The ex vivo and in vivo MR Study of Glioma Targeted Contrast Agents

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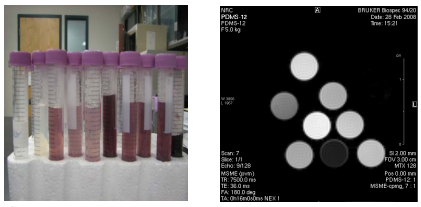
Introduction

Brain tumors are one of the most devastating cancers. Among patients with high grade gliomas, mean survival over the past 30 years has remained unchanged, at only 50 weeks [1]. Standard clinical MRI fails to detect gliomas in their early development stages [2]. Therefore the application of molecular sensing technologies would ensure earlier and more accurate diagnosis, individualized therapies and improve monitoring of the patient's response to treatment. An ideal contrast agent (c.a.) is delivered only to a specific cell and provides strong changes in relaxation times, that are detectable even in small concentration [3]. The fundamental features of contrast agents used in MRI are superparamagnetic nanopaticles (NPs). Their exterior shell can be modified to render such contrast agents multi-modal (i.e. MR and IR [4]), to reduce its potential toxicity and to enable conjugation to biological objects, such as viruses, peptides or antibodies. While NPs can be used in a passive way for glioma imaging, their specificity can be increased by conjugating them to an antibody (sdAb) that targets tumor-specific proteins. Before the targeted contrast agents can be used in humans they must be carefully selected considering their composition, toxicity, efficacy and specificity. This study focuses on the application of *ex vivo* high field MRI for the selection of the most efficient NPs followed by *in vivo* study of their targeting capabilities, thus potential suitability for the early detection of gliomas in the brain.

Methods

For both *in vivo* and *ex vivo* study we used 9.4T/21cm Bruker (Germany) MRI system. NPs were manufactured and provided by NRC, IMI, Canada.

We tested iron oxide (Fe_3O_4) and iron cobalt (FeCo) core (5-15nm) NPs with SiO_2 and Au shells (1-40nm). To render NPs targeted high grade glioma specific single domain antibodies (NRC, IBS, Canada) were conjugated with the NPs and IR marker (Cy 5.5.) directly or using amine-functionalized PEG coating (NP-PEG-NH_2). To assess the effect of composition and size of the c.a. on magnetic properties, the NPs were synthesized, mixed with 1% agarose to ensure homogenous distribution of NPs, poured into small glass tubes and imaged. The T_2 and T_1 values were assessed (Fig .1) using MSME (TR = 7ms, 128 echoes, 4ms apart, 1.3mm slice, FOV = 3 × 3 cm, 128 × 128) and TRUE FISP (TR/TE=3/1.5ms, 128 × 128, FOV 3 × 3cm , 1.3 mm slice) pulse sequence respectively.



A B

Fig 1. Phantoms with different c.a. placed in tubes with agar solution (A); MRI image of the tubes; MSME: TR/TE=4s/36ms (B).

For *in vivo* study of the selected contrast agents we used the intracranial injection of U87MG deltaEGFR cells to nude mice, as a model of human high grade glioma. U87MG deltaEGFR cells were grown in culture, harvested and implanted (50,000 cells) into the left striatum in nude mice. The glioma specific sdAb (IGFBP7(Ab)) was developed in house and synthesized with MR and IR markers. The IR and T_2-weighted MR images were collected before, 30, 60, 90 and 120 min after the injection of the contrast agent via the tail vein. T_2 values of the tumor tissues were measured at each time point using single exponential fitting of the echo train.

Results and conclusions

Ex vivo MR measurements (Fig 2) demonstrated that larger core NPs had a greater effect on T_2 values as compared with smaller NPs. The addition of a shell decreased effect on T_2. When comparing the chemical composition of the coating, results showed that SiO_2 impeded the effect on T_2 values more than gold. The FeCo core based NPs had a stronger effect on T_2 than those with Fe_3O_4 core. As expected, for all iron oxide compounds, T_1 values remained unchanged. To compare the efficacy of the synthesized nanoparticles with commercially available iron based compounds, an equal (0.3mg/ml iron) concentration of Feridex® (Bayer HealthCare Pharmaceuticals) was used. Results showed, that the efficacy of Feridex® in affecting the T_2 value was comparable to Fe_3O_4 coated with SiO_2.

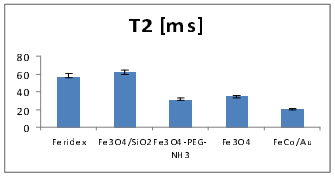
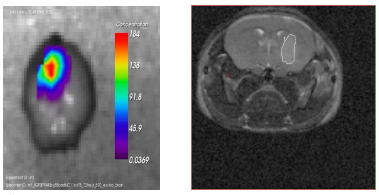


Fig. 2. T_2 values of agar solution in the presence of different c.a. The same concentrations (0.3 mg/ml) were used.

We were able to conjugate gold coated FeCo to sdAb, that could provide better contrast to noise ratio in MRI than Fe_3O_4 NPs. However, considering their unknown toxicity, we selected Fe_3O_4 based NP (Genovis, Sweden) for the preliminary study of glioma specific c. a. and we functionalized them with IGFBP7(Ab).

The accumulation of the targeted c.a. (IGFBP7(Ab)-Cy5.5-Fe_3O_4-PEG-NH_2) in the tumor (mouse glioma model) using IR imaging is shown in Fig. 3A. The corresponding T_2-weighted MR image obtained with the same targeted c.a. is shown in Fig. 3B. The T_2 of the healthy brain tissue remained unchanged (within the error value). The MRI *in vivo* results using the c.a. showed about 5% decrease in T_2 over the tumor area 2 hours after the contrast injection (Fug. 4).

Our study showed, that the changes in T_2 depend on both the core and shell of the NP as well as their sizes. Using IR and MRI we have shown the efficacy of a new contrast agent. Further studies, including the toxicity, of the NPs based on FeCo core and Au shell are needed to develop more effective glioma targeted MRI c.a.



A B

Fig 3. Mouse brain IR imaging using IGFBP7(Ab)-cy5.5-PEG-NH_2-Fe_3O_4) contrast agent (A); MRI (TR/TE = 4sec/60ms) obtained with the same c.a. (B)

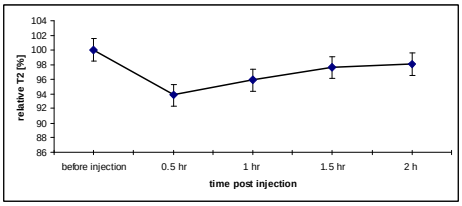


Fig 4. Changes in the T_2 of the glioma after the injection of the targeted c.a. (IGFBP7(Ab)-Fe_3O_4-Cy 5.5-PEG-NH_2).

References:

1. Salcman, M et al. Neurosurgery 34:213-20, 1994. 2. Moller-Hartman et al., Neuroradiology, 44:371, 2002. 3. Lee at al., Nature Medicine, 13(1): 95-99, January 2007. 4. Ntziachristos et al., Neoplasia. 4:347– 354, 2002.

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