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Daily Administration of Ibuprofen Modifies Neuroinflammation Gene Expression, but not Neuroplasticity Gene Expression in Traumatic Brain Injured Rats

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Introduction

Each year there are at least 1.4 million civilian traumatic brain injury (TBI) incidents, with most as a result of a moderate level of injury and being treated in the emergency department (Langlois and Sattin 2005). The post-traumatic morbidity is likely to result from secondary molecular, biochemical and cellular events that cause additional neuronal, glial and vascular injuries across multiple brain areas (Thompson, Gibson et al., 2006; Farkas and Povlishock 2007).

One of the primary histopathological consequences associated with TBI is microglial activation and ensuing neuroinflammation(Csuka, Hans et al. 2000). Inflammation in neurodegenerative conditions can be both detrimental and beneficial(Gensel, Nakamura et al. 2009). Inflammation can clear dead or dying tissue. On the other hand, previous studies in spinal cord injury and Alzheimer diseases have shown that microglia activation, especially the alternative activation of microglia, can be one factor that promotes neuroplasticity and potentially affect the recovery of the patient (Donnelly and Popovich, 2008; Popovich and Longbrake, 2008; Colton, 2009; Colton and Wilcock, 2009; Gensel, Nakamura et al., 2009; Kigerl, Gensel et al., 2009). We hypothesize that the primary mechanical forces of TBI initiate microglia activation, and therefore anti-inflammatory treatments would reduce microglia activation, helping to control post-traumatic neuroplasticity. Here, we examine the post-traumatic microglia activation states: classical activation associated with neurotoxicity, alternative activation associated with recovery, and acquired deactivation associated with anti-inflammatory response (Colton and Wilcock, 2009; Gensel, Nakamura et al., 2009). Also, we examine the ability of the anti-inflammatory ibuprofen to modulate microglial activation and neuroplasticity gene expression using real-time PCR.

Experimental Design

Adult male Sprague-Dawley rats (350–375 g) were subjected to midline fluid percussion injury (FPI) consistent with methods described previously (Lifshitz, Kelley et al., 2007; Lifshitz, 2008; Hosseini and Lifshitz, 2009; McNamara, Lisembee et al., 2010). A craniotomy was performed and the injury hub was secured on the skull of rats under isoflurane anesthesia. As each rat's reflex response returned, a sham injury (the animal is connected to the injury device, but no injury introduced) or a moderate (1.9-2.0 atm) FPI was introduced to each rat. Uninjured (n=12) and injured rats (n=16) were treated with daily administration of either saline or ibuprofen (20mg/kg; i.p. injection). At 7 days post-injury, each animal was euthanized and tissue biopsies of the somatosensory cortex and thalamus were taken from the coronal brain section. From the tissue, the mRNA was isolated (FastPrep®-24 sample preparation system, M.P. Biomedicals and MagMax™ Express, Applied Biosystems Inc.), quantified (NanoDrop ND-1000 spectrophotometer), and then converted to complementary DNA (cDNA; High Capacity RNA-to-cDNA Kit, Applied Biosystems Inc.). The cDNA was used as a template for quantitative real-time PCR using commercially-available gene expression assays (Taqman® Gene Expression Assay, Applied Biosystems). Within each animal, expression of microglia activation marker (TSPO), classical activation marker (CD45), alternative activation marker (Arg1), acquired deactivation marker (TGFß1) and neuro-plasticity markers (GAP43, Synaptophysin) were normalized to the gene expression level of endogenous control, 18s rRNA (Colton, 2009; Colton and Wilcock, 2009; Cook, Vink et al. 2009; Gensel, Nakamura et al., 2009; Harris, Reeves et al., 2009; Kigerl, Gensel et al., 2009). Gene expression in each group was averaged and normalized to the sham-vehicle treated group using $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

Gene expression in all other groups was expressed as the percentage change of sham-vehicle group. Peirce's criterion

