Does repeated injections of an iron-based contrast agent lead to an accumulation of iron in the brain parenchyma?

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Introduction

Angiogenesis was found to be a major factor in predicting the aggressiveness of different tumor types [1-Weidner M *et al*]. The measurement of angiogenesis is complicated by the fact that it is a dynamic process and most studies have focused to date on analysing it at a particular point in time. VSI imaging is a powerful tool for assessing vessel size distribution for detecting small changes between different regions of the brain [2-Tropres I *et al*]. VSI imaging is based on the measurement of the change of R_2 (ΔR_2) and R_{2^*} (ΔR_2^*) induced by the injection of the intravascular contrast agent iron-based Sinerem®. However, half-life of Sinerem® is 182+/-32 min in the blood and 11 and 15 days in the liver and in the spleen, respectively. Since VSI depends on numerous factors, one could interrogate if these factors are altered by repeated injections of the contrast agent. The aim of the study was to verify if the VSI imaging could be performed on the same animal over time and to analyse if Sinerem® accumulates in the brain.

Material and Methods

The C6-glioma was used as a brain tumour model (n=5). VSI was measured on anesthetised rats (isoflurane 2% in oxygen-enriched air) by MRI (at 2.35T, SMIS console; FOV 35mm/Thk 1mm; matrix 128*128) with a diffusion sequence and with a multi gradient echo spin echo sequence before and after intravenous injection of the Sinerem® (200µmol Fe/Kg). MRI exam was performed every four or five days (total duration of one exam: 95 min) following tumor cell implantation (a total of 6 MRI experiments per animal represent about 384 µmol of iron). At each time point, plasma samples were collected before and after MRI and the plasma T_2 was measured with a multi-spin echo sequence. Rats were euthanized 22 days post-implantation and organs were harvested (brain, liver, lung, kidney, spleen) for subsequent histological analysis (Prussian blue staining). Student *t*-test was used for statistical analysis.

Results

In the contralateral side, we observed that T_2^* before injection, ADC, ΔR_2, ΔR_2^* and VSI remained stable over time despite of repeated injections of Sinerem® (Fig. 1 and Fig. 2). Prussian blue staining showed no iron clusters in any regions of the parenchyma (even in the tumor region); in contrast to other organs such as the spleen (Fig. 3).The plasma T_2 (before injection) remained also stable over time. These results suggest that no Sinerem® accumulates in the brain parenchyma or in the vascular compartment over time. In the tumor region, we observed a ΔR_2 decrease as a function of time, a slight ΔR_2^* decrease but an increase of the ADC and of the VSI as early as 8 days post-implantation.

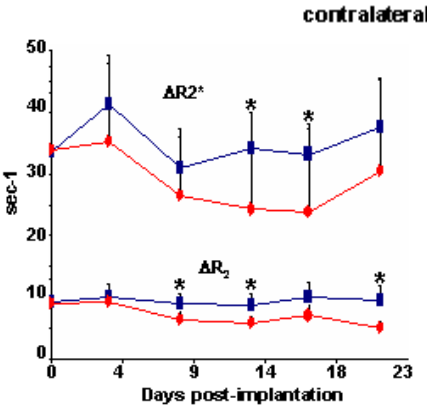

Fig 1 :Evolution of ΔR_2 and ΔR_2^* as a function of tumor growth in two regions. Mean +/- SEM, *p<0.05 as compared to the other side.

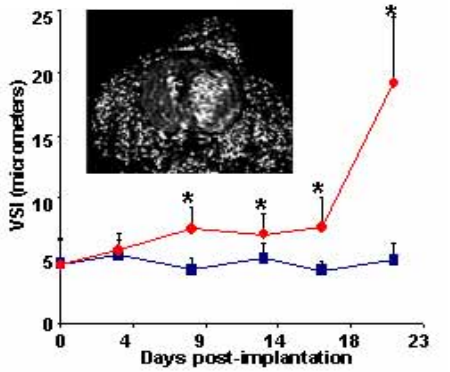

Fig 2 :Evolution of VSI as a function of tumor growth in two regions. Inset shows an example of a VSI map at 22 days. *p<0.05 as compared to the other side.

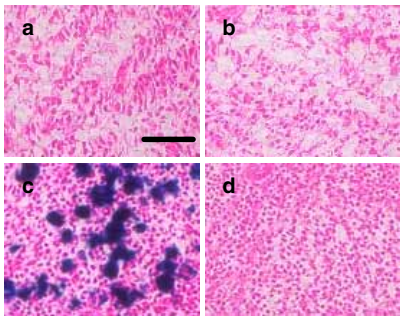

Fig 3 :Prussian Blue detection of iron clusters in a Sinerem injected rats (**a**: brain; **c**: spleen) or in a control rat (**b**: brain; **d**: spleen) scale bar: 40µm.

Conclusion

The results obtained in this study suggest that the VSI imaging could be used to follow on the same animal the evolution of the vasculature as a function of time. Actually, in the control side, we didn't observe variations of the VSI despite of repeated injections of Sinerem®. This assumption was confirmed by the results obtained on the plasma T_2 or with histology which suggests that no Sinerem® accumulates in the brain. Results obtained in the tumor side show an increase, on a same animal, in the VSI concomitant with the development of the tumor size, which is in accordance with previous results obtained on different animals [3-Remy C *et al*]. As a conclusion, this study demonstrates that the VSI imaging technique is applicable to follow the evolution on a same animal of the vasculature.

References

[1] New Engl J Med (1991)324:1-8. [2] Magnetic Resonance in Medicine (2001)45:397-408. [3] ISMRM Workshop (2003).