# Quantification of Cerebral Vascular Volume After Stroke in a Rat Model Using an Intravascular Contrast Agent and Steady State MRI


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## INTRODUCTION

We are studying angiogenesis and angioregression during stroke evolution. In order for brain tissue to be salvaged after stroke, there needs to be a functional vasculature present to supply nutrients to recovering cells or implanted precursor cells. We developed an MRI method to quantify cerebral blood volume as a marker of vascular volume and integrity. The method, Steady-STAte quantification of the change in $R_2$ ($SSTAR_2$) [1], involves quantification of $R_2$ before and after infusion of contrast. Since it is based on a $T_2$ method with intravascular contrast, the technique is differentially sensitive to the volume of the microvasculature [2]. It has the advantage over dynamic contrast enhanced MRI in that the imaging time is not limited, and so one can achieve higher spatial resolution and a time-course of an arterial input function is not required.

## METHODS

Infarcts were induced using a clip model with one hour of reversible MCA occlusion [3]. MR imaging on Wistar rats (n=5) was conducted at 9.4T using a Bruker console and a 35 and 45mm quadrature birdcage coil 1 day after transient ischemia. A multi-echo spin echo sequence was used to quantify $R_2$ *in vivo* (TR=1.5s, TE=3ms, 128 echoes, FOV=3x3 cm, matrix=128x128 pixels, slice=1.5 mm, NT=4). The *in vivo* calibration was done by sequentially infusing Combidex® (Advanced Magnetics, MA) in a serial does for Fig 2A and CBV studies were done with 10mg/kg for Fig 2B. Multi-echo spin echo (SE) images were obtained before and after infusion. Blood samples were taken for hematocrit (Hct) and to quantify serum $R_2$ using the same parameters as were used *in vivo* after a 10 fold dilution with saline. CBV was calculated as $\Delta R_2t/\Delta R_2b$, where Δ=the difference in $R_2$ before and after contrast, t=cortical tissue and b=blood. Blood values are calculated from serum(s) and Hct using $\Delta R_2b = \Delta R_2s \cdot (1\text{-Hct})$. Alternately, the CBV was modeled using the change in susceptibility (deltachi) caused by the contrast agent [1,4]. Rats were infused with FITC-dextran. Brains were immersion fixed and cryoprotected with sucrose before freezing. Sections were imaged for vascular structure using a confocal microscope and the stacks collapsed to show Fig 3.

## RESULTS

The volume of stroke induced damage was measured using T2w images (Fig 1). Damage was largely cortical and averaged 121±30mm$^3$ (mean±SD). Contrast agent readily reached the infracted area but did not show signs of leaking into the parenchyma. The change in $R_2$ with contrast agent concentration *in vivo* and *in vitro* was proportional (Fig 2A). The measured CBV values in the contralateral side were 1.09±0.23% volume/volume (mean±S.D, n=5) and 0.71±0.11% in the stroke region representing a statistically significant decline in volume of 35%. Modeling of susceptibility change showed similar changes in volume. Confocal imaging of infarcted areas from rats that had been infused with fluorescent plasma markers also showed a reduction in functional vessels (Fig 3).

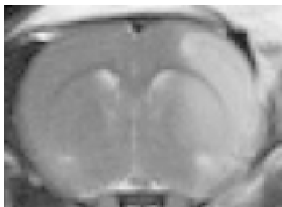
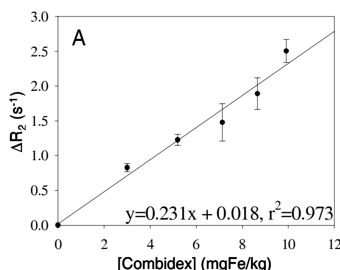


**Figure 1:** Example infarct shown by T2w MRI and manually contrasted to highlight infarct.



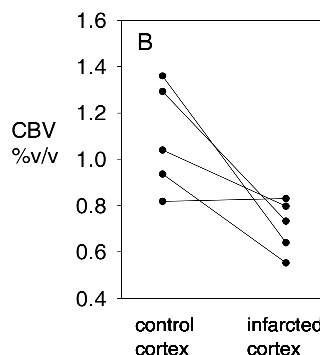




**Figure 2:** In vivo contrast agent studies. A) Calibration of relaxivity using a single exponential analysis (mean±SD, n=3). An *in vitro* curve was also linear with a slope of 1660s-1*(mg/kg)$^{-1}$. B) The difference in CBV between the control and ischemic cortex. Each line represents one animal with the CBV of the normal cortex on the left and the ischemic region on the right. CBV in all animals except one were significantly reduced in the stroke region.

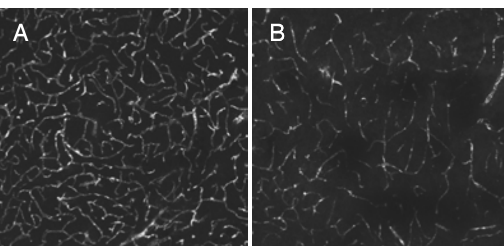



**Figure 3**: Vessel integrity 24 hrs after ischemia visualized with fluorescent microscopy. A) Contra-lateral cortex. B) Infarcted cortex showing the presence of perfused vessels, although with a reduced area and number.

## DISCUSSION

This model has large strokes that will evolve to necrotic regions. Even so, 24 hrs after transient ischemia the CBV had declined, but only by 35%. These data show that MRI with stable plasma agents can be used to assess vascular volume after stroke. There is a significant decline in functional vasculature (defined as those vessels which had sufficient flow that they were filled with contrast). The presence of significant vascular volume indicates that there may be sufficient vasculature remain 24 hours after a reversible ischemia to support tissue regeneration.

## REFERENCES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