Development of Gadolinium Based Contrast Agents to Detect Tumor Apoptosis *in vivo* using MRI

A. Krishnan[1], A. Neves[1], M. Kettunen[1], D-E. Hu[1], M. de Backer[1], K. M. Brindle[1]
[1]Biochemistry, University of Cambridge, Cambridge, United Kingdom

Introduction

Apoptosis can occur in tumors following chemotherapy, where its extent and speed of onset are good prognostic indicators for the outcome of treatment[1]. An early event in apoptosis is the exposure of phosphatidylserine (PS) on the cell surface. The C_2A domain of Synaptotagmin I (C_2A) is a 14kD protein that shows Ca^{2+}-dependent high affinity (nM) binding to PS on apoptotic cells. When conjugated to a superparamagnetic iron-oxide nanoparticle, (C_2A-SPIO) this protein can be used to detect apoptotic cells, both *in vitro* and *in vivo*, in a murine lymphoma tumor model, using T_2-weighted MRI[2]. We are currently developing a new generation of smaller contrast agents (CAs), based on conjugation of C_2A to Gd^{3+}-chelates[3], which can be used to give positive contrast in T_1-weighted images. This new class of agents should improve detectability in tumors, which are irregular structures that can show heterogeneous and time varying contrast.

Materials and Methods

GST-tagged C_2A was directly conjugated via its lysine residues to a gadolinium-DTPA complex to produce a 95 kDa CA, with a T_1-relaxivity of ca. 60 $mM^{-1}s^{-1}$ (at 9.4 T).

Experiments *in vitro* were conducted with mouse lymphoma (EL4) cells that had been treated with 15 µM etoposide to induce apoptosis. Sixteen hours after induction of apoptosis, samples of 10^7 cells were incubated with increasing concentrations of contrast agent in HEPES buffered saline (pH 7.4), either in the presence or absence of 2 mM Ca^{2+}. T_1-weighted spin echo images (TR=250ms, TE=6ms) and T_1 maps (IR-FLASH, 15 TIs between 50ms and 15s, 15s delay between images, TR=5.5ms, TE=2ms) were acquired at 9.4 T.

MRI experiments *in vivo* were performed using the same tumor model. C57BL/6 mice were implanted with 10^6 EL-4 cells and at 12-14 days after implantation, when the tumors had reached a mean size of 1.5 cm diameter, the animals were treated, by i.p. injection, with etoposide (67 mg/kg) and cyclophosphamide (100 mg/kg). Sixteen hours later, 200 µl of a 0.5 mM solution of C_2A-GST-Gd (r1 = 60 $mM^{-1}s^{-1}$) was injected i.v., into treated and untreated animals, and images acquired at 10 mins, 4 hours and 24 hours after injection. A site directed mutant of C_2A (Asp230 to Asn) (iC_2A), which showed no binding to PS was used as a control. In this case, 200µl of a 0.3 mM solution of the mutant protein (iC_2A-GST-Gd; r1 = 100 $mM^{-1}s^{-1}$) was injected into treated and untreated animals. T_1-weighted spin echo images (TR=450ms, TE=8ms) and T_1 maps (11 TIs between 50ms and 10s, 10s delay between images, TR=5.5ms, TE=2.5ms) were acquired at 9.4T.

Results

Measurements on cells *in vitro*, showed an increase in signal intensity in T_1-weighted images with increasing amounts of C_2A-GST-Gd (Fig 1). This was proportional to the decrease in T_1 relaxation time of the samples (Fig 2). Experiments *in vivo* showed a statistically significant decrease in tumor T_1 over 24 hours in treated animals that had been injected with active C2A-GST-Gd (TA), compared with controls that had not been drug-treated (UA) or had been injected with the inactive material (TI and UI) (Fig 3). In the controls (UA,TI,UI), the T_1 relaxation times after 24 hours were similar to those immediately post-injection

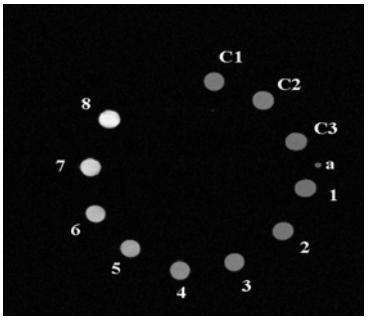


Figure 1. T_1-weighted spin echo images of apoptotic EL4 cells incubated with increasing amounts of C_2A-GST-Gd (1-8 increasing from 0 nmol to 20 nmol). Controls were incubated with 5 nmol C_2A-GST-Gd. C1=apoptotic cells without Ca^{2+}, C2=viable cells with Ca^{2+}, C3=viable cells without Ca^{2+}

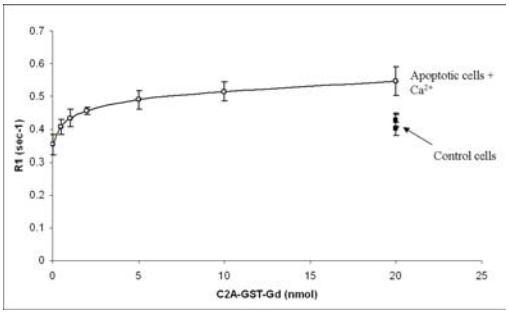
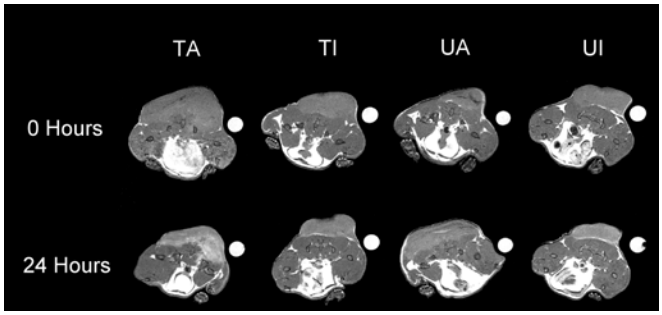


Figure 2. Change in T_1 relaxation rate (R_1) with increasing concentration of C_2A-GST-Gd. A linear relationship up to 2.5 nmol was obtained, with a plateau in the response at higher concentrations. This indicates saturation of PS, which agrees with the amount of exposed PS reported in the literature[4].

Discussion

These data suggest that Gd^{3+}-based contrast agents have the potential to detect cell death *in vitro* and *in vivo.* Treated ar
C_2A, showed a progressive decrease in tumor T_1 relaxation times over 24 hours. This indicates that active C_2A accumulates in tumors undergoing cell death. In the control tumors, this progressive decrease did not occur. However even in these tumors, the T_1 did not fully recover, remaining similar to the post injection T_1. This may be explained by some non-specific trapping and binding of the agents in the tumors. These results indicate that these Gd^{3+}-based contrast agents are promising, although their sensitivity and specificity still need to be fully evaluated.



References

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2. Zhao et al (2001) Nat Med **7**: 1241-4
3. Jung et al (2004) Bioconj Chem **15**: 983-7
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Figure 3. T_1-weighted spin echo images of treated and untreated tumors before injection of contrast and after 24 hours. The treated tumor injected with active C_2A-GST-Gd shows increased signal intensity compared to the control tumors. TA=Treated Active; TI= Treated Inactive; UA= Untreated Active; UI= Untreated Inactive