

IN VIVO MRI EVIDENCE OF CRANIAL NERVE INFLAMMATION AFTER CORNEAL DAMAGE

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Introduction

This study was designed to put in evidence the inflammatory activity within a cranial nerve, the trigeminal ganglion (TG), induced by a peripheral damage of the cornea. To detect inflammatory cells infiltration *in vivo*, we used *in vivo* MRI in combination with uspio contrast which has been demonstrated as a sensitive marker of macrophage activity.

Methods

Animal model: A corneal injury was induced by topical application of a paper disc soaked in 1 N NaOH for 10 seconds in the right eye of CD1 mice (6-8th weeks old male) deeply anesthetized. The ocular surface was then washed with normal saline. All procedures were done under slit-lamp examination.

***in vivo* MRI:** On day 4 and 8 post corneal damage, mice were analyzed by MRI (n=6-8) before and post administration of uspio contrast (9.7 μmol iv. of P904 from Guerbet) using a 7T scanner. As control, MRI was repeated in 3 animals without contrast treatment. Animals were kept under gas anesthesia during the entire exam. To assess uspio uptake, T2 relaxation time was measured using a multi-spin-echo-multi-echo (MSME) with TR= 3000ms, a train of 12 echos from 8 to 100ms, 6 averages, a spatial resolution of $82.3 \times 125 \mu\text{m}^2$ with coronal sections of 0.8mm in thickness. The distribution of T2 values were analyzed in both cornea and along the TG. Difference in T2 values pre and post contrast ($\% \Delta T2$) were calculated across the cornea along lines drawn on each eyes (Figure A). To determine the presence of uspio along the TG, T2 value histogram was analyzed in both left and right TG in transversal sections (n=5-7). For each ROI, the percentage of pixels ($\% \text{Area}$) with a T2 inferior of 43ms was calculated. This threshold was defined from the analysis of T2 before contrast administration, for which values were found around 52 and 60ms and the percentage of pixels with a $T2 < 43\text{ms}$ was between 0 and 5%.

Histology: At the end of MRI, animals were sacrificed and eyes and ganglions were removed and frozen in OCT. Longitudinal cross-sections of TGs and corneas were processed for immunostaining to characterize inflammatory cells. Prussian blue staining was used to detect iron particles. Percentage areas of positive pixel staining per field were calculated for CD45 staining in both cornea and TG.

Results

Compared to healthy eyes, corneal damage was clearly observed on T2 weighted (Figure A) as it is thickened. Uspio uptake was observed as a reduction of T2 values which was significant on day 4 post injury (Figure B). Interestingly, T2 reduction was also measured in the right TG (Figure C) at both time points with a particular increase on day 8 (Figure D). Prussian blue staining was found on both cornea and TG of the damaged eye while the contralateral parts were negative. Those iron positive cells were also positive for markers of inflammatory cells (CD45) and in particular of M2 macrophages (F4/80 and CD206). Furthermore, we found a strong correlation in the $\% \text{area}$ of CD45 positive cells with the $\% \text{area}$ of low T2 in the TG of the damaged eye with a similar trend of increase on day 8 post injury.

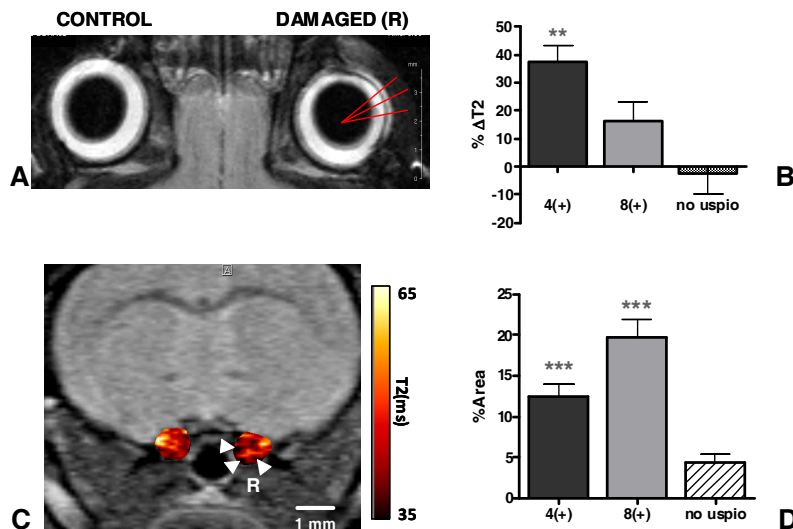


Figure A: T2 weighted image of both damaged and contralateral eyes. T2 variation between pre and post uspio administration were measured by analyzing the plot profiles of lines (red) crossing the cornea on each eyes.

Figure B: T2 difference pre-post uspio in the cornea on 4 and 8 days injury. No significant T2 variation was found in animals without uspio administration. ($**$ $p < 0.002$, t-test vs no-uspio).

Figure C: T2 weighted image at the level of the trigeminal ganglion (T2 map, color coded) acquired 24hrs post uspio showing areas of low T2 values (head arrows).

Figure D: $\% \text{Area}$ of pixels with low T2 ($< 43\text{ms}$) within TG of the damaged eye showing a significant uspio uptake on day 4 which increased on day 8 post injury. ($***$ $p < 0.001$, t-test vs no-uspio).

Conclusion

This study demonstrated the potentiality of MRI combined with uspio contrast to track inflammatory cells infiltration as M2 macrophages. In particular, this infiltration was found in the trigeminal ganglion far away from the site of injury (cornea) which may have significant clinical implications for treatment strategies considering that M2 macrophages produce anti-inflammatory cytokines and should promote tissue repair.