MRI study in rat to evaluate the effect of cyclooxygenase inhibition on Blood Brain Barrier disruption following intracerebral injection of Tumor Necrosis Factor-[alpha]

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Introduction

Disruption of the blood-brain barrier (BBB) occurs during the neuroinflammatory response to cerebral ischemia. Proteases are an important component of that response. Proteases of the serine and matrix metalloproteinase (MMP) gene families have been shown to degrade proteins of the basal lamina around blood vessels contributing to the proteolytic breakdown of the BBB. Several pro-inflammatory cytokines induce the expression of MMPs in the injured brain. Among these molecules, recombinant human tumor necrosis factor α (rhTNF-α) is reported to be a potent stimulator of MMP secretion and expression in the brain. RhTNF-α is elevated in a variety of neuropathologies including multiple sclerosis and HIV encephalitis. In ischemic stroke, several studies indicate that rhTNF-α is involved in BBB disruption and the initiation of inflammatory response in the brain. Additionally, metabolism of arachidonic acid through cyclooxygenase (COX) plays a key role in neuroinflammatory events [1] and has been shown to increase BBB permeability. In this study, an MRI technique for estimating barrier permeability coefficient based on a multiple time graphical analysis method [2] has been used for investigating the effects of COX isoform inhibition using indomethacin, a non-selective COX inhibitor on BBB permeability. This technique involves quantifying temporal distribution of Gd-DTPA in the brain tissue and fitting the data to a unidirectional tracer kinetic model. Thus, the aim of the study was to investigate the effects of COX inhibition on BBB permeability in a well characterized animal model of rhTNF-α induced BBB disruption using MRI.

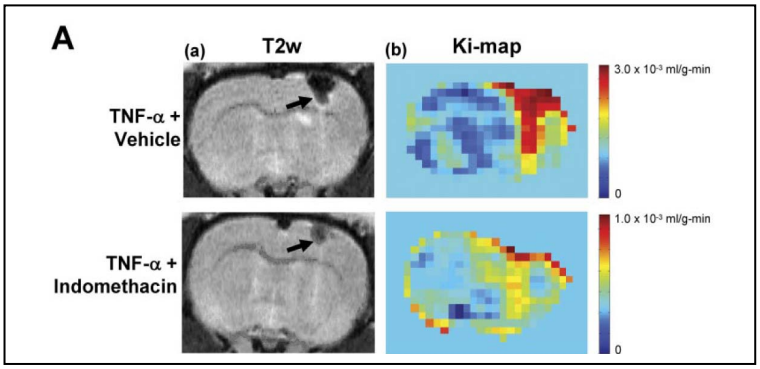
Materials and Methods

Male Wistar rats (N=8) weighing 280-320 g were used in this study. The rats were randomly divided into two groups: control group (N=4) included rats injected intracerebrally with saline and treated with vehicle (5% Cremophor EL in saline) and the drug treated group that included rats (N=4) treated with indomethacin (a non-selective COX inhibitor, Cayman chemical). In the drug treated group, animals were treated after rhTNF- injection and again at 8h. Animals were anesthetized with 1.5% halothane in 70:30 N_20:O_2. Animals were positioned in a stereotaxic headholder (Kopf Instruments, Tujunga, CA) and burr holes were made in the right hemisphere, 3 mm from midline, at the bregma. A 23-gauge infusion needle was inserted into the caudate. Single bolus of TNFα injection in 10 µL of sterile saline (5000 U/rat; rhTNFα; Upstate, Lake Placid, NY) was injected in the right striatum, 5 mm below the dura over a duration of 10 min with a microsyringe (Hamilton, Reno, NV). MR Imaging was performed on a dedicated research 4.7T Biospin® MR scanner (Bruker, Billerica, MA) on each rat at 24 hrs post rhTNF-α injection to acquire T2-weighted, diffusion weighted and multi-slice multi-echo (for T2 maps) images. During the entire duration of the study, animals were maintained under 1.5% isoflurane anesthesia and physiological parameters were monitored. Following the preliminary scans, a rapid T1 mapping protocol was implemented to acquire one pre-Gd-DTPA baseline data set. After acquiring baseline images (reference), 200 µL of Gd-DTPA equivalent to 0.2 mM/Kg was injected intravenously as a bolus into the femoral vein via an indwelling catheter, following which a time series of inversion recovery MR images were acquired over 45 minutes (14 times points) using fast T1 mapping technique. The following optimized MRI parameters were used: 2D IR-SE-EPI, TR/TE 8.0s/19.4ms, FOV 4.0 X 4.0 mm, slice thickness 2 mm, # averages 2, scan time 3minutes and 12s. Data was transferred to an offline workstation for further processing. All data processing was performed using in-house software. T1 map for each slice for each time point was constructed using a three parameter least square fit to pixel signal intensity values in the inversion recovery images. Data was post processed pixel-wise to generate Gd-DTPA concentration and permeability color maps. Permeability values were estimated from the permeability color maps.

