LIVER INFLAMMATION EXACERBATES INTRASPINAL TISSUE LOSS AFTER SPINAL CORD INJURY

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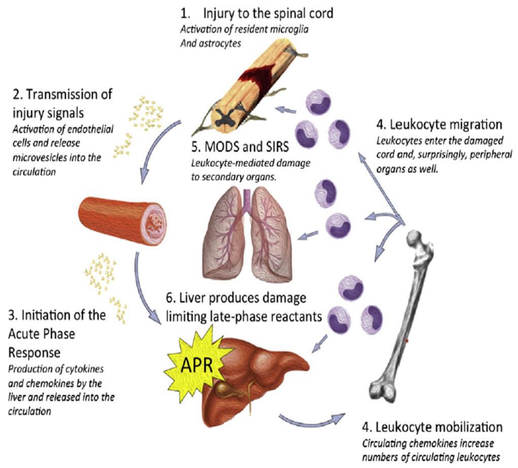
Background

Traumatic spinal cord injury (SCI) is a devastating condition that significantly reduces a patient's sensory and motor abilities. SCI can cause multiple organ system dysfunction that contributes to chronic health impairments. Prior work from our lab showed that immune system cells called Kupffer Cells (KCs), which are resident macrophages of the liver, initiate a pro-inflammatory response that persists long after SCI. Additionally, some reports suggest that liver inflammation may exacerbate lesion pathology in the spinal cord after SCI, though this effect has not been directly tested. Therefore we employed a bile duct ligation (BDL) surgical model to induce liver inflammation prior to a thoracic spinal cord contusion to test our hypothesis that inducing liver inflammation before SCI will increase spinal inflammation, lesion pathology, and functional deficits.

Methods

Adult Sprague-Dawley rats received a total BDL and were sacrificed at 1 and 5 days. Fresh liver samples were used to examine pro-inflammatory gene expression in the liver. Other rats were given a T8 spinal cord contusion five days after BDL and were sacrificed 28 days post BDL. Control animals received sham BDL surgery followed by T8 contusion. Serum was assessed for changes in triglycerides, non-esterified fatty acids (NEFAs), and insulin levels. Liver Kupffer cells (KCs) and spinal cord macrophages were examined with OX42 immunohistochemistry. Lipid accumulation and morphological changes within the liver were examined with Oil Red O and Hematoxylin and Eosin (H&E) staining, respectively. White matter and axonal damage within the spinal cord lesion were visualized with Eriochrome Cyanine (EC) and neurofilament (NF) labeling, respectively. Hindlimb motor function was determined using the Basso, Beattie, Bresnahan (BBB) locomotor scale.

The innate immune system response following spinal cord injury



Anthony & Couch (2014)

BDL increases pro-inflammatory gene expression in the liver

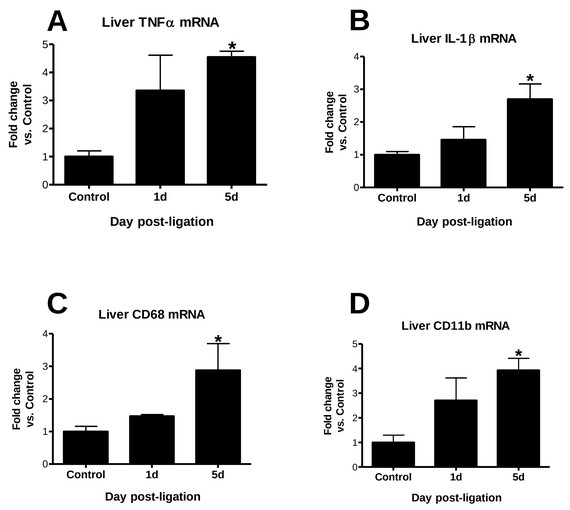


Figure 1: RT-PCR of liver tissue samples at 1 and 5 days post-BDL for **(A)** TNFα, **(B)** IL-1β, **(C)** CD68 and **(D)** CD11b. Expression of all pro-inflammatory genes examined were significantly increased by 5 dpi.

