Cellular Labeling with Gadolinium containing Imaging Probes. A method for checking the release of Gd3+ ions in the cellular environment.

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Introduction.
"In vivo" MRI tracking of labeled cells is currently a topic of huge interest. In most cases the label is represented by iron-oxide based agents that enable the visualization of the labeled cells as dark spots in the MR images as a consequence of the largely dominant T_2^* effects induced by the presence of the magnetic particles (1,2). Recently it has been shown that the incubation of stem and tumor cells in the presence of Gd(III) chelates leads to the internalization of relatively high amounts of Gd(III) chelates that are sufficient for their *in vivo* visualization (3-5). The internalization occurs via pinocytosis and the contrast agents are entrapped into endosomic vesicles. In alternative electroporation, that distributes the Gd probes directly into the cytoplasm, can be used. Since the cells (i.e. stem cells, pancreatic islets...) have to be monitored up to many days after transplantation, it is necessary to know whether the integrity of the complex is maintained or not in the cellular environment. In fact, free Gd^{3+} ions are toxic also at low concentrations and very little is known about the eventual transmetallation of the complex in the intracellular compartements.(6) Furthermore, the effect of the binding to macromolecular carriers (i.e. LDL, HDL, liposomes...) on the Gd^{3+} release has also to be checked out. In this communication a method based on ICP-MS, MS and NMR has been tested on Gd complexes characterized by different thermodynamicand kinetic stabilities and internalized into cells by different internalization procedures.

Methods.
The internalization of Gd-complexes (GdHPDO3A, GdAAZTA, GdDTPA, GdDTPA-BA) into different tumor cell lines (HTC, Neuro2a, C6) 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). Cell internalization by receptor mediated endocytosis has also been investigated. MR-images were acquired on a Bruker Avance300 spectrometer (7 T) equipped with a Micro 2.5 microimaging probe. The total amount of Gd internalized was determined by ICP-MS (Element-2, Thermo-Finnigan, Rodano (MI) Italy). The amount of intact complexes internalized were determined by Mass Spectrometry (Waters Micromass ZQ) and by high resolution ^{1}H-NMR (Bruker Avance600, 14T) by using the Eu(III) complexes .

Results.
It has been found that, when complexes characterized by a high stability are used, all the internalized Gd^{3+} ions are in the intact complexes also if they are localized in low pH compartements (endosomes or lysosomes). Longitudinal studies showed that the total amount of internalized Gd decreases with time as a consequence of cells proliferation, and the complexes remain intact upon time till their elimination occur (likely, in part, by exocytosis). On the contrary, the use of less stable complexes such as Gd-DTPA bisamide derivatives (with a logKa of ca 17) showed that, upon cellular uptake, the amount of intact complexes decreases dramatically to represent only a few percent of the total Gd^{3+} .

Conclusions.
A method, based on ICP-MS, MS and NMR, to assess the mainteinment of the integrity of Gd complexes upon cell internalization has been set up. The obtained results show that Gd-complexes with an high thermodynamic stability remain intact after cell internalization with no release toxic Gd3+ ions also after a long time after the labeling step. We can conclude that these Gd-based imaging probes can be used safely for cell tracking by MRI. Moreover these results prompt new studies aimed at finding new Gd-based agents in order to improve the sensitivity of this type of labeling procedure.

References.
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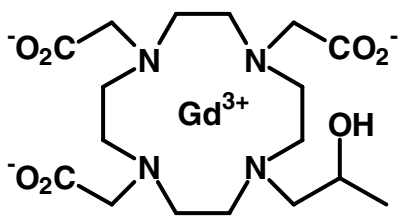
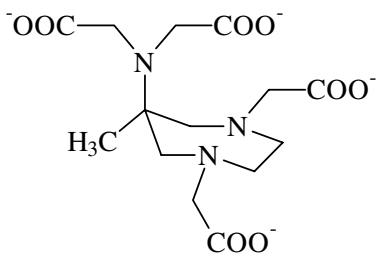
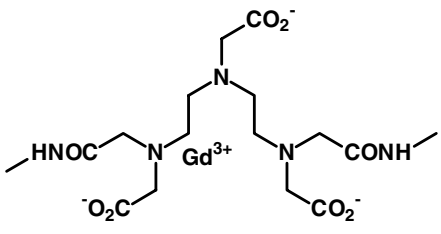


Gd-HPDO3A



Gd-AAZTA



Gd-DTPA-BA