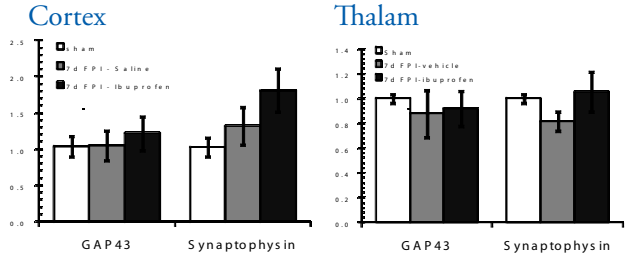

Figure 2: Neuroplasticity gene expression was not affected by brain injury or ibuprofen treatment in the cortex or thalamus (n=5-6/group). The sham vehicle-treated and sham ibuprofen-treated groups were combined into a single sham group after confirming no significant difference between groups.

was used to identify outliers in the data sets (Ross 2003). To maximize statistical power, sham-saline treated and sham-ibuprofen treated groups were combined into one sham group after confirming no significant differences between the two groups by student t-test. All statistical assessments were performed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test using GraphPad InStat statistical software. Results are considered significant if $p < 0.05$. All data are presented as mean ± standard error of the mean (SEM).

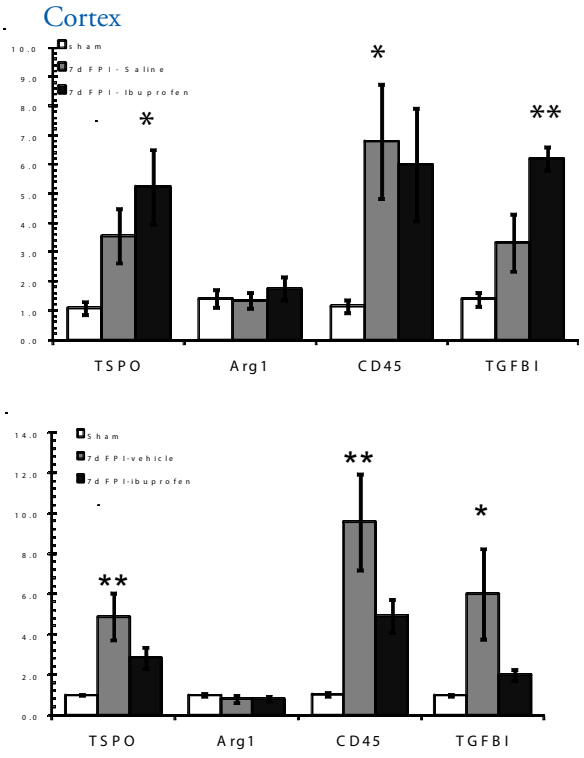

Figure 1:Post-traumatic inflammatory gene expression after anti-inflammatory treatment. 7 days after fluid percussion injury (FPI) are compared between sham, injured-saline treated, and injured-ibuprofen treated rats (n= 5-6 per group). In the cortex, gene expression of both TSPO and TGFB1 increased after ibuprofen treatment compared to sham. Injury-induced increases in CD45 gene expression at 7 days post-FPI was not affected by ibuprofen treatment. In the thalamus, gene expression indicates significantly increased classical (TSPO and CD45) and anti-inflammatory (TGFB1) microglial activation at 7 days post-FPI. Ibuprofen treatment reduced gene expression levels TSPO, CD45 and TGFB1 such that they are no longer significantly different from sham. *, $p < 0.05$; **, $p < 0.01$ compared to sham.
TSPO: translocator protein 18 kDa
CD45: protein tyrosine phosphatase receptor type C
TGFßI: transforming growth factor ß1

Result

Gene expression in the two brain regions responded differently to anti-inflammation treatment. In the cortex, daily ibuprofen administration was ineffective in attenuating the injury-induced increase in classical microglia activation and acquired microglia deactivation (Figure 1). TSPO gene expression remained significantly elevated above sham in the ibuprofen-treated brain-injured animals ($F(2,15) = 5.123$; $p = 0.0201$).TGFß1 gene expression was increased significantly in injured ibuprofen-treated animals compared to sham animals ($F(2,12) = 6.749$; $p = 0.0109$), suggesting increased anti-inflammatory gene expression following ibuprofen administration. Injury-induced increases in CD45 gene expression at 7 days post-FPI was not significantly affected by ibuprofen treatment, whereas ibuprofen treatment increased TSPO and TGFß1 gene expression.

