complexing efficiency was determined by measuring the radioactivities after complex formation (= 100%) and after column elution (= x% complexed), taking the dilutions into account.

In vitro incorporation stability of the metal complexes was monitored by chromatography of 0.5 ml aliquots of the liposomes through BioGel P 6-DG columns (5 × 1 cm) 5 days after prepara-

tion. Concentrations of remaining incorporated complexes were calculated from radioactivity counting (cf. Table 1).

Determination of liposome size and population homogeneity was performed by laser light scattering as described previously (Milsmann et al., 1978; Von Schulthess et al., 1976).

Pharmacokinetics and organ distribution of Mn- and Gd-DTPA-SA liposomes in rats

Pharmacokinetic parameters and organ distribution of labelled Mn- and Gd-DTPA-SA liposomes were determined as follows: male or female rats (ZUR : SIV rats, Institut für Zuchthygiene, University of Zürich) weighing 160–240 g were used. They were fasted for 24 h before the experiments.

Liposomes (0.5–1.5 ml), corresponding to 0.03 mM Me-DTPA-SA/kg b.wt. were injected intravenously into the exposed external jugular vein. Groups of 3 animals per time point were used and killed by ether narcosis, followed by cervical dislocation after 0.5, 1, 2, 4, 24, 72 and 192 h. Blood, liver, lung, spleen, kidney, small intestine, colon and, at some time points, bone (femur), brain and muscle tissue were removed. Faeces were collected from the 24, 72 and 192 h groups. Whole weighed organs or aliquots as well as the faeces were counted for ^{54}Mn or ^{153}Gd activity.

Percents of injected dose and absolute amounts of the metal complexes in the corresponding organs were calculated as shown in Figs. 1 and 2 and Tables 2–4. The pharmacokinetic parameters as listed in Table 5 were calculated with the use of a least-squares parameter estimation program (Rstrip, MicroMath).

Magnetic resonance imaging

For the imaging experiments, the rats were anaesthesized with Inovar-vet (0.4 ml/animal, i.m.) and 0.03–1.5 mM/kg b.wt. of the corresponding Me-DTPA-SA liposomes were injected via the external jugular vein. MR imaging experiments were performed with a Philips Gyroscan S15 imager at 1.5 T field strength. Spin-echo (SE) images with a repetition time (T_R) of 200 ms and echo times (T_E) of 20 or 30 ms were recorded. T1-weighted relaxation images were calculated from a mixed mode sequence (T_R = 500 ms, inversion time (T_I) = 700 ms, and T_E = 30 or 60 ms). Imaging time was approximately 2 h.

Results and Discussion

The practically insoluble lipophilic derivative, DTPA-SA was solubilized with sodium-cholate at a 3 : 1 M detergent-to-DTPA-SA ratio in warm ethanol. The liposomes were prepared by the addition of solubilized DTPA-SA to the mixture of the lipids (SPC : cholesterol : α-tocopherol = 10 : 2 : 0.1 mol parts).

Dialysis of the micelles with the capillary dialysis system allowed fast and reproducible preparations of 100 ml batches of the liposomes containing either 0.5, 1.0, or 1.5 mg DTPA-SA/ml (20, 40, 60 mol%).

The physicochemical properties of the DTPA-SA and the metal complex liposomes are shown in Table 1. The incorporation of DTPA-SA reduces the hydrodynamic diameter from 66 nm of reference liposomes without DTPA-SA to 28–36 nm when 20–60 mol% DTPA-SA are incorporated. This effect of size reduction may be compared to the effects of addition of increasing amounts of cholesterol to the phospholipid membrane, which condenses the spatial packing of the lipid molecules in the bilayer and thus reduces vesicle diameters (Yeagle, 1985). The formation of small vesicles might also be caused by a negative net vesicle surface charge, provided that DTPA-SA is at least partially deprotonated at pH 7.4.

Complex formation of Gd^{3+} and Mn^{2+} with liposomes containing 20, 40 and 60 mol% of DTPA-SA does not result in an increase of vesicle size. Liposome population homogeneity, however, is somewhat decreased as compared to the reference liposomes. The preparations with 20 and 40 mol% DTPA-SA, and correspondingly the liposomes complexed with Gd^{3+} and Mn^{2+} were stable over the 45-day observation period. The incorporation of 60 mol% of the complexing agent (1.5 mg DTPA-SA/ml liposomes) yields less stable preparations.

