An improved route for the visualization of Stem Cells labelled with a Gd-/Eu-chelate as dual (MRI and fluorescent) Agent

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Introduction

Pluripotent stem cells deserve a great therapeutic potential for their capacity of regenerating damaged tissues in the presence of a number of pathologies.(1) In particular several therapeutical applications have already been envisaged for blood-derived endothelial progenitor cells (EPCs). The possibility of their in vivo visualization will allow to monitor the fate and localization of the transplanted cells. Until now, only one Gd-based agent has been used to label stem cells. It is a system containing a dextran polymer backbone which contains between 9 to 12 Gd-chelates per dextrane. (2) It bears also a tetramethyl rodamine functionality for fluorescence microscopy detection. Gd3+ ions inside such a large macromolecule (MW=16.6 KDa) appear to act essentially as a T_2-agent thus compromising the basic advantage of T_1-Gd agents that is to generate hyperintense spots in T1-weighted images.

For these reasons we thought of interest to explore alternative routes to label stem cells based on the use of a well tolerated, small-sized paramagnetic Gd (III) chelate. Moreover the close analogy between Gd^{3+} and Eu^{3+} ions suggests the development of a dual probe thanks to the fluorescent properties of the latter one.(3-4) Indeed, Gd^{3+} and Eu^{3+} chelated by the same ligand display the same chemical/biological behaviours and therefore they may be used to detect the localization and migration of stem cells by either MRI and Fluorescence Microscopy, respectively.

Results and discussion

In figure 1, the amount of internalized Gd (expressed in terms of 1 mg of total protein) is plotted against the concentration of Gd-HPDO3A in the incubation medium (incubation time = 24 h). As expected for this type of internalization process, no saturation effect is detected and the amount of uptaken Gd is linearly proportional to the concentration of Gd-HPDO3A in the incubation medium.Gd-chelate distribution cannot be observed by the microscopic techniques currently used in cellular biology. However the Gd-neighbour in the periodic table is Eu that owns excellent fluorescent properties. As a characteristic feature of lanthanide(III) ions is their remarkably analogous chemical properties, one expect that Gd-HPDO3A and Eu-HPDO3A have an identical behaviour in the cell internalization process. Thus the fluorescent response of Eu-HPDO3A can be exploited for histological confirmation of cell distribution. Confocal Microscopic Images of EPCs incubated with Eu-HPDO3A show endosomic vescicles containing Eu-HPDO3A are clearly detected in the cytoplasmatic region, around the nucleus. Actually, EPCs can be incubated in a medium containing both GdHPDO3A and EuHPDO3A in order to have cells suitably labelled for the two imaging modalities. The proposed method is of general applicability and is simpler than the use of the recently reported GRID (Gadolinium rhodamine dextran) agent. In an "in vivo" mouse model of angiogenesis, we implanted EPCs subcutaneously within a matrigel plug. One day after implantation hyperintense spots well distributed in the matrigel were clearly detected in the resulting MR image (figure 2). Seven days after implantation a fine network of bundles was "in vivo" imaged within the matrigel. MRI images parallels histologic findings and the hyperintense signal persisted after 14 days.

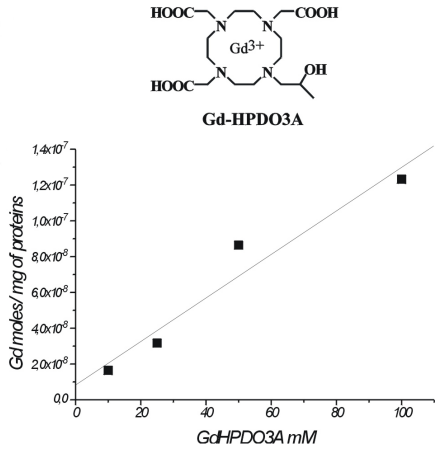

Figure 1

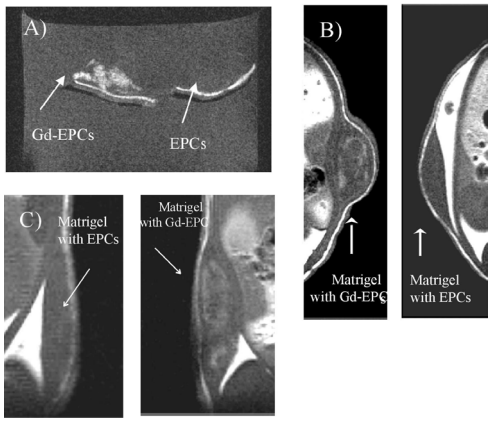

figure 2

In summary, the labelling procedure herein discussed for EPCs appears of a very general applicability as all cell types tested to date showed an analogous efficient uptake of Gd(III)-chelates via the pynocitotic route, with no apparent citotoxicity. Likely the compartimentalization of Lanthanide(III)-HPDO3A complexes into membrane-bound cytoplasmatic perinuclear endosomes prevents any impact on relevant cellular processes meanwhile maintaining an efficient accessibility to cytoplasmatic water molecules. Finally, it has been shown that the related Eu-HPDO3A complex is an excellent probe for Fluorescence Microscopy which allows a good match with the corresponding MR images based on the distribution of the Gd-chelate for an efficient localization of the transplanted cells.

References.

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