Mass spectrometry detection of loss of structural integrity of Gd-DTPA entrapped in J774 machrophages

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Introduction

In vivo MRI tracking of labelled cells is currently a topic of huge interest. Frequently the cells are labelled with Iron Oxide nanoparticles in order to exploit the high T_2* shortening effect promoted by these negative contrast agents. However, in order to get positive contrast, Gd-chelates have also been used. The threshold for MRI visualization of a cell has been determined to be in the order of 10^7-10^9 Gd-chelates per cell.[1] Such a high payload of stable Gd(III)-chelates seems to be well tolerated by the cellular machinery as low cytotoxicity has been reported for several cell types. Cell viability tests are usually carried out at short times after the end of cellular uptake. It seems now of primary importance the study of the "fate" of Gd-complexes at longer times. The knowledge of the "in cellulo" behaviour of Gd-complexes may also provide some insight into the understanding of the upsurge of pathologies apparently related to the release of Gd ion reported for NSF (Nephrogenic systemic fibrosis). In this work we investigated how Gd-complexes endowed with different thermodynamic stabilities can be transformed when entrapped into living cells.

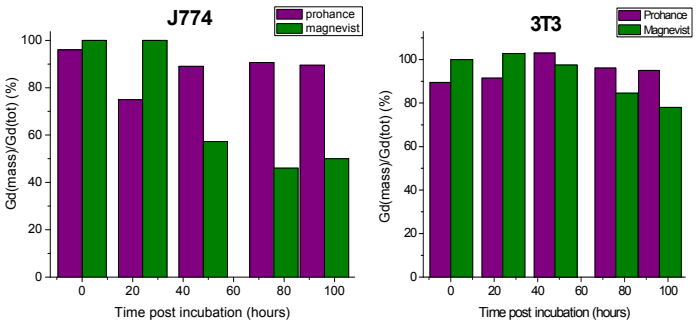
Methods

J774 (murine macrophages) and NIH-3T3 (murine fibroblasts) cells were incubated with two commercially available contrast agents (Magnevist and Prohance) for 5h or 18h at 37°C and 5% CO_2 atmosphere at a concentration of 50 mM. Then cells were extensively washed and reincubated with fresh incubation media for 1, 2, 3 and 4 days. At the end of these times, cells were resuspended in a small volume of water and lysed. On cells lysates the total amount of Gd(III) was determined through a relaxometric method and mass spectrometry analysis was carried out. The mass spectrometric quantification of the Gd-complexes was obtained through the use of a standard curve obtained by adding to a lysate of untreated J774 or NIH-3T3 cells aliquots of an appropriate internal standard (Tm-HPDO3A or Tm-DTPA) to a final concentration of 20 μM and aliquots of the Gd-complexes to a final concentration ranging from 5 to 100 μM. The same amount of internal standard was then added to unknown samples before mixing them to the matrix solution (CH_3CN/H_2O 1:1). Before analysis samples were centrifuged at 14000 rpm for 10 min and filtered. A Waters Micromass ZQ (ESCI ionization mode) was used both in positive or negative acquisition mode. Fragments with the higher intensities corresponding to the intact Gd-complexes were integrated with respect to the same fragments of the internal standards (Tm-complexes). Samples were analyzed in quadruplicate. The relaxometric characterization of the treated cell lysates was performed through the registration of the NMRD profiles on a fast field-cycling Stelar relaxometer over a continuum of magnetic field strengths from 0.00024 to 0.47 T (corresponding to 0.01-20 MHz proton Larmor frequencies). Additional data points in the range 20-70 MHz were obtained on the Stelar Spinmaster relaxometer.

Results

Herein we report a mass spectrometric method for accurate detection and quantification of a Gd-complex after its internalization in living cells. Quantification has been possible by using as an internal standard the corresponding Tullium complex of the same ligand of the analyte. A lanthanide ion was chosen in order to maintain an high similarity with respect to the analyte.

A net difference in the behavior of Gd-DTPA and Gd-HPDO3A has been observed when the Gd-complexes are internalized into J774 macrophages. As shown in the figure, whereas Gd-HPDO3A appears to maintain largely its integrity also after four days, only ca. half of the Gd(III) present in these cells is under the form of intact complex when the internalized complex is Gd-DTPA. Conversely when entrapped into NIH-3T3 fibroblasts both complexes appear to largely maintain their structural integrity upon long incubation time. The NMRD profiles of the lysates from J774 incubated with Gd-DTPA complex are currently under study, in order to get insights for the characterization of endosomal/lysosomal transformation of Gd-DTPA.



Conclusions

Being both complexes internalized through a pathway which leads to their endosomal/lysosomal confinement, the observed behaviour clearly reflects the different ability of the enzymatic armoury to attack the xenobiotic molecules. Macrophages display an improved ability to transform Gd-DTPA in respect to fibroblasts. To a first glance the observed behaviour appears related to the different stability and rigidity of the two systems being Gd-HPDO3A more robust and therefore less prone to be dismantled in the endosomal microenvironment.

References

1) Aime S., Cabella C. , Colombatto S., Geninatti Crich S., Gianolio E., Maggioni F. J. Magn. Reson Imag. 2002, 16, 394-406.
2) Newton B.B. and Jimenez S.A. J. of Magn. Reson. Imag. 2009, 30, 1277-1283.