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The sepsis as cause of acute kidney injury: an experimental model

A SEPSE COMO CAUSA DE LESÃO RENAL AGUDA: MODELO EXPERIMENTAL

LA SEPSIS COMO CAUSA DE LA LESIÓN RENAL AGUDA INDUCIDA: MODELO EXPERIMENTAL

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ABSTRACT

Sepsis associated with multiple organ failure such as acute kidney injury (AKI) shows a high mortality rate in critically ill patients. This study investigated the sepsis induced AKI in experimental models. Adult, males, Wistar rats divided into the following groups: Control-surgical control and Sepsis-sepsis induction for the cecal ligation and puncture (CLP). Physiological parameters (rectal temperature, mean arterial pressure-MAP, serum glucose and urinary flow); renal function (creatinine clearance); oxidative stress (urinary peroxides and thiobarbituric acid reactive substances-TBARS) and kidney histological analysis were evaluated. That study concludes that sepsis induces AKI by endothelial injury with hemodynamic dysfunction, release of inflammatory mediators and reactive oxygen species (ROS) generation by tubular cells, in an association of renal vasoconstriction due to hemodynamic and inflammatory disturbances.

DESCRIPTORS

Acute kidney injury
Sepsis
Inflammation
Oxidation
Animal experimentation

RESUMO

A sepse associada à falência de múltiplos órgãos como a lesão renal aguda (LRA) demonstra alta taxa de mortalidade no paciente crítico. Este estudo investigou a LRA induzida pela sepse em modelo experimental. Foram utilizados ratos da raça Wistar, adultos e machos divididos nos seguintes grupos: Controle – controle cirúrgico e Sepse – indução da sepse pela ligadura e punção do cécon (LPC). Foram avaliados os parâmetros fisiológicos (temperatura retal, pressão arterial média – PAM, glicemia sérica e fluxo urinário); a função renal (clearance de creatinina); o estresse oxidativo (peróxidos urinários e substâncias reativas com ácido tiobarbitúrico – TBARS) e realizada a análise histológica renal. O estudo conclui que a LRA induzida pela sepse caracteriza-se por lesão endotelial com disfunção hemodinâmica, liberação de mediadores inflamatórios e geração de espécies reativas de oxigênio (EROs) por células tubulares, caracterizando-se como uma associação de vasoconstrição renal de origem hemodinâmica e inflamatória.

DESCRITORES

Lesão renal aguda
Sepse
Inflamação
Oxidação
Experimentação animal

RESUMEN

La sepsis asociada la insuficiencia múltiple de órganos como la lesión renal aguda (LRA) muestra una alta tasa de mortalidade en pacientes críticos. Este estudio investigó los mecanismos implicados en la LRA inducida por la sepsis en modelo experimental. Fueron utilizados ratones Wistar, adultos y machos distribuidos en los grupos: Control - control de cirugía, Sepsis - inducción por l ligadura y cecon punción (LPC). Se evaluon los parámetros fisiológicos (temperatura rectal, presión arterial media - PAM, glucosa en suero y flujo de orina), función renal (clearance de creatinina), estrés oxidativo (peróxidos urinarios y sustancias reactivas al ácido tiobarbitúrico - TBARS) y histología renal. El estudio concluye que LRA inducida por la sepsis caracteriza por una lesión endotelial con disfunción hemodinámica, liberación de mediadores inflamatorios y generación de especies reactivas del oxígeno (EROs) por las células tubulares que caracteriza como una asociación de la vasoconstricción renal causas hemodinámica e inflamatorias.

DESCRIPTORES

Lesión renal aguda
Sepsis
Inflamación
Oxidación
Experimentación animal

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INTRODUCTION

Acute kidney injury (AKI) is as an abrupt reduction in glomerular filtration (GF) and the first diagnostic test of kidney function loss is clinically evidenced by an increase in serum creatinine levels, criteria that are imprecise and tardy[1-2]. In 2004, to standardize the AKI diagnostic criteria, the ADQI (Acute Dialysis Quality) group proposed the AKI severity classification: RIFLE (Risk, Injury, Failure, Loss, End)[3]. Furthermore, another research group, AKIN (Acute Kidney Injury Network), changed the RIFLE classification and defined AKI as an increase in serum creatinine levels by 0.3 mg/dl or more or a percentage increase of 1.5 times the baseline value or more in the last 48 hours. Additionally, the decreased urine output criteria came to exert a marker function. This diagnostic consensus are the most accepted today[4].

AKI can occur in different clinical situations, including sepsis. A classic multicenter research in intensive care units, involving 29,269 patients, confirmed the incidence of AKI in approximately 6% of critical patient, with sepsis and its severe form, septic shock, corresponding to 50% of cases[2]. Other studies present incidence between 45% and 70% for sepsis-associated AKI[5].

