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Original Article

SEPSIS MODEL TO INDUCE SYNDROME OF MULTIPLE ORGAN DYSFUNCTION: AN EXPERIMENTAL STUDY IN RATS

Síndrome de disfunção de múltiplos órgãos induzida por sepse: estudo experimental em ratos

João Batista de Area **LIMA**, Thelma Larocca **SKARE**, Osvaldo **MALAFAIA**, Jurandir Marcondes **RIBAS-FILHO**, Tiago **MICHAELIS**, Fernanda Marcondes **RIBAS**, Rodrigo de Almeida Coelho **MACEDO**

From Medical Research Institute of the Curitiba Evangelical Hospital, Evangelical University of Paraná, Curitiba, Paraná, Brazil

ABSTRACT - ***Background*** - The leading cause of death in patients with sepsis in surgery is syndrome of multiple organ dysfunction. Thus, experimental models that simulate organic changes of sepsis in humans are required. ***Aim*** - To present two models that induce the syndrome of multiple organ dysfunction and to compare, the changes induced, by intravenous injection of lipopolysaccharide or cell 36UE of viable *Escherichia coli* in relation to mortality and survival, level of lipopolysaccharide, release of tumor necrosis factor alpha ; hematological, liver and kidney function. ***Method*** - The study lasted seven days and it was used on it 50 male Wistar rats divided into three groups: control, lipopolysaccharide and *Escherichia coli*. The experimental groups were inoculated and divided into two subgroups, with inocuation with 24 or 48 hours. On the seventh day were proceeded blood collection and histopathologic analysis of liver, kidneys and lungs. ***Results*** - There was a survival of ten animals in the control group; zero in bacteria group of 24 hours and six in 48 hours; ten of lipopolysaccharide in 24 hours and six in 48 hours. In the experimental groups, levels of endotoxin, tumor necrosis factor alpha, leukocytes, platelets, renal and liver levels were higher than the control group. There were histopathological changes in the bacterial group. ***Conclusion*** - The two models of sepsis induced multiple organ dysfunction syndrome; yet the administration 36UE endotoxin every 48 hours could be utilized in advantage over the other for not induce death in significant numbers during the period of seven days.

HEADINGS - Sepsis. Multiple organ dysfunction syndrome. Sepsis models. Lipopolysaccaride . *Escherichia coli*.

Correspondence:
João Batista de Area Lima,
e-mail: jobalclinica@bol.com.br

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RESUMO – ***Racional*** - A principal causa de morte em pacientes com sepse em cirurgia é a síndrome de disfunção de múltiplos órgãos. Assim, modelos experimentais que simulem alterações orgânicas da sepse em humanos são necessários. ***Objetivo*** - Apresentar dois modelos que induzem a síndrome de disfunção de múltiplos órgãos e comparar as alterações induzidas por inoculação endovenosa de 36UE de lipopolissacarídeo ou célula viável de *Escherichia coli*, em relação à: mortalidade e sobrevivência; nível de lipopolissacarídeo; liberação de fator de necrose tumoral alfa; alterações hematológicas e das funções hepática e renal. ***Método*** - Este estudo teve duração de sete dias e utilizou-se nele 50 ratos Wistar machos, divididos em três grupos: controle, lipopolissacarídeo e *Escherichia coli*. Os grupos experimento eram inoculados e separados em dois subgrupos, com inoculação a cada 24 ou 48 horas. No sétimo dia eram procedidas coletas de sangue e análise histopatológica de fígado, rins e pulmões. ***Resultados*** - Houve sobrevivência de dez animais no grupo controle; zero no bacteriano de 24 horas e seis no de 48 horas; dez no lipopolissacarídeo de 24 horas e seis no de 48 horas. Nos grupos experimentais, os níveis de lipopolissacarídeo, fator de necrose tumoral alfa, leucócitos, plaquetas, bastonetes e as alterações renais e hepáticas foram superiores ao grupo controle. Houve alterações histopatológicas no grupo bacteriano. ***Conclusão*** - Os dois modelos de sepse induziram síndrome de disfunção de múltiplos órgãos, contudo a administração de 36UE de endotoxina a cada 48 horas pode ser utilizada com vantagens sobre os demais por não induzir morte em número significativo durante o período de sete dias.

