The Effect of Combined Therapy with L-NAME and Vasoactive Amines on Acute Endotoxemia in Rats.

Cristiano Corrêa Batista[1]; Jorge Alberto Castro Benitez[2], Ana Luiza Muccillo Baisch [3]

ABSTRACT

Introduction: *Sepsis and its potentially lethal complication, septic shock, are the most frequent causes of mortality, especially, in intensive care units. Cardiovascular changes are often don't respond to conventional therapy, also the mechanisms by which sepsis leads to hemodynamic disturbance are not fully understood. A common cause of septic shock is gram negative bacterial infection. Nitric Oxide is a key mediator of hypotension, peripheral vasodilatation and vascular hyporeactivity to vasoconstrictor agents in endotoxemia.*

Objective: *The aim of the present study is to verify the hemodynamic and biochemical responses to therapy with dopamine and dobutamine with or without a non-selective inhibitor of Nitric Oxide Synthase (NOS) in rats with shock-like syndrome.*

Hypothesis: *We hypothesized that the inhibition of nitric oxide synthesis along with the intravenous infusion of vasoactives amines such as dopamine and dobutamine would reverse the hemodynamic status, optimizing heart performance without resulting in significant damage to platelet, renal and hepatical functions.*

Materials and Methods: *An experimental model of endotoxic shock was induced in rats by means of intravenous infusion of bolus of lipopolyssaccharide derived from Escherichia coli (5 mg/kg). We evaluated mean arterial blood pressure, heart rate, respiratory rate and urine output. Biochemical data such as platelet count, hepatic enzymes, urea and creatinine were also analysed. The treatment was carried out with the intravenous infusion of dopamine and dobutamine, both in doses of 10 µg/kg/min, through the use of an infusion pump with or without the intravenous bolus infusions of NG-nitro-L-arginine methyl ester (100 mg/kg).*

Results: *The results showed that the use of vasoactive amines dopamine and dobutamine, along with the infusion of bolus of NG-nitro-L-arginine methyl ester, improved the problem of hypotension caused by lipopolyssaccharide. It did not result in heart rate reduction, change in respiratory responses, or an increase in hepatic and renal disfunction.*

Conclusions: *The inhibition of nitric oxide through by non-selective nitric oxide synthase pathways along with the infusion of dopamine and dobutamine can be a useful therapy in the treatment of septic shock.*

Key Words: *shock-like syndrome, nitric oxide, L-NAME, rats, vasoactive amines.*

Sepsis and its potentially lethal complication, septic shock, are the most frequent causes of mortality, especially, in intensive care units. Moreover, the incidence of this condition is steadily increasing. Many factors underlie this tendency: extended longevity with its accompanying susceptibility to infections for people in developed countries; wider use of immunosuppressive therapy; complex surgical procedures which prolong the lives of patients who might have otherwise die of such causes as cancer, extensive trauma, burns, extensive blood loss, and others.

Septic shock is characterized by hipotension with normal or high cardiac output, low systemic vascular resistance, and inadequate tissue perfusion[1]. Cardiovascular changes often don't respond to conventional therapy and the mechanisms by which sepsis leads to hemodynamic disturbances are not fully understood. Present mortality is 50-75%[2]. Decreased responsiveness to catecholamines has also been observed in animal models of sepsis[3]. A common cause of septic shock is gram negative bacterial infection. Furthermore, intravenous administration of bacterial lipopolysaccharide in humans and animals produces a shock-like syndrome[4].

