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Mixed micelles containing lipophilic gadolinium complexes as MRA contrast agents

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Abstract

Mixed micelles for MRA are multicomponent systems containing a phospholipid, a biocompatible non-ionic surfactant (e.g. Synperonic® F-108) and a lipophilic gadolinium complex. A variety of lipophilic gadolinium complexes were designed taking into account features such as: (i) nature of ligand (cyclic versus acyclic); (ii) lipophilic moiety; (iii) global charge of the complex; and (iv) nature of bond connecting the complex to the lipophilic moiety. All the lipophilic gadolinium complexes after formulation as mixed micelles show high relaxivities in water and in blood (rat). Mixed micelles containing gadolinium complexes bearing only one aliphatic chain cannot be used as MRA contrast agents because they have a high haemolytic effect. Furthermore, in rats they are quickly eliminated from the blood stream. These drawbacks are completely circumvented using gadolinium complexes bearing two aliphatic chains. Mixed micelles containing such complexes show high relaxivities, no haemolytic effect and long blood permanence. This makes them promising candidates as MRA contrast agents. However, elimination, which occurs exclusively through the liver, is not complete, even after 7 days. Complexes containing labile (e.g. ester) bonds between the lipophilic moieties and the chelate subunit are eliminated through both the liver and the kidneys. However, elimination is still not complete after 7 days.

Keywords: Blood pool agents; Contrast agents; Lipophilic gadolinium complexes; Mixed micelles; MR angiography; MR imaging

1. Introduction

In the last years, great effort has been devoted to the discovery of blood pool contrast agents for MRI. Indeed, the gadolinium complexes currently available on the market (e.g. Magnevist®) are all low molecular weight species that rapidly equilibrate between the intra and extravascular spaces after intravenous administration. For this reason, intravascular contrast agents are more desirable for coronary artery imaging [1,2] and for the assessment of other important features such as: relative blood volume of tissues, relative blood flow and endothelial permeability [3].

In order to obtain a long permanence in blood of a gadolinium complex, three main different approaches have been proposed. The first approach is based on the use of a low molecular weight gadolinium complex (e.g. Angiomark®, Epix Medical or MP-2269, Mallinckrodt) [4,5] which is able to reversibly bind to endogenous blood components, e.g. serum albumin. The second approach takes advantage of gadolinium containing macromolecules like dendrimes (e.g. Gadomer17, Schering) [6] or polymers (e.g. Gd-DTPA-mer, Nycomed) [7] which, because of their large molecular size, remain confined in plasma. The third approach is based on the use of large supramolecular systems like liposomes [8] or micelles [9].

According to this third approach, we found mixed micelles very promising as MRA contrast agents. The term mixed micelles typically refers to three component aggregates of: (i) a non-ionic surfactant containing a polyoxyethylene chain; (ii) an amphipatic compound (e.g. a phospholipid); and (iii) a lipophilic gadolinium complex (Fig. 1).

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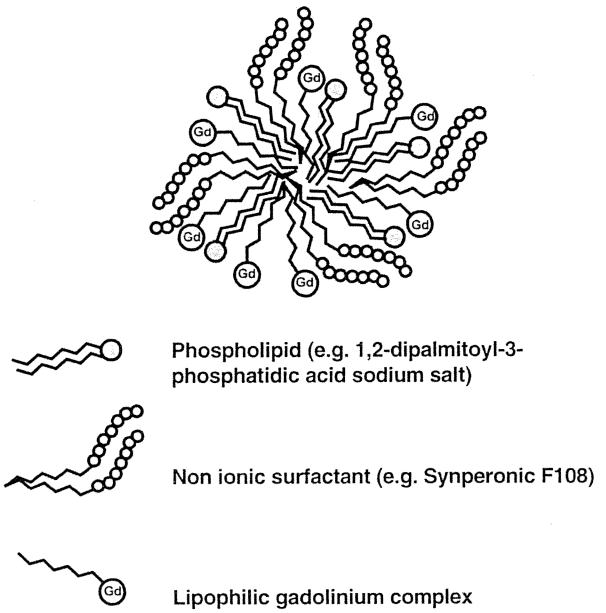

Fig. 1. Typical composition of a mixed micelle.