DESCRITORES - Sepse. Síndrome de disfunção de múltiplos órgãos. Modelos de sepse. Lipopolissacarídeo. *Escherichia coli*.

INTRODUCTION

Over the past decade, advances in the fields of cell and molecular biology have facilitated the understanding of the factors involved in sepsis, making clear both the role of bacterial invasion and the inflammatory host response.

The study of bacteria and their harmful effects on human body has made great progress in the late nineteenth century, when bacteriologists studied the substances secreted by these microorganisms. These substances, called exotoxins, had a common point: inactivation by heating. During this period it was discovered that a substance released by bacterial lysis was resistant to heat, and proved ability to produce important biological effects such as fever and circulatory shock. This substance was called endotoxin[1]. They are composed of polymerized sugars, fatty acids and complex proteins. As the protein portion has pathological performance, the term lipopolysaccharide (LPS) has been used as a synonym of endotoxin[1,2,9]. It is a component of the outer wall of gram-negative and composed of a polysaccharide and a lipid portion[1,2,9].

The polysaccharide portion is divided into two parts. The outer with antigenic properties, induces the formation of specific antibodies against this portion of the molecule[1,5,9]. The innermost, the core, is divided into internal and outer core. In the internal, are acid molecules of 3-deoxy-D-man-2-octulosonic (Kdo) which are found only in LPS, and may point to future forms of therapy[1].

The lipid portion, known as lipid A, has small structural variation and lies within the outer membrane of bacteria, and is responsible for the toxicity of gram-negative bacteria[9,12]. The lipid A consists of glucosamine, phosphate and fatty acid. For the occurrence of his release from the bacterial wall, it is necessary that the molecule becomes active, a phenomenon that occurs when bacteria die or multiply[1].

Among the many models proposed to reproduce in animals the signs of sepsis seen in humans are: inoculation of bacteria or endotoxin intravascular or intraperitoneal cecal ligation and perforation, soft tissue infection, pneumonia and models of meningitis[13]. Hundreds of different models of sepsis have been described, and it is clear, therefore, that there is no single model to suit all applicationsThe ideal model for sepsis should have the following criteria[8]:1. ability to predict the positive and negative outcomes of new therapeutic agents; 2. low cost; 3. be reproducible within a laboratory; 4. be reproducible in different laboratories; 5. be humanitarian; 6. be able to predict the extent of mortality; 7. reproduce the hemodynamic, hematologic and biochemical parameters similarly to observed in human sepsis.

The models of sepsis using intravenous administration of LPS or bacteria represent models without a prior infectious focus[8,10], with advantages over the precise control of infusion dose and reproducible. They have the disadvantage, however, to induce sepsis very quickly, unlike sepsis in humans, where the process begins slowly[21]. The main shortcomings identified in models of sepsis are the use of young animals, with no associated disease, induced generally by a single gram-negative bacteria. This is the opposite of what occurs in most cases of human sepsis, where patients are elderly with concomitant diseases, which is not always known or the causative agent can sometimes be caused by more than one bacteria, fungus or virus[6].

So, to help in the study of sepsis, this study aims to present two models that induce the syndrome of multiple organ dysfunction and to compare the changes induced by intravenous injection of lipopolysaccharide or cell 36UE of viable *Escherichia coli* in relation to mortality and survival level, lipopolysaccharide, release of tumor necrosis factor alpha, hematological, liver and kidney function.

METHODS

Was used 50 rats (*Rattus norvegicus albinus, Rodentia mammalia*), male, Wistar, aged between 123 to 138 days and weighting 236.2 ± 11.4 g and divided in groups (Figure 1).

Groups	Subgrups		Procedures
	Inoculum	N	
Lipopolysaccharide	LPS-1	10	Injection every 24 hours for 7 days
	LPS-2	10	Injection every 48 hours for 7 days
Escherichia coli	EC-1	10	Injection every 24 hours for 7 days
	EC-2	10	Injection every 48 hours for 7 days
Control		10	Without manipulation

FIGURE 1 - Study design

The animals were separated into groups of five, housed in boxes of polypropylene disinfected with sodium hypochlorite 1% containing shaver sterilized by autoclaving - changed every 48 hours - in temperatures between 19 to 23ºC in cycle lighting automatically regulated every 12 hours in a room with forced air extraction. Received a diet specific to the species (Nuvilab - Nuvital®) and water acidified to pH 4.0 ad libitum. During this period tests were conducted for parasites, inspection of the coat for investigation of parasites, excluding the carrier animals. After the screening stayed for two weeks under observation regime before the experiments started.