Excessive production of proinflammatory cytokines (such as tumor necrosis factor-α (TNFα) and interleukin-1 (IL-1)) plays a prominent role in

MD, Intensive Care, Ms. Internal Medicine, professor at Universidade Católica de Pelotas, Brazil.[1]
MD, PhD Physiology, professor at Fundação Universidade Federal do Rio Grande, Brazil.[2]
Nurse, PhD Medical Sciences, professor at Fundação Universidade Federal do Rio Grande, Brazil.[3]
Place where the researsh was developed: Federal University Foundation of Rio Grande; Departament of Physiological Sciences; Section of Pharmacology.
Address: Rua Eng.Alfredo Huch 475, Rio Grande, RS, Brazil
Correspondent author: Cristiano Corrêa Batista
Address: Rua Três de Maio 700, Pelotas, RS, Brazil, Zip code: 96010-620
e-mail: cbatista.sul@zaz.com.br
Phone: 053-2279806 Celular Phone: 053-9825792

the pathogenesis of the sepsis syndrome[5]. These mediators stimulate vascular endothelial cells and induce the production of vasoactive substances (such as nitric oxide (NO), endothelin and prostacyclin by vascular endothelial cells). NO has been found to exert an important role in regulating blood flow on the vascular system since the endothelium relaxing factor and excess NO are produced in the body during sepsis and endotoxin shock[6].

The aim of the present study is to verify hemodynamic and biochemical responses to therapy with dopamine and dobutamine with or without a non-selective inhibitor of Nitric Oxide Synthase (NOS) in rats with shock-like syndrome. We hypothized that the inhibition of nitric oxide synthesis, along with the infusion of vasoactives amines such as dopamine and dobutamine, would reverse the hemodynamic status, optimizing heart performance without resulting in significant damage to platelet, renal and hepatical functions.

MATERIALS AND METHODS

This study was performed at the Federal University Foundation of Rio Grande (Rio Grande, RS, Brazil), in the Pharmacology laboratory of the Physiological Sciences Departament. The experiments were performed in male Wistar rats (n = 24), weighting 512,08 ± 43,21g. The animals were purchased from the Biotron of the Federal University Foundation of Rio Grande. All rats had free access to water and food ad libitum until 12 hours before the experiment. At this time they fasted. The animals were anesthetized with an intraperitoneal injection of urethane 1,5 g/kg (Merck, Germany)[7]. A polyethylene cannula was placed in the left carotid artery for monitoring blood pressure and taken blood samples for biochemical analysis[8]. A second one was inserted into the left jugular vein for administration of drugs and saline replacement[9]. A tracheostomy was performed and a cannula, aboutcath n° 18, (Johnson & Johnson Medical, INC-EUA/Ethicon SPA- Italy) was placed in the upper airway to offer oxygen (O2) (0,5 l/min) through a T-tube system. Animals were allowed to breathe spontaneously[7;10]. The laparotomy, an abdominal midline incision of aproximately 3 cm was made. The urinary bladder was then cannulated with a polyethylene catheter by means of a cystostomy for urine collection. The incision was then closed around this catheter[9;11]. The electrocardiogram was also recorded (Birtcher Cardio Tracer 375-A1) with electrodes placed into the subcutaneous skin of the arms and legs to measure heart rate using lead II[12]. The animals were placed in a thermostatically controlled heated environment to keep body temperature above 36,5°C. A clinical thermometer (Thermoflat °C NBD Brazil G007) was used to measure rectal temperature[10].The catheter inserted into the carotid artery was connected via a transducer (Isotec Pressure Transducer version 1:1, Hugo Sachs Elektronik) to an amplifier (Two-Channel Bridge Amplifier Type 301, Hugo Sachs Elektronik) and to a graphic recorder (ECB model RB-201) to register mean arterial blood pressure. The catheter inserted into the jugular vein was connected via a three way tap to two infusion pumps (Lifemed FARS 600, Lifemed Pesquisas Médicas Indústria e Comércio LTDA) for drug applications and saline replacement, 6ml/h[13]. Urine output was determined gravimetrically on an analytical precision scale (Sartorius Basic: Electronic Analytical and Precision Scales).

