^{19}F Imaging Assessment of Labeled Macrophage Accumulation in a Mouse Brain Following Experimental Traumatic Brain Injury

L. M. Foley[1], T. K. Hitchens[1,2], J. A. Melick[3], C. Ho[1,2], and P. M. Kochanek[3,4]

[1]Pittsburgh NMR Center for Biomedical Research, Carnegie Mellon University, Pittsburgh, PA, United States, [2]Department of Biology, Carnegie Mellon University, Pittsburgh, PA, United States, [3]Safar Center for Resuscitation Research, University of Pittsburgh School of Medicine, Pittsburgh, PA, United States, [4]Departments of Critical Care Medicine, Pediatrics and Anesthesiolgy, University of Pittsburgh School of Medicine, Pittsburgh, PA, United States

INTRODUCTION

Neuroinflammation is widely known to have both a beneficial and detrimental role after traumatic brain injury (TBI): beneficial in repairing injured tissue, and detrimental via the elaboration and release of neurotoxic substances that exacerbate damage. Recent studies suggest that macrophages may play a role in mediating both early detrimental and delayed beneficial effects of inflammation [1]. Therefore, the ability to detect the macrophage response *in vivo* after TBI may lead to a greater understanding of the mechanistic underpinnings of both secondary injury and repair. Here we report the use of an MRI ^{19}F tracer agent that is taken up by macrophages *in vivo* to detect the response to experimentally induced TBI produced by controlled cortical impact (CCI) in a mouse model.

MATERIALS AND METHODS

The mice used in this study were C57Black/6J between 11-15 weeks of age. Mice were divided into one of three groups for MRM assessment, 24, 48, or 72 hrs after trauma. Mice were anesthetized with isoflurane in N_2O:O_2 (1:1) via a nose cone and a femoral venous catheter was surgically placed. The mouse CCI model was used as previously described [2] with minor modifications [3]. Mice 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 min, a vertically directed CCI was delivered at 5.0m/sec with a depth of 1.0mm. The bone flap was replaced, sealed with dental cement and the incision closed. The fluorine agent given was delivered in a 200 µl bolus of 30% v/v perfluoro-15-crown-5 ether nanoemulsion (VS-580H, Celsense Inc., Pittsburgh, PA) at either 30 min post CCI (24 hr group), 24 hr post CCI (48 hr group) or 48 hr (72 hr group). At 24, 48 and 72 hr post CCI mice were euthanized, the brains perfused, fixed in 4% paraformaldehyde and removed to be imaged *ex vivo* at 11.7 T. A spin-echo sequence is used to detect the ^{19}F-labeled cells.

RESULTS AND DISCUSSION

Preliminary results indicate presumptive macrophage infiltration at the site of injury in the brain (Figure 1). The ^{19}F signal intensity is directly correlated with both the amount of label administered [4] and the pattern of macrophage accumulation—as assessed by immunohistochemical methods at 72 hr post CCI. The ^{19}F signal distribution and intensity also corroborates a recent study using micron-sized iron oxide particles to label macrophages after TBI [5]. Fluorine-based tracer agents provide spin-density weighted signal in ^{19}F images that have the advantage, in some cases, of improving the positive identification of labeled cells. Due to the fact that there is no endogenous ^{19}F signal, fluorine-labeled cells provide a positive signal only where the labeled cells are found, therefore labeled cells are unambiguously identified and amenable to quantification (Figure 1). Future work involves applying this approach to *in vivo* imaging studies.

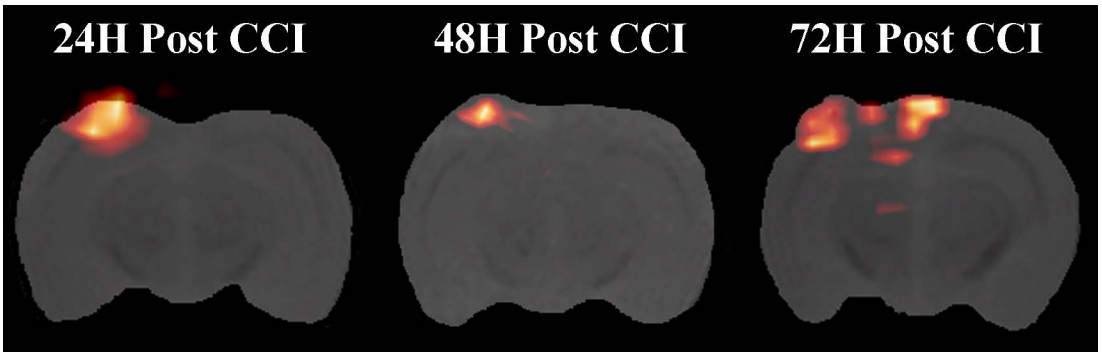

Figure 1: The *ex vivo* ^{19}F image is rendered in hot-iron pseudo color which is overlaid on the corresponding ^{1}H anatomical image (2 mm slice thickness, TR/TE = 2000/10 ms, 128 x 128 matrix and 2 averages for ^{1}H, and 64 x 32 matrix and 1500 averages for ^{19}F).

REFERENCES

1. Gris D, Marsh DR, Oatway MA, Chen Y, Hamilton EF, Dekaban GA, and Weaver LC. J Neurosci **24**, 4043–4051 (2004).
2. Smith DH, Soares HD, Pierce JS, Perlman KG, Saatman KE, Meaney DF, Dixon CE, and McIntosh TK. *J Neurotrauma* **12**, 169-178 (1995).
3. Whalen MJ, Carlos TM, Dixon CE, Schiding JK, Clark RS, Baum E, Yan HQ, Marion DW, and Kochanek PM. *J Neurotrauma* **16**, 299-309 (1999).
4. Srinivas M, Morel PA, Ernst LA, Laidlaw DH, Ahrens ET. *Magn Reson Med* **58,** 725-34 (2007).
5. Foley LM, Hitchens TK, Ho C, Janesko-Feldman KL, Melick JA, Bayir H, and Kochanek PM. *J Neurotrauma* **26**, 1509-1519 (2009).