Here we report on a series of lipophilic gadolinium complexes which were designed taking into account features such as: type of ligand (cyclic versus acyclic), number and kind of lipophilic moieties, charge of the complex and type of bond connecting the complex to the lipophilic moiety.

The physico–chemical and pharmacokinetic properties of the mixed micelles prepared with such lipophilic gadolinium complexes are also discussed.

2. Materials and methods

2.1. Chemicals

All organic and inorganic reagents were purchased from Fluka A.G. (Buchs, Switzerland) except for: gadolinium acetate (Alfa Aesar, Karlsruhe, Germany), 1,2-epoxyoctadecane (Aldrich, Milan, Italy), 1-propanephosphonic acid cyclic anhydride (Lancaster, Mühlheim am Main, Germany), 1,2-dipalmitoyl-glycero-3-phosphatidic acid sodium salt and 1,2-distearoyl-glycero-3-phosphatidylethanolamine-polyethyleneglycol 2000 (Genzyme/Sygena, Liestal, Switzerland).

2.2. Synthesis of lipophilic gadolinium complexes

Building blocks easily synthetizable, like DO3A tris(*t*-butyl) ester **1** [10], DTPA tetra(*t*-butyl) ester **2** [11], DTPA dianhydride **3** [12] and DOTA tribenzyl ester **4** [13], were used as starting materials for the synthesis of the lipophilic gadolinium complexes, by reaction with synthons containing C_{16}–C_{18} aliphatic chains. The synthetic pathways followed to obtain the

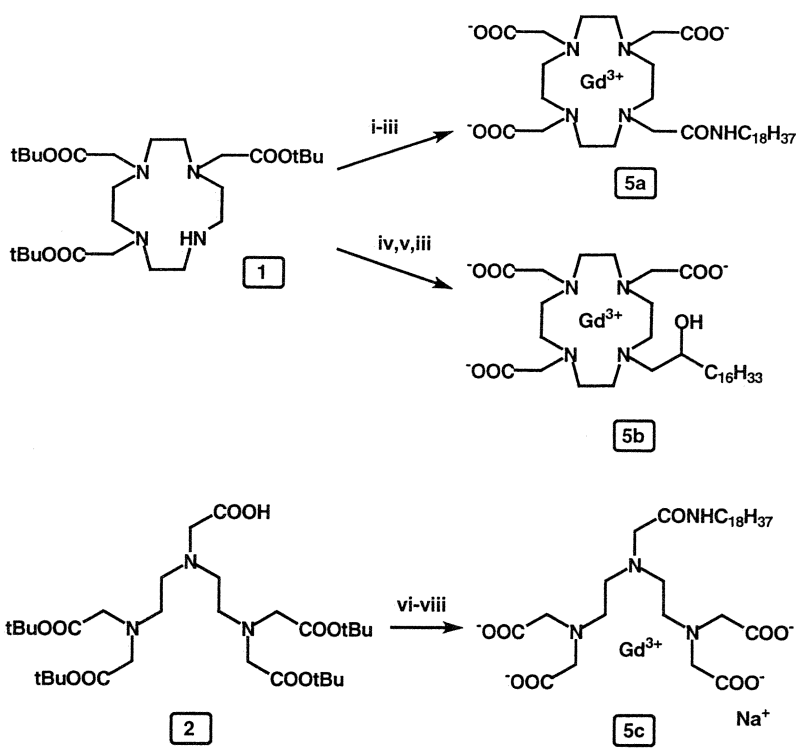

Fig. 2. Synthesis of lipophilic gadolinium complexes (first generation). Conditions: (i) $BrCH_2CONHC_{18}H_{37}$ [14], EtOH, reflux; (ii) CF_3COOH; (iii) Gd_2O_3; (iv) 1,2-epoxyoctadecane, EtOH, reflux; (v) 5 M HCl; (vi) isobutyl chloroformate, $C_{18}H_{37}NH_2$, Et_3N, THF; (vii) 0.5 M H_2SO_4, dioxane, 90°C; (viii) $GdCl_3$, 1 M NaOH, H_2O, CH_3CN.