Experimental protocols

After the surgical procedure, a recovery period was allowed for hemodynamic stabilization. A 60 min. control period followed, after which the animals were observed for fours hours. At the end of this period, blood samples were taken by means of a cannula placed in the left carotid artery for biochemical analysis[14]. Platelet count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine were measured using a Cobas Mira Plus machine and a Cobas Micros machine (Roche Diagnostic Systems 31-6699 and 6090T-2672). To produce endotoxic shock, an i.v. infusion in bolus of lipopolysaccharide (derived from Escherichia coli (LPS) (026:B6, Sigma,St.Louis, MO, USA)) was administered (5 mg/kg in 1 ml saline)[10]. Shock was considered in effect when mean arterial blood pressure fell about 30% below the baseline. Mean arterial blood pressure, heart rate and respiratory rate were checked every 30 minutes during four hours.

The animals were divided in four groups. Group 1 (n = 6) a control group. The animals in this group received an intravenous infusion of saline (6ml/h), during the entire experiment (four hours). At the end of which blood samples were taken for biochemical analysis. The animals in Group 2 (LPS + saline) (n = 6) received saline (6ml/h) throughout the experiment, and as bolus a intravenous injection of LPS (5mg/kg at 0 minutes). These animals were then observed for four hours. At the end of which blood samples were taken for biochemical analysis. The animals in Group 3 (n = 6) (LPS + saline + dopamine and dobutamine) received an intravenous infusion of saline (6ml/h)

throughout the experiment, and as bolus a intravenous injection of LPS (5mg/kg at 0 minutes). When shock was detected, these animals received by means of a pump continuons intravenous infusion of dopamine 10 µg/kg/min, (Eurofarma), and dobutamine 10 µg/kg/min, (Bedford Laboratories). At the end of four hours which blood samples were taken for biochemical analysis. The animals in Group 4 (n = 6) (LPS + saline + dopamine and dobutamine + L-NAME) received an intravenous infusion of saline (6ml/h) throughout the experiment, and as bolus a intravenous injection of LPS (5mg/kg at 0 minutes). When shock was checked these animals received a continuons intravenous infusion of dopamine and dobutamine by means of pump and as bolus a intravenous injection of NG-nitro-L-arginine methyl ester (L-NAME) (Sigma, St. Louis, MO, USA, 100 mg/kg, dissolved in 1 ml of saline)[12]. At the end of four hours which blood samples were taken for biochemical analysis.