Preparation of the inoculum of LPS

Was followed the methodology proposed by Morrison and Leive[19] and Fink[8], the solution being prepared daily, two hours before inoculation. Was done by weighing the LPS® Sigma Chemical (Article L-4130 Lot 30k4063), lyophilized, obtained from strain 0111: B4 from *Escherichia coli* coming from the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, in sterilized glass jars and kept in apirogenic refrigeration between 4º C and 8º C, as needed for daily schedule of inoculations. Next step was to solubilize aliquots of LPS in glass bottles of 10 ml with saline solution immediately before their inoculation; an aliquot of the solution for determination of endotoxin by the LAL method (Lymulus Amebocyte lysate) was separated and, also, for standardization of the inoculum diluted to contain 36 units endotoxic/ml (EU/ml).

After identification, sedation with isoflurane (Foran® Abbott) was made taking care not to induce anesthesia, and inoculation of 36 EU of LPS solution was done using disposable insulin syringe in the dorsal penile vein, and held a slight pressure on the puncture site to prevent reflux and observe them for 30 minutes.

All animals were assessed daily, always at the same time, noting in a protocol the following parameters: prostration, piloerection, bloody blepharitis, hematuria, death. In case of death, necropsy was done and taken samples of kidney, liver and lungs for histopathological evaluation (hematoxylin-eosin).

Preparation of inoculum of *Escherichia coli*

Was followed the methodology proposed by Isenberg[15], and the suspension prepared daily two hours before inoculation based on cultivation of *Escherichia coli* 0111: B4 (ATCC 12,015 coming from the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil in brain and heart agar infusion, 24 hours before its preparation; from 18 hours in oven at 37ºC incubation and two hours prior inoculation, samples were collected from the colonies developed in the culture and suspended in saline in the test tube, comparing its turbidity with the ones with the tubes of barium chloride, based on the scale nephelometric Mac-Farland. Defined the concentration, it was proceeded the dilution, in another test tube with saline solution until obtaining a concentration of 10^6 CFU (10^6 CFU/ml), separated an aliquot of *Escherichia coli* for determination of endotoxin by the LAL method. A new culture process with *Escherichia coli* in the same brain and heart infusion agar was done for the next day. After that, it was diluted to contain 36 units of endotoxin/ml (EU/ml).

Inoculation

After checking the box and the identification of each rat, sedation was made in an under saturation isoflurane (Foran® Abbott) atmosphere, taking care not to induce anesthesia. After sedation, inoculation of LPS or 36UE solution of *Escherichia coli* with disposable insulin syringe in the dorsal penile vein was done, and made slight pressure on the puncture site to prevent reflux. The site was observed on inoculation point for 30 minutes.

Blood sampling

On the seventh day of the experiment the rats were anesthetized the same way and realized an intra-cardiac puncture; blood was collected in a 20 ml syringe using 25x8 mm apirogenic needle aspirating at least 10 ml, sufficient for the induction of cardiopulmonary arrest. The blood was separated into aliquots of 2 ml tubes in different measurements for the following tests: 1.leukocytes, platelets and rods; 2. liver function tests - direct bilirubin, total bilirubin, AST and ALT; 3. kidney function - urea and creatinine; 4. doses of endotoxin and tumor necrosis factor alpha (TNF-α); 5. prothrombin time (TAP).

For the determination of endotoxin, was used the method of *Lymulus Amebocyte lysate* (Endosafe® 1073), described by Bertók[4], which has in principle the ability of amoebocytes extracted from crustacean *Limulus polyphemus* gelling in the presence of endotoxin (LPS). This gelation is a consequence of activation of the enzyme serin-protease zymogen in the presence of divalent cations that produce coagulogen *Limulus*

Immunosorbent assay for the determination of plasma levels of tumor necrosis factor alpha (TNF-α) has employed the quantitative enzyme immunoassay[23], in which a specific monoclonal antibody anti-TNF is fixed at TNF plate for later Terazaki capture antigen TNFα. Was used set of reactive Biotra Tumor Necrosis Factor ELISA System (Amersham Pharmacia code RPN 2718®).