Results & Discussion

T2w images demonstrated the lesion in relation to the anatomical detail in the rat brain (Fig. A). On the T2 maps, a 12% reduction in T2 values was observed on the ipsilateral lesion side in the indomethacin treated group compared with rhTNFα + vehicle due to reduction in edema. There was no significant reduction in ADC values in the indomethacin-treated group compared the rhTNFα + vehicle group. On the permeability coefficient color maps, there is no region of high permeability visible in the indomethacin treated group compared to the rhTNFα + vehicle group. Fig. B shows a graph of mean permeability coefficient values in rhTNFα + indomethacin group and rhTNFα + vehicle estimated using MRI. In the rhTNF-α + vehicle group, the permeability coefficient estimates in the ipsilateral and contralateral hemisphere were $0.41 \pm 0.18 \times 10^{-3}$ ml g^{-1} min^{-1} and $0.31 \pm 0.18 \times 10^{-3}$ ml g^{-1} min^{-1} respectively. The permeability coefficient estimates in rhTNF-α + indomethacin rats were $5.47 \pm 1.0 \times 10^{-3}$ ml g^{-1} min^{-1} and $0.38 \pm 0.26 \times 10^{-3}$ ml g^{-1} min^{-1} in the ipsilateral and contralateral hemisphere respectively. On applying ANOVA test, there was a significant difference in permeability coefficient values between the groups. Furthermore, a significant difference ($p<0.05$) in the permeability coefficient values between the lesion and non lesion side in rhTNF-α + vehicle rats was observed. However, the mean permeability coefficient estimates between the ipsilateral and contralateral side in rhTNF-α + indomethacin rats were not significantly different. There was a significant ($p<0.05$) reduction observed in mean permeability coefficient values on the ipsilateral side in the Indomethacin treated rats as compared to the untreated rats. Initial results suggest that indomethacin, a non-selective COX inhibitor, may be working to reduce BBB leakage.

References: [1] Candelario-Jalil E et al, J. Neurochem 2003. 86:545. [2] Ewing, et al, MRM 2003. 50:283.



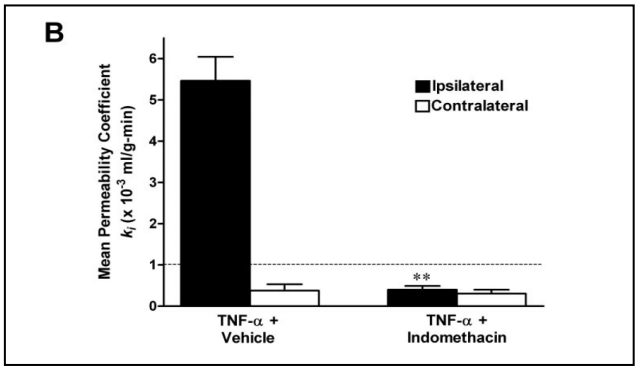


Fig. A. (a) T2w image; (b) color coded permeability map for rhTNF-α + vehicle and rhTNF-α + indomethacin. The anatomical T2w images clearly demonstrate the extent of the lesion in both untreated and treated groups. However, visually, the extent of the lesions appears to be limited in the treated groups compared to the untreated group. Color coded permeability maps demonstrate clearly the regions of high (arrow) and low permeability in treated and control rats. In the treated group, indomethacin affects permeability by blocking BBB leakage. Note the different color scales used for the permeability maps. Fig. B. A plot of mean permeability coefficient estimates in the rhTNF-α + vehicle (n=4) and rhTNF-α + indomethacin (n=4) obtained using MRI. The dashed line represents the upper limit of the range ($0\text{-}1 \times 10^{-3}$ ml g^{-1} min^{-1}) of the permeability coefficient values in healthy tissue. Rats treated with indomethacin demonstrated a significant reduction in permeability coefficient values on the side of the rhTNF-α injection as compared with untreated rats, **, $p < 0.001$ with respect to vehicle ipsilateral. No significant difference was observed on the contralateral side between the control and treated rats. Bars, mean ± S.E.M.