Complex formation with Fe^{3+} leads to drastic changes in liposome characteristics. At 20 mol%

TABLE 1

Physicochemical characteristics of Gd-, Mn- and Fe-DTPA-SA liposomes [a]

Liposomes	Complexed metal (%)	Leakage 5 days (%)	Liposome characteristics after [b]			
			1 day		45 days	
			D (nm)	H (%)	D (nm)	H (%)
20% DTPA-SA	–	–	28	59	35	53
40% DTPA-SA	–	–	28	55	46	37
60% DTPA-SA	–	–	36	63	54	48
20% Gd-DTPA-SA	60	ND	26	46	31	46
40% Gd-DTPA-SA	77(10) [c]	0.4	24	50	28	54
60% Gd-DTPA-SA	47	ND	36	60	70	81
20% Mn-DTPA-SA	23	ND	26	52	34	54
40% Mn-DTPA-SA	59 [d]	1.8(0.1) [c]	26	57	37	66
60% Mn-DTPA-SA	ND	ND	33	60	38	64
20% Fe-DTPA-SA	54	ND	38	57	634	76
40% Fe-DTPA-SA	52	5	62	64	1920	30
60% Fe-DTPA-SA	ND	ND	140	66	1500	58
0% DTPA-SA	–	–	66	33	69	30

[a] Liposome composition: SPC/cholesterol/α-tocopherol/DTPA-SA (10 : 2 : 0.1 : x mol%, x = 0–20–60 mol%)
[b] Laser light scattering, sample dilution 1 : 6 v/v in PB. D, diameter; H, homogeneity parameter: H = 20–40%, very homogeneous, H = 41–60% homogeneous and H > 60% heterogenous vesicle population (Milsmann et al., 1978).
[c] Mean of 3 values, S.D. in parentheses. ND = not determined.
[d] Mean of 2 values.

DTPA-SA the complexation with Fe^{3+} results in a small increase of vesicle size from 28 to 38 nm when determined one day after preparation. A significant increase of size, together with the formation of a less homogeneous vesicle population was observed 45 days after liposome preparation. More drastic changes of vesicle sizes were found with the 40 and 60 mol% DTPA-SA liposomes,

TABLE 2

Organ distribution of Mn-DTPA-SA liposomes (40 mol% DTPA-SA) after intravenous injection in rats

Organ	Concentration of ^{54}Mn-DTPA-SA (nmol/g) [a]					
Time (h):	0.5	1	2	4	24	72
Blood	112 (19) [b]	78 (10)	21 (4)	9 (3)	0.2 (0.1)	0.1 (0.01)
Liver	285 (10)	229 (45)	168 (26)	154 (23)	34 (2)	18 (1.0)
Lung	46 (6)	33 (1)	17 (1)	13 (1)	3 (0.3)	2 (0.1)
Spleen	87 (18)	65 (10)	48 (6)	37 (6)	6 (1)	3 (0.4)
Kidney	60 (4)	67 (3)	52 (9)	38 (3)	14 (0.5)	9 (0.3)
Small intestine	73 (2)	131 (26)	252 (16)	130 (38)	9 (1)	3 (0.7)
Colon	ND	ND	ND	ND	20 (16)	4 (1.0)
Recovery % [c]	70 (2.5)	65 (2)	66 (3)	41 (7)	10 (2)	4.6 (0.5)

[a] Mn-DTPA-SA liposomes (0.5–1 ml) corresponding to 0.03 mM Mn-DTPA-SA/kg b.wt. were injected i.v. into groups of 3 ZUR : SIV rats (160–250 g) via the jugular vein. The animals were killed at the time points indicated and the ^{54}Mn activity measured in a gamma-counter.
[b] Mean of 3 animals with S.D. in parentheses. ND = not determined.
[c] Count recovery is given in % of the injected dose as mean of 3 animals.

