Development of New Contrast Agents for Imaging Function and Metabolism by Magnetic Resonance Imaging

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ABSTRACT: Liposomes are interesting nanosystems with a wide range of medical application. One particular application is their ability to enhance contrast in magnetic resonance images; when properly loaded with magnetic/superparamagnetic nanoparticles, this means to act as contrast agents. The design of liposomes loaded with magnetic particles, magnetoliposomes, presents a large number of possibilities depending on the application from image function to metabolism. More interesting is its double function application as theranostics (diagnostics and therapy). The synthesis, characterization, and possible medical applications of two types of magnetoliposomes are reviewed. Their performance will be compared, in particular, their efficiency as contrast agents for magnetic resonance imaging, measured by their relaxivities r_1 and r_2 relating to their particular composition. One of the magnetoliposomes had 1,2-diacyl-sn-glycero-3-phosphocholine (soy) as the main phospholipid component, with and without cholesterol, varying its phospholipid to cholesterol molar ratios. The other formulation is a long-circulating liposome composed of 1,2-diacyl-sn-glycero-3-phosphocholine (egg), cholesterol, and 1,2-distearoyl-sn-glycerol-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000]. Both nanosystems were loaded with superparamagnetic iron oxide nanoparticles with different sizes and coatings.

KEYWORDS: Magnetoliposomes, SPION, contrast agents, relaxivities, MRI

Introduction

The large variety of systems now available in the nanometric scale, opened a door to a close proximity with cells or even parts of cell. This represents a clear advantage in diagnostic and therapy of some of the most challenging human pathologies. Different nanoparticles such as iron oxide nanoparticles, quantum dots, gold nanoparticles, carbon nanotubes, silica nanoparticles, to name a few, are under study as interesting platforms for diagnostics, therapy, or both functionalities, theranostics.[1] Drug delivery systems are being intensively studied essentially due to their properties as efficient vehicles to carry superparamagnetism to be applied in magnetic resonance imaging (MRI), in hyperthermia, or in applications that involve light emission. In addition, due to their capacity to couple with radioactive systems, they can be used in dual imaging methodologies, such as MRI and single-photon emission computed tomography or positron emission tomography,[2] and even to carry ionizing radiation close to the target volume in cancer treatment.[3–5] In this context, superparamagnetic iron oxide nanoparticles (SPIONs) are frequently incorporated in liposomes and the resultant magnetoliposomes are used as theranostic vehicles.[6] In this concise review, two different magnetoliposomes[7–10] will be reviewed for their capacity to enhance contrast in MRI. Their ability to act as negative contrast agents (CA), that is, to mainly shorten the transverse relaxation time and in one of the cases their efficiency to detect liver ischemia-reperfusion injuries (IRI),[8] will be discussed in a comparative approach.

The design of magnetoliposomes with high r_2 and r_2 / r_1 is a complex problem that involves both the magnetic properties and coating of the SPIONs and also the composition of the liposome.[11,12] Because their interaction with cell membranes is of great importance, the effect of SPIONs on stability and properties of the magnetoliposomes such as fluidity and phase transition temperature of the liposomal bilayer has attracted some attention.[13] The efficiency of the magnetoliposome as negative CA is also related to the microenvironment of the SPIONs.[10,14] All variables will be addressed in this review and discussed relating to the two contributions usually accepted for proton relaxation in the presence of superparamagnetic particles: the inner-sphere and the outer-sphere contributions. Briefly, in the inner-sphere contribution, the proton is close to the superparamagnetic magnetic moment, and in the outer-sphere contribution, the relaxation occurs by dephasing of water protons moving in the large magnetic field gradient generated by the superparamagnetic particle.[15,16]

Magnetoliposomes

Two different types of magnetoliposomes are considered and represented in Figure 1. Figure 1A shows the liposomes
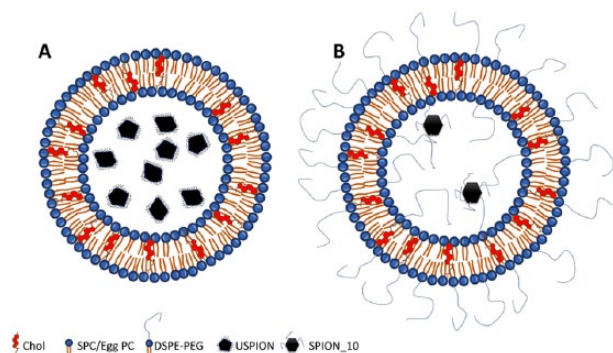
Figure 1. Schematic representation of two different types of magnetoliposomes: (A) liposomes prepared with SPC and Chol with a magnetic core of magnetite nanoparticles stabilized by TMAOH (USPIONs) and (B) liposomes prepared with Egg-PC, Chol, and DSPE-PEG with a magnetic core of superparamagnetic nanoparticles (SPION_10). Chol indicates cholesterol; DSPE-PEG, 1,2-distearoyl-sn-glycerol-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000]; Egg-PC, 1,2-diacyl-sn-glycero-3-phosphocholine (egg); SPC, 1,2-diacyl-sn-glycero-3-phosphocholine (soy); SPION, superparamagnetic nanoparticle; TMAOH, tetramethylammonium hydroxide; USPIONs, ultrasmall iron oxide nanoparticles.