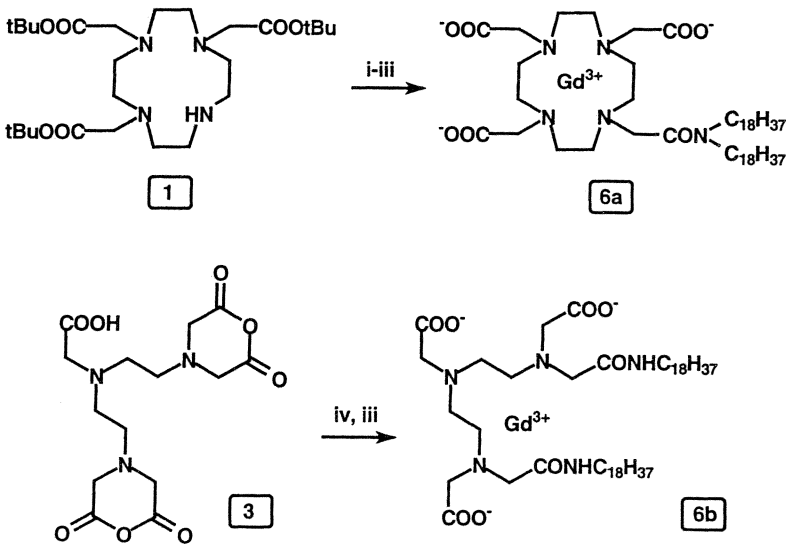

Fig. 3. Synthesis of lipophilic gadolinium complexes (second generation). Conditions: (i) $BrCH_2CON(C_{18}H_{37})_2$ [13], EtOH, reflux; (ii) 5 M HCl; (iii) $GdCl_3$, 1 M NaOH, EtOH, reflux; (iv) $C_{18}H_{37}NH_2$, DMF, 80°C.

lipophilic complexes are shown in Figs. 2–4. Compounds **6a** and **7b** labelled with ^{153}Gd were obtained by complexation with $^{153}GdCl_3$ (NEN Life Science Products, Switzerland) of the corresponding ligands.

2.3. Mixed micelles formulations

Two different formulations were used. The first formulation was prepared by mixing the lipophilic complex (20 mg ml^{-1}), Synperonic® F-108 (20 mg ml^{-1}) and 1,2-dipalmitoyl-glycero-3-phosphatidic acid sodium salt (DPPA-Na) (20 mg ml^{-1}) in buffer at pH 7.3 (Tris 1 g l^{-1}, 0.3 M glycerol), sonication at 70°C (Branson sonicator 250) for 30 min and filtration through 0.45 μm membrane filter. The second formulation was prepared in the same way using the lipophilic complex (20 mg ml^{-1}) and 1,2-distearoyl-glycero-3-phosphatidylethanolamine-polyethyleneglycol 2000 (PE-PEG) (20 mg ml^{-1}) [17]. In the latter case, PE-PEG acts both as the phospholipid and the surfactant.

2.4. Relaxivities

The relaxation times T_1 and T_2 were measured at 39°C in buffer (Tris 1 g l^{-1}, 0.3 M glycerol) and rat blood using a Minispec PC120 (Bruker, 0.47T) and using, respectively, the 'inversion recovery' method for T_1 and the Carr–Purcell–Meiboom–Gill (CPMG) technique for T_2.