Sepsis is characterized as an intense inflammatory response associated with severe systemic bacterial infection and presented high mortality in intensive care units, particularly in cases of multiple organ failure, like AKI, which attain rates between 20% and 35%[1,5]. Although AKI is described as a reversible syndrome, the development of chronic kidney failure or terminal kidney diseases should be considered in sepsis cases[7]. Various pathophysiological studies have been proposed to describe sepsis-induced AKI, including vasodilation that induces glomerular hypoperfusion, inflammatory process, oxidative injury and tubular dysfunction(8). The exact mechanism involved in sepsis-induced AKI is not clearly understood, due to difficulties to obtain histological data or biochemical kidney function marks in the different sepsis phases in clinical studies[9].

Although AKI is described as a reversible syndrome, the development of chronic kidney failure or terminal kidney diseases should be considered in sepsis cases.

Experimental studies involving animal models are fundamental to better define and characterize the pathophysiological phases and to suggest treatment and monitoring strategies that alter the unfavorable statistics of sepsis-induced AKI[9]. Animal models of sepsis induction are defined in three categories: lipopolysaccharide (LPS) induced inflammation model. LPS is a gram-negative bacteria cell membrane component, the infusion results in a systemic inflammatory process that reflects the initial phase of clinical sepsis and includes the induction of pro-inflammatory cytokines like the tumor necrosis factor (TNF-α) and interleukin-1 (IL-1); administration of exogenous bacteria in the animal and reproduction of infectious state, a technique e used in large animals for the hemodynamic study of a specific organ; and the cecal ligation puncture (CLP) technique, which is widely used for sepsis induction in animal models, characterized by the distal ligation of the ileocecal valve and needle puncture and ligated cecum cause leakage of fecal contents into the peritoneum of the animal, subsequent to bacteremia and sepsis[9].

Sepsis is a severe syndrome with multiple causes. Clinical reactions are extremely aggressive and few pharmacological interventions have been successful, as its evolution is rapid and uncontrolled, without signs of the best clinical moment to intervene. The study of sepsis in humans is restricted for ethical reasons and the speed at which negative outcomes happen.

Therefore, this study departs from the hypothesis that the animal sepsis model will serve as an important instrument to describe mechanisms that can clarify the pathophysiology of this inflammatory syndrome.

OBJECTIVES

To characterize mechanisms involved in the physiopathology of sepsis-induced AKI in rats.

To standardize the experimental sepsis model in animals.

METHOD

All procedures in this study are in compliance with Ethical Principles of Animal Care adopted by the Brazilian College of Animal Experimentation – COBEA and received approval from the Ethics Committee on Animal Experimentation at the Institute of Biological Sciences of the University of São Paulo, protocol 92 on page 106, book 02 for the use of animals in tests. All animals had free access to water and food and were kept in thermal conditions with day and night cycles during the experiment.

Adult male Wistar rats were used, weighing between 250 and 300 grams, divided in the following groups: *Control* – surgical control with surgical simulation without the accomplishment of the CLP technique; *Sepsis* – sepsis induction through the CLP technique.

The animals were anesthetized with the intraperitoneal (i.p.) injection of 40-50 mg/kg of sodium thiopental, submitted to laparotomy performed with LPC technique and placed in metabolic cages for 24-hour urine collection. After this period, the animals were anesthetized with 60 mg/kg of sodium thiopental for a new laparotomy with abdominal aorta puncture and blood collection for renal function (RF), tubular function and oxidative stress measurements. The left kidney was removed and prepared for histological sections and hematoxylin-eosin

was used to stain the renal tissue. Physiological parameters were evaluated in this last phase: rectal temperature, mean arterial pressure (MAP), serum glucose and urinary output.

Renal function (RF): Creatinine clearance, using Jaffé's method to measure plasma, and urine creatinine were evaluated[10].

Oxidative stress: Urinary hydrogen peroxide (UHP) measurement using the FOX-2 method. The measurement of UHP levels is considered a biomarker of hydrogen peroxide production and an oxidative stress predictor in experimental models *in vivo* [11].The determination of urinary TBARS (thiobarbituric acid reactive substances) levels is an analytic method used to detect thiobarbituric acid reactive aldehydes. Malondialdehyde (MDA) is one of the aldehydes that is frequently analyzed in lipid peroxidation measurement methods[12].

Serum glucose: serum glucose was measured in a blood sample obtained from the abdominal aorta at the end of the experiment.

Statistical Analysis: Univariate Anova and Bonferroni multiple comparison test were used. Statistical significance was set as $p < 0.05$.