Statistical evaluation

The results were compared by ANOVA using the method of Tukey-Kramer. For comparisons between groups used the Student t test. In both evaluations it was adopted $p < 0.05$ as the minimum index of statistical significance.

RESULTS

Table 1 shows the occurrence of mortality among the animals of each group through daily monitoring until the seventh day.

After death, the animals were autopsied and samples of kidney, liver and lungs for histopathological evaluations.

The ones inoculated with LPS died with

TABLE 1 - Survival and mortality of the animals in each subgroup during the seven days of observation

Groups	Subgroups	Total rats	Daily Monitoring														Total number of survivors on the seventh day
			1º day		2º day		3º day		4º day		5º day		6º day		7º day		
			D	S	D	S	D	S	D	S	D	S	D	S	D	S	
LPS	LPS-1	10	0	10	0	10	0	10	0	10	0	10	2	8	2	6	6
	LPS-2	10	0	10	0	10	0	10	0	10	0	10	0	10	0	10	10
Escherichia coli	EC-1	10	0	10	0	10	6	4	2	2	2	0	+	+	10	0	0
	EC-2	10	0	10	0	10	4	6	0	6	0	6	0	6	0	6	6
Control		10	0	10	0	10	0	10	0	10	0	10	0	10	0	10	10

LPS = lipopolysaccharide M = dead, S = survivors, (+) no survivors

prostration and had only piloerection. The necropsy showed no alterations in the kidneys, and liver and lungs appeared congested.

Rats that received inoculations of *E. coli* were prostrated in times prior to death, with piloerection and bloody blepharitis. Macroscopic evaluation of kidneys from this group (subgroup EC-1) revealed a cortical edema and hemorrhagic foci in the medullary region with characteristics of tubular necrosis (Figure 2A). Microscopically was observed tubular congestion and inflammatory infiltrate (Figure 2B).

In Figure 2, also can be seen macro and microscopic appearance of the lung, being prominent intense macroscopic hemorrhage (2C), confirmed by microscopy (2D).

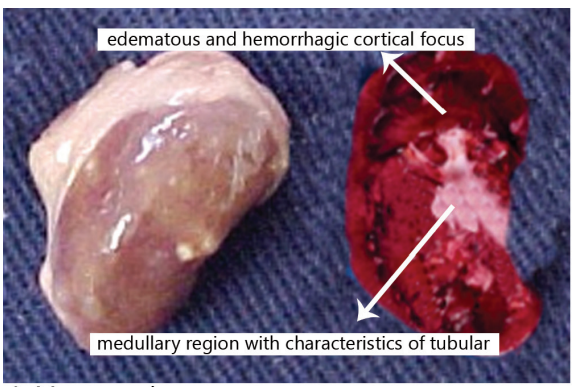
The evaluations reported below refer to groups LPS-1, LPS-2, EC-2 and Control, and not proceeded in group EC-1 since all the rats died during the experiment.

So, on the seventh day of observation of 36 EU inoculation of viable cells of *Escherichia coli* each 24 hours (EC-1) did not allow the survival of animals until the seventh day of the study; inoculation and every 48 hours (EC-2) allowed the survival of six of the ten rats inoculated. The same amount of endotoxin administered every 48 hours (LPS-2) enabled the survival of the ten rats, and in cases of injection every 24 hours (LPS-1) there was a survival of six of ten rats.

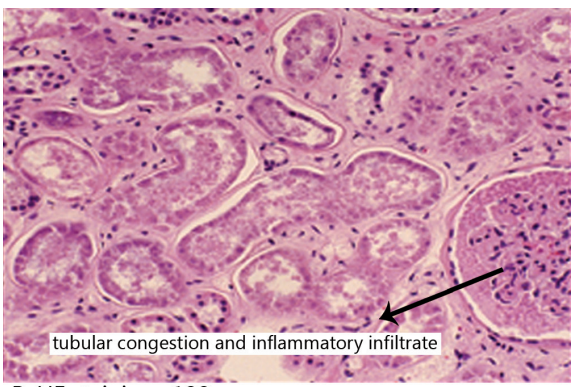
Hematological evaluation

Leukocytosis in the groups inoculated with LPS and *Escherichia coli* (Table 2), white blood cell counts in these groups were higher than in control group. Among the groups inoculated with *Escherichia coli* and LPS no significant leukocytes differences were found.

