DEVELOPMENT OF A RODENT MODEL FOR THE STUDY OF ABDOMINAL COMPARTMENT SYNDROME

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Background: The clinical pertinence of increased abdominal pressures has gained attention with the high correlation with damage control surgery in trauma. Increasing abdominal compartmental pressures have been associated with an increased rate of multiple organ dysfunction in several studies. This led to the 2004 consensus conference of the World Society on Abdominal Compartment Syndrome (WSACS) where abdominal compartment syndrome (ACS) was defined as an intra-abdominal pressure of ≥ 20 mmHg associated with the new onset of organ failure.

Various animal models have been developed to simulate ACS employing methods including carbon dioxide insufflation, gelatin infusion, intra-abdominal balloons and crystalloid infusion. Unfortunately each is fraught with methodological weakness such as an unequal distribution of pressure in the abdominal compartment or disruptions to the hemodynamic changes that would usually occur, impairing the simulation of ACS. To the best of our knowledge, the pathophysiology of the tissue and microvascular injury remains incompletely understood. Using intravital videomicroscopy, we hope to further understand the microvascular changes and tissue perfusion deficits that occur in ACS, using the liver primarily to quantify cell death and the inflammatory response.

Objective: The objective of our study was to develop a small animal model that is easily financially feasible and reproducible to study the mechanisms of tissue and microvascular injury associated with abdominal compartment syndrome.

Methods:
Nine male Wister rats were randomized into either a control group (no ACS – 5 rats), or a group subjected to elevated intra-abdominal pressure (ACS – 4 rats). Experimental rats were anesthetized with 5% inhalational isoflurane; anesthesia was maintained at 2% isoflurane. Core temperature was maintained at 37°C; mean arterial pressure was maintained between 95-100 mm Hg with mild fluid replacement titrated to a carotid arterial line. Intra-abdominal hypertension was established with CO₂ insufflation with an associated fitted abdominal cast to account for the distensibility of the rat skin and subcutaneous tissue. A second intra-abdominal port was used to monitor intra-abdominal pressures, allowing for titration to 20 mmHg. This was maintained for 2 hours. Control animals (n=5) were subjected to the same preparation; however, CO₂ insufflation was not performed and control levels of intra-abdominal pressure were maintained for the duration of the experiment.

A midline laparotomy was performed in order to expose the abdominal cavity and organs. Animals were then placed onto the stage of an inverted microscope (Nikon Diaphot 300) where the liver was externalized onto a slide moistened with saline and diluted propidium iodide dye. Time from laparotomy to the first microscopy reading was carefully minimized. Intravital videomicroscopy analysis was utilized to visualize a complete hepatic vascular unit (peri-portal area, sinusoids, pericentral area) within the liver. A 60-second video recording under 700x magnification was taken for each field of view obtained. The same method was performed on six randomly chosen fields of view containing no complete microvascular unit. These views provided large numbers of sinusoids for volumetric flow analysis and allowed for visualization of cellular injury. Six more randomly chosen

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fields of view were taken for approximately 45 seconds to observe leukocytes in a post-sinusoidal venule. Using a final magnification of 1400x, leukocyte rolling and adherence were observed in post-sinusoidal venules and quantified. For our injury analysis propidium iodide, an intercalating agent (membrane impermeable and will only stain DNA of dead cells) was used and cell death was examined and expressed as the number of PI-labeled cells per $10^3\, \text{mm}^3$. The degree of cellular injury and leukocyte rolling/adherence was compared between groups using a Student’s t-test. A one-way analysis of variance was employed in comparing perfusion differences between control and experimental groups. The sample size was calculated for a power of 80% or greater with significance of all statistical analyses set at an alpha level of 0.05. Data is presented as mean ± standard error of the mean (SEM).

Results: All rats were examined at 2-hours. When rats were dissected post-sacrifice, an extensive amount of pulmonary contusion was noticed at the overall morphological and histological levels. The percentage of continuously perfused sinusoids was found to be significantly lower in experimental rats (85.1±3 %) as compared to the control group (99.6 % ± 0.50) with an increase in nonperfused and intermittently perfused sinusoids (p=0.002). Although the number of leukocytes present in sinusoids was not significantly different between both groups (p=0.84), the number of rolling leukocytes identified in post-sinusoidal venules (PSV) was found to be significantly higher in experimental rats (8.7±3.53) compared with control rats (1.2±1.6; p=0.04). The number of adherent leukocytes was not found to be significantly different between both groups (p=0.12). Furthermore, although a trend towards increased cell death can be seen (Figure 3C), this failed to reach significance (p=0.084).

Discussion: This study demonstrates the development of a small animal model of abdominal compartment syndrome as evidenced by the eventual respiratory failure of these animals and the initial stages of the inflammatory cascade observed in the livers of the experimental group. Direct imaging of capillaries also confirms early microvascular responses to the intra-abdominal pressure applied. We saw a decrease in the number of continuously perfused sinusoids with a concomitant increase in the number of intermittently perfused and nonperfused sinusoidal units. This demonstrates a significant decrease and complete lack of movement in blood flow in the intermittently perfused and nonperfused sinusoids, respectively. Furthermore, we see initial evidence at the microvascular level of the inflammatory cascade with increases in leukocyte rolling. Although a trend towards increased leukocyte adherence was observed in the experimental group, the lack of an observed significant difference could be secondary to the early stage at which rats were assessed (45 minutes after the 2-hour insult period). The changes in sinusoidal perfusion associated with the increase in leukocyte rolling all suggest a proinflammatory environment. Furthermore, we see direct evidence of a trend towards an increase in cell death in the experimental group of rats.
Figure 1. Comparisons of (A) adherent and rolling leukocytes in post-sinusoidal venules, (B) sinusoidal perfusion categorized as continuously perfused (CPS), intermittently perfused (IPS) and non-perfused sinusoids (NPS) and (C) cell death as a function of propium iodide stained hepatocytes, between control and experimental rats exposed to 2 hours of increased intra-abdominal pressure (20 mmHg).


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