Evaluation of Renal Function in Septic Spontaneously Hypertensive Rat

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Abstract: Sepsis is a characteristic set of systemic reactions in response to a severe infection that causes multiple organ injury and hemodynamic changes. Experimentally, eighteen hours after cecal ligation and puncture (CLP) in a group of spontaneously hypertensive rats (SHR) and WKy rats caused a progressive reduction in urinary excretion and in glomerular filtration rate. Caused the increase to 120 minutes of evaluation for fractional excretion of potassium and sodium with rejection by the proximal tubular renal in groups and reduction of blood pressure in rats. Additional studies are underway to examine renal function, blood pressure changes and mechanisms that contribute to sepsis in SHR aimed at distinguishing the systemic hemodynamics phase of hyper- and hypodynamic states.

Keywords: SHR; Renal Function; Blood Pressure; CLP model; Lithium Clearance

INTRODUCTION

Sepsis is a characteristic set of systemic reactions to severe infection, which are accompanied by a decline in immunological function and by multiple organ injury. In addition to inflammation and immunological dysregulation, a number of different mechanisms contribute to sepsis in distinct phases of systemic
hemodynamics that develop from hyperdynamic to hypodynamic states. However, an intra-abdominal abscess can cause such a condition as well as devitalized tissue, both common sources in septicemic patients and animal models. In summary, the animal models for studies can be divided into three categories: - (i) infusion or instillation of exogenous bacteria; - (ii) injection of an exogenous toxin with lipopolysaccharide (LPS); - (iii) alteration of the animal’s endogenous protective barrier, such as intestinal leakage by the cecal ligation and puncture (CLP) or colon ascendens stent peritonitis (CASP). However, all display some limitation in the experimental protocol with regard to the stimulus, site, administration route, and kinetics of the release of inflammatory mediators. However, CLP is currently the most widely used animal model of sepsis, because it mimics the clinical conditions of meningococcal sepsis or postsurgical peritonitis in humans. The model is ligation distal to the ileocecal valve and puncture of the ligated cecum, causing leakage of fecal contents into the peritoneum, with subsequent polymicrobial bacteremia and sepsis. This surgical manipulation, while not well standardized, allows the severity to be adjusted by the length of ligated cecum and the size and/or number of the punctures leading to the infection picture together with the hemodynamic alterations. There has been much effort to clarify the metabolic and renal function changes in sepsis. On the order hand, there has been interest in hemodynamic instability, such as studies on arterial hypertension, for a long time. Arterial hypertension is recognized as one risk factor and/or accelerator of the progression of renal injury when in advanced stages, mainly when comparing the responses obtained to the same maintenance treatment and hemodynamics and metabolic support of arterial hypertensive and normotensive patients becoming theme of the times. In 1963, Okamoto and Aoki introduced an animal experimentation model to study arterial hypertension without the need of any physiological, pharmacological or surgical intervention: the spontaneously hypertensive rat (SHR) allowed a great advance for the better understanding of the illness. The objective of this work was to elucidate the possible hemodynamic alterations and/or complications associated with physiological mechanisms of renal function (glomerular filtration and renal water-electrolyte handling) in septic SHR using the CLP model.

