EFFECT OF THE INTRACELLULAR LOCALIZATION OF A Gd-BASED IMAGING PROBE ON THE RELAXATION ENHANCEMENT OF WATER PROTONS

E. Terreno[1], S. Geninatti Crich[1], S. Belfiore[1], L. Biancone[2], C. Cabella[3], G. Esposito[1], A. Manazza[4], S. Aime[1]

[1]Chemistry IFM, University of Torino, Torino, Italy, Italy, [2]Department of Internal Medicine, University of Torino, Torino, Italy, Italy, [3]Bioindustry Park, Bracco Imaging, Colleretto Giacosa (To), Italy, Italy, [4]Department of Oncology, University of Torino, Torino, Italy, Italy

Introduction

MRI visualization of cells labeled with Gd-based imaging probes appears a promising route for pursuing novel applications in the field of cellular and molecular imaging.(1) In fact, it has been shown that the problems associated with the intrinsic low sensitivity of MRI can be overcome by the intracellular accumulation of a high number of paramagnetic Gd(III) chelates. A method to entrap 10^8-10^9 Gd-chelates per cells consists of incubating the cells with the imaging agent at 25-50 mM concentration.(1) By this route, Gd-chelates are entrapped into endosomic vesicles, but it has been noted that the observed relaxivity is "quenched" in the presence of high amounts of internalized Gd-chelates.(2,3) This drawback clearly represents a limitation to the proposed method of cell labeling and needs to be investigated in more detail. In order to ascertain whether the observed relaxivity "quenching" is dependent upon the localization of the paramagnetic agent inside the endosomes, parallel internalization experiments have been carried out by using the electroporation procedure in which the imaging probe is localized into cytosol.

Methods

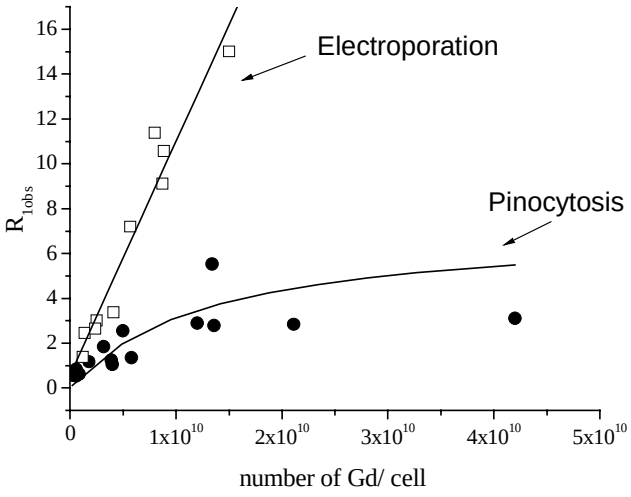
The internalization of Gd-HPDO3A (ProHance®, Bracco Imaging) into a rat hepatocarcinoma cell line (HTC) was carried out by pinocytosis (the cells were incubated for 16-24 hours in culture media containing the Gd-complex) or by electroporation (an electric pulse is applied to a cell suspension containing the Gd-complex in order to induce the membrane permeation). T_1 of water protons were measured on the resulting cellular pellets at 0.5 T and 25°C on a Stelar Spinmaster spectrometer. MR-images were acquired on a Bruker Avance300 spectrometer (7 T) equipped with a Micro 2.5 microimaging probe. The time dependence of longitudinal magnetization has been analyzed according to theoretical models(4) which describe the relaxometric behavior of multi-compartment systems.

Results

As shown in figure 1, the relaxation efficiency of Gd-HPDO3A internalised by pinocytosis shows a drastic decrease upon increasing the amount of complex entrapped into endosomes. Conversely, the relaxation rate measured for cells labelled by the electroporation route increases linearly with the amount of the entrapped Gd. The different relaxation enhancement displayed by the two internalization route was confirmed by acquiring MR images of phantoms containing the labeled cells dispersed in agar. These experiments allow us to establish that the minimum number of HTC cells labeled with GdHPDO3A detectable by MRI is *ca.* 500 cells/μl in the case of electroporation and *ca.* 5000 cells/μl for pinocytosis. A quantitative analysis of the relaxation data shows that the lower relaxation enhancement is a consequence of the water exchange across the endosome membrane which sensibly reduces the relaxation rate of the cytosolic water protons, if compared with the same amount of the agent dissolved in the cytosol.

Conclusions

The obtained results remark the importance of the procedure used for labeling cells and demonstrate that the cytosol confinement of the agent yields a higher relaxation enhancement, thus allowing the MRI detection of a smaller number of cells with respect to the entrapment into endosomes.



References

1) Pathak AP, Gimi B, Glunde K, Ackerstaff E, Artemov D, Bhujwalla ZM. Molecular and functional imaging of cancer: advances in MRI and MRS. Methods Enzymol. 2004;386:3-60.

2) Geninatti Crich S, Biancone L, Cantaluppi V, Duo D, Esposito G, Russo S, Camussi G, Aime S. Magn Reson Med. 2004;51:938-44.

3) Lewin M, Clement O, Belguise-Valladier P, Tran L, Cuenod CA, Siauve N, Frija G. Invest Radiol 2001; 36: 9-14

4) Landis CS, Li X, Telang FW, Molina PE, Palyka I, Vetek G, Springer Jr. CS. Magn. Res. Med. 1999; 42:467-478.