In VPM, the fourfold significant increase in TSPO gene expression in brain-injured saline-treated animals was down-regulated by the ibuprofen administration to the level where no significant change was found compared to the sham group ($F(2,14) = 6.754$; $p = 0.0088$). Similar results were obtained for the gene expression of CD45 ($F(2,13) = 8.160$; $p = 0.0051$) and TGFß1 ($F(2,12) = 4.844$; $p = 0.0287$); the gene expression increased significantly with saline treatment, while administration of ibuprofen down-regulated gene expression. As found previously, arginase 1 gene expression was not significantly different between sham animals, FPI-injured animals and FPI-injured ibuprofen-treated animals (S1BF: $F(2,14) = 0.4881$, $p = 0.6239$; VPM: $F(2,14) = 0.6358$, $p = 0.5441$)

Even though anti-inflammatory treatment resulted in various changes in both classical activation and acquired deactivation in cortex and thalamus, no significant change in neuroplasticity gene expression was observed in either brain region as the result of ibuprofen administration (Figure 2). Classical and acquired activation of microglia may not be directly associated with neuroplasticity gene expression.

Discussion

Results of TSPO gene expression suggest there is increased microglia activation in the cortex and reduced microglial activation in the thalamus when ibuprofen is administered. In the cortex, increased TGFß1 gene expression occurs concomitantly with an increase in TSPO gene expression, suggesting interplay between inflammatory and anti-inflammatory responses. Also the ibuprofen treatment did not affect the CD45 gene expression significantly in the cortex. The result suggests ibuprofen treatment is not able to reduce classical activation of microglia in the cortex, and thus neurotoxic effect of microglia is still present. Higher doses

may have a positive outcome on cortical gene expression. On the other hand, in the thalamus, ibuprofen not only reduced CD45 gene expression but also reduced TGFß1 gene expression, suggesting reduction in both classical activation and acquired deactivation of microglia. Higher doses may have a negative effect on thalamic gene expression. The contrasting responses in the two brain regions to ibuprofen are unknown, but highlight different microenvironments in the two brain regions.

Only the arginase 1 gene expression did not change as the result of the FPI or ibuprofen treatment, indicating that arginase 1 gene expression is not affected by traumatic brain injury at 7 days time point and it is independent of the COX2 pathway. An experiment in spinal cord injury has shown that there is an increase in arginase 1 gene expression at 1 and 3 days (Kigerl, Gensel et al. 2009), which may hold true for TBI. By 7 days post-injury, arginase 1 gene expression might have already subsided. We have found, however, the alternative activation is not likely to be present at 7 days post-injury and the alternative activation process seems to be independent of COX2 pathways.

TGFß1 can reduce expression of TNFß, IL1ß and other proinflammatory cytokines. From this and previous studies, TGFß1 gene expression has correlated well with TSPO and CD45 gene expression . This result suggests that the expression of TGFß1 might be a compensatory mechanism to reduce classical activation process. In each experiment, gene expression of TGFß1 is shown to increase as CD45 and TSPO gene expression increases and vice versa.