prepared with 1,2-diacyl-sn-glycero-3-phosphocholine (soy) (SPC) and cholesterol (Chol) with SPC:Chol ratios varying from 1:0 to 1:1 and named SPC:Chol 1:0, SPC:Chol 1:0.5, and SPC:Chol 1:1, respectively.[7] Their magnetic core was ultrasmall iron oxide nanoparticles produced by a coprecipitation method and stabilized with tetramethylammonium hydroxide, each magnetite particle is coated with this surfactant[7,17] and their hydrodynamic diameter was in the range of 180 to 200 nm. Figure 1B shows the liposomes prepared with 1,2-diacyl-sn-glycero-3-phosphocholine (egg) (Egg-PC), 1,2-distearoyl-sn-glycerol-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000] (DSPE-PEG), and Chol with a total composition of Egg-PC:Chol:DSPE-PEG 1.85:1:0.15 and named pegylated magnetoliposomes. Their magnetic core was superparamagnetic nanoparticles (SPION_5 and SPION_10) with 5 ± 1 nm and 10 ± 1 nm diameter (manufacture transmission electron microscopy results) coated with short-chain-length polyethylene glycol and with magnetizations at 4500 Oe greater than 25 emu/g and 45 emu/g, respectively. These magnetoliposomes named MagLipos_5 and MagLipos_10 presented a 130 nm hydrodynamic diameter.[8]

Relaxivity measurements

SPC and Chol magnetoliposomes. As presented in Carvalho et al,[9] for the SPC:Chol magnetoliposomes with three different lipid:cholesterol ratios, the relaxation times T_1 and T_2 were measured at 25 °C in a Bruker Avance III nuclear magnetic resonance spectrometer, in a 7 T magnetic field (300 MHz for proton). The T_2 values were obtained both by high-resolution

spectroscopy and MRI microimaging methods (imaging gradient field up to 160 G/cm). For all the studied samples and within the investigated concentration range, the T_1 relaxation time showed no variation. This was explained by the lack of effect of the presence of the magnetoliposomes in the high-frequency spectra of proton dynamics responsible for the T_1 relaxation.[16]

The obtained transverse relaxation time T_2 allowed the usual fitting:

$$\frac{1}{T_2} = \frac{1}{T_{20}} + r_2[c] \tag{1}$$

where T_{20} is the transverse relaxation rate, in the absence of magnetoliposomes and r_2 is the transverse relaxivity and $[c]$ is the iron concentration.[16] The fits results are presented in Table 1.

For the SPC:Chol r_2 correlation, coefficients range from 0.90926 to 0.99951, and for the MagLipos the r_2 correlation, coefficients range from 0.98663 to 0.99793.

Pegylated magnetoliposomes. In the study by Martins et al,[8] the procedure to obtain the longitudinal and the transverse relaxivities for the pegylated magnetoliposomes MagLipos_5 and MagLipos_10 is detailed. Briefly, the relaxation times were obtained in a Bruker Avance III spectrometer in a magnetic field of 7 T at 25 °C, and the magnetoliposome samples used to study the relaxivities were prepared by dilution in a physiological compatible saline solution. The T_1 relaxation time was obtained using an inversion recovery sequence, and the transverse relaxation rate was obtained using a Carr-Purcell-Meiboom-Gill (CPMG) sequence. It was possible to fit equation (1) to T_1 values, and the longitudinal relaxivities obtained were 3.95 and 3.69 (mM s)⁻¹ with correlation coefficients 0.99948 and 0.9990 for the MagLipos_5 and MagLipos_10, respectively.

Relatively to T_2 values, it was possible to fit equation (1) and obtain the transverse relaxivity for MagLipos_5 and MagLipos_10 magnetoliposomes, and the results are presented in Table 1. The MagLipos presented r_2/r_1 high ratios of 50.3 and 57.1 for the MagLipos_5 and MagLipos_10, respectively.[10]

Ischemia/reperfusion injury detection by MRI

The pegylated magnetoliposomes loaded with SPION_10 coated with a short-length polyethylene glycol presented a long residence time in bloodstream that leads to interesting applications.[8] It was clearly demonstrated by 7 T MRI their capacity to enhanced the detection of lesions in liver ischemia–reperfusion injuries(IRI). Recently, the same liposome with antioxidant enzyme superoxide dismutase covalently linked to the distal end of DSPE-PEG showed an enhanced therapeutic result in the IRI.[18] This opens the possibility to access diagnostic and therapy with the same nanoplatform.

Table 1. Transverse relaxivity for the SPC and Chol and for the pegylated magnetoliposomes.

SAMPLE	SPC:Chol 1:0.0	SPC:Chol 1:0.5	SPC:ChoL 1:1.1	MagLipos_5	MagLipos_10
r_2 (mM s)$^{-1}$	143.7	61.7	33.5	198.0	210.5
Model contributions	Inner	Inner	Inner	1/4 outer + 3/4 inner	1/4 outer + 3/4 inner

Abbreviations: Chol, cholesterol; SPC, 1,2-diacyl-sn-glycero-3-phosphocholine (soy).

