

# The effect of interleukin-1 on local brain temperature during focal cerebral ischemia in the rat: a $^1\text{H}$ magnetic resonance spectroscopic imaging study

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## Introduction

The pro-inflammatory cytokine interleukin-1 (IL-1) plays a key role in exacerbating experimental cerebral ischemia and represents a promising target for novel therapies.<sup>1</sup> Precise mechanisms of action have remained elusive but are likely to be multiple including effects on glia, neurons, endothelial and immune cells and alterations in physiological parameters.<sup>2</sup> Brain temperature critically influences outcome from cerebral ischemia and given that IL-1 is a major pyrogen, this has clear mechanistic implications. Previous work suggests that IL-1 exacerbates cerebral ischemia independently of rectal temperature.<sup>3,4</sup> However, it is temperature in critical ischemic brain regions that influences outcome and rectal temperature is likely to be a poor surrogate marker. Proton magnetic resonance spectroscopy ( $^1\text{H}$ -MRS) allows non-invasive measurement of absolute brain temperature from the water/*N*-acetylaspartate (NAA) chemical shift difference.<sup>5</sup> To test the hypothesis that IL-1 exacerbates ischemic brain damage in part by increasing ischemic brain temperature, proton magnetic resonance spectroscopic imaging ( $^1\text{H}$ -MRSI) was used to produce brain temperature maps in rats receiving recombinant human IL-1 $\beta$  (rhIL-1 $\beta$ ) or vehicle at the onset of focal experimental ischemia.

## Methods

Male Wistar rats (290-350g) were anesthetized with halothane (1-2%) delivered in a 70/30% mixture of  $\text{N}_2\text{O}/\text{O}_2$  during surgery and MRI. Animals underwent 90 minutes of transient right middle cerebral artery occlusion (MCAo)<sup>6</sup> and received 1mg of rhIL-1 $\beta$  i.p. (n=6) or vehicle (n=7) at occlusion. MRI experiments were carried out using a 4.7T horizontal magnet interfaced to a Varian UNITY INOVA console. A quadrature half volume coil was used as a transmitter and receiver. Diffusion imaging was undertaken from 25-35 minutes of occlusion and  $^1\text{H}$ -MRSI from 42-80 minutes of occlusion. Both sequences were repeated during early reperfusion (Diffusion imaging, 90-100 minutes;  $^1\text{H}$ -MRSI, 100-138 minutes) and at 24 hours (with additional  $\text{T}_2$ -weighted spin echo multi-slice imaging to determine lesion volume). Maps of  $\frac{1}{3}$  of the trace of the diffusion tensor ( $\text{D}_{av}$ ) were obtained using a spin-echo sequence (TR, 1500ms; TE, 55ms; FOV, 40mm x 40mm; matrix, 128 x 64; single slice, thickness, 1.5mm) with four bipolar gradient pairs in each direction at 3 b-values (0, 469, 1056  $\text{s}/\text{mm}^2$ ).<sup>7</sup>  $^1\text{H}$ -MRSI was undertaken at a short echo time (TE, 2ms) using pre-localisation by outer volume suppression (OVS) and stimulated echo acquisition mode (STEAM) (pre-localisation 6-8mm x 10-12mm x 4mm; FOV, 20mm x 20mm; matrix, 16 x 16; voxel size 6.25mm<sup>3</sup>; spectral width, 2000Hz; number of points, 1024) with partial water suppression.<sup>8</sup> To determine the chemical shifts, peaks of water, NAA, creatine and lactate were manually selected and a total number of 7 points around the peak value were chosen to fit the second order polynomial function  $y = ax^2 + bx + c$  [1] in magnitude spectra. The condition  $a < -500$  was used to ensure a suitable line width for temperature estimation. Thus, each voxel had 0 to 3 derived temperatures (using none, 1, 2 or all of NAA, creatine and lactate as references to the water peak) which were averaged for the final calculated voxel temperature. Tissue was considered ischemic if  $\text{D}_{av}$  in the right hemisphere fell by  $\geq 0.11 \times 10^{-3} \text{mm}^2 \text{s}^{-1}$  relative to the mean  $\text{D}_{av}$  in the left hemisphere. This level has previously been shown to correspond to blood flows of less than 15% of normal.<sup>9</sup> The mean temperatures in ischemic and non-ischemic brain were then derived from co-registered temperature maps. The mean  $\text{D}_{av}$  of ischemic tissue was also calculated for each time point. All data are given as mean  $\pm$  S.D.