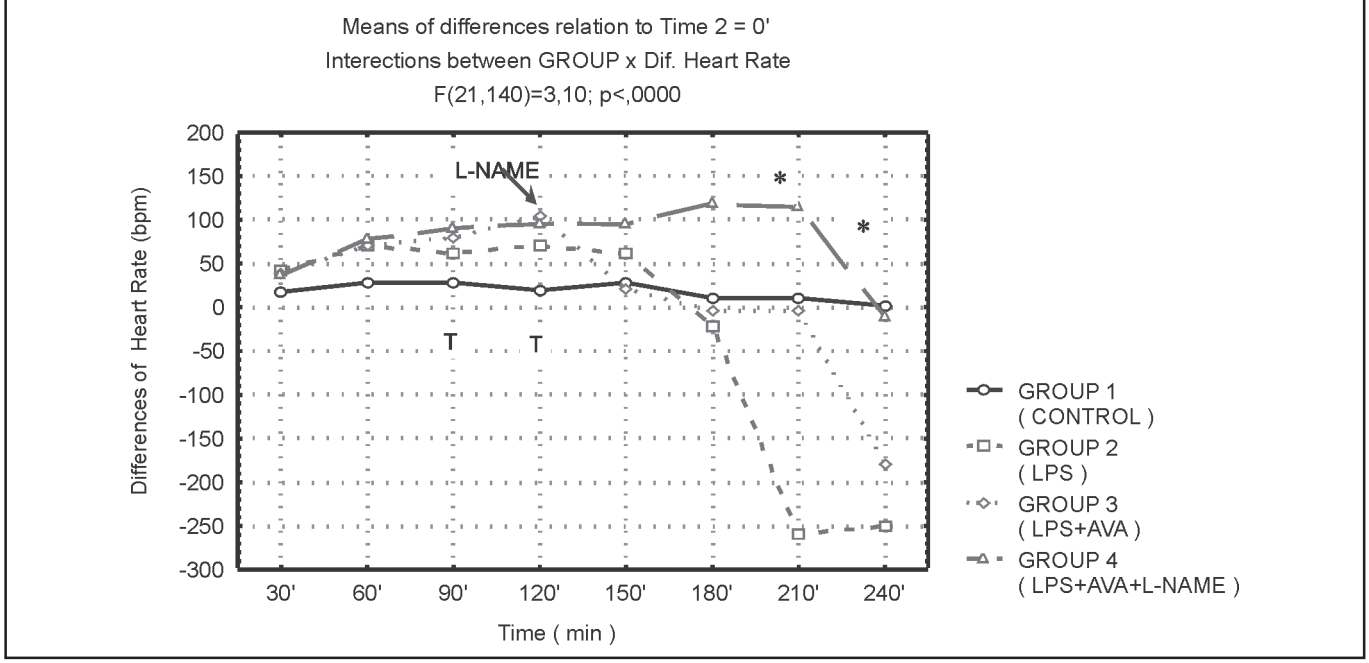
STATISTICAL ANALYSIS

Biochemical data was analyzed using a simple analysis of variance (ANOVA). A significant ANOVA was followed by Tukey's test to compare the means. Hemodynamic data was analyzed using the analysis of variance for repeated measurements. The average of difference in the decrease of MABP, HR and RR were applied to each group, at interval 30 minutes in relation to time two (the starting point of the study = 0'). Data are reported as mean ± SEM. The level of significance was set at $p \leq 0,05$.

RESULTS

It was observed that, in relation to MABP, the groups did not behave in the same manner throughout the same time period. There was a effect of group, and a time effect, for instance, a group-time interaction ($p < 0,05$). This means that, as time progressed, MABP varied amongst groups. Not all differences between groups were significant at all times (fig. 1). Group 1 (control: 0,9% saline 6 ml/h) kept its MABP stable throughout the duration of the experiment. All animals survived the observation period, stipulated at four hours. Group 2 (0,9% saline 6 ml/h + LPS 5 mg/kg), presented an accentuated decrease in MABP, which lead to hemodynamic shock and subsequent death. Four deaths were registered during the observation period, the first of which happened during the third hour of observation and the others during the last hour. In comparing groups 1 and 2 , it was observed that the average difference in the decrease of MABP was inicially the same. That is, there was no significant statistical difference between the two

Fig. 1- Evolution of the average differences of mean arterial blood pressure decreasing in studied groups throughout the observation period in relation to time 2= 0'. LPS= lipopolysaccharide; L-NAME= NG-nitro-L-arginine methyl ester; VA= vasoactive amines * p < 0,05 (between Group 4 and Groups 2 and 3); B p < 0,05 (between Group 1 and Group 2).



groups (30' p = 0.3526, 60' p = 0.2391). As time evolved, however, they began to differ. From 90 minutes on, the decrease MABP in Group 2 was significantly superior to that of Group 1 (p<0.05). Group 3 (0,9% saline 6 ml/h + LPS 5 mg/kg + dopamine and dobutamine 10 ìg/kg/min) developed in a manner similar to that of Group 2. Despite the infusion of vasoactive amines, there was no significant statistically difference, throughout the four hours, in the decrease in the MABP of others. Three deaths were registered during the observation of four hours: one rat died during the third hour of observation, and two at the end of the fourth hour. Group 4 (0,9% saline 6ml/h + LPS 5 mg/kg + dopamine and dobutamine 10 ìg/kg/min + L-NAME 100 mg/kg) presented, as the time passed, an interruption in the decrease of MABP, followed by an immediate recovery of arterial blood pressure levels, reaching values above those registered at the beginning of observation. This fact coincided with the intravenous infusion of L-NAME, normally given two and a half hours after the LPS. In addition, only one death was registered in this group, during the forth hour of observation. A comparison of Group 4, with combination of Groups 2 and 3 revealed, significantly different changes in MABP (p< 0,05). Groups 2 and 3 presented an accentuated decrease in MABP during the observation period, whereas, in Group 4, the decrease was interrupted, and was subsequentially followed by a complete recovery of arterial blood pressure levels.

