Characterization and potential of Gd-ACX, a new contrast agent for Magnetic Resonance Neuroimaging

H. Lahrech[1,2], A. T. Perles-Barbacaru[1,2], S. Aous[3], R. Farion[1,4], J-C. Debouzy[3], A. Gadelle[5], P. H. Fries[5]

[1]INSERM, U594, Grenoble, F-38043, France, [2]Univ Grenoble 1, Grenoble, F-38043, France, [3]Laboratoire de Biophysique Cellulaire et Moléculaire CRSSA, La Tronche, F-38702, France, [4]Univ Grenoble, Grenoble, F-38043, France, [5]CEA/DSM/Département de Recherche Fondamentale sur la Matière Condensée, Laboratoire de Reconnaissance Ionique/Service de Chimie Inorganique et Biologique (UMR-E 3 CEA-UJF), Grenoble, F-38054, France

Introduction

The (3,6-per anhydro) α-cyclodextrin derivative (ACX) [1] (flat disc, 1.2 kDa) has been synthesized in order to capture toxic metallic ions. In this work, it is shown that ACX also encloses Gd^{3+} ions, resulting in a MRI contrast agent (CA) Gd-ACX. The *in vivo* toxicity, the r_1- and r_2- relaxivity, the Nuclear Magnetic Relaxation Dispersion (NMRD) profiles (R_1 relaxation rate vs. magnetic field), as well as the biodistribution in glioma bearing rats are investigated. In addition, a MRI application of Gd-ACX is demonstrated.

Methods

Gd-ACX is obtained by adding $GdCl_3$ to ACX in 1:2 stoichiometry. Toxicity tests were carried out on CD1 mice from Charles River, France, by intraperitoneal injection of up to 0.54 mmol kg^{-1} Gd-ACX (in terms of Gd^{3+}-content), and up to 1.08 mmol kg^{-1} ACX. A daily examination for weight loss, tegument alterations and abnormal behavior was performed during one week, followed by renal histology. The hemolytic activity of Gd-ACX and ACX was measured in human blood samples and compared to the hemolysis induced by natural α-cyclodextrins. NMRD profiles were obtained at larmor frequencies of 0 to 35 MHz on a fast field-cycling relaxometer at 25 and at 37°C and at 100 and 400 MHz on high-resolution spectrometers at 25°C. The r_1-relaxivity of Gd-ACX in normal saline solution and in human plasma as well as the r_2-relaxivity in normal saline solution were measured at 100 MHz and 20°C. Also at 100 MHz, MRI was performed on a C6 glioma model in rats (n = 6) using a T_1-weighted spin echo sequence to monitor the signal enhancement in different cerebral regions for 60 min after a unique Gd-ACX injection at a dose of 0.05 mmol/kg. At the end of this study, a last acquisition was performed following an injection of 0.1 mmol kg^{-1} Gd-DOTA for comparison. Gd-ACX at a dose of 0.05 mmol kg^{-1} was also used for cerebral blood volume quantification in healthy rats (n = 4) using the Rapid Steady State T_1 method [2].

Results

The $LD_{50\%}$ (= dose corresponding to the death of 50% of the mice) is higher than 0.5 mmol kg^{-1}, 10 times the dose usually employed in *in vivo* MRI studies. No histological sign of nephrotoxicity was observed. Hemolysis never exceeded 5% even at the maximum soluble concentration (27 mM Gd, 54 mM ACX). At 2.35T and 20°C, the r_1-relaxivity in human plasma up to 1.5 mM Gd-ACX is 10.66 $mM^{-1}s^{-1}$ (Fig. 1). In normal saline solutions the r_1-relaxivity and, to a lesser extent, the r_2-relaxivity are not constant but higher for low Gd-ACX concentrations (data not shown), and 8.61 and 10.11 $mM^{-1}s^{-1}$ respectively for concentrations exceeding 3 mM. The NMRD profiles (Fig. 2) have a surprisingly flat appearance up to high magnetic field strengths, contrary to other paramagnetic CAs. These NMRD profiles also show that the R_1 relaxation rate is independent of the temperature. Fig.3 shows the *in vivo* signal enhancement in specific regions of interest (ROIs). A mean vascular enhancement of 170% was observed one minute after Gd-ACX injection and the intensity remains high throughout the experiment, demonstrating that Gd-ACX is a blood pool CA. No significant enhancement was observed in tumor ROIs with Gd-ACX, whereas about 90% enhancement was obtained with Gd-DOTA. However, progressive or delayed signal enhancements with Gd-ACX were detected in small tumor regions, which require further investigations. The average cerebral blood volume fraction obtained with Gd-ACX in normocapnic healthy rats is 2.2 ± 0.4%, and consistent with values obtained with other CA.