TABLE 3

Organ distribution of Gd-DTPA-SA liposomes (40 mol% DTPA-SA) after intravenous injection in rats

Organ	Concentration of ^{153}Gd-DTPA-SA (nmol/g) [a]						
Time (h):	0.5	1	2	4	24	72	192
Blood	300 (5) [b]	246 (32)	216 (13)	91 (7)	19 (7)	1.5 (0.4)	1.5 (0.2)
Liver	119 (12)	164 (34)	358 (15)	459 (56)	342 (56)	219 (62)	127 [c]
Lung	69 (5)	58 (2)	60 (4)	27 (2)	9 (0.5)	8 (1)	9 (2)
Spleen	45 (5)	43 (5)	89 (4)	73 (14)	75 (32)	51 (3)	138 (74)
Kidneys	53 (10)	42 (13)	40 (2)	18 [c]	8 (1)	8 (1)	13 (1)
Small intest.	10 (14)	15 (6)	26 (0)	22 (7)	2 (4)	11 (2)	9 (1)
Colon	4 (1)	2 (0.5)	5 (1)	9 (1)	40 (20)	18 (2)	6 (1)
Bone	30 (2)	23 (3)	21 (1)	21 (3)	14 (2)	ND	23 [c]
Recov. % [d]	80 (2)	74 (8)	84 (2)	82 (1)	64 (4)	42 (8)	22 [c]

[a] Gd-DTPA-SA liposomes (0.5–1 ml) corresponding to 0.03 mM Gd-DTPA-SA/kg b.wt. were injected i.v. into groups of 3 ZUR : SIV rats (160–250 g) via the jugular vein. The animals were killed at the indicated time and the ^{153}Gd activity measured in a gamma-counter.

[b] Mean of 3 animals with S.D. in parentheses. ND = not determined.

[c] Mean of 2 values.

[d] Count recovery is given as % of the injected dose as mean of 3 animals.

surprisingly without concomitant decrease of population homogeneity. Because of these persistent changes in liposome size, the Fe-DTPA-SA liposomes were not further used.

The efficiency of complex formation of Gd^{3+}, Mn^{2+} and Fe^{3+} was on the average 53 ± 16%. This indicates that only the DTPA-SA molecules situated on the outer liposomes membrane surface are accessible for the metal ions for complex formation.

The divergence from 50% can be explained by a distribution ratio of DTPA-SA which is different from 1 : 1 between inner and outer lipid bilayer moiety. Since it is unlikely that multivalent ions permeate through a lipid membrane (Szoka and Papahadjopoulos, 1980), the DTPA-SA molecules which are incorporated within the inner bilayer membrane part are not accessible for metal-complex formation which again might explain the determined half-saturation of DTPA-SA.

Leakage of the metal ions from the liposomes is generally low and occurring within the first hours after complexation (data not shown). As listed in Table 1, at most 5%, in the case of Fe^{3+}, are lost after re-chromatography of the liposomes, 5 days after preparation. Precipitates, occasionally formed after complex formation, originated from the excess of metal-chloride added to the liposomes. They could easily be removed by filtration or centrifugation.

Organ distribution and pharmacokinetics of the DTPA-SA-liposomes

Tables 2 and 3 summarize the organ distribution of Mn- and Gd-DTPA-SA (40 mol% DTPA-

TABLE 4

Organ elimination half-lives of Gd- and Mn-DTPA-SA liposomes in rats [a]

Organ	Gd-DTPA-SA		Mn-DTPA-SA	
	$t_{1/2}(1)$ [a] (h)	$t_{1/2}(2)$ [b] (h)	$t_{1/2}(1)$ [a] (h)	$t_{1/2}(2)$ [b] (h)
Blood	2.07	24.6	0.8	46.2
Liver	–	61 [c]	0.25	10.2
Spleen	–	105	0.77	9.2
Kidneys	2.0	580	3.5	89.0
Lung	2.7	580	0.6	13.6
Bones	–	26	–	–
Small intest.	–	–	2.16 [d]	–

[a,b] Half-lives of mono- (a) and biphasic (b) elimination curves were calculated by least-squares fitting.

[c] Calculated from 4 to 192 h.

[d] Calculated from 2 to 72 h.