In relation to heart rate (figure 2), the groups did not behave in the same manner throughout the duration of the experiment. Group-time interaction was verified, (p< 0,05). As time passed, heart rate varied among the groups. In the animals of the control group (Group 1), heart rate was maintained: despite small oscillations, heart rate remained unchanged until the end of the observation period. In relation to the animals of Group 2, despite tachycardia, there was no significant alteration in heart rate untill half way through the second hour, when a gradual decrease in heart rate began to be observed, at this time animals started to die. In Group 3, where animals received vasoactive amines as treatment, evolution of heart rate was similar to that of Group 2. No significant statistical difference was verified between the two groups. Group 4, to which vasoactive amines and L-NAME were given, showed an evolution similar to that of Group 2. No significant statistical differences being observed until 180 minutes. From that point on, both groups began to differ, the decrease in heart rate in Group 2 being significantly superior to that of Group 4 (p< 0.05).

There were no significant statistical differences in heart rate between Groups 4 and the Groups 2 and 3 combined. However, exceptionally, at 210 minutes,

Fig. 2: Evolution of the average differences of heart rate decrease in studied groups throughout the observation period, in relation to time 2 = 0'. LPS = lipopolysaccharide; L-NAME = NG-nitro-L-arginine methyl ester; VA = vasoactive amines; * p < 0,05 (between Group 4 and Groups 2 and 3); B p < 0,05 (between Group 1 and Group 4).

