Preclinical Studies for the Treatment of Blast-related Injuries

Cytokine Responses in a Rat Model of Blast-induced Mild Traumatic Brain Injury

Chemokines and cytokines play early pivotal roles in the inflammatory cascades underlying blast-induced injuries and are promising targets for therapeutic interventions. To effectively pursue this therapeutic avenue, the timing of the interplay among these responses must be characterized to identify the key participants and the optimal therapeutic windows for intervention. Researchers at the Walter Reed Army Institute of Research (Silver Spring, Maryland) are conducting a study that includes longitudinal screening of cytokine levels in plasma and brain at varied times after blast exposure using immunoassay arrays based on newly developed Luminex® bead technology. The arrays (R&D Systems Inc.) are used to simultaneously and precisely quantify very small concentrations (picomolar) of up to 17 rat specific cytokines across a single 96 sample well plate. Thus, this method is highly time- and cost-effective versus data yield. Analyses to date of plasma and brains collected from blast-exposed rats reveal significant increases (2-fold or less) in pro-inflammatory cytokines (Chemokine (C-X-C motif) ligand 2 (CXCL2), interleukin (IL) -1-α, IL-18, and tumor necrosis factor (TNF) -α) along with counter elevations in inflammation resolving cytokines (IL-4 and tissue inhibitor of metalloproteinases (TIMP) -1) up to seven days post-exposure (*DeMar et al. 2016b, 2016a*; Figure 1). MRI has shown that extensive immune cell infiltration occurs within brain and retina by three days post-exposure (*DeMar et al. 2017b, 2017a, Foley et al. 2017*). These findings have been corroborated by immunohistochemistry of brain and eye sections using biomarkers for activated immune cells (*DeMar et al. 2017b, 2017a, Foley et al. 2017*). Cytokines can act as recruitment factors for macrophages into tissues, and in turn are excreted by these immune cells as signaling molecules that further trigger protein-pathways involved in apoptosis of neurons (e.g., caspases). Based upon these response profiles, interventions with existing compounds targeting these mediators are likely to be most effective during subacute or acute phases of injury. Notably, since rats maintained on a diet supplemented with long chain omega-3 fatty acids (i.e., fish oil) appear to have lowered (23 percent or less) pro-inflammatory cytokines (CXCL3, intercellular adhesion molecule (ICAM) -1, IL-1-α, IL-6, IL-18, and TNF-α) in the plasma and brain (Figure 2); these data point to the potential utility of these diets as nutritional anti-inflammatory countermeasures to blast-induced traumatic brain injury (*DeMar et al. 2016b, 2016a*).

By defining important neurobiological underpinnings of blast injuries, these findings point to potential therapeutic countermeasures that can lessen permanent debilitations suffered by Service members experiencing blast-induced injuries.

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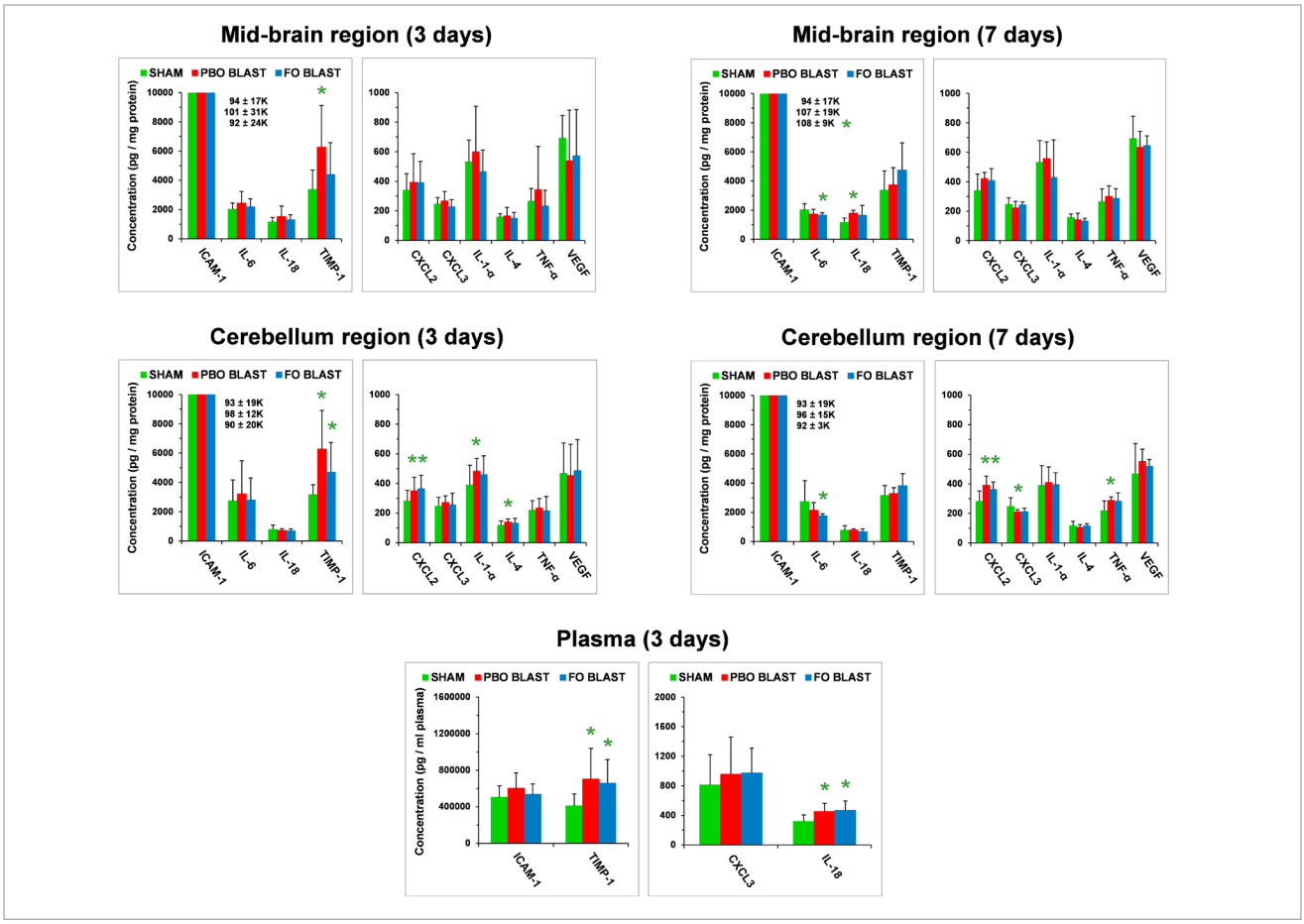