MATERIAL AND METHODS

The experiments were carried out in groups (n=5) of WKy and SHR male rats (twelve weeks of age); they were allowed free access to tap water and standard rat chow (Nuvilab Radiated- Nuvital Nutrientes S/A, Brazil). The rats were housed under controlled climatic conditions in accordance with current international bioethics and biosafety norms for animal experimentation and with guidelines of the Brazilian College of Animal Experimentation (COBEA). Cecal ligation and puncture (CLP) – The procedure for induced sepsis as previously described by Wichterman and coworkers in 1980 is outlined in Figure 1. Renal function and blood pressure were assessed in SHR and WKy eighteen hours after sepsis.
induction using the CLP model. Tail blood pressure (mmHg) – This parameter was measured using an electrophysgmanometer (Narco Bio-System, Austin, TX, USA). This indirect approach permits repeated measurements with close correlation with direct intra-arterial recording. Renal test - Fourteen hours prior, 60 mmol LiCl/100 g body weight was given by gavage. The unanesthetized rats were subsequently housed individually in metabolic cages with free access to tap water but no food. The experiment was performed at the same time in each group. At 8:00 a.m., each rat received a tap water load (5% of body weight) by gavage followed by a second load of the same volume 1 h later. Twenty minutes after the second load, spontaneously voided urine was collected over periods of 30, 60, 90 and 120 minutes. The voided urine passed through the funnel in the bottom of the cage into a graduated centrifuge tube. At the end of the experiment, blood samples were drawn by cardiac puncture. Biochemical analysis - Plasma and urine sodium, potassium and lithium concentrations were measured by flame photometry, while creatinine levels were determined spectrophotometrically by the alkaline picrate method. Statistics and calculations – The results are reported as Means ± SD per 100 gram body weight. Urinary excretion was assessed in each rat in microliters per minute per 100 gram (VU µl/min/100 g) for control of renal water handling. Creatinine clearance (C_{CR}) was used to estimate glomerular filtration rate (GFR), and lithium clearance (C_{Li}) was used to assess proximal tubule output. Fractional sodium excretion (FE_{Na}) was calculated as C_{Na}/C_{Cr}, where C_{Na} is sodium clearance and C_{Cr} is creatinine clearance. The fractional proximal (FEP_{Na}) and post-proximal (FEPP_{Na}) sodium excretion rates were calculated as C_{Li}/C_{Cr} × 100 and C_{Na} / C_{Li} × 100, respectively. Fractional potassium excretion (FE_{K}) was calculated as (C_{K}/C_{Cr}) where C_{K} is potassium clearance and C_{Cr} is creatinine clearance. The renal data were expressed as a percentage of WKy and SHR values obtained at 30, 60, 90 and 120 minutes. Statistical analysis – The data evaluated by repeated measures ANOVA. Bonferroni’s post-hoc analysis was used to determine the extent of the differences. p ≤ 0.05 was considered significant and indicated by an asterisk (*).

RESULTS AND DISCUSSION

All CLP rats showed lethargy and piloerection. Eighteen hours after, following the slight decrease in pressure levels in the two groups, there was a gradual decrease in urinary excretion and glomerular filtration rate, which was more substantial in SHR. At 120 minutes assessment, there was an increase in fractional excretion of potassium and sodium with rejection by the proximal tubule. For better understanding, it is necessary to consider some physiological mechanisms that can account for a decline in renal function, considering the capacity of fine adjustment of excretion of sodium in order to keep the constant cell volume. GFR can diminish due to decreased capacity of plasma flow and glomerular hydraulic pressure caused by the the vasoconstriction of the glomerular arterioles and mediated by vasoconstrictors. Under such conditions, if not controlled, plasma creatinine levels increase inversely with the magnitude of GFR decline. Despite
the reduction in urinary flow and GFR, the fractional excretion levels of sodium and potassium continued to be the same up to 120 minutes observation. At this assessment time, there was an increase in the fractional excretion of potassium (FEK) and sodium (FENa) with rejection by the proximal tubule (FEPNa) in WKy and SHR, indicating the beginning of functional failure of nephrons in this evaluation period. However, it has been reported that small physiological changes can occur 2 hours after the induction of disease in an experimental model [7]. Anyway, it can be suggested that the response of renal function is associated with an interaction of various physiological mechanisms, including hormonal mechanisms and renal arteriolar vasoconstriction [28]; overactivity of the sympathetic nervous system [29], and direct effects on the kidney tubules [19]. Moreover, one has to accept that in hypertensive rats increased peripheral vascular resistance, dependent on neurogenic mechanisms, contributes to the disorder of the central mechanisms affecting blood pressure over the glomerular filtration rate and renal tubular sodium balance as shown (Figure 2 and Table 1). It is known that in WKy the autoregulation of renal blood flow is much more efficient than in SHR [1]. Studies have shown that kidneys of SHR require a higher arterial pressure than kidneys of normotensive rats to excrete the same amount of sodium under basal conditions [5] as can be seen in our results showing that blood pressure was still high after 18 hours of sepsis induction. Since glomerular perfusion pressure was reduced down to that observed in normotensive rats, urinary sodium excretion in SHR may be more affected and reduced [25] but also reflect on the abnormalities in intracellular electrolyte balance with increased sodium in the SHR strain [10]. However, this slight decrease in blood pressure in the animals may be indicative of the manifestation of a hypodynamic phase of sepsis independent of animal species in the CLP model. However, at the cellular level, the presence of bacterial products in the systemic circulation activates the inflammatory cells infiltrating the kidney tissue, causing a release of oxygen free radicals, proteases and inflammatory cytokines. This can lead to injury and activation of resident renal cells (endothelial, mesangial and tubular), prompting them to cause metabolic changes, producing and releasing locally the same and other mediators of hemodynamic and systemic inflammatory responses, such as angiotensin II (Ang II) [18], endothelin-1 (ET-1) [27], platelet activating factor (PAF) [32], tumor necrosis factor (TNF) [20], leukotrienes (LTS) [2], nitric oxide (NO) [22,35] and thromboxane A2 (TXA2) [15], contributing extensively to the acute renal dysfunction observed in septic animals.