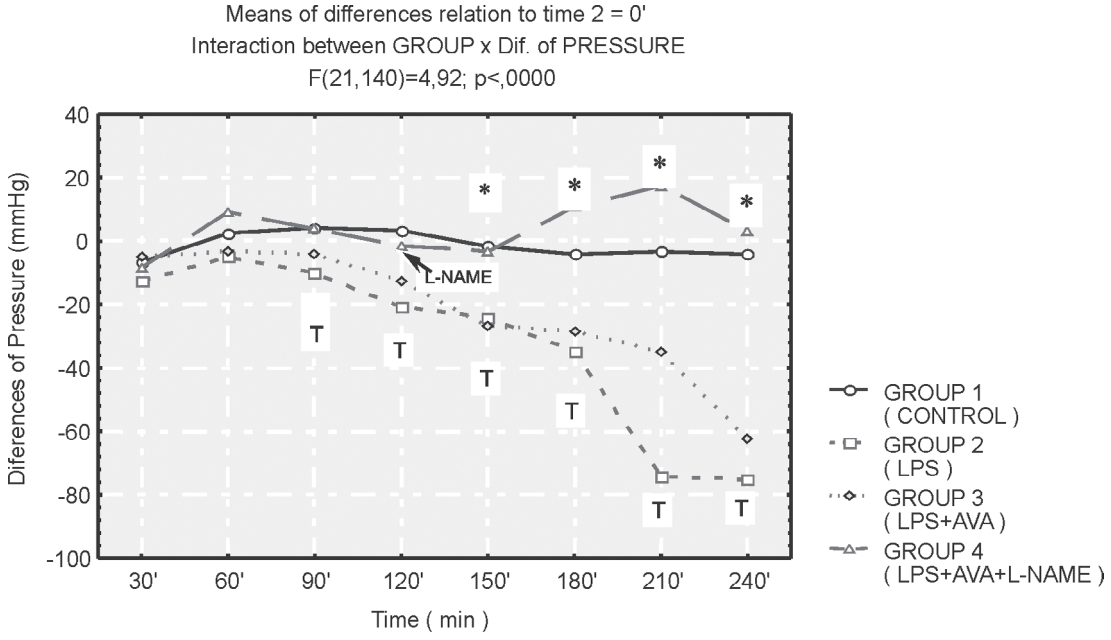
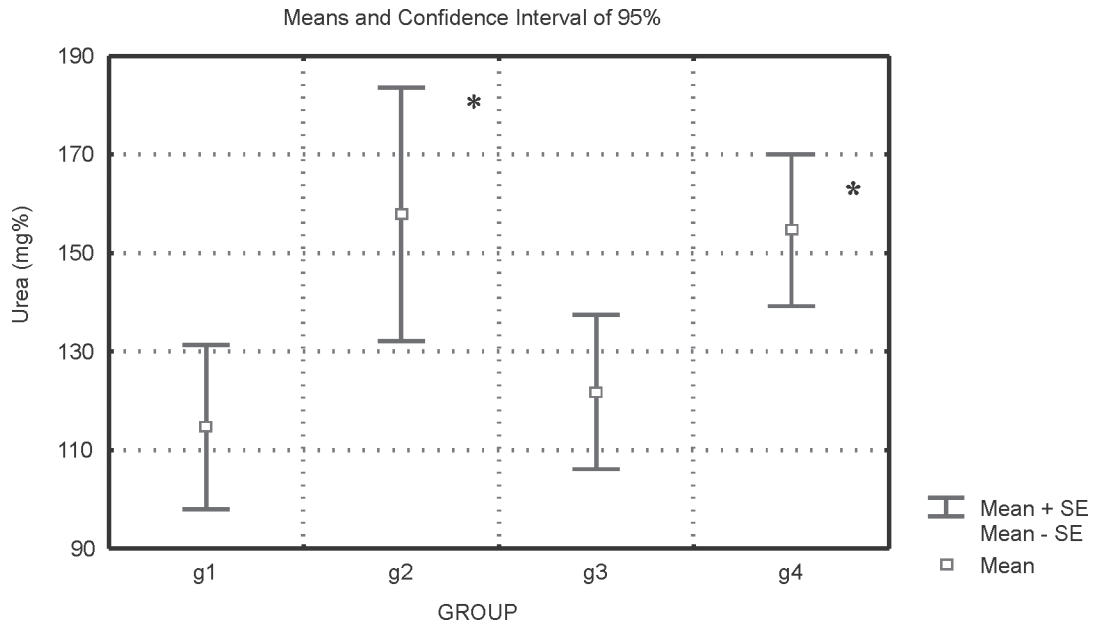


Fig. 3: Evolution of the average differences in respiratory rate decrease in studied groups throughout the observation period, in relation to time 2 = 0'. LPS = lipopolysaccharide; L-NAME = NG-nitro-L-arginine methyl ester; VA = vasoactive amines; * p < 0,05 (between Group 1 and Group 2).



the decrease in heart rate in Group 4 was inferior to that of Groups 2 and 3 combined (p< 0.05).

