Induced sepsis by cecal ligation and puncture in a rat model: hepatic and renal histological features

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Abstract:

Animal models have been developed in an attempt to test potential therapeutic agents. The study aims to determine the hepatic and renal histological features induced by sepsis following cecal ligation and puncture (CLP) in a rat model. Regarding the material, we used 20 adult-male Wistar rats (control group 1, n=10, and group 2, n=10, that underwent CLP protocol). The experimental protocol was approved by the National Sanitary Veterinary and Food Authority, Cluj (Romania), project number 116/11.05.2018. The endpoint of the experiment was pre-set to 10 hours post-surgery. A complete necropsy survey was performed. The main renal lesions detected histologically were: glomerular congestion and edema, hyalinization of glomerular mesangium, presence of hyaline in the urinary space with associated compression atrophy of vascular glomerular tuft, granules of hyaline in the lumen of cortical tubules, and reduction of the urinary space. The hepatic lesion identified histologically was represented by isolated miliary necrotic foci.

Keywords: histology, sepsis, model, puncture, rat.

Introduction

Sepsis is a severe condition defined as a “life-threatening organ dysfunction that is caused by a dysregulated host response to infection”. The immune response that is initiated in sepsis by an invading pathogen, fails to return to homeostasis and instead continues to develop, ultimately reaching a state of sustained excessive inflammation and immune suppression (Van Der Poll *et al.*, 2017). Traditionally, the immune response to sepsis was thought to be composed of two separate phases: an initial hyperinflammatory phase lasting several days, followed by a more prolonged immunosuppressive phase. However, recent evidence suggests that early in the pathogenesis of sepsis, both pro-inflammatory and anti-inflammatory responses develop simultaneously (Hotchkiss *et al.*, 2013), and based on the direction of imbalance, the pro-

cess can result in inflammation and tissue damage, immunosuppression and infection, or recovery (Ettinger *et al.*, 2016).

Animal models have been developed in an attempt to create reproducible systems, in order to study the pathogenesis of sepsis and to test potential therapeutic agents (Buras *et al.*, 2005), since staging human sepsis models presents multiple difficulties (Fink and Heard, 1990; Deitch, 1998). In light of this evidence, the current study aims to determine the main hepatic and renal histological features that can be detected in induced sepsis by cecal ligation and puncture (CLP) in a rat model.

Materials and methods

In the study, we used 20 adult-male Wistar rats that were bought from the Faculty of Human

Medicine and Pharmacy Iuliu Hațieganu Cluj-Napoca. During this period, the rats were kept in boxes in groups of ten, at 20-26°C, with room humidity at around 40-70%, in a 12h light/dark cycle and fed with standard rat food and water *ad libitum*. The experimental protocol was approved by the National Sanitary Veterinary and Food Authority, Cluj (Romania), project number 116, on May 11th, 2018. The experimental study was conducted at the Faculty of Veterinary Medicine Cluj-Napoca (Romania). Ten rats represented the control group (i.e., group no. 1) and did not go under any procedure. The individuals from the second group (no. = 10) underwent CLP protocol for sepsis induction (Wichterman et al., 1980; Parker and Watkins, 2001). Basically, the CLP protocol involves the perforation of the cecum with a 21G needle (following anesthesia and visualization of the cecum by standard abdominal surgery). Before cecal perforation, a ligature was applied distal to the ileocecal valve at 1 cm from the tip of the cecum. The needle penetrated the caecum on both sides performing 2 holes in the organ. The cecum was gently squeezed until its contents began to exude through the perforations and then it was repositioned in the abdominal cavity. Following the CLP procedure, the second group was maintained in normothermia (35.9-37.5°C). The endpoint of the experiment was pre-set to 10 hours post-surgery, during this time the rats were clinically monitored. At the end of the experiment, the rats were euthanized using a high dose of Isoflurane followed by cervical dislocation. The sepsis was confirmed microbiologically.

Eventually, the necropsy exam was performed and the tissue samples (liver and kidney) were collected, underwent fixation in 10% buffered formalin and processed by classical paraffin technique and stained later by the H&E method. The examination of the histological slides achieved was performed using an Olympus BX41 light microscope, and the images were captured with an Olympus E-330 photo camera, attached to the microscope.

Results and discussion

In contrast to the previous experimental studies which have only found minor histological changes (Brooks *et al.*, 2007; (Buras *et al.*, 2005; Deitch, 1998; Lerolle *et al.*, 2010), in our experiment we observed renal injury that

affected large portions of the kidneys. In group 2, the rats showed glomerular congestion and edema, as well as a significant reduction of the urinary space associated with hyalinization of glomerular mesangium (Figure 1) and discrete intraglomerular infiltrate composed of young neutrophils. In addition, the tubular epithelium of the cortical tubules showed cloudy to vacuolar degeneration (Figure 2). Hyaline was also observed in the lumen of cortical tubules (Figure 3), and corticomedullary hyperemia was present. In some individuals (n=3), the deposits of hyaline from the urinary space have led to compression atrophy of vascular glomerular tuft. In contrast to Lerolle *et al.* (2010) and Takasu *et al.* (2013), which found that most nephrons appeared normal, we observed that these changes affected the majority of corpuscles in the cortex.

Regarding the liver state in group 2 individuals, the lesions were represented by necrotic miliary foci (Figure 4). Each miliary focus of necrosis appeared to be coagulative necrosis of a small group of hepatocytes. Differently, from the findings published by Muftuoglu *et al.* (2006), the inflammatory reactions identified in liver samples from rats in the current study were only negligible.

The host barrier disruption model such as the CLP model is widely used and even considered the “golden standard” for sepsis research (Wichterman *et al.*, 1980; Parker and Watkins, 2001). CLP in rats mimics the fundamental clinical features of human sepsis including the following development of multi-organ dysfunction. Therefore, it appears to be the best currently available animal model used in sepsis research. In the CLP model, the infective selection is uninterrupted due to the presence of a complete range of enteric pathogens in the septic focus. Hence, CLP resembles the clinical condition of bowel perforation, with the consequential infection caused by a bacterial mix originating in the intestine (Brooks *et al.*, 2007). Several characteristics have been shown to exist in both rat CLP models and humans with sepsis, including progressive bacteremia, fever, increased metabolism and changes in blood flow (Wichterman *et al.*, 1980).

Conclusion

The histological findings described in a range of sepsis-induced protocols are scarce. The main renal lesions detected histologically in CLP rat

Figure 1. Glomerular congestion and edema (black arrow); hyalinization of glomerular mesangium associated with a reduction of the urinary space (grey arrows); Kidney, H&E stain, ob. 40x.

Figure 2. Cloudy to vacuolar nephrosis (black arrows) of the urinary epithelium of the nephron's tubules; Kidney, H&E stain, ob. 40x.

Figure 3. Presence of discrete proteinaceous material (hyaline) in the lumen of cortical tubules (black arrows); Kidney, H&E stain, ob. 40x.

Figure 4. A miliary focus of hepatic necrosis with a negligible inflammatory reaction (black arrow); Liver, H&E stain, ob. 40x.

model of sepsis were: glomerular congestion and edema, hyalinization of glomerular mesangium, presence of hyaline in the urinary space with associated compression atrophy of vascular glomerular tuft, discrete granules of hyaline in the lumen of cortical tubules, and reduction of the urinary space. The hepatic lesion identified histologically was represented by isolated miliary necrotic foci.

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