A new route to cellular labelling

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Introduction

MRI is the technique of choice for tracking cells in vivo. Several methods have been applied to label cells by using either Gd(III) complexes and iron oxide particles. The soluble Gd(III) complexes can be entrapped into cells by pynocitosis, electroporation or receptor mediated endocytosis. It has been found that the threshold for MRI visualization corresponds to a number of Gd/cell of $N\sim10^9/r_{1p}$ (1) and upon increasing the amount of entrapped Gd(III), the relaxation enhancement is "quenched" when the Gd(III) complexes are confined in the endosomal vesicles.(2) Iron Oxide particles can be entrapped spontaneously into cells endowed with phagocytotic capabilities. Furthermore, it has been shown that the cellular uptake can be improved by applying a weak electroporating pulse or by using transfecting agents. Some concern has been raised about the metabolic fate of the internalized iron. This contribution deals with an alternative route to label cells based on anchoring a micelle containing almost 10^3 Gd(III) atoms to a cell through a properly designed linker.

Methods

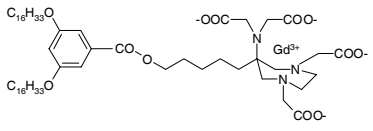
Paramagnetic micelles consisting of a self assembling Gd-AAZTA lipophilic derivative endowed with two C16 aliphatic chains (complex I) have been prepared. Relaxometric measurements were carried out on a Spinmaster Relaxometer operating at variable frequencies between 20 and 80 MHz and on a Stelar Field Cycling Relaxometer in the frequency range 0.01-40 MHz. The size of the micellar systems were measured by Dynamic Light Scattering on a Malvern Zeta-sizer Nano ZS instrument. Cell labeling was carried out on a murine neuroblastoma (NEURO-2a) cell line and MR-Imaging was performed on a Bruker300 spectrometer equipped with a micro-imaging probe operating at 7.1T.

Results

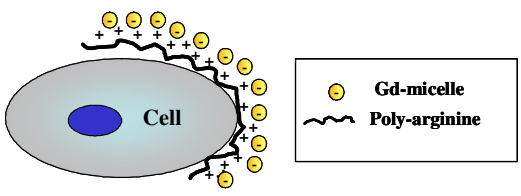
To visualize cells it is necessary to use assemblies consisting of a high number of Gd(III) centers. The herein reported method deals with the use of a tightly assembled micelle that displays on its surface a high number of negative charges that represent the recognition sites for a positively charged linker (i.e. Polyarginine) that is responsible for the anchoring to the cell's surface. A tightly assembled micelle has been obtained with the lipophilic Gd-complex I that contains two saturated alphatic chain and a coordination cage that allows two water molecules to enter the inner coordination sphere of the paramagnetic metal ion. Complex I forms micelles (cmc < 10^{-5} M) that endow each Gd center with a relaxivity of ca. 25 $mM^{-1}s^{-1}$ (20MHz, 298K). Fig. 1 reports the NMRD profile of complex I. From Light Scattering measurements a micelle diameter of ca 80 nm and an aggregation number of ca 850 have been determined. The outer surface of cell membrane is known to present an excess of negative charges and therefore there is a limited interaction with the Gd-containing micelle (Fig. 2). To attain a strong binding interaction between the cell and the micelle, a basic polypeptide (Poly-Arg, n=173) has been used. It has been found that each cell can load up to ca. 1.2×10^7 micelles. This corresponds to ca. 1×10^{10} Gd/cell i.e. well above the threshold for MRI visualization.

Conclusions

The herein reported results show that cellular labelling can be pursued by linking a negatively charged Gd-containing micelle to cellular membranes through a basic polypeptide that acts as a "leasch" between the two entities. One may envisage a variety of applications for the proposed methodology. For instance one may think of designing a "leasch" such that can be selectively cleaved by enzymes at the target site; i.e. the cells act as vectors and the payload can be released at the diseased tissue by the cleavage of the linker between the cell and the micelles. Finally, other supramolecular systems besides micelles can be considered as well as imaging drug-delivery processes may be pursued by implementing procedures analogue to that herein reported.



Gd-complex I



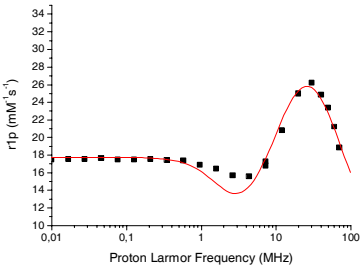


Fig. 1- NMRD Profile recorded at 298K and neutral pH

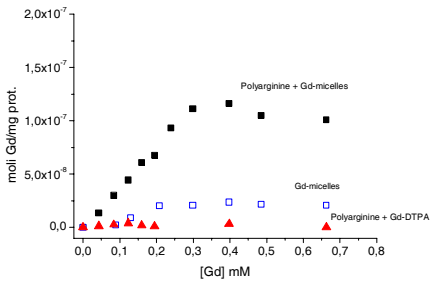


Fig. 2- Labelling of NEURO-2A cells with negative charged systems

References:

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2) Terreno E, Geninatti S, Belfiore S, et al Magnetic Resonance in Medicine 55 (3): 491-497 2006