Effect of iNOS on Cerebral Blood Flow after Head Trauma

L. M. Foley[1], T. K. Hitchens[1], P. M. Kochanek[2], J. A. Melick[2], and C. Ho[1]

[1]Pittsburgh NMR Center for Biomedical Research, Carnegie Mellon University, Pittsburgh, PA, United States, [2]Safar Center for Resuscitation Research, University of Pittsburgh School of Medicine, Pittsburgh, PA, United States

INTRODUCTION

In traumatic brain injury (TBI), secondary events mediate the progression of damage and repair, providing therapeutic opportunities. A likely mediator of both beneficial and deleterious secondary events after TBI, is the induction of inducible nitric oxide synthase (iNOS). iNOS is one of three key enzymes that generate nitric oxide (NO) using L-arginine as a substrate. Once expressed, which is typically not until hours or days after injury, iNOS produces large amounts of nitric (NO) for sustained periods, limited only by substrate and co-factor availability. iNOS is expressed in neutrophils, macrophages, glia, neurons and in the microcirculation in the peri-trauma region [1] and is induced by cytokines, such as interleukin-1, via mechanisms promoted by protein kinase C and other transcription factors. iNOS is intimately involved in the tissue injury response. Since NO is a powerful cerebrovasodilator, we hypothesize that delayed production of NO by iNOS would contribute to recovery of cerebral blood flow (CBF) after TBI.

MATERIALS AND METHODS

Male C57Black/6J iNOS wild type (WT, n = 28) and knockout (KO, n = 25) mice aged between 11-15 weeks were used throughout these studies. Animals were divided into one of three groups for MRI assessment, naïve, 24 or 72 hours after trauma. Mice were anesthetized with isoflurane in N_2O:O_2 (1:1), intubated and mechanically ventilated; then femoral arterial and venous catheters were surgically placed. The mouse controlled cortical impact (CCI) model was used as previously described [2] with minor modifications [3]. Animals were placed in a stereotaxic holder and a temperature probe was inserted through a burr hole into the left frontal cortex. The parietal bone was removed for trauma. Once brain temperature reached 37˚C and was maintained at this temperature for 5 minutes, a vertically directed CCI was delivered at 4.0m/sec with a depth of 1.0mm. The bone flap was replaced, sealed with dental cement and the incision closed.

MR studies were performed on a 4.7-Tesla, 40 cm bore Bruker AVANCE-DBX system, equipped with a 15 cm diameter shielded gradient insert and a home-built saddle-type RF coil. For all imaging experiments, FOV = 4 cm and slice thickness = 2 mm. Maps of T_{1obs} [4] were generated from a three-parameter exponential fit to a series of spin-echo images with variable *TR* (*TR* = 8000, 4300, 2300, 1200, 650, 350, 185, 100 msec, 2 averages, 128 x 70 matrix). Perfusion spin-echo images were acquired in duplicate using the arterial spin-labeling technique [5] (*TR*/*TE* = 2000/10, 20, 30, summation of 3 echoes, 2 averages, 128 x 70 matrix) with labeling applied ± 2 cm from the imaging plane. CBF maps were generated from: $CBF = \lambda \cdot (T_{1obs} \cdot 2\alpha)^{-1} \cdot (M_C - M_L) \cdot (M_C)^{-1}$, where M_C and M_L are the magnetization intensities from the control and labeled images, respectively. A spatially constant value of 0.9 mL · g^{-1} was assumed for the blood brain partition coefficient for water (λ). The spin labeling efficiency (α) [6] was determined in each study with gradient echo images on the carotid arteries and spin-labeling applied at ± 6 mm (*TR*/*TE* = 100/9.6 msec, 45˚ flip angle, 8 averages, 256 x 256 matrix). Body temperature was maintained at 37 ± 0.5 ˚C using warm air, regulated with a rectal temperature probe. Prior to, and after each MRI study, $PaCO_2$, PaO_2, MABP, HR and rectal temperature were recorded. CBF was quantified for 6 anatomical regions within each hemisphere.

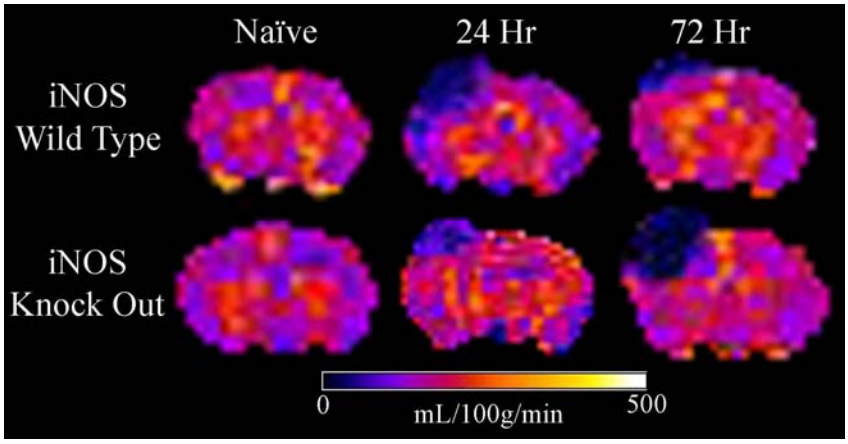

Figure 1: Representative CBF maps of iNOS wildtype (WT) and knockout (KO) mice without injury, naïve, and 24 and 72 hours post CCI.

RESULTS AND DISCUSSION

Figure 1 shows that there was no significant difference between the WT and KO animals either with no injury or at 24 hours post CCI. 72 hours after trauma however, CBF in the ipsilateral hippocampus, amygdala and thalamus was significantly lower in the KO mice compared to the WT. At and beyond 72 hours after TBI iNOS has been shown to confer beneficial effects. Specifically at 72 hours after CCI iNOS induction is maximal, and NO derived from this induction could possibly act as endogenous antioxidant [7]. iNOS expression mediates protein nitrosylation of substrates, including caspase-3, cytoskelatal proteins and glutamate receptors, that could have beneficial effects. Studies of long-term outcome have shown that despite identical performance on the Morris water maze before injury, iNOS KO mice showed dramatically worse performance than WT at 14-20 days after CCI [8]. The findings of this report suggest that enhanced recovery of CBF by iNOS may either represent an additional beneficial effect or reflect reduced tissue injury with coupled CBF recovery.

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