2.5. Pharmacokinetic and biodistribution studies in rats

Blood kinetics were studied by measurement of relaxation times T_1 and T_2 in rat blood. The rats (Sprague–Dawley, ≈250 g) were weighed and the total amount of blood calculated (6% of body weight). The theoretical Gd concentration (mM) in blood at time $t = 0$, corresponding to 100% of the injected dose (ID), was calculated for each rat. The rats ($n = 2$) were sacrificed at 10, 30, 60 and 90 min and 2, 4, 7 and 24 h after intravenous injection of 1 ml of the formulation/rat (≈ 75 μmol Gd^{-1} kg^{-1}). At each time t, 5 ml of blood were collected and $T_1(t)$ and $T_2(t)$ were measured. The Gd concentration (mM) in the blood at time t was calculated using the following formula:

$$[Gd] = [1/T_i(t) - 1/T_i(0)]/r_i \quad i = 1 \text{ or } 2$$

where $T_i(t)$ is the relaxation time of the blood measured at time t; $T_i(0)$ is the relaxation time measured in fresh blood; and r_i is the relaxivity of the tested formulation in blood. The results were then normalized (injected dose = 75 μmol Gd^{-1} kg^{-1}) and expressed as percent of ID.

Body distribution and urinary and faecal elimination in rats were obtained by ^{153}Gd radioactivity (γ-counter Packard Minaxi Autogamma® 5000 series) or by inductively coupled plasma atomic emission spectrometry (ICP-AES; ARL 3410+, Redox Laboratories, Monza, Italy) measurements.

The urinary and faecal eliminations of gadolinium were studied after single administration of **6a**, **7a** and **7b** to male Sprague–Dawley rats (≈250 g) ($n = 3$ per group) at a dose of 1 ml per rat of formulation. Urine and faeces were collected daily in metabolism cages for up to 7 days after the injection. Both urine and faeces were immediately frozen and maintained at −20°C until assayed. Three animals were sacrificed at 24 and 72 h and 7 days after administration and blood, liver, spleen, femur and kidneys were collected to evaluate residual content of gadolinium in organs.

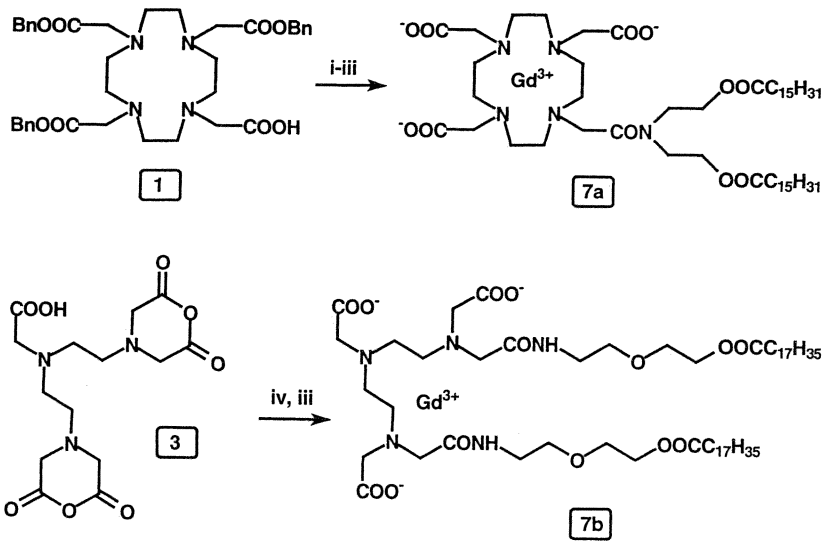

Fig. 4. Synthesis of lipophilic gadolinium complexes (third generation). Conditions: (i) $(C_{15}H_{31}COOCH_2CH_2)_2NH$ [15], 1-propanephosphonic acid cyclic anhydride [16], Et_3N, CH_2Cl_2; (ii) H_2, Pd/C, EtOH; (iii) $(CH_3COO)_3Gd$, $CHCl_3$, MeOH, H_2O; (iv) $C_{17}H_{35}COOCH_2CH_2OCH_2CH_2NH_2$ [13], DMF, 60°C.

