Relaxation behavior of paramagnetic liposomes in cell cultures

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Introduction

Recent developments in the field of molecular MR imaging, which aims to detect sparse concentrations of molecular epitopes, have given rise to a continuous demand for more effective T_1 lowering contrast agents. A contrast agent composed of paramagnetic liposomes may be a good candidate, because liposomes can carry a large payload of Gd-chelate in the lipid bilayer, leading to a very high relaxivity per particle. This lowers the detection limit of these contrast agents dramatically, compared to conventional contrast agents such as for example Gd-DTPA [1]. The relaxivity of these paramagnetic liposomes in an aqueous solution might differ considerably from the relaxivity *in vivo*, where the environment is far from homogeneous, such as when for example the liposomes are associated with a vessel wall or are taken up by cells. There is also much interest in labeling cells, *e.g.* monocytes, stem cells, T-lymphocytes, and leukocytes, with T_1 lowering contrast agents for *in vivo* cell tracking with MRI [2]. It is for these reasons essential to have knowledge about the relaxivity of the contrast agent in cells, especially for quantitative molecular imaging applications.

The aim of this work was therefore to characterize the relaxation behavior of paramagnetic liposomes *in vitro* in cell cultures. Relaxation rates were measured for endothelial cells incubated with liposomes for different durations. The relaxation rates of mixtures of incubated and non-incubated cells were determined, to investigate whether a change in T_1 can be used to quantify the number of labeled cells in a certain volume. Finally, we report on the detection of a cell monolayer following incubation with paramagnetic liposomes, which mimics labeling the endothelial surface of a vessel wall.

Materials & Methods

Liposome preparation: Pegylated paramagnetic liposomes consisted of appropriate amounts of DSPC, cholesterol, DOTAP, and Gd-BSA (Gd-DTPA-bis(stearylamide)), as previously described [1]. The liposomes were prepared by lipid film hydration and sized by extrusion through 400 and 100nm filters.

Cell cultures: Human umbilical vein endothelial cells (HUVEC) in culture flasks, containing approximately 10^6 cells, were incubated with the liposomes for 15, 30, 60, and 180 minutes. The concentration of Gd-ions for the 180 minutes incubation was of the order of 1mM. The cells were subsequently harvested and mixed with non-incubated cells in percentages of 0, 33, 67, and 100%. HUVEC monolayers on 9-wells culture plates were incubated with liposomes for 0, 5, 10, 15, 30, 60, 120, and 180 minutes.

MRI: Experiments were performed on a 6.3 T MR scanner. T_1 maps of the cell pellets were made with an inversion recovery spin-echo sequence with the following parameters: inversion-time = 8ms to 10s (exponential array of 12 points), TR = 18s, TE = 9ms, NSA = 4, slice thickness = 0.7mm, FOV = 1.5×1.5cm^2, matrix = 64×64. T_2 maps of the cell pellets were made using a spin-echo sequence with: TE = 7.5ms to 200ms (exponential array of 10 points), TR = 3s, NSA = 4, slice thickness = 0.7mm, FOV = 1.5×1.5cm^2, matrix = 64×64. T_1 maps of the HUVEC monolayers were made with an inversion recovery spin-echo sequence using the following parameters: inversion-time = 10ms to 12s (exponential array of 15 points), TR = 20s, TE = 10ms, NSA = 4, slice thickness = 1mm, FOV = 3×3cm^2, matrix = 256×128.

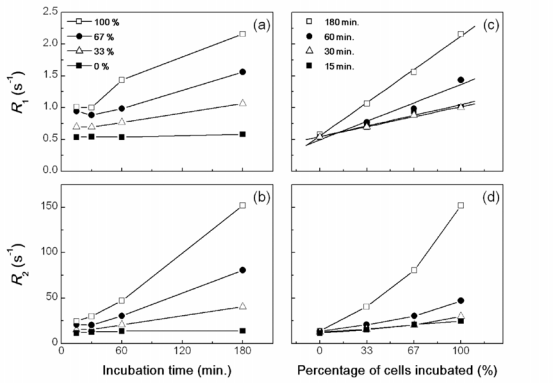

Figure 1: (a,b) Relaxation rate of the cell pellets as function of the incubation time for different mixtures as indicated. (c,d) Relaxation rate of the cell pellets as function of the percentage of incubated cells for different incubation times as indicated. Solid lines in (c) are linear fits.

Results & Discussion

In figure 1 the longitudinal and transverse relaxation rates R_1 and R_2 for the pellets of cells incubated with the paramagnetic liposomes are shown as function of the incubation time (a,b) and as function of the percentage of cells incubated (c,d). Qualitatively, the R_1 increases proportional to the incubation time, which shows that the cells take up the liposomes. There is a linear relationship between the R_1 and the percentage of incubated cells (figure 1c, solid lines with R^2>0.96 for all curves). This shows that, when starting from a known number of labeled cells, *e.g.* in a cell tracking experiment, the R_1 can be used to quantify the number of cells in a certain voxel. As expected, the transverse relaxation rate R_2 also increases with longer incubation times. The relative increase, however, is much larger. For the pellets with 100% incubated cells this leads to a dramatic increase of the R_2/R_1 ratio from 24.3 to 70.3 for 15 min. and 180 min. incubation, respectively. In comparison, for an aqueous solution of liposomes with comparable Gd-ion concentration the R_2/R_1 at this field strength is about 6. Figure 1d shows that R_2 is not a linear function of the percentage of incubated cells. The high R_2/R_1 ratio is disadvantageous, since strong T_2 effects hinder quantification based on T_1 contrast, especially when using gradient echo sequences.

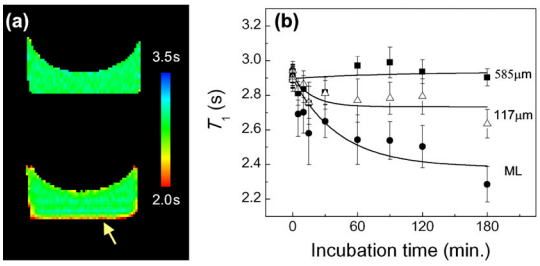

Figure 2: (a) T_1 maps of the cell monolayer for (top) not incubated and (bottom) 180min. incubated with liposomes. (b) T_1 relaxation as function of the incubation time for the HUVEC monolayers for pixels in the monolayer (ML) and 117 and 585µm above. The solid lines are guides to the eye.

In figure 2a the T_1 maps are shown of two wells with a monolayer HUVEC at the bottom, not incubated (top) and incubated for 180 minutes (bottom) with the paramagnetic liposomes. At the bottom of the lower well a clear decrease in the T_1 can be observed (pixels in red), indicated with the arrow, which demonstrates that the monolayer of cells can be detected. Figure 2b shows the decrease in T_1 for the pixels at the bottom of the well, which contain the monolayer (ML) and 117 and 585µm above, showing that the decrease in T_1 is a local effect, almost completely restricted to the HUVEC monolayer itself.

Conclusions

We have characterized the relaxation behavior of paramagnetic liposomes in cell cultures. The longitudinal relaxation is linearly dependent on the percentage of incubated cells, which makes quantification feasible, while the transverse relaxation increases very strongly upon higher concentrations. This poses a limit to the useful liposome concentration for target detection and cell tracking. We have demonstrated that a monolayer of cells can be detected based upon T_1 contrast.

References

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[2] Vuu et al., Proc. Intl. Soc. Mag. Reson. Med. 11 (2004).