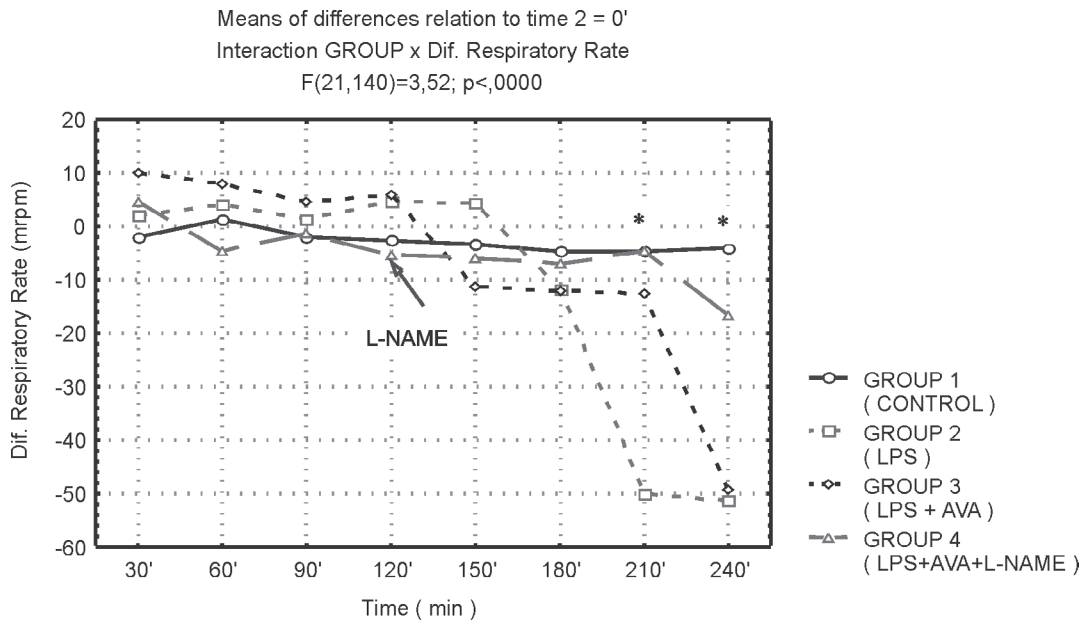
When analyzing respiratory rate (figure 3), different behaviour between groups throughout the experiment was detected. Again, a group-time interaction (p< 0,05) was present. This means that, as time progressed, respiratory rate varied among groups. In Group 1 (control) a stable respiratory rate was maintained. In Group 2 (LPS), as in Group 1 there was no statistically significant difference between the two groups untill 180 minutes. From that point on, both groups began to differ. The respiratory rate in Group 3 (LPS + vasoactive amines) was similar to that of Group 2. A statistical difference between the two groups was verified only at the beginning of the fourth hour of observation, when the decrease in respiratory rate of Group 3 was inferior to that of Group 2 (210 minutes p< 0.05). This fact is also attributed to the larger number of deaths registered in Group 2. Group 4 (LPS + vasoactive amines + L-NAME), developed in a similar way to the control group. When the latter was compared to Groups 2 and 3, it was observed that during most of the experiment Groups 2 and 3, in relation to respiratory rate, were statistically undistinguishable. However at 60 minutes (p< 0.05) and 120 minutes (p< 0.05), the decrease in respiratory rate of Group 4 was superior to that of Groups 2 and 3. At 150 minutes, however, and up untill the end of the experiment, the three groups are again statistically indistinguishable.

Concerning the serum levels of transaminases (AST and ALT), platelet and creatinine, the results showed no significant statistical difference between the groups (AST p = 0.117159, ALT p = 0.191826, platelet p = 0,241400, creatinine p = 0,206317).

With respect to urine output, there were no significant statistical difference between groups studied (urine output p=0.379947).

When analyzing serum levels of urea, a significant statistical difference was verified (p< 0,05) (figure 4). A comparison of Group 1 to Group 2 revealed that plasmatic urea was significantly greater in Group 2 than in Group 1 (p < 0.05). Comparing Groups 1 and 3, no significant statistical difference was verified (p > 0.05). A comparison of Group 1 to Group 4 revealed that the urea in Group 4, was significantly superior to that of Group 1 (p < 0.05). Groups 2 and 4 didn't demonstrate any significant statistical difference (p > 0.05). A result similar to that encountered when comparing Groups 3 and 4 (p > 0.05), and Groups 2 and 3 (p > 0.05). In spite of this difference, the increase in plasmatic urea in Group 4 did not differ statistically from that of 2 and 3, which suggests that the use of NOS inhibitors, in this model of endotoxic shock, did not increase kidney damage previously established by the endotoxin. It should be

Fig. 4: Serum levels of urea encountered among studied groups at the end of experimantation time 240'. G1 = control Group; G2 = LPS Group; G3 = LPS + vasoactive amines Group; G4 = LPS + vasoactive amines + L-NAME Group. Data are presented as mean ± SEM. * p < 0,05.



noted, however, that the use of vasoactive amines, without associating L-NAME, revealed plasmatic urea levels indistinguishable from those of the control group.