RESULTS

Table 1 – Physiological parameters

Groups	Rectal Temperature (°C)	MAP (mmHg)	Capillary Glucose (mg/dl)
Control (n=6)	36.4±0.1	88±3	148±55
Sepsis (n=6)	34.3±1.1*	60±2*	250±44

MAP – mean arterial pressure *p < 0.05 *versus* Control

Table 1 shows that the sepsis induced animals presented reduction of rectal temperature ($p < 0.05$) and mean arterial pressure ($p < 0.05$), with a non significant increase in capillary glucose levels, and maintained urinary output.

Table 2 – Renal function

Groups	Urea (mg/dl)	Urea (mg/dl)	Urinary Output (ml/min)	*Creatinine Clearance/100g (mg/dl)*
Control (n = 6)	48±8	0.41±0.33	0.007±0.002	0.65±0.15
Sepsis (n = 6)	99±6	0.70±0.36	0.010±0.008	0.21±0.10*

*p < 0.001 *versus* Control

Table 2 demonstrates the RF of the groups: the Sepsis group presented no statistically significant increase in urea and creatinine plasma levels and maintained urine output. A significant decrease in creatinine clearance was observed in the Sepsis group, confirming sepsis induced no oliguric renal injury.

Table 3 – Oxidative stress

Groups	Urinary Hydrogen Peroxides (nmol/mg creatinine)	Urinary TBARS (nmol/mg creatinine)
Control (n = 6)	2.2±2.3	4.3±2.2
Sepsis (n = 6)	12.5±2.2*	13.0±3.4*

TBARS – Thiobarbituric acid reactive substances
*p < 0.05 *versus* Control

Table 3 shows that animals of the Sepsis group increased urinary hydrogen peroxide and TBARS levels compared to the Control group, confirming the redox imbalance and release of higher levels of aldehydes, such as MDA (urinary TBARS), and the presence of oxidative injury by lipid peroxidation.

Figure 1 illustrates the histological sections for group. The Control group (**A**) demonstrated no tubular alterations in the histological sections. On the other hand, the Sepsis group (**B**) was characterized by edema and diffused interstitial inflammatory infiltrate, flattened tubular cells with of tubular lumen dilatation and denuded basement membrane in the cortical region.

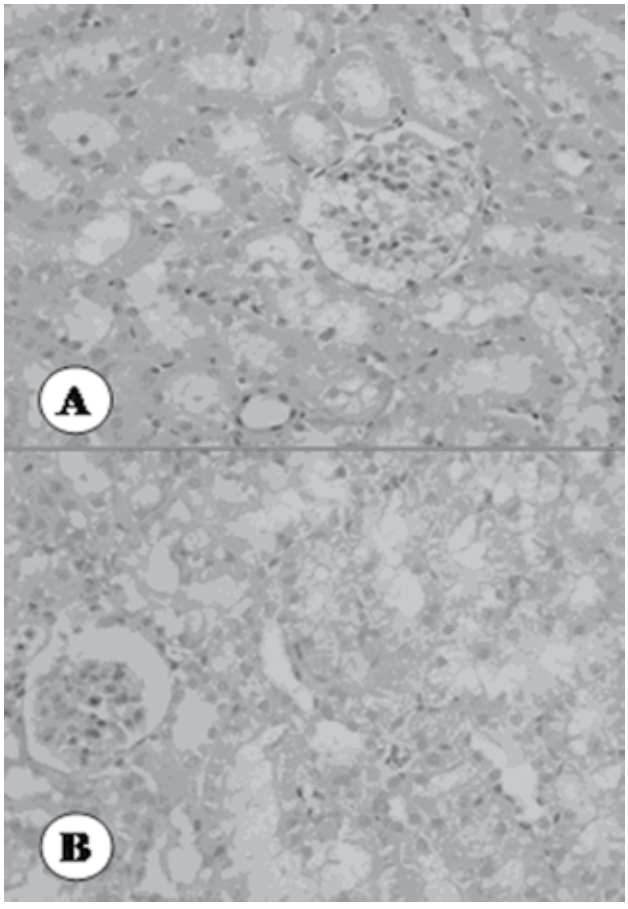

Figure 1 – Histological sections for group analysis (400X increase): Control (**A**) and Sepsis (**B**).

DISCUSSION

Sepsis is characterized as a complex, nonlinear syndrome with great variations and criteria and definitions divided in large groups: sepsis, severe sepsis and septic shock[7,12]. It is also defined as an inflammatory response and immunological dysfunction that leads to a hyperdynamic state in early phases to a hypodynamic state in late phases[8].

The first clinical signs of sepsis are tachycardia, hypotension, hyperthermia or hypothermia, gradually evolving to multiple organ failure[9,13]. The characteristics of the Sepsis group include hypothermia, decrease in MAP to 60 mmHg, AKI confirmed by increased urea and serum creatinine levels with a reduction in creatinine clearance.