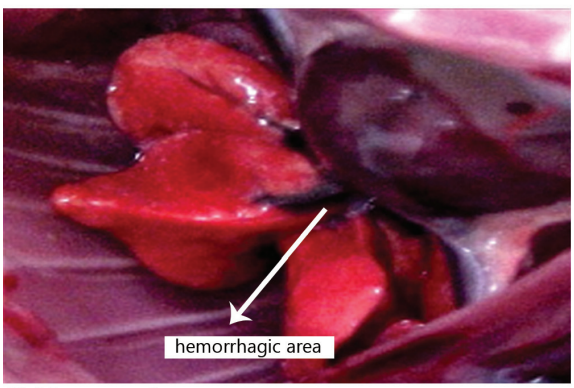
Platelet counts showed significant thrombocytopenia on inoculation groups of *Escherichia*



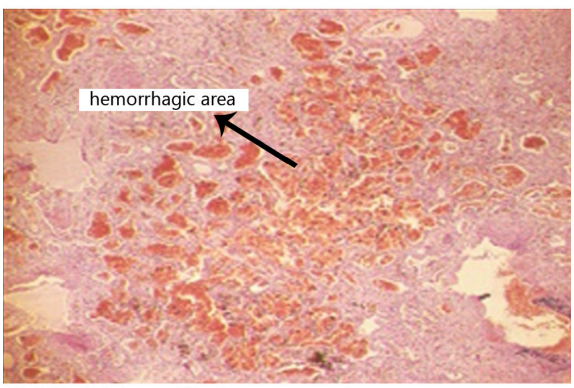
A: Macroscopic



B: HE staining x100



C: Macroscopic



D: HE staining x40 - Lung necrosis

FIGURE 2 - Macroscopic and microscopic kidney (A and B) and lung (C and D) of rat of group EC-1 (*Escherichia coli*)

TABLE 2 - Results on blood, liver and kidney parameters

Hematologic reviews			LPS-1	LPS-2	EC-2	Control
Leukocyte counts (x103/ml)			12,7±3,1	9,9±1,4	10,38±2,9	5,4±1,1
Platelet counts (x103/ml)			67,8±34,0	41,4±22,1	39,1±24,2	381,4±83,7
Counts of rods (%)			29,5±7,7	22,5±9,7	12,2±5,3	0,2±0,6
Groups compared			Leukocyte Count		Platelet Count	Counting rods
Control	X	LPS-1	<0,0001		<0,0001	<0,0001
	X	LPS-2	<0,0001		<0,0001	<0,0001
	X	EC-2	<0,0001		<0,0001	<0,0001
LPS-1	X	LPS-2	0,0267		0,0784	0,1553
	X	EC-2	0,2040		0,1227	0,001
LPS-2	X	EC-2	0,6786		0,8472	0,032
Hepatic review			LPS-1	LPS-2	EC-2	Control
Measurements of SGOT (UI/L)			509,7±131,1	766,3±214,1	588,0±136,4	152,5±60,4
Measurements of ALT (UI/L)			293,8±100,8	315,6±112,8	194,5±23,7	66,1±21,4
BT dosages (mg/dl)			2,0±0,3	1,9±0,3	2,3±0,5	0,3±0,1
Evaluation of TAP (s)			19,3±0,8	17,2±1,6	19,4±1,7	15,9±0,7
Groups compared			TGO	TGP	BT	BT
Control	X	LPS-1	<0,0001	<0,0001	<0,0001	<0,0001
	X	LPS-2	<0,0001	<0,0001	<0,0001	<0,0001
	X	EC-2	<0,0001	<0,0001	<0,0001	<0,0001
LPS-1	X	LPS-2	0,0196	0,7039	0,9230	0,9230
	X	EC-2	0,3344	0,0405	0,2424	0,2424
LPS-2	X	EC-2	0,0906	0,0226	0,1513	0,1513
Renal review			LPS-1	LPS-2	EC-2	Control
Doses of urea (mg/dl)			141,8±39,4	193,2±22,6	97,8±14,4	66,1±17,3
Measurements of creatinine (mg/dl)			1,7±0,43	2,0±0,55	1,2±0,51	0,3±0,12
Groups compared			Doses of urea		Doses of creatinine	
Control	X	LPS-1	<0,0001		<0,0001	
	X	LPS-2	<0,0001		<0,0001	
	X	EC-2	0,0021		0,0001	
LPS-1	X	LPS-2	0,0047		0,2762	
	X	EC-2	0,0278		0,0889	
LPS-2	X	EC-2	<0,0001		0,0109	

coli and LPS against the control group. Among the groups inoculated with *Escherichia coli* and LPS no significant differences were found.