Whether increased or decreased, ibuprofen was successful in modulating inflammation-related gene expression in FPI-injured rats. Despite the changes in the inflammation gene expression, neuroplasticity genes were not modulated by ibuprofen administration. Lack of neuroplasticity gene expression change is possibly correlated with lack of alternative activation of microglia (Gensel, Nakamura et al., 2009; Kigerl, Gensel et al., 2009). The result might be different if neuroplasticity gene expression is measured earlier. In conclusion, at this point we have not found a relationship between neuroplasticity gene expression and microglia activation process. These experiments will be followed-up on by comparing changes of microglia activation and neuroplasticity protein quantity in ibuprofen-treated and ibuprofen-non-treated animals.

Works Cited

Colton, C. A. (2009). "Heterogeneity of microglial activation in the innate immune response in the brain." J Neuroimmune Pharmacol **4**(4): 399-418.

Colton, C. A. and D. M. Wilcock (2009). "Assessing Activation States in Microglia." CNS Neurol Disord Drug Targets.

Cook, N. L., R. Vink, et al. (2009). "Validation of reference genes for normalization of real-time quantitative RT-PCR data in traumatic brain injury." J Neurosci Res **87**(1): 34-41.

Csuka, E., V. H. Hans, et al. (2000). "Cell activation and inflammatory response following traumatic axonal injury in the rat." Neuroreport **11**(11): 2587-2590.

Donnelly, D. J. and P. G. Popovich (2008). Inflammation and its role in neuroprotection, axonal regeneration and functional recovery after spinal cord injury. Experimental Neurology: 378-388.

Farkas, O. and J. T. Povlishock (2007). "Cellular and subcellular change evoked by diffuse traumatic brain injury: a complex web of change extending far beyond focal damage." Prog Brain Res **161**: 43-59.

Gensel, J. C., S. Nakamura, et al. (2009). "Macrophages promote axon regeneration with concurrent neurotoxicity." J Neurosci **29**(12): 3956-3968.

Harris, J. L., T. M. Reeves, et al. (2009). "Injury modality, survival interval, and sample region are critical determinants of qRT-PCR reference gene selection during long-term recovery from brain trauma." J Neurotrauma **26**(10): 1669-1681.

Hosseini, A. H. and J. Lifshitz (2009). "Brain injury forces of moderate magnitude elicit the fencing response." Med Sci Sports Exerc **41**(9): 1687-1697.

Kigerl, K. A., J. C. Gensel, et al. (2009). "Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord." J Neurosci **29**(43): 13435-13444.

Langlois, J. A. and R. W. Sattin (2005). "Traumatic brain injury in the United States: Research and programs of the Centers for Disease Control and Prevention (CDC) - Preface." Journal of Head Trauma Rehabilitation **20**(3): 187-188.

Lifshitz, J. (2008). Fluid Percussion Injury. Animal Models of Acute Neurological Injuries. Z. C. X. Jun Chen, Xiao-Ming Xu, Jhon H. Zhang. Totowa, NJ, The Humana Press, Inc.

Lifshitz, J., B. J. Kelley, et al. (2007). "Perisomatic thalamic axotomy after diffuse traumatic brain injury is associated with atrophy rather than cell death." J Neuropathol Exp Neurol **66**(3): 218-229.

Livak, K. J. and T. D. Schmittgen (2001). "Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method." Methods **25**(4): 402-408.

McNamara, K. C., A. M. Lisembee, et al. (2010). "The Whisker Nuisance Task Identifies a Late Onset, Persistent Sensory Sensitivity in Diffuse Brain-Injured Rats." J Neurotrauma.

Popovich, P. G. and E. E. Longbrake (2008). Can the immune system be harnessed to repair the CNS? Nature Review, Neuroscience. **9**: 481-493.

Ross, S. M. (2003). "Peirce's criterion for the elimination of suspect experimental data." Journal of Engineering Technology **20**(2): 38-41.

Thompson, S. N., T. R. Gibson, et al. (2006). "Relationship of calpain-mediated proteolysis to the expression of axonal and synaptic plasticity markers following traumatic brain injury in mice." Exp Neurol **201**(1): 253-265.