2.6. Coronary imaging in micropigs

The potential of compounds to improve contrast-enhanced magnetic resonance coronary angiography (ceMRCA) was tested with an inversion-recovery 3D gradient-recalled echo sequence (IR 3D GRE). This technique uses a segmented 3D GRE sequence. In order to minimise the effect of cardiac motion, the data acquisition window is gated to mid-diastole, which in turn is determined by cine-imaging in a short axis plane through the left ventricle. The acquisition window is $\approx 10-15\%$ of the RR interval in the ECG trace. For RR intervals of 600–700 ms, the data acquisition window is < 100 ms. Respiratory motion was controlled by acquiring navigator-echo 3D coronary angiography on the anaesthetised and artificially-ventilated animals. Depending on the extent of heart coverage and on spatial resolution, the total acquisition times varied between 8 and 15 min.

For all post-contrast imaging, an inversion pulse preceded the data acquisition segment to null the myocardium signal. This step helps to maximise the contrast-to-noise ratio (CNR) for the coronary arteries. The time interval (TI) between the inversion pulse and the beginning of the acquisition segment is ideally chosen in a way to null the myocardium signal with the blood having returned to the fully relaxed condition (maximum signal) at the same time point.

In order to assess the ability of mixed micelles formulations to cope with these requirements, the T_1 in the blood of three domestic pigs (≈ 20 kg body weight) after administration of cumulative doses of **6a** (from 0.025 to 0.2 mmol Gd kg^{-1}) was measured in vivo, 10 min after injection, using the above techniques on a 1.5 T Siemens Symphony® scanner (TR/TE/TI/Flip angle: 3.35/1.81/200 ms/25°; Matrix: 125 × 256 × 32; F.O.V.: 125 × 256 × 64 mm^3; Spatial resolution: 1 × 1 × 2 mm^3) at Northwestern University (Department of Radiology, Chicago, IL).

3. Results

The mixed micelles formulated with complexes **5a–c**, containing one aliphatic chain (first generation), all showed high relaxivities (r_1 16–26 mM^{-1} s^{-1}) but a very poor blood permanence. For example, after 10 min of injection only 30% of ID of **5a** was present in plasma (Fig. 5). Moreover, for all complexes **5** a strong haemolytic effect was noticed both in vitro and in vivo.

The mixed micelles which were formulated with complexes **6a,b** (containing two aliphatic chains, second generation) showed high relaxivities (r_1 18–23 mM^{-1}

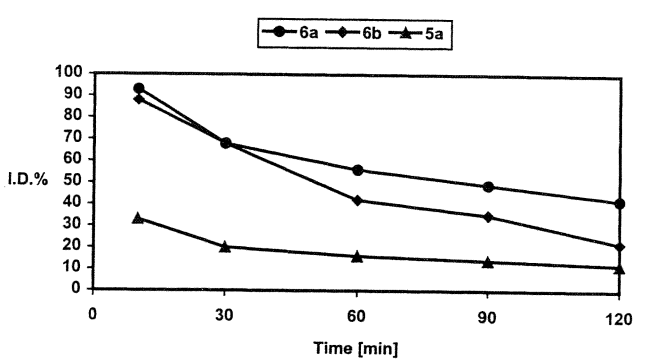

Fig. 5. Blood elimination profiles of **5a**, **6a** and **6b** as evaluated on the basis of T_1 measurements (formulation: complex/DPPA-Na/Synperonic® F108 = 20/20/20 mg ml^{-1}; injected dose = 1 ml per rat)

Table 1
Organ distribution (ID%) of PE-PEG formulation of **6a**, **7a** and **7b** in rats 7 days after administration (dose = 1 ml per rat)

	6a[a,b]	**7a**[c]	**7b**[a,d]
Liver	21.0 ± 1.7 (20.8 ± 0.5)	9.1 ± 1.1	16.8 ± 5.3 (14.4 ± 1.9)
Bone	8.4 ± 2.1 (14.7 ± 2.7)	2.5 ± 0.10	8.7 ± 0.5 (4.6 ± 0.8)
Spleen	2.6 ± 0.2 (8.7 ± 1.0)	0.46 ± 0.06	0.31 ± 0.07 (0.40 ± 0.03)
Kidneys	0.33 ± 0.03 (0.19 ± 0.01)	0.27 ± 0.03	0.53 ± 0.02 (0.61 ± 0.05)

