TIME-COURSE QUANTIFICATION OF CHRONIC HYPOXIA-INDUCED ANGIOGENESIS USING ΔR_2 ($SSTAR_2$) WITH MION INFUSION

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INTRODUCTION

We used **S**teady-**STA**te quantification of ΔR_2 in tissue and blood with infusion of the intravascular contrast agent MION ($SSTAR_2$) to measure cerebral blood volume (CBV) as a marker of angiogenesis [1]. We studied the time-course of in individual animals over a 17 day time-course of exposure to chronic hypoxia. This work will provide a framework for a non-invasive link between molecular biological changes and morphological responses to chronic hypoxia induced angiogenesis.

METHODS

CBV was measured in wistar rat before acclimation, and after 3, 7 and 17 days of acclimation to 1/2 atm (equivalent to 10% FiO_2) as well as a normobaric control group. NMR imaging was conducted at 7T using a Varian console and a 4.5 cm quadrature birdcage coil. A multi-echo spin echo imaging sequence was used to quantify *in vivo* R_2 before and after infusion of 10 mg/kg of MION (monocrystaline iron-oxide nanoparticals, 0-0.3 mg Fe/ml) [2]. Imaging parameters were: TR=1.5 s, TE=0.011 s with 0.011 s inter-echo spacing, 14 echoes, FOV=3x3 cm, matrix=128x128, slice =1.5 mm, 4 transients. CBV was calculated as $\Delta R_2 t/\Delta R_2 b$, where Δ=the difference in R_2 before and after MION infusion, t=cortical tissue and b=blood. Serum samples were obtained for quantification of serum ΔR_2, using the same collection parameters and temperature (37°C) used for *in vivo* measurements. $\Delta R_2 b$ was corrected for hematocrit (Hct) by $\Delta R_2 s \cdot (1\text{-Hct})$, where s=serum. A separate group was analysed for microvascular densities using confocal microscopy and a fluorescent based detection method [1].

RESULTS

During time course studies CBV increased to a maximum level by 3 days of acclimation. CBV at day 0 was 3.52±1.05v/v% (n=5; mean±SD; $p \leq 0.05$) and increased to 5.81±1.60 after 3 days. At 17 days, CBV was 6.85±1.35, but was not significantly difference from day 3. Morphological analysis did not detect significant changes in L_V after 3 days of hypoxia (1316.77±156.46 and 1281.01±180.27 mm/mm3); however, capillary radii were significantly increased 4.07±1.12 to 4.64±1.43μm. (n=958; mean±SD; $p \leq 0.05$). Based on mean values of CBV, a significant increase was quantified by both NMR imaging and morphological studies (calculated according to $\pi r^2 L_V$) between day 0 and 3 (3.52 to 5.81 – 65% versus 1.71 to 2.17 – 27%).

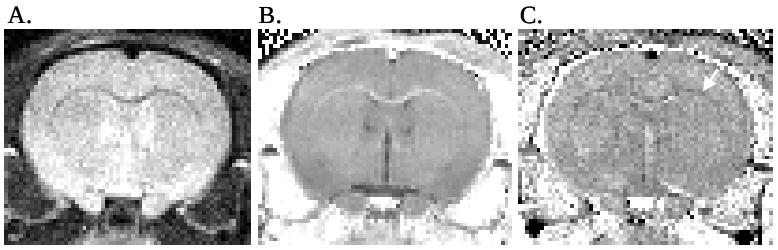

Fig. 1. Quantification of MION in rat brain. A. pre-MION T_2 image B. is a pre-MION R_2 map C. ΔR_2 map calculated from pre-and post-MION R_2 images. Each voxel in the ΔR_2 map is proportional to the CBV. Arrow indicates dark area of white matter, with a low CBV.

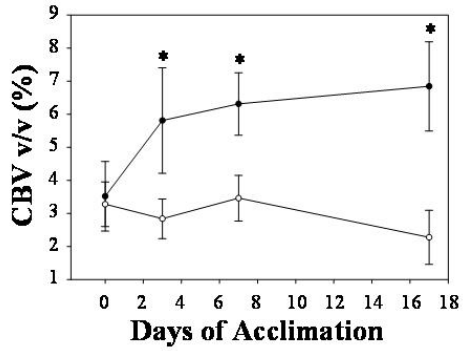

Fig. 2. Time-course of changes in CBV as a marker of angiogenesis during hypoxic exposure. Closed circles: acclimated animals, open circles, control animals. Stars indicate difference from 0 days, $p<0.05$, n=5.

DISCUSSION AND CONCLUSIONS

This study demonstrates that $SSTAR_2$ can be used to quantify CBV in the same animal over a time-course study. Our data indicate that chronic hypoxia-induced changes in CBV occur by 72 hrs. NMR imaging and morphological investigations detected a morphological component to the CBV after 3 days of acclimation. Literature evidence suggests that capillary density is not significantly increased at 3 days [3] while our histological method did show an increase. These data indicate that significant angiogenesis is possible within 3 days of stimulus onset.

REFERENCES

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