Discussion/Conclusion

Gd-ACX is an intravascular MRI CA with r_1- and r_2-relaxivities at least twice those of Gd-DOTA. A better understanding of the molecular properties of this Gd^{3+}-complex is necessary to explain the unusual relaxivity dependence on its concentration, which was confirmed by several repeated measurements by different experimenters. Although its high relaxivity at high magnetic field strengths is not yet fully understood, inner-sphere interactions seem to be dominant. The present work shows that even when the blood brain barrier is impaired, the vascular permeability to this molecule is very low, and therefore, Gd-ACX may be used for angiographies. To assess its potential as an intravascular CA for cerebral perfusion quantification in the presence of blood brain barrier lesions, further experiments including MRI, histology, and permeability tests are necessary.

[1] A. Gadelle, J. Defaye, Angew. Chem. Int. Ed. Engl. (1991) 30: 78-79.
[2] AT. Barbacaru, H. Lahrech, Venice (Italy). ISMRM Workshop (March 2004)

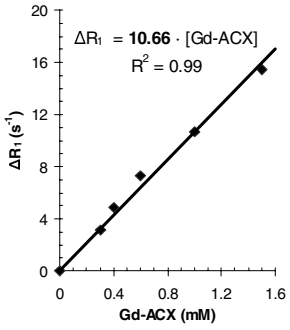

Fig. 1: r_1-relaxivity of Gd-ACX in human plasma, 2.35 T, 20 °C

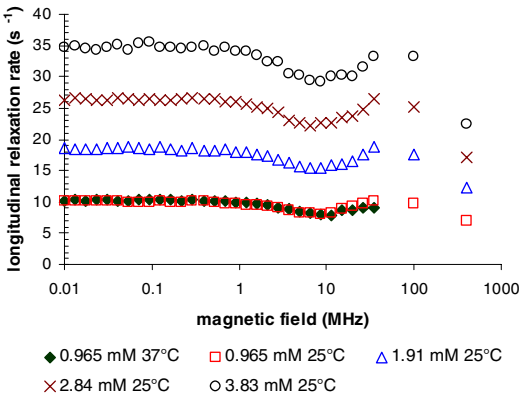

Fig. 2: NMRD profiles at 25 and 37 °C

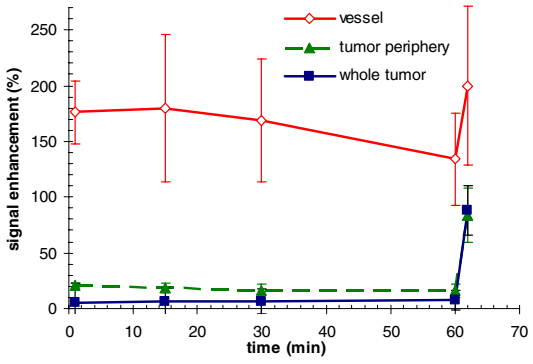

Fig. 3: signal enhancement after 0.05 mmol/kg Gd-ACX injection to a C6 glioma bearing rat. Spin-echo sequence with TE = 20 ms, TR = 500 ms