Liver fat deposition and Kupffer Cell activation are altered after BDL and SCI

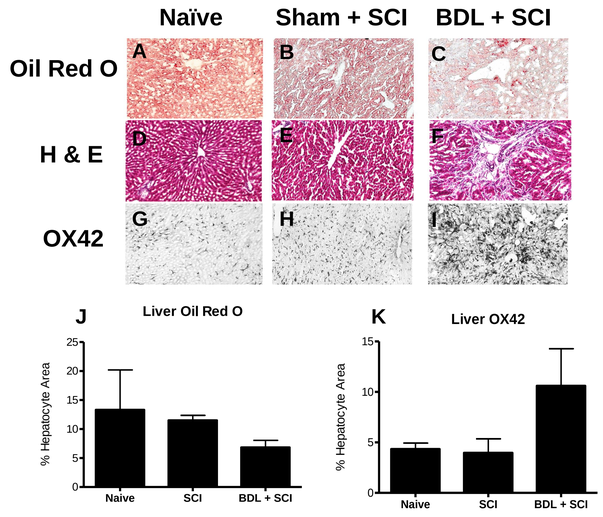


Figure 2: Decreased Oil Red O+ staining showing fat deposition **(A-C, J)**, H & E for morphological changes **(D-F)** and increased OX42+ Kupffer Cell activation **(G-I, K)** 28 days post BDL and 23 days post SCI.

Serum fatty acid and insulin levels are altered following both injuries

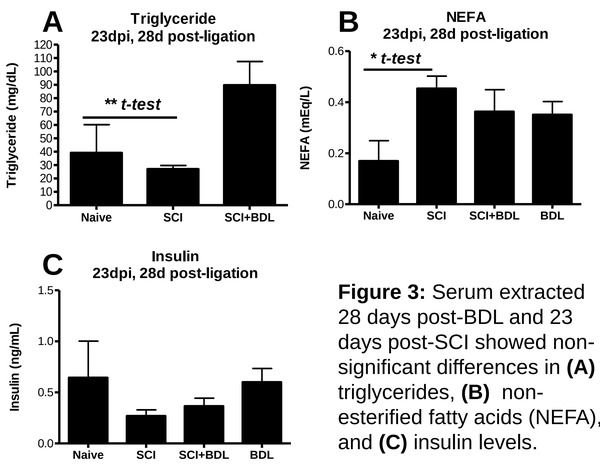


Figure 3: Serum extracted 28 days post-BDL and 23 days post-SCI showed non-significant differences in **(A)** triglycerides, **(B)** non-esterified fatty acids (NEFA), and **(C)** insulin levels.

BDL preceding spinal contusion leads to increased tissue damage

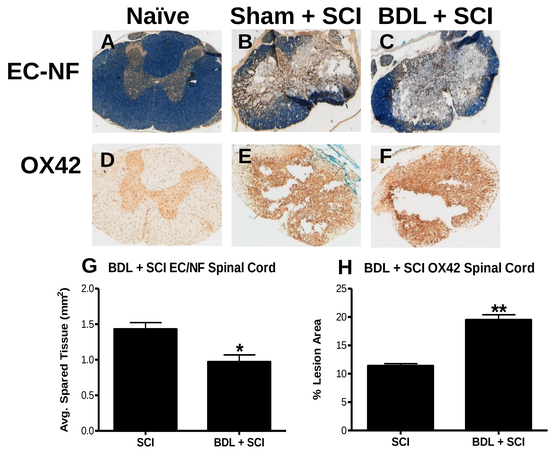


Figure 4: (A-C, G) EC-NF staining revealed increased lesion pathology and tissue loss in the spinal cord 28 days post-BDL and 23 days post-SCI. **(D-F, H)** Similarly, OX42 revealed significantly increased macrophage and microglia activation.

Hepatic inflammation has no significant effect on locomotor recovery

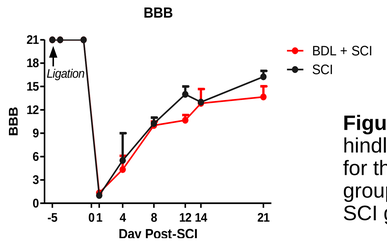


Figure 5: Reduced hindlimb motor scores for the BDL + SCI group compared to the SCI group.

Conclusions

Pathological effects of SCI are exacerbated when preceded by induced liver inflammation.

Significant findings include:

- Increased lesion tissue in the injured spinal cord
- Elevated levels of activated macrophages in lesion epicenter
- Changes in circulating fatty acids