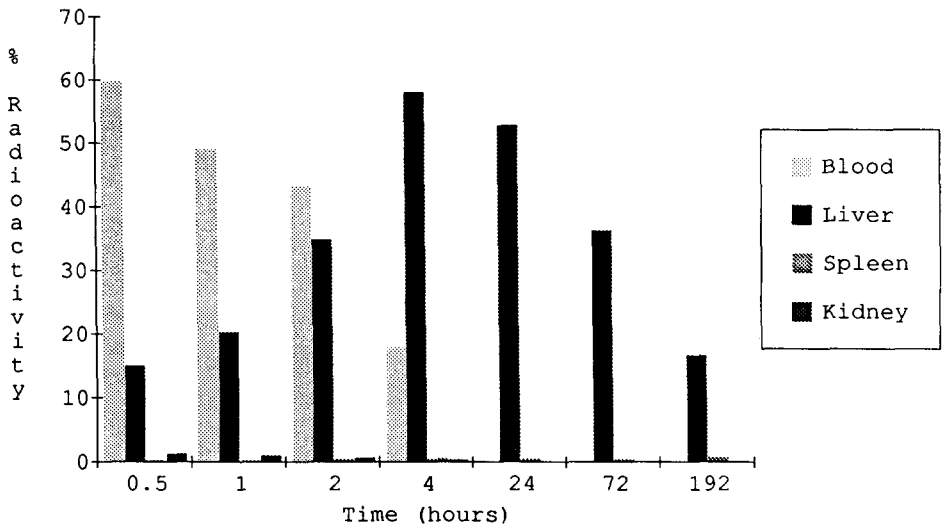

Fig. 1. Organ distribution in percent per organ of Gd-DTPA-SA liposomes after intravenous injection (0.03 mM Gd-DTPA-SA/kg b.wt.) into rats. The bars represent the mean of 3 values per time point.

SA) liposomes after intravenous injection. Highest concentrations of the metal complexes were found in the liver. Mn-DTPA-SA liposomes showed a peak absorption in the liver 30 min after injection, whereas for Gd-DTPA-SA the highest concentration was found only after 4 h.

Correspondingly, blood clearance was also faster for the manganese liposomes, as shown in Table 4, where the elimination half-lives from the most important organs are listed.

The differences between the organ elimination of Mn-DTPA-SA and Gd-DTPA-SA are prominent. Blood clearance and organ elimination are significantly slower for the gadolinium complex. This striking difference between the two metal complexes cannot be explained from the data ob-

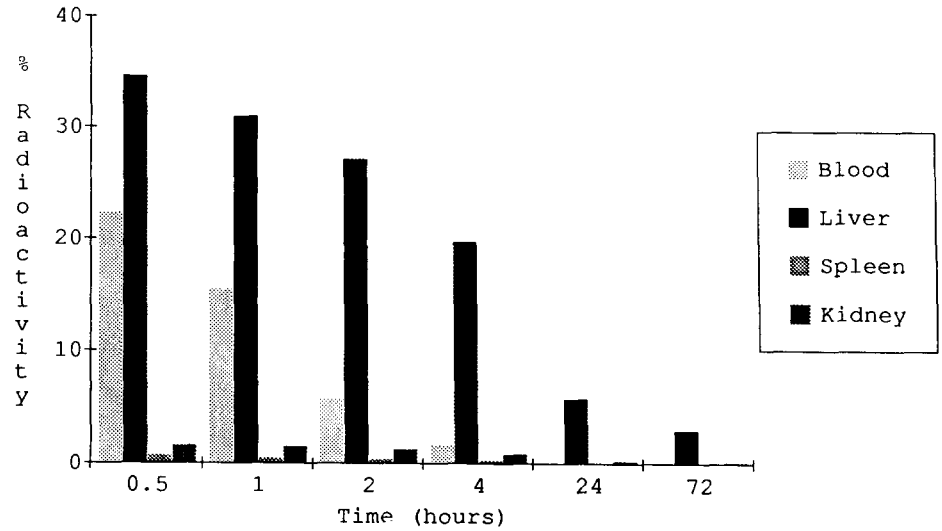

Fig. 2. Organ distribution in percent per organ of Mn-DTPA-SA liposomes after intravenous injection (0.03 mM Mn-DTPA-SA/kg b.wt.) into rats. The bars represent the mean of 3 values per time point.