Transverse Relaxivities and Magnetoliposome Formulation

The transverse relaxivity values obtained at a static magnetic field of 7T for the studied magnetoliposomes, SPC:Chol and MagLip, allows an interesting discussion on the two different contributions, inner sphere and outer sphere, for the r_2 of a system of protons subjected to a superparamagnetic agent.[16]

The SPC:Chol magnetoliposomes showed a strong effect of cholesterol in the transverse relaxivity.[9] For the SPC:Chol 1:0, SPC:Chol 1:0.5, and SPC:Chol 1:1, the main contribution for r_2 comes from the inner sphere because the T_2 relaxation time shows invariance for different echo times in the CPMG pulse sequence, within the experimental error. In the case of strong outer-sphere contribution, the dephasing of water protons is related to diffusion in the strong magnetic field gradient created by the superparamagnetic particles; this implies that the effect of different echo times on T_2 is weighting differently the diffusion of protons. For the SPC:Chol magnetoliposomes, the transverse relaxivity is a function of water exchange rate which is inversely proportional to the cholesterol present in the magnetoliposome bilayer. Water exchange rate has been identified as an important parameter for the efficiency of magnetoliposomes as negative CA.[19,20]

The pegylated magnetoliposomes MagLipos_5 and MagLipos_10 as referred in the study by Carvalho et al[10] presented the following ratios: 10 μmol of lipid/mL of solution and 40 μg Fe/mL and 11 μmol of lipid/mL of solution and 14 μg Fe/mL, respectively, this means 40 μg Fe/11 μmol of lipid for the MagLipos_5 and 14 μg Fe/11 μmol for the MagLipos_10. The pegylated magnetoliposomes with a hydrodynamic diameter of 130 nm had a superficial area of $4\pi(65)^2$ nm^2. The MagLipos_5 was loaded, as previously referred with 5 nm SPIONs and the MagLipos_10 with 10 nm SPIONs, assuming for both a density of 5.1 g/cm^2, it was possible to estimate 2.4×10^{-19} g Fe/5 nm SPION and 1.9×10^{-19} g/10 nm SPION. Following the study by Hak et al,[14] taking an average superficial area for the lipids (DSPE-PEG and Egg-PC) of 0.68 nm^2, it was possible to obtain about 2 SPIONs of 5 nm/MagLipos_5 and 1 SPION of 10 nm/MagLipos_10.

Hak et al[14] presented a very interesting study in a related nanosystem composed of nanoemulsions with controlled diameter and loaded with SPIONs of 5 nm diameter. In this system, clearly, the inner-shell contribution is negligible. For the outer-shell contribution, four models were implemented, three of them in the known fast diffusion regime, that is, in the timescale of a T_2 measurement, diffusion average out the nanoparticle effect, and rephasing has no effect and one in the static dephasing regime, where in the opposite the proton resides near the nanoparticle magnetic moment, this is induced by the presence of water entrapped in the PEG coating of the nanoemulsions. In the fast diffusion regime, three different situations were considered: a isotropic model, a loose aggregate model, and a dense one. For our pegylated magnetoliposomes, it is clear that the appropriated model is the loose aggregate one because we estimated 2 SPIONs of 5 nm/MagLipos_5 and 1 SPION of 10 nm/MagLipos_10 average. The r_2 model results[14] for a nanoemulsion loaded with 1 to 3.7 SPIONs are 0.5 to 2.4 (mM s)$^{-1}$, although the measured values were 40 to 53 (mM s)$^{-1}$, an order of magnitude deviation that could be explained by water protons that reside in the nanoemulsions' PEG coating and that exchange with the bulk water ones. In our case, pegylated magnetoliposomes presented r_2 two orders of magnitude higher (~200 (mM s)$^{-1}$) than the ones predicted by the fast diffusion model in the loose aggregate geometry. Following the same line of though, one could attribute the same weight as in the study by Hak et al[14] for the measured r_2 of the MagLipos; this accounts roughly for 25% of the transverse relaxivity. The rest can be attributed to protons inside the aqueous bilayer of the liposomes near the also pegylated SPIONs. The relaxation of these protons is described also by a static dephasing regime but in an inner-shell approach.

Conclusions

The CA efficiency of a magnetoliposome is a complex issue not only related to the magnetic properties of the SPIONs (magnetic saturation) and the nanoparticle coating but also to the SPION microenvironment.

The permeability of the liposome is of the utmost importance because it controls the inner-shell contribution to the transverse relaxivity.

The presence of PEG, both in the magnetoliposome bilayer and in the SPIONs coating, enhances the static dephasing regime in an inner-shell and outer-shell perspective, allowing the design of high transverse relaxivity systems similar to the ones reviewed.

In addition, the pegylated magnetoliposomes showed a capacity to enhance liver IRI in an MRI examination, but other medical applications such as drug delivery and tissue targeting or hyperthermia could be considered.

Author Contributions

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