Endosomal escape of Gd-agents activated by external photochemical stimulus

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Introduction

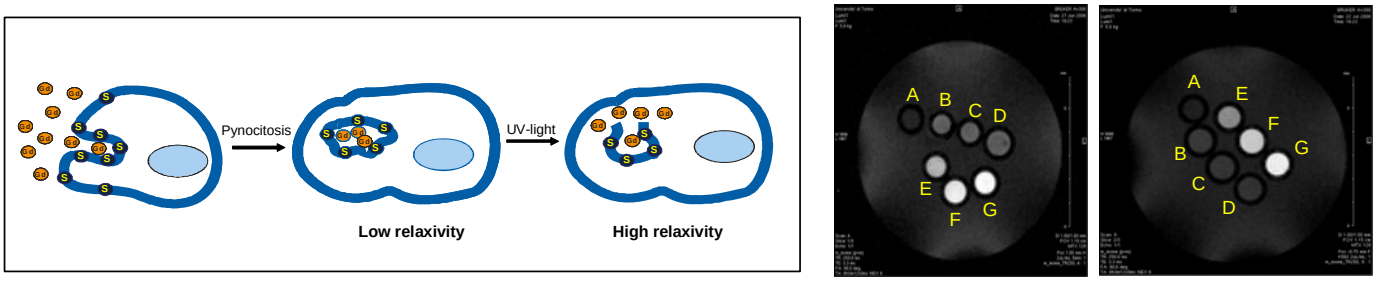
Several routes to cellular labeling through the entrapment of Gd(III) complexes have been reported, namely pinocytosis, phagocytosis and receptor mediated endocytosis. (1) The accumulation of paramagnetic complexes in endosomial vesicles leads to the quenching of the relaxivity when the number of entrapped Gd(III) complexes is higher than 10^9-10^{10} units per cell. (2) This drawback occurs because the exchange rate of water molecules across the endosomial membrane is slower than the difference of relaxation rates in the endosome and cytoplasm compartments, respectively. It was shown that such limitation does not hold when the same or higher amounts of Gd(III) complexes are distributed in the cytoplasm as it happens when the used labeling procedure is based on the electroporation of the cells. The herein reported results show that is possible to remove the relaxivity quenching effect thanks to the use of a novel photochemical procedure (PCI) that promotes the endosomal escape of entrapped molecules into the cytoplasmatic compartment.

Methods

The Photosensitizer $TPPS_{2a}$ (LumiTrans®) and the Lumisource® lamp were provided by PCI Biotech AS laboratories (Oslo, Norway) while Gd-HPDO3A (ProHance®) complex was kindly provided by Bracco Imaging S.p.A (Colleretto Giacosa, Torino, Italy). For cellular labeling, ca. 3-4×10^6 HTC or K562 cells were incubated for 18 hours at 37°C with different concentrations (5-15mM) of Gd-HPDO3A in the absence and in the presence of the Photosentisizer (2µl/ml). After this incubation time, the medium containing the excess of Gd-HPDO3A and LumiTrans® was removed, cells were washed three times with fresh growth medium and incubated for additional 4 hours at 37°C in a CO_2 incubator to assure that most of the LumiTrans® bound to plasma membrane is washed out or internalized into the cell before irradiation. Then the medium was removed, the cells were washed one more time, fresh medium was added and cells containing the Photosentisizer were exposed to LumiSource light for 5 minutes. Then cells were detached by the addition of Tripsine/EDTA, washed two times with PBS, collected in 50 µL of PBS , transferred into glass capillaries that were centrifuged at 1500g for 5 minutes and placed in an agar phantom.
Two different viability test assays (Tripan Blue and WST-1 tests) were used in order to determine PDT citotoxicity. Determination of intracellular Gd^{3+} content has been made by reported relaxometric method. MR Images of capillaries containing the cells pellets, were recorded on a Bruker Avance300 spectrometer operating at 7.1T equipped with a microimaging probe using a standard T_1 weighted multislice multiecho sequence.

Results

A marked enhancement in the labeling efficiency has been observed upon application of photochemical stimulus to cells entrapping Gd-HPDO3A and $TPPS_{2a}$. The cellular labeling (HTC, hepatocarcinoma and K562, limphoblastoma) has been pursued by pynocitosis (18h at 37°C) at different concentration of Gd-HPDO3A (5,10 and 15 mM) in the presence and in the absence of PDT (Photodynamic therapy). As shown in the figure E,F and G cellular pellets (undergone to the PDT treatment) are markedly more hyperintense than B,C,D ones (incubated at the same concentration of Gd-HPDO3A than E,F,G). This considerable gain in signal intensity achieved when cells are processed with PCI tehnology is due to the release of Gd-units from endosomes to cytosol, with the consequent removal of the "quenching" effect on the attainable relaxivity. The average calculated relaxivity (r_{1p} – 7.1T, 298K) is 4.1 $mM^{-1}s^{-1}$ and 5.1 $mM^{-1}s^{-1}$ for HTC and K562 cells respectively i.e. values much higher than those ones found in the case of endosome compartimentalized Gd-HPDO3A (ca. 1 $mM^{-1}s^{-1}$). At the employed concentration of the Photosensitizer cell viability appears very good (98 %).



T_1-weighted spin echo images of agar phantoms containing HTC and K562 cells labeled with Gd-HPDO3A. The concentration of Gd-probe in the incubation medium was: 0 (A), 5 mM (B, E), 10 mM (C,F) and 15 mM (D,G). In both cases phantoms E, F and G contain cells treated with the PCI technology (Lumitrans® 2 µl/ml with a light exposure of 5 minutes) while phantoms B,C and D contain cells simply labeled by pynocitotic uptake.

Conclusions

The novel Photochemical internalisation (PCI) technology has been succesfully adapted to MRI applications as the release of endocytosed paramagnetic probes into the cytosol traduces in a great enhancement in signal intensity if compared with the case of endosomes compartimentalized agents.

(1) Aime, S; Barge, A; Cabella, C; Geninatti Crich, S; Gianolio, E, Targeting cells with MR imaging probes based on paramagnetic Gd(III) chelates, CURRENT PHARMACEUTICAL BIOTECHNOLOGY, 5, 509-518, 2004.
(2) Terreno, E; Geninatti Crich, S; Belfiore, S; Biancone, L; Cabella, C; Esposito, G; Manazza, AD; Aime, S, Effect of the intracellular localization of a Gd-based imaging probe on the relaxation enhancement of water protons, MAGNETIC RESONANCE IN MEDICINE, 55, 491-497,2006.