Renal ischemia/reperfusion mechanism is described as the main cause of sepsis-associated AKI. The reduction in renal blood flow and hypoperfusion result in low oxygen demands, which induce the tubular epithelial cell injury, apoptosis and acute tubular necrosis in prolonged hypoperfusion[14]. Other factors associated with the pathophysiology of sepsis, such as absolute hypovolemia, vasoplegia and capillary extravasations with interstitial edema contribute to the reduced oxygen transportation[15]. Renal failure happens when the organ function is compromised, with signs of water and electrolyte homeostasis imbalance and accumulation by reduction of the metabolic excretion of nitrogenous products, including serum urea and creatinine [9,14].

The study confirmed the reduction in creatinine clearance in Sepsis animals, highlighting the renal dysfunction. However, no significant reduction was demonstrated. The sepsis animal presented reduction of urine output. When applying the severe AKI classification system, AKIN criteria, mentioned in the introduction, this group would categorize in category 1 (risk), due to the serum creatinine levels of 0.3 mg/dl or higher or increase in baseline levels by 1.5-2 times. The increase in serum creatinine and reduced GF rate have been described as secondary events by the decreased renal vascular resistance (RVR) in an experimental study using a sepsis induction animal model[16]. The presence of endotoxins, inflammatory response with release of TNF-α, interferon-γ (IFN-γ) and IL-1 induce the expression of inducible nitric oxide synthase enzymes (iNOS) in the renal medulla, mesangial glomerular cells and endothelial renal artery cells, including the intense and continuous release of nitric oxide (NO) concentrations that induced vasodilation and reduced RVR[6,9]. At the same time, the acidosis deriving from septic shock causes low cellular ATP levels in the endothelial smooth muscle cells, enhancing cell hyperpolarization and the exit of the potassium ion through the ATP-dependent membrane channels, and perpetuates the vasodilation effects, systemic hypotension and resistance to catecholamines and angiotensin II[9].

Endothelial injury in sepsis-induced AKI generates the formation of intracellular adhesion molecules (ICAM-1), adhesion molecules (VCAM) and P and E-selectins, which promote leukocyte-endothelial interaction, platelet adhesion and mechanical obstruction of the renal microvasculature[17]. Endothelial dysfunction generates the formation of proinflammatory cytokines by the tubular cells that intensify the inflammation process and induces apoptosis, mainly of tubular cells, and oxidative injury through the production of reactive oxygen species (ROSs)[9,17].

In this study, the analysis of the oxidative stress component for the Sepsis group suggests oxidative injury by the increased of intermediary hydrogen peroxide and urinary MDA as the end product of lipid peroxidation. The description of oxidative stress in sepsis is related to the increased production of ROSs and reduction in antioxidant levels[18]. Another factor that intensifies the oxidative injury during sepsis is the inflammatory cascade and nitric oxide (NO) generation, considered a free radical that can interact with ROSs and form toxic molecules, such as peroxynitrite, even more toxic effects by oxidation and injury cell membrane proteins [9].

The histological analysis of the renal cortex showed edema and diffused interstitial inflammatory infiltrate, flattened tubular cells with tubular lumen dilatation and denuded basement membrane in the cortical region for the Sepsis group. Studies involving histological analysis in sepsis-induced AKI models demonstrated heterogeneity, with relevant inflammatory infiltrate and altered morphology of tubular cells, including loss of brush borders and apoptosis. Other alterations are also described, including intercellular junction dysfunction, which enhances tubular fluid reflux through the renal epithelium, the basement membrane that continues cellular detachment and formation of debris in the tubular lumen, accompanied by the sediments and tubular cylinders in the urine, but were not identified in this study[19].

In summary, adhesion molecules are active in the endothelium in sepsis cases, resulting in leukocyte-endothelial integration. The active leukocytes increase the release of inflammatory mediators and the generation of ROSs by tubular cells, contributing to the progression of the injury and organ failure, characterizing sepsis-induced AKI. These more intimate injury mechanisms were not evaluated in this study, but the lipid peroxidation results in the Sepsis group presuppose that this cellular and molecular disorganization was present.

The renal function response and precise manifestation of redox imbalance in this study characterized the noxious effect of sepsis on the kidney and clarified that the production of free oxygen species, probably associated with the consumption of the endogenous antioxidant reserve, are the main mechanisms triggering this complication. These data reinforce the property of the experimental sepsis model as a promising alternative for research on this theme.

CONCLUSION

This animal model of sepsis-induced AKI is characterized by endothelial injury with hemodynamic dysfunction, release of inflammatory mediators and ROSs generation by tubular cells. It should be reminded that multiple pathophysiological mechanisms and associated factors are involved in this AKI. Understanding this whole dynamics is necessary to put in practice strategies that favorable affect the epidemiology of this syndrome.

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