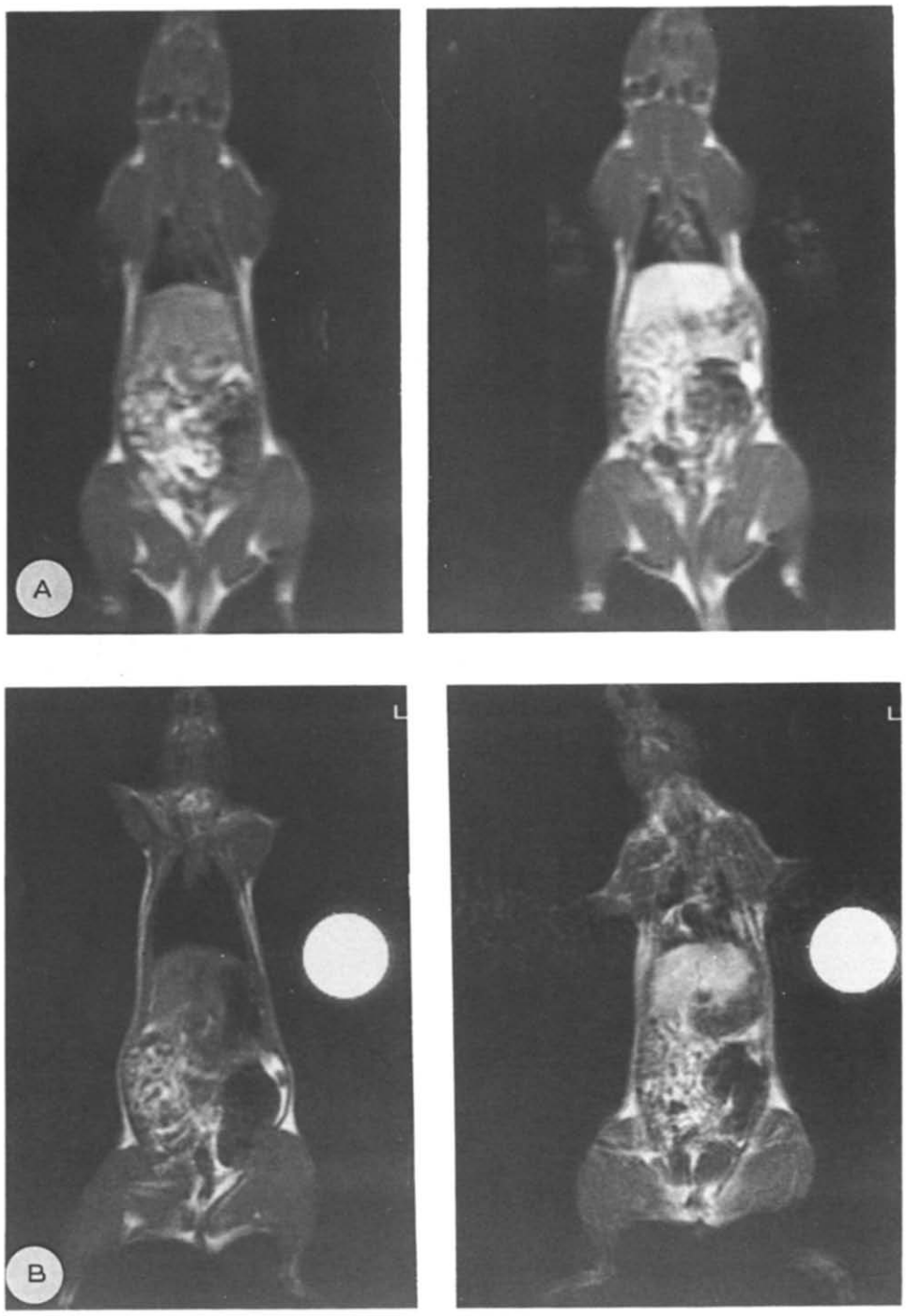

Fig. 3. Coronal MR images of rats. A (left): image obtained before liposome injection. A (right): 45 min after i.v. injection of Mn-DTPA-SA liposomes (0.03 mmol/kg b.wt., 40 mol% DTPA-SA, $T_R = 200$ ms, $T_E = 20$ ms). B (left): image obtained before liposome injection; B (right): 75 min after i.v. injection of Gd-DTPA-SA liposomes (0.09 mmol/kg b.wt., 40% DTPA-SA, $T_R = 200$ ms, $T_E = 30$ ms). The bright circles represent the contrast of a $CuSO_4$ (0.1 mM) control solution.

