MRI evaluation of the effect of a COX-2 inhibitor on BBB permeability in a rat stroke model

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Introduction

The blood-brain barrier (BBB) is a system of tissue sites that restrict and regulate molecular movement between the systemic circulation and the central nervous system (CNS). BBB is a neurovascular interface made up of cerebral endothelial cells, astrocytes and pericytes, along with the extracellular matrix that maintains the integrity of the brain tissue. Ischemic stroke, resulting from loss of blood supply to the brain tissue due to a thrombus or embolus, is a leading cause of mortality and morbidity. The first therapeutic approach in management of a patient with ischemic stroke is intravenous thrombolysis within the first 3h, thereby restoring blood flow to the ischemic brain tissue. Recent research has demonstrated that brain damage occurring after focal ischemic insult, occurs over a period of time. Post-ischemic inflammation has been proposed as an important underlying mechanism involved in the brain damage. An increase in expression of inflammation related enzymes such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) has also been found to play an important role in secondary events that amplify brain damage post-ischemia. COX-2 mRNA has been shown to be increased within neurons and vascular cells in both experimental animal models of cerebral ischemia and infarcted human brain tissue. Additionally, metabolism of arachidonic acid through COX pathway has been shown to increase BBB permeability. This observation has resulted in search for an alternate therapeutic strategy targeting the late phase of the damage with COX-2 inhibitors. Nimesulide, a non-steroidal anti-inflammatory drug with potent COX-2 inhibitory effects, has been proposed as a candidate to reduce late phase damage in ischemic stroke [1]. 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-2 inhibition using nimesulide, 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 nimesulide induced COX inhibition on BBB permeability in a well characterized animal model of ischemic stroke using MRI [2] and the results are compared with the well established 14-C sucrose technique.

Materials and Methods

MR imaging study was performed in a rat brain 2h Middle Cerebral Artery Occlusion (MCAO) model of ischemic stroke. The study was approved by the Local Animal Research Committee and conformed to the NIH guidelines for the use of animals for research. Male Wistar rats with N=16 and N=12, weighing 280-320 g were used for the MRI and 14-C sucrose technique in this study. For the MRI study, the rats were randomly divided into two groups: control group (N=8) treated with the vehicle and the drug treated group that included rats (N=8) treated with nimesulide. In the drug treated group, 12 mg/kg of nimesulide was injected intraperitoneally immediately after stroke and additional doses were given at 12h intervals for 48 h period. MR Imaging was performed on a dedicated research 4.7T Biospin® MR scanner (Bruker, Billerica, MA) on each rat at 48 hrs post MCAO to acquire T2-weighted and diffusion weighted 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 and the total area of leakage were estimated from the permeability color maps. The MRI results were compared with findings from the 14-C sucrose technique.

Results & Discussion

There was a significant ($P<0.05$) decrease in BBB permeability coefficient and area of leakage in drug treated rats compared to the control group suggesting that nimesulide may be working to reduce BBB damage by COX-2 inhibition. However, this effect was observed only in the cortex region of the brain. There was good agreement between MRI results and findings from the 14-C sucrose technique regarding effect of nimesulide on BBB permeability coefficient. Initial results suggest that nimesulide is a promising candidate for reducing BBB permeability and may be beneficial in reducing late phase brain damage post stroke. **References:** [1] Candelario-Jalil E et al, Brain Research 2004. 1007:98. [2] Ewing, et al, MRM 2003. 50:283.

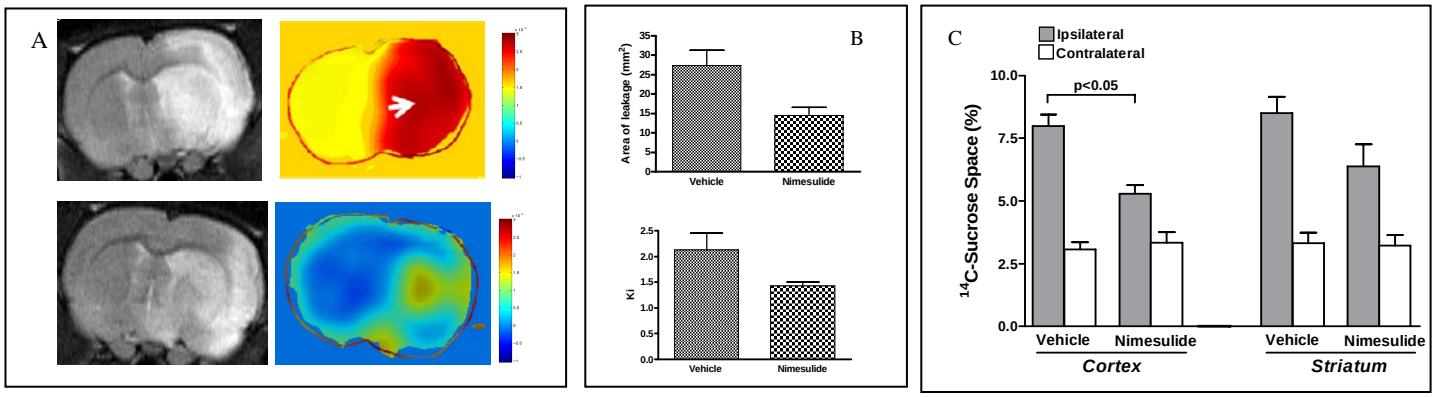

Fig. A. shows T2w images for control (left top) and treated (left bottom) rat and color coded permeability map for control (right top) and drug (right bottom) treated rat. The T2w images show the extent of the lesion in relation to anatomic detail. In the permeability coefficient maps reconstructed using MRI data, pixel signal intensity values are proportional to permeability coefficient values. Pixels with high and low signal intensity correspond to pixels with high and low permeability coefficient values respectively. Before analyzing the permeability maps i.e. drawing ROI, intracranial volume was differentiated from skull and image background by thresholding signal intensity from recognized anatomical structures identified on DW and T2w images. Image analysis was performed on permeability coefficient maps using ImageJ. The permeability map for each slice was matched with DW image and ADC map to identify normal and ischemic regions. The lesion was identified on T2w and DW images as regions with long T2 relaxation time and low ADC values respectively. ROI was drawn manually by a single operator around regions with high signal intensity on the ipsilateral side and the same ROI was placed on the contralateral side. Analysis was repeated for the data sets obtained from control and treated rats. Color coded permeability maps demonstrate clearly the regions of high (arrow) and low permeability in control and treated rats respectively. In the treated group, nimesulide affects permeability by blocking BBB leakage via inhibition of COX-2 enzyme. Fig. B. A plot of area of leakage and mean permeability coefficient estimates obtained from the ipsilateral hemisphere in the vehicle (n=8) and nimesulide treated group (n=8) obtained using MRI. A significant decrease in area of leakage and mean permeability estimates were observed in cortex region (data shown), while in the striatum there was no significant change observed (data not shown). Bars, mean ± S.E.M. The permeability coefficient values for normal healthy tissue are in the range 0-1 x 10^{-3} ml/g-min. Fig. C shows the BBB permeability results for control (N=6) and drug treated group (N=6) obtained using 14-C sucrose technique. There was good agreement between results obtained from MRI and 14-C sucrose technique.