[a] Assessed by radioactivity (^{153}Gd).
[b] Values in parentheses refer to the DPPA/Synperonic® F108 formulation.
[c] Assessed by ICP-AES.
[d] Values in parentheses were assessed by ICP-AES.

s^{-1}) and good blood permanence, without haemolytic effect. Compound **6a** was still present in plasma at 70% of ID 30 min after the administration (Fig. 5), while its elimination from plasma was complete (< 1% of ID) after 24 h, as evaluated by ^{153}Gd radioactivity studies. A 50–58% of ID was found in faeces and < 1% in urine 7 days after injection. The biodistribution data for two different formulations of **6a** are reported in Table 1.

The pig was chosen as an animal model for demonstration of the potential of **6a** to improve ceMRCA for the size and anatomy of the coronaries that are similar to those in humans. Multiplanar reconstructions (MPR) from a typical 3D experiment showing the right coronary artery (RCA) are displayed in Fig. 6. High blood signal-to-noise ratio (SNR) and almost total myocardial signal suppression is maintained for almost 1 h after administration.

Compounds **7a,b** (third generation) contain labile (e.g. ester) bonds which connect the gadolinium complex to the aliphatic chains. For mixed micelles prepared using **7a,b** relaxivities (r_1 18–22 mM^{-1} s^{-1}), blood permanence and haemolytic effect are very similar to those of mixed micelles containing compounds **6a,b**. A significant difference was found in the elimination route and the case of **7b** is a good example: 32% of ID was found in faeces and 36% in urine 7 days after injection. (In the case of **7a**, a 40% of ID was found in faeces and 27% in urine 7 days after injection). A comparison of the biodistribution of the PE-PEG formulations of **6a**, **7a** and **7b** is reported in Table 1.

4. Discussion

Mixed micelles that contain gadolinium complexes bearing a single aliphatic chain cannot be used as MRA contrast agents because they are quickly eliminated from the blood stream. Moreover, they are strong haemolytic agents, independently of either the charge or the ligand structure (cyclic or acyclic). They apparently behave on erythrocyte membranes like surfactants do. Indeed, single chain surfactants are commonly used to disrupt biological membranes [18]. An empirical explanation of their disruptive power could be the mismatch between their intrinsic geometry and that of the phospholipids, the main components of biological membranes [19].

Mixed micelles containing gadolinium complexes with two aliphatic chains do not show any haemolytic effect. This feature, together with high relaxivities and, more importantly, a long permanence in blood, make these systems very efficient MRA contrast agents. The