Deviation to the left was significant in the groups inoculated with *Escherichia coli* and LPS, higher than the control group. Among the subgroups inoculated with LPS-1 and LPS-2 no significant difference was noticed. The score of the subgroup EC-2 was significantly lower than the count in subgroups LPS-1 and LPS-2

Evaluation of liver function

Was observed marked increase of dosages of glutamic-oxalacetic transaminase in the groups inoculated with *Escherichia coli* and LPS compared with the control group. There were significantly greater numbers in LPS-2 in relationship to LPS-1. There was no significant difference between subgroups inoculated with LPS-1 and EC-2, and between LPS-2 and EC-2.

Was observed marked increase in plasma levels of glutamic pyruvic transaminase in the groups inoculated with *Escherichia coli* and LPS compared with the control group. There was significant difference between higher numbers on LPS-1 and EC-2, and between LPS-2 and EC-2.

Was observed marked increase in plasma levels of total bilirubin in the groups inoculated with *Escherichia coli* and LPS compared with the control group. Among the subgroups inoculated with LPS or *Escherichia coli* no significant differences were found.

An increase was observed in the evaluations of the TAP in groups inoculated with *Escherichia coli* and LPS against control group. Among inoculated subgroups, LPS-1 subgroup was higher than LPS-2 and, this one, lower than the EC-2 subgroup.

Among the evaluation tests of liver function GOT determinations differentiated groups LPS-1 and LPS-2; TGP between LPS-1 and EC-2, and LPS-2 and EC-2; TAP was useful between LPS -1 and LPS-2, and LPS-2 and EC-2 (Table 2).

Assessment of renal function

Was observed marked increase in urea levels in the groups inoculated with *Escherichia coli* and LPS compared with the control group. A significant increase was also observed on subgroups LPS-1 and LPS-2 relatively to the subgroup EC-2, and LPS-2 in relationship to LPS-1.

Significant increase of serum creatinine in the groups inoculated with *Escherichia coli* and LPS compared with the control group. There was a significant increase in LPS-2 subgroup compared to the subgroup EC-2 (Table 2).

Doses of endotoxin

In the control group there was no detection of endotoxin, therefore it was not possible to compare this result to groups inoculated with *Escherichia coli* and LPS. Among the subgroups no significant differences of dosages were found, showing that serum levels of endotoxin were similar (Table 3).

Thus, in relation to endotoxemia induction, the three subgroups showed levels higher than the control group and no significant difference between endotoxin administration every 24 or 48 hours (sub-1 LPS and LPS-2 respectively) or viable cells of *Escherichia coli* per 48 hours (CE-2) was found.

Measurements of tumor necrosis factor alpha

Was observed marked increase of serum TNΦ-α in the groups inoculated with *Escherichia coli* and LPS compared with the control group. Among the subgroups inoculated, LPS-1 showed significantly higher values than the subgroup EC-2 (Table 3).

36UE administration of endotoxin every 24 hours (LPS-1) did not differ from administration every 48 hours (LPS-2) for seven days.

TABLE 3 - Doses of endotoxin (EU/ml) and tumor necrosis factor-alpha (pg/ml)

Reviews			LPS-1	LPS-2	EC-2	Control
Doses of Endotoxin (UE/ml)			0,098±0,064	0,109±0,060	0,117±0,060	-
Groups compared			**Doses of Endotoxin**			
LPS-1	X	LPS-2	0,7191			
	X	EC-2	0,5994			
LPS-2	X	EC-2	0,8062			
Reviews			**LPS-1**	**LPS-2**	**EC-2**	**Control**
Dosages of TFN-alpha (pg/ml)			0,117±0,060	1.047,30±354,88	895,83±204,01	32,40±7,06
Groups compared			**Dosages of TFN alpha**			
Control	X	LPS-1	**<0,0001**			
	X	LPS-2	**<0,0001**			
	X	EC-2	**<0,0001**			
LPS-1	X	LPS-2	0,1910			
	X	EC-2	**0,0086**			
LPS-2	X	EC-2	0,3594			

