Assessing early effects of angiogenesis inhibitors using MR molecular imaging

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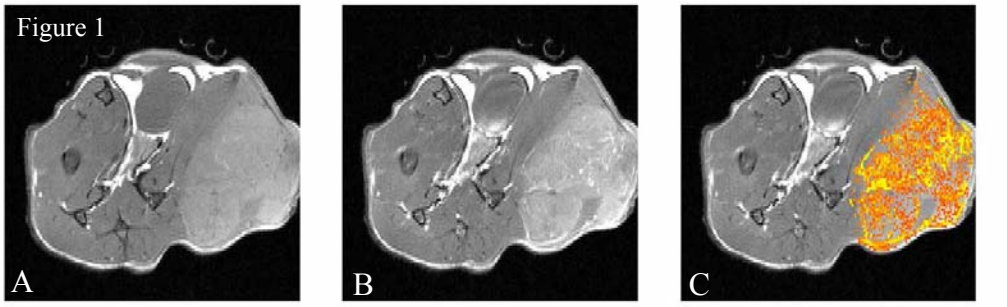
Introduction

Angiogenesis, the formation of new blood vessels, is an important process in tumor growth. Inhibition of angiogenesis could therefore be of therapeutic use. Angiogenesis inhibitors are developed to inhibit two processes promoting angiogenesis, namely endothelial cell (EC) growth and adhesion. In order to investigate the angiogenic processes and to monitor the effect of therapy we developed paramagnetic and fluorescent RGD-liposomes that are specific for activated endothelial cells by targeting to αvβ3-integrin. This contrast agent was previously tested *in vitro* on endothelial cells and *in vivo* on tumor bearing mice and was shown to exclusively target tumor endothelium[1]. In this study we assessed the potential of the RGD-liposomes to evaluate the early effects of anti angiogenesis therapies. To that aim, mice that were treated for 3 days with the angiogenesis inhibitors Endostatin[2] or Anginex[3], and untreated mice were imaged with MRI before and after injecting the contrast agent. The fraction of voxels within the tumors that showed contrast enhancement was compared between the 3 groups.

Material and Methods

Paramagnetic and fluorescent RGD-liposomes were prepared as described previously[1]. For the therapy study osmotic pumps with either Endostatin (n=4, 4 mg/kg) or Anginex (n=5, 8 mg/kg) were implanted subcutaneously in tumor bearing mice. Mice (n=4) that were not treated served as a control group. After 3 days of treatment mice were anesthetized with an isoflurane/air mixture and an infusion line was brought into the tail vein. Next, the mice were placed in a 6.3 T MRI scanner. First, multislice T2-weighted (TR = 2000 ms, TE = 30 ms, matrix size 128 x 128, slice thickness 1.0 mm, NEX = 4, FOV = 3 x 3 cm2) images were acquired to localize the tumor. Next, T1-weighted (TR = 800 ms, TE = 9 ms, matrix size 128 x 128, slice tickness 1.0 mm, NEX = 16, FOV = 3 x 3 cm2) images (31 consecutive slices) were generated before the contrast agent was injected. Dynamic contrast enhanced MRI was performed during the injection to monitor the administration of the contrast agent. At two time point (15 and 45 minutes) after injecting the contrast agent T1-weighted images with the same parameters as above were acquired. The MR-data were analyzed with Mathematica. In short, the tumors were segmented by manually drawing a ROI around the tumor area for all slices in the T2-weighted images. At each time point after injection of the contrast agent the pixel intensities in the tumor ROIs were compared to the pre-contrast intensities. A pixel was considered significantly enhanced when its intensity was increased by at least 3 times the mean noise level. Next, the number of signal enhanced pixels in the tumors, in terms of percentages, was averaged for the 3 groups.



Results and Discussion

In Figure 1 a MRI slice through the tumor of a mouse from the control group is depicted before contrast agent injection (A) and 45 minutes after injecting the contrast agent (B). In Figure 1C pixels in the tumor with signal enhancement of at least three times the noise level are color coded according to the pseudo-color scale: 0 30% signal enhancement. Next, all MRI slices through the tumors of the three groups were analyzed and the percentage of voxels that were significantly contrast enhanced were quantified (Figure 2). Both treated groups showed a lower percentage of contrast enhanced voxels within the tumor as compared to the control group, but only the Endostatin treated group showed a significant decrease. This decrease is indicative for a reduction of the αvβ3 expression induced by the therapy. The length of the treatment was chosen in such a way that no decrease of the microvessel density was expected. This leads us to propose that the reduction of the percentage of contrast enhanced pixels can be fully ascribed to a reduction of the expression of αvβ3 and not to a limited supply of the contrast agent to the tumor.

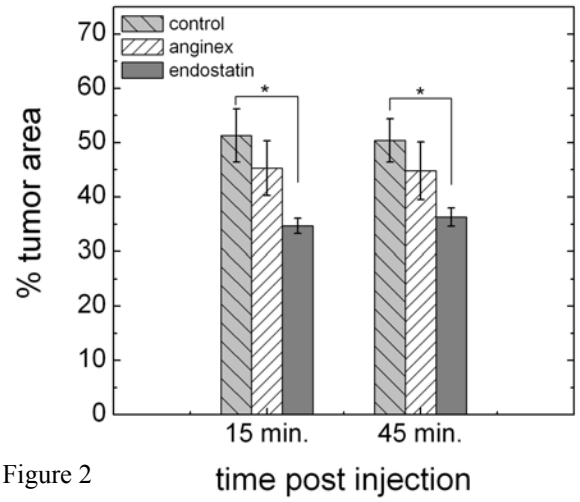


Figure 2

Conclusions

The use of paramagnetic and fluorescent RGD-liposomes allows in vivo MR molecular imaging of angiogenesis. Angiogenesis inhibitors mainly act on the endothelium and therefore the RGD-liposomes were used to assess early effects of Endostatin and Anginex therapy. It was shown that the Endostatin treatment resulted in a significantly reduced contrast enhancement as compared to the control group.

References

1. Mulder *et al*. FASEB J. 2005 Oct 4.

2. O'Reilly *et al*. Cell. 1997 Jan 24;88(2):277-85.

3. van der Schaft *et al*. FASEB J. 2002 Dec;16(14):1991-3.