**CONCLUSION**

Although the results include mechanisms for direct associations linked to hypertension, the CLP and lithium clearance technique proved effective in investigating the development of sepsis and the changes in renal water-electrolyte handling in animals, especially with excellent correlations to the clinic. However, progress continues in an additional study associating the hemodynamic response
Evaluation of renal function

of renal function, evaluating time-response relations after sepsis induction by CLP, with renal function and hypertension in these rat strains.

FIGURE 1 – RODENT CLP MODEL: WKy and SHR were weighed using a digital electronic balance. They received pre-induction anesthetic with atropine (0.02 to 0.04 mg / kg, ip) and after 10 minutes, ketamine (75-100 mg / kg body weight, ip) + xylazine (5-10 mg / kg body weight, ip), inserting the needle at an angle of 20 degrees in the lower left quadrant to preserve the cecum that is above. (a) After the abolition of the corneal-palpebral and foot reflexes, the animals were placed on a surgical thermal table and immobilized with clamps. A gel eye shield was then applied during anesthesia. After shaving the abdomen, ventral surface, followed by antisepsis of the region with polyvinylpyrrolidone iodine, (b) an incision was made approximately two centimeters into the abdomen including skin, muscle-aponeurotic plane and peritoneum, (c) exposing the viscera, (d) identifying and externalizing the cecum. (e) The cecum was ligated with 0-0 silk below the ileocecal valve with total obstruction in order to increase the total pressure within this segment of the intestine without causing ischemia but not allowing the free passage of the contents of the small intestine into the large intestine. (f) After ligation, the cecum was punctured five times with a 21-G venipuncture needle and gently pressed to verify leakage of feces into the abdominal cavity and put back into abdomen. (g) The suture was performed with 4-0 nylon monofilament, 2 planes: peritoneum-muscle-aponeurosis and skin. The rats received analgesic, buprenorphine (0.05-0.1 mg / kg, sc, 12-12 hours). They were placed under an incandescent light and, after recovery from anesthesia, returned to their boxes with free access to food and water.
TABLE 1 - RENAL FUNCTION STUDY AND TAIL BLOOD PRESSURE: 18 hours after induction of sepsis (CLP-model) in rats of WKy and SHR strains. The results are presented as the means (M) standard deviations (SD) obtained at 30, 60, 90, 120 minutes for assessment of urine volume in microliters per minute per 100 gram of body mass of the animal (VUμl/min/100g), creatinine clearance (CCr), fractional sodium excretion (FE\textsubscript{Na}) - proximal (FEP\textsubscript{Na}) - post-proximal (FEPP\textsubscript{Na}), fractional potassium excretion (FEK) and tail blood pressure (mmHg). The number of animals in each group is n =5 and values of the average computation of means and standard deviation (M ± SD). p≤ 0.05 was considered significant and indicated by an asterisk (*).
Evaluation of renal function

**FIGURE 2- RENAL FUNCTION STUDY:** 18 hours after induction of sepsis (CLP-model) in rats of WKy and SHR strains, which indicates the results obtained in periods of 30, 60, 90, 120-minute assessment of urine volume in microliters per minute per 100 gram of corporal mass of the animal (VUμl/min/100g), creatinine clearance (CCr), fractional sodium excretion (FE_{Na}) - proximal (FEP_{Na}) - post-proximal (FEPP_{Na}), fractional potassium excretion (FE_{K}). p≤ 0.05 was considered significant and indicated by an asterisk (*).

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Evaluation of renal function


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