## Results

An example of a temperature map from a naïve animal is shown in Fig.1. During MCAo, rhIL-1 $\beta$  increased cortical damage at 24 hours (vehicle,  $111 \pm 26 \text{mm}^3$  vs.

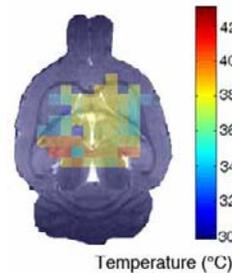


Fig. 1: Temperature map, naïve male Wistar rat.

IL-1,  $212 \pm 78 \text{mm}^3$ ) leading to a significant increase in total infarct volume from  $202 \pm 29 \text{mm}^3$  to  $315 \pm 95 \text{mm}^3$  (Fig.2). No difference in rectal temperature was seen between the two groups during acute cerebral ischemia (Vehicle,  $37.1 \pm 1.0^\circ\text{C}$ ; IL-1,  $37.1 \pm 1.0^\circ\text{C}$ ). Given this, brain temperature was expressed as deviation from rectal temperature in each animal. Significant differences in ischemic brain temperature were seen between groups. In animals receiving vehicle, ischemic brain was  $0.6 \pm 0.6^\circ\text{C}$  less than rectal temperature at occlusion and was  $0.6 \pm 0.9^\circ\text{C}$  higher during early reperfusion (Fig.3). In animals receiving IL-1, a greater fall in ischemic brain temperature was seen at occlusion ( $1.2 \pm 0.9^\circ\text{C}$  less than rectal temperature) and during early reperfusion ischemic brain temperature remained below rectal temperature by  $0.6 \pm 1.0^\circ\text{C}$ . In IL-1 and vehicle treated animals, ischemic brain was  $1.4 \pm 1.2$  and  $1.2 \pm 0.5^\circ\text{C}$  higher than rectal temperature, respectively, at 24 hours. The mean  $\text{D}_{av}$  of ischemic tissue was initially slightly higher in the vehicle group and in contrast to the IL-1 treated animals, a partial normalization on reperfusion was observed (Fig.4).

## Conclusions

Contrary to the stated hypothesis that IL-1 increases damage through a rise in ischemic brain temperature, a reduction in temperature is seen compared to vehicle both at occlusion and during early reperfusion. The greater depth of ischemia in the IL-1 group may account for the fall in temperature through the combined effects of reduced metabolic activity (less heat production) and reduced external heat influx from the circulating blood. IL-1 may act to reduce cerebral blood flow, most notably during early reperfusion and does not increase local brain temperature during occlusion and early reperfusion.

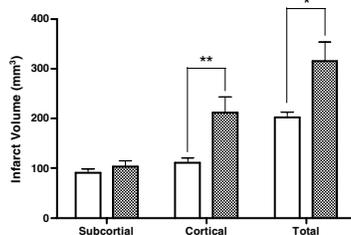


Fig.2: IL-1 exacerbates ischemic damage \* $p=0.01$ , \*\* $p=0.008$  (unpaired t-test)

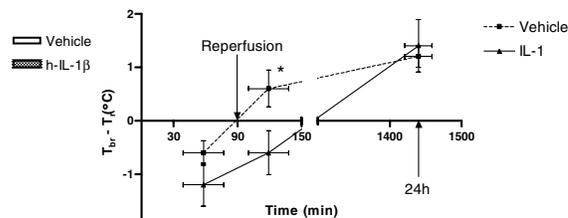


Fig.3: Ischemic brain temperature is lower in IL-1 treated animals. \* $p < 0.05$ , vs. vehicle (ANOVA)

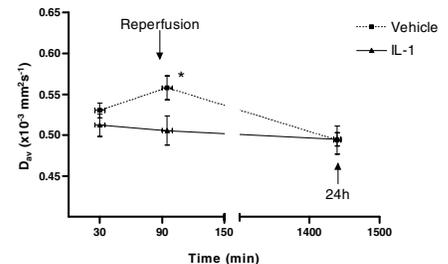


Fig.4:  $\text{D}_{av}$  in ischemic brain does not increase on reperfusion in IL-1 treated animals. \* $p=0.05$ , vs. vehicle (ANOVA)

## References

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**Acknowledgements:** Supported by the Medical Research Council, UK, The Academy of Finland and The Sigrid Juselius Foundation, Finland.