DISCUSSION

It is important to note that our study had the treatment, not the pre-treatment, as its aim. This study demonstrated that the use of non-selective NOS inhibitors promoted recovery from the state of hypotension established by the endotoxemia, and that the associate with of an NOS inhibitor with vasoactive amines dopamine and dobutamine promoted recovery from hypotension, not causing bradycardia nor altering the already established respiratory rate, thus increasing the survival rate of the animals.

Although we are aware that we are dealing with an biologic experimentation model, unidentical to a human one, this model allowed us to imitate human endotoxic shock conditions. During the four hour period of observation it was possible to monitor hemodynamic variables involved in endotoxic shock, such as: MABP, heart rate, respiratory rate and urine output through an analysis of hemodynamic variables, as well as a biochemical analysis. The repercussion of the treatment of endotoxic shock was also observed through using a nitric oxide synthase inhibitor, in association with the infusion of vasoactive amines, on the functioning of the liver, kidneys, and heart, on blood circulation, and on the coagulation. Lai and Komarov[15] demonstrated that intravenous infusion in bolus of LPS results in a gradual decrease in MABP, leading to conditions similar to those of septic shock. They demonstrated, as well, that after the infusion of LPS there is a production of NO via NOS, producing, as a result, L-arginine. Fatehi-Hassenbad et al.[16], during an observation period of nine hours, they verified progressive hypotension after an intravenous infusion of LPS in rats. In the group that received LPS many deaths were registered, at different moments. In the control group (which received saline), however, all animals survived, maintaining stable blood pressure. In the present study, by means of an intravenous bolus infusion of LPS (5mg/kg), a state of hemodynamic shock was verified when Group 2 was compared to the control group, which maintained stable blood pressure. Several deaths were also registered during the observation period of the group that received LPS and was left untreated, demonstrating the shock inducing role of endotoxin. Data in the literature show that the infusion of LPS is followed by a twenty-three-fold increase in nitrate concentration levels, and that, in animals pre-treated using NOS inhibitors at a dose of 100mg/kg this increase is inhibited[17]. In the present

study, L-NAME was used in association with an infusion of dopamine and dobutamine, and a significant increase in tensional levels was verified in relation to the groups that received LPS, associated or not to the vasoactive amines. This demonstrates that the blocking of NO synthesis plays an important role in the pathogenesis of endotoxic shock, and that L-NAME proved itself efficient in increasing the tensional levels in these conditions. Mayer et al.[18], reverted the state of hypotension through the use of L-NAME in models of endotoxic shock utilizing sheep. In addition, they demonstrated that, following the infusion of L-NAME there was a reduction in oxygen delivery, and a normalization of the oxygen extraction ratio without a decrease in oxygen consumption, which would indicate adequate tissue perfusion of metabolically active organs. Studies with surviving sepsis patients revealed na increase in systemic vascular tone, na improvement in ventricular function performance, as well as increase in MABP. Blood pressure and ventricular function are shown to be the best survival indicators in patients suffering from septic shock[19]. Pathologic production of NO during sepsis has been described as the mediator responsible for the loss of vascular tone, also known as vasoplegia, and as a participant in myocardial depression[20]. The present study demonstrated that the use of a NOS inhibitor associated to vasoactive amines promoted an improvement in the state of hypotension probably caused by the reversion of vasoplegia, established by the application of LPS.

Concerning heart rate, it was observed that the use of vasoactive amines dopamine and dobutamine associated to L-NAME did not contribute to bradycardia, but, on the contrary, helped maintain the heart rate already established by the hyperdynamic state related to the endotoxin. Amongst cytokines the most crucial are TNFα and IL-1, which mediate septic shock and, among other functions, have an influence on vascular permeability and resistance, on cardiac function, and on cardiac output[21]. The mechanisms through which the cytokines led to ventricular damage include: the death of myocardical cells by necrosis or apoptosis with progressive myocardical fibrosis, systemic endothelial dysfunction, and