DISCUSSION

Was chosen here to induce sepsis through intravenous methodology because it is simple and easily reproducible. Its disadvantage is the inexistence of infection site, resulting in installation of the septic process of abrupt onset. To circumvent this problem was chosen to use induction maintained for seven days, with doses every 24 or 48 hours and not only one inoculation, with large dose, as in other experiments[9]. A way to simulate the sequence of events that occur secondary to an outbreak of infection was tried to be obtained here. The material used in LPS dose was 36 EU/ml solution of lipopolysaccharide, with 2 mg/kg, which is considered low dose, existing literature reports on the use of up to 10 mg/kg[18].

In the case of using *Escherichia coli*, the suspension has also been standardized to contain the same amount, ie 36 units per milliliter of endotoxin. Thus, the two systems used, the EC and LPS contained the same concentration of toxin, bypassed possible bias.

In analyzing the methods used to induce sepsis in groups LPS and EC, it was seen that both groups are controlled models, easily reproducible, but that the EC group required several steps to prepare the inoculum. These bacteria must be grown on brain heart agar for 24 hours, collected and submitted to the determination of the concentration in CFU/ml and subsequently diluted so that the solution may contain 36 EU/ml. Preparation for the LPS group was methodologically simpler, because the procedure required basically weighing and dissolution at the time of inoculation. However, the LPS group had the disadvantage of higher cost.

It is known that sepsis can be triggered by inoculation of viable cells or only the cellular component actually responsible for the outbreak of sepsis, lipopolysaccharide[20]. These two forms of induction are now reflected in the models tested. As performed by the inoculation of micro-living organism, we chose to use *Escherichia coli*, because the gram-negative bacteria are the main related infection and sepsis in humans.

For identification of success on the induction of sepsis in animals, were dosed markers such as the level of endotoxin and TNF-α release. Akamine[1] 1999 reported that endotoxin is related to the triggering of the basic pathophysiological processes in septic shock and that injection of amounts of lipopolysaccharide is able to produce septic shock and death in experimental animals.

Apart from the clinical, hemodynamic and conventional laboratory, several studies have shown to be possible to characterize SIRS through the presence or absence of certain biological markers associated with inflammation and infection[22]. Indeed, a striking characteristic and invariable of SIRS is the induction and release of various cytokines and acute phase proteins, both pro and anti-inflammatory, whose serum levels rise rapidly during the inflammatory response. Hubl, et al.[14], showed that TNF-α and its receptor TNF-R5 (5 receptor of tumor necrosis factor alpha) are consistently increased in sepsis. According to vam Der Pollt e Sauerwein[24], TNF-α it is seen as an important mediator in sepsis from the following evidence: it is the first cytokine which appears in sepsis, its administration in animals induced syndrome with features of sepsis and treatment with anti-TNF therapy against the lethal effects of LPS in several animal models.

Regarding the level of endotoxin not been detected in the control group, it is not possible to compare these results to those inoculated with LPS and *Escherichia coli*. There was an increase in LPS and EC groups and between subgroups no significant difference of dosages, showing that serum levels of endotoxin were similar to characterize sepsis in all groups.

Was observed marked increase of serum TNF-α in groups LPS and *Escherichia coli* in the control group, showing that the two groups were in sepsis by this marker. So, the two models were efficient in presenting both elevation of TNF-α and endotoxin.

Among the rats of EC-1 there were six deaths on the third day, two on the fourth and two on the fifth. Among the subgroup of the EC-2 (inoculation every 48 hours for seven days), four deaths occurred on the third day. Thus, it stands out on the third day of evolution, the highest number of deaths among the rats inoculated with *Escherichia coli*. Those who died within the study were submitted to necropsy and histological evaluation of vital organs like liver, kidneys and lungs. Was checked the same macroscopic findings at necropsy and microscopy of kidneys, lungs and liver of a sub-EC and EC-2.

