On the fate of MRI Gd-complexes in cells. Evidence for extensive degradation of linear complexes.

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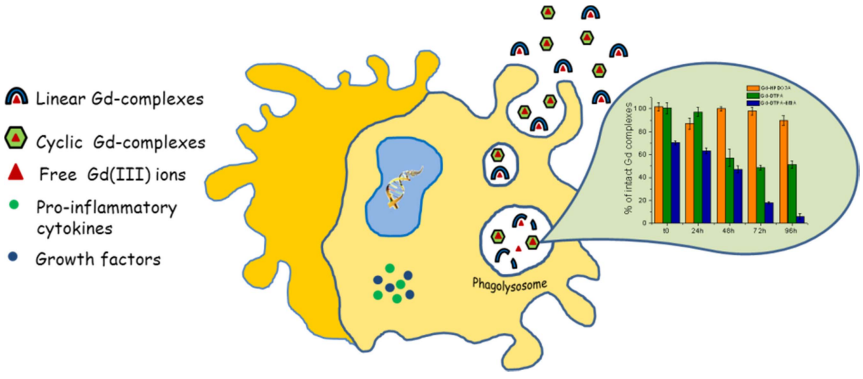
Purpose: Gd-containing complexes are used as MRI reporters in cellular labeling procedures as they are internalized into endosomes by pinocytosis.[1] The herein reported work aims at assessing the relative stability of three common Gd-containing agents upon their uptake in fibroblasts and macrophages. Whereas cell viability tests are usually carried out, in order to access toxicity issues, at short times after incubation, no study of the "fate" of cell internalized Gd-complexes at longer time has yet been reported.

Methods: J774(murine macrophages) and NIH-3T3(murine fibroblasts) cells were incubated with three commercial contrast agents (Gd-DTPA, Gd-DTPA-BMA and Gd-HPDO3A). Once loaded with the Gd-agents the cells were extensively washed and reincubated for 1-4 days in a Gd-free medium. The total amount of internalized Gd(III) was determined by ICP and relaxometric methods whereas the amounts of intact Gd-complexes were determined by mass spectrometry using a calibration curve obtained with an appropriate internal standard (Tm-DTPA, Tm-DTPA-BMA or Tm-HPDO3A). Moreover, In order to get more insight into the occurring processes, NMRD profiles of the lysates obtained from the Gd-loaded cells were acquired and analyzed.

Results: For both types of cells the total amount of measured Gd is under the form of the intact complex (up to 96 h of incubation) only for Gd-HPDO3A. Conversely, Gd-DTPA has maintained its integrity only in 3T3-NIH cells whereas in the macrophages it has been extensively degraded (ca. 55% at 48 h). Finally, Gd-DTPA-BMA is the least robust as it is partially degraded in both cell types already at the end of the first incubation period and is <50% recovered after further 48h of incubation.

Insight on the intracellular transformation of Gd-DTPA and Gd-DTPA-BMA have been gained by analyzing the NMRD profiles of lysates of cells treated with the contrast agents and reincubated for different time intervals. The profiles were compared with the ones obtained by adding the three contrast agents to cell lysates at the same concentration. Whereas for the systems that maintain their integrity upon internalization, there is an almost complete overlap between the two profiles, in the presence of an extensive degradation of the Gd (III)-complexes, the NMRD profiles display a characteristic dispersion at low frequency (*ca.* 0.1 MHz). As an analogous dispersion has been observed in LPS activated macrophages, it has been suggested that this relaxometric behavior can be associated to a high inflammatory state.

Discussion: The herein reported results pinpoint a dramatic instability of Gd-DTPA-BMA upon entrapment in both fibroblast and macrophage cells. Gd-DTPA appears to be largely unaffected in fibroblasts but the most aggressive enzymatic armory of macrophages is definitively able to prompt its degradation after few hours from its entrapment. Only the macrocyclic based Gd-HPDO3A is robust enough to be found fully unaltered up to 96h from its entrapment into both fibroblasts and macrophages.



Conclusion: The observed behaviour can be accounted in terms of the different stability and rigidity of the three investigated systems being Gd-HPDO3A the most robust and stable. The degradation of linear Gd-complexes in macrophages calls for more attention in the cell labeling and cell-targeting procedures with Gd(III) complexes.

References:

1) Aime S., Cabella C. , Colombatto S., Geninatti Crich S., Gianolio E., Maggioni F. J. Magn. Reson Imag. 2002, 16, 394-406.