Gd(III)-based probes for MR Imaging of exofacial protein thiols

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Introduction. Cells within aerobic tissues can maintain a reduced intracellular redox state even though the microenvironment where they live can be subjected to large fluctuations of the redox conditions. While there are several mechanisms controlling the intracellular redox state (antioxidant systems coupled with redox buffers), the redox state of the extracellular milieu appears to be more susceptible to several biophysical/biochemical factors, including oxygenation level, blood flow, acidosis or presence of reactive oxygen species arising from inflammation processes. In the last few years, the view that the extracellular redox microenvironment is sensed by the proteins exposed on the extracellular side of the plasma membrane has gained popularity as a regulatory or signaling mechanism. It has been reported that the function of cell surface receptors, ectoenzymes and adhesion molecules is controlled by the cleavage/formation of intramolecular disulfide bonds[1]. Exofacial Protein Thiols (EPTs) can assume the reduced –SH form or several reversibly oxidized forms, such as disulfide bridges, mixed disulfides (S-thiolation), cysteic acid or S-nitrosothiols. The shift of the equilibrium between the oxidized and reduced forms is strongly dependent upon the redox state of the extracellular environment. Therefore, imaging of EPTs would give information about the extracellular redox state, that is in turn indicative of patho-physiological alterations. For instance, malignant tumors are characterized by hypoxic conditions leading to a very reducing extracellular microenvironment [2]. In this work we present a new class of MR probes for the imaging of reduced EPTs. These probes are based upon a Gd(III) chelate containing a 2-pyridyl-dithio group, that can promptly react with free thiols to form mixed disulfides. Figure 1 shows the prototype of this class of MRI probes [3], called GdDO3AS-Act.

Methods. Cultured K562 cells (5 millions) were incubated with the EPTs targeted probe (up to 5 mM) in different conditions (0.5-4 hrs incubation time, 4-37 °C). The amount of uptaken Gd(III)-complex was measured by relaxometric techniques. The number of EPTs exposed on K562 cells was measured by means of the DTNB spectrophotometric assay. Labeled cells were imaged at 7T (Bruker Avance300 microimaging system) using standard T_1-weighted sequences.

Results. EPTs are expressed in K562 cells in the range 5-20 nmol(SH) per 10^6 cell, offering a large number of potential anchorage points for labeling agents. Upon formation of –SS– linkages between EPTs and the paramagnetic probe, cells become Gd(III) labeled to an extent proportional to the concentration of EPTs. We have found that the labeling of EPTs with these Gd(III) chelates is very efficient, as the number of paramagnetic centers accumulated per cell is largely above the threshold for visualization by MRI (Fig. 2). Although the extent of labeling is proportional to the available reactive EPTs, the amount of Gd(III) uptaken is larger than the number of reactive (titrable with Elman's reagent) EPTs. Rather unexpectedly, this indicates that the probe is at least partially internalized. Apparently, the formation of the disulfide bond between EPTs and the probe triggers an efficient active mechanism for the internalization of the complex.

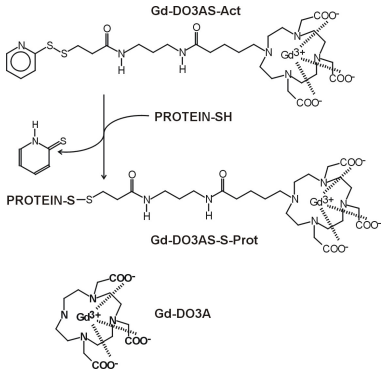
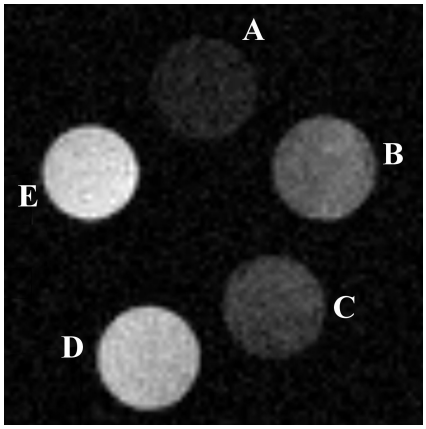


Figure 1: structure and reactivity of Gd(III) based MRI probes targeted to EPTs.



Sample	T1(ms)
A- Buffer solution	2200
B- GdDO3A 2mM	1020
C- Cellular Pellet	1800
D- GdDO3AS-Act 1mM	317
E- GdDO3AS-Act 2mM	192

Figure 2. T_1-weighted spin echo image of a phantom containing K562 cells labeled with GdDO3AS-Act (GdDO3A is the control). The buffer solution and the cellular pellet are negative control

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