FIGURE 1: Bar graphs for the mid-brain (top 2 panels), cerebellum (middle 2 panels), and plasma (bottom panel) cytokine levels of placebo versus fish oil treated rats, at 3 and 7 days following double blast exposure, as well as those for sham controls (shams = green, PBO; placebo = red, and FO; fish oil = blue; n = 15, 11, and 10 and 15, 4, and 4, as by day, respectively). For each tissue, the data is broken into two rescaled frames to allow visualization of less abundant cytokines. Tissue concentrations (per mg total protein or ml plasma) for up to 10 cytokines are shown, i.e., CXCL2, CXCL3, ICAM-1, IL-1-α, IL-4, IL-6, IL-18, TIMP-1, TNF-α, and VEGF. There were no significant differences detected between dietary treatment groups for blasted animals. *p < 0.05; significant difference between shams and blasted rats, as by t-test. (Figure used with permission from the authors)

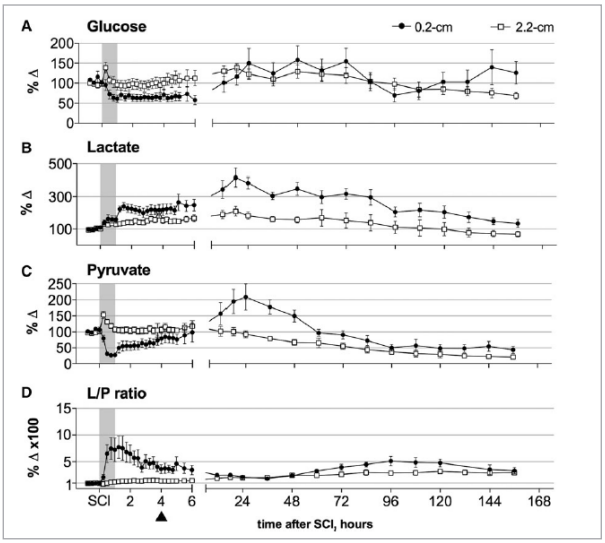

FIGURE 2: Microdialysis measurements of intraparenchymal glucose, lactate, and pyruvate (%D) in response to SCI at 0.2 and 2.2 cm from injury. The percentage change (%Δ) is calculated using the average of measurements obtained through 60 minutes of baseline recordings just prior to the SCI. (A) Glucose, (B) lactate, (C) pyruvate, and (D) lactate to pyruvate (L/P) ratio responses before, during, and after 1-hour spinal cord contusion/compression (gray shading). At the 0.2-centimeter position (●), glucose values decreased significantly upon SCI, and subsequently returned to baseline by Day one. Within minutes after SCI, we observed an increase in lactate, a decrease in pyruvate, and a resulting increase in L/P ratio. After decompression, glucose, pyruvate and lactate increased while L/P ratio declined to 200 percent above baseline at 24 hours. Thereafter, both lactate and pyruvate levels decreased again, although pyruvate fell proportionately more, resulting in a subsequent rise in L/P ratio till the end of the experiment (500 percent above baseline). At the 2.2-centimeter position (□), a slight increase in glucose levels was observed within the first 24 hours after SCI; however, levels retuned to baseline thereafter. Within hours after SCI, researchers observed a slow but steady rise in lactate while pyruvate levels remained unchanged, producing an increase in L/P ratio. After 24 hours, researchers observed a drop in lactate and a simultaneous and disproportionately greater drop in pyruvate, resulting in a continuous increase in L/P ratio to 500 percent above baseline at Day seven. The dashed line at the four-hour post-SCI mark (▲) represents the discontinuation of anesthesia and ventilation at the end of the surgical procedure. BSL, baseline; SCI, spinal cord injury. (Figure from Streijger et al. (2017) used with permission from the authors)

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