Ten rats in subgroup EC-1 and four in the EC-2, who died in seven days, showed significant

histopathological changes. In the kidneys there were edema, hemorrhage, congestion and tubular necrosis and even inflammation. In the liver there were congestion, hemorrhage, edema and necrosis. In the lungs, copious bleeding. These findings are consistent with SIRS due to infection, mainly by gram-negative bacteria.

In rat inoculated with LPS had lower mortality rates than those inoculated with *Escherichia coli* , and LPS only in group-1 (injection every 24 hours) there were two deaths on the sixth day and another two in the seventh. Unlike what was observed in animals inoculated with *Escherichia coli*, which had prostration, piloerection, bloody blepharitis just prior to death, the latter died presenting only prostration and piloerection.

In the four rats inoculated with LPS, died every 24 hours over seven days, the autopsy showed no changes in the kidneys and liver, and lungs appeared congested. The death of rats in this group may be explained by acute coagulation disorder, or heart conditions, without objective evidence for confirmation, but discussed and confirmed by Fink e Heard[9], when evaluating the sepsis induced by intravenous infusion of lipopolysaccharide in rats.

In terms of survival, was highlighted the difference observed in terms of inoculation of LPS-2 subgroup, in which they received 36 units endotoxin every 48 hours for seven days. In this group there was no mortality at all. Other groups with a lower mortality rate were the LPS-1 and EC-2, each with six survivors.

In the present study, was leukocytosis in both groups, both in rats inoculated with LPS as with *E. coli* with nuclear deviation to the left. As described[3,24], in an extensive review of mediators in experimental sepsis, the white blood cell counts greater than total blood 12.000/mm^3 or less than 4.000/mm^3 or with more than 10% immature forms are related to installation of the systemic inflammatory response syndrome in sepsis, together with other findings.

Platelet counts showed significant decrease but not significant difference between groups LPS and EC. In rats studied at the moment before death, was found bloody blepharitis in the autopsy, congestion and hemorrhage in the kidneys, liver and lungs, which is justified by significant thrombocytopenia in groups LPS and CE.

The elevation of alanine aminotransferase and aspartate aminotransferase is relatively common in sepsis and may be the result of ischemic liver injury or after re-perfusion, drug toxicity, systemic inflammation or direct action of pathogenic infectious agent, as a sign of cell injury hepatocytes and in some cases, mitochondrial dysfunction[16,17].

The elevation of direct bilirubin and enzymes of membrane, such as gamma glutamyltransferase and alkaline phosphatase indicate cholestasis inflammatory drug or obstruction. The increase in indirect bilirubin may indicate ineffective hematopoiesis, microangiopathic hemolysis or effect of drugs, alloimmunization leading to autoimmune hemolytic anemia, acute onset of defects underlying the membrane, such as glucose-6-phosphate dehydrogenase, among others. Was observed marked increase in plasma levels of bilirubin in the groups inoculated with *Escherichia coli* and LPS compared with the control group. Hepatic dysfunction may occur due to direct alteration of the functioning of the liver cell or tissue hypoperfusion. It may accentuate the inflammatory response by decreasing the clearance of endotoxins and/or cytokines[11] and is translated by hyperbilirubinemia with increased direct way. This is not the excretion of bilirubin in the sinusoidal pole.

The activity of prothrombin time is often used as a marker of liver function and its increase was observed in groups LPS and *Escherichia coli* in relationship to the control group.

In recent years, sepsis has become the leading cause of acute renal failure, especially in the intensive care environment. In a prospective study, the incidence was 19% in septic patients, 23% in those with severe sepsis and 51% in patients with septic shock[7]. It is usually a component of multiple dysfunction syndrome organs and systems, and their presence adversely affects the prognosis. Urea and creatinine are used as markers of renal function. It was observed increase in urea and creatinine in groups LPS and *Escherichia coli* in relationship to the control group.

In this study it was found that the rats had sepsis by changes in markers TNF-α and lipopolysaccharide. Indicators of the presence of the syndrome of multiple organ dysfunction included the finding of two or more organs - such as liver, kidneys and lungs. Also was considered the existence of alterations in coagulation. So, these findings here reproduced shown the induction of sepsis in rats.

CONCLUSIONS

The two models of sepsis induced syndrome of multiple organ dysfunction; but the administration of 36UE endotoxin every 48 hours could be utilized with advantage by not inducing death in significant numbers during the period of seven days.

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