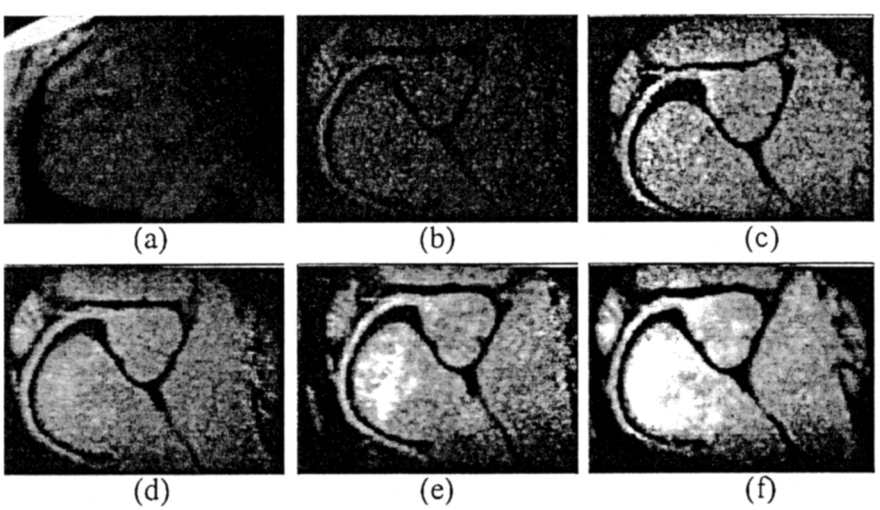

Fig. 6. Inversion recovery Nav' Echo 3D MR coronary angiography in pigs after cumulative doses of **6a** at 1.5 T. (a) Precontrast, blood T_1: 1200 ms; (b) 0.025 mmol Gd kg^{-1}, blood T_1: 147 ms; (c) 0.05 mmol Gd kg^{-1}, blood T_1: 82 ms; (d) 0.1 mmol Gd kg^{-1}, blood T_1: 48 ms; (e) 0.15 mmol Gd kg^{-1}, blood T_1: 40 ms; (f) 0.2 mmol Gd kg^{-1}, blood T_1: 30 ms.

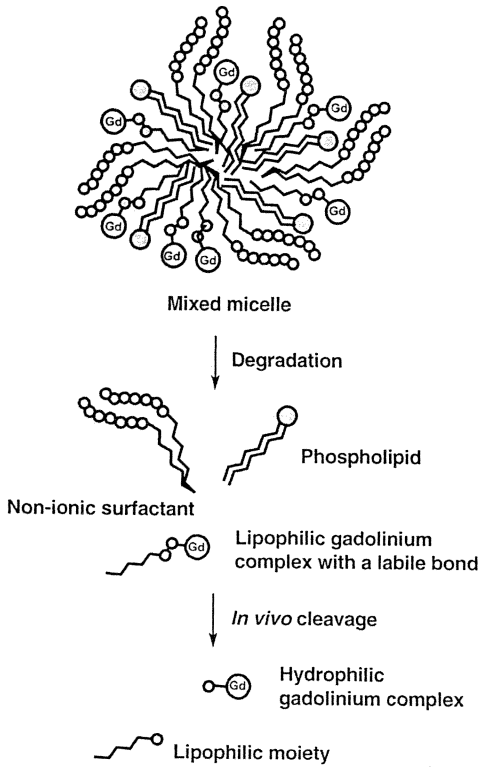

Fig. 7. Rationale of the in vivo cleavable lipophilic complexes.

images taken from pigs (Fig. 6) using a mixed micelle formulation of **6a** clearly show the usefulness of this product in the detection of blood vessels, such as the right coronary artery (RCA). Compound **6b** [20] is also promising, although the relaxivities and the permanence in blood are a little inferior to those of **6a**.

The main problem of compounds such as **6** is the elimination. Compound **6a** is eliminated, although not completely through the hepatic route. After administration to rats of micelles labelled with ^{153}Gd, a significant amount of radioactivity is found in some organs, even after 7 days (Table 1). Better results, especially for residual radioactivity in spleen and bone, are obtained with the PE-PEG formulation (Table 1). This is an important issue because even better results, in terms of elimination, could be achieved simply investigating different formulations of the mixed micelles.

The synthesis of compounds containing an in vivo labile bond between the gadolinium complex and the lipophilic moiety was taken into account to overcome the elimination issue. The idea was that, after degradation of the mixed micelle, the lipophilic gadolinium complex, exposed to the biological environment, should be converted into a small, hydrophilic, easily eliminable gadolinium complex and a lipophilic counterpart, preferably endogenous or with a known metabolic pathway (Fig. 7). The body of mammals is rich in enzymes capable of hydrolysing esters (i.e. esterases are practically ubiquitous all over the body) and for this reason, an ester was chosen as the cleavable bond [21], while fatty acids were chosen as lipophilic moieties.

Compounds **7a,b** are eliminated, not only through liver but also through kidneys and this is an indirect evidence of a partial metabolisation of the products. Unfortunately, significant amount of gadolinium is still found in liver and bone of rats 7 days after injection (Table 1).

The present study shows that mixed micelles containing lipophilic gadolinium complexes are promising candidates as MRA contrast agents. The synthesis of new classes of labile gadolinium complexes and the study of different formulations are in progress in order to improve the elimination.

Acknowledgements

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