TABLE 5

T1 relaxation time reduction in rat livers after intravenous injection of Mn- or Gd-DTPA-SA liposomes [a]

Time (ms)	Dose of metal complex injected (mM/kg b.wt)							
	0.01		0.03		0.09		0.15	
	Mn	Gd	Mn	Gd	Mn	Gd	Mn	Gd
Before inj.	398	399	441	435	470	455	ND	418
Post 1 h	234	308	168	286	111	193	ND	156
Post 2 h	238	304	169	ND	120	174	ND	122

[a] Mean of 2 individual experiments.
ND = not determined.

tained. Since the mean diameter of both liposome preparations was identical (cf. Table 1), a vesicle size effect must be excluded. It is known that lanthanides can effectively block Kupffer cell activity at low concentrations (Roerdink et al., 1981). If small amounts of gadolinium are released from the liposomal complex, or if the Gd-DTPA-SA complex itself exerts a blocking activity, such a Kupffer cell blockade might explain the slow elimination of gadolinium. However, it has also been found that the activity of the parenchymal cells which are responsible for the uptake and processing of small liposomes is not affected by lanthanides (Spanjer et al., 1986; Rahman et al., 1980).

Both complexes are predominantly eliminated by the hepato-biliary route. The concentrations found in the kidneys were very low at all time points monitored. Figs. 1 and 2 show bar graphs of the radioactivities of blood, liver, spleen and kidneys, expressed in percents of the injected dose. With the Gd-DTPA-SA liposomes the decrease of blood concentration proceeds with the increase in liver concentration. The Mn-DTPA-SA liposomes, on the other hand, show parallel elimination courses from blood and liver. The metal concentrations in the liver 72 h after administration were 3% for manganese and 36.5% for gadolinium and after 8 days 17% of the gadolinium concentration still remained in the liver.

For Gd-DTPA-SA the distribution in the brain was only 0.2% and in bone (femur) 0.6%, both determined 30 min after injection. In the collected faeces 24 h after injection 14.7% of Mn-DTPA-SA but only 0.73% Gd-DTPA-SA were found. Seventy-two hours after injection, the excretion of the Gd-complex increased to 8.5%, whereas for the Mn-complex no marked change was found with 16.6% excreted. The cumulative excretion of Gd-DTPA-SA over 5 days was 16%, with 12% excreted after the first 2 days.

MR imaging of the RES with Mn- and Gd-DTPA-SA liposomes

The reduction of T1 relaxation times of the liver in rats after intravenous injection of various concentrations of Mn- or Gd-DTPA-SA liposomes (40 mol% DTPA-SA) is listed in Table 5. In particular, the Mn-DTPA-SA liposomes at 0.03 mM/kg b.wt. gave a contrast enhancement, such that the liver appeared almost as bright as fat tissue on T1 weighted images. With the corresponding gadolinium liposomes, an equally strong effect was not obtained.

The significant enhancement of liver contrast of both liposome preparations is shown in Fig. 3 where coronal sections of rats are shown. The Mn-DTPA-SA liposomes produce a very bright liver signal 30–60 min after injection at 30 μmol/kg b.wt. (Fig. 3A, right), whereas with Gd-DTPA-SA liposomes at 0.09 mmol/kg b.wt. 75 min after injection a weaker, but still significant, signal of the liver is obtained (Fig. 3B, right).

Conclusions

Small unilamellar liposomes containing paramagnetic metal chelates with DTPA-SA prove to be effective contrast agents for MRI of the RES.

Of the metal complexes studied, Mn-DTPA-SA gave the best results in terms of liposome stability as well as in vivo MR signal enhancement of the liver. Their fast absorption, followed by a favorable fast hepato-biliary excretion makes these liposomes particularly well suited for the use as a contrast agent. Furthermore, DTPA-SA added in trace amounts to liposomes may serve as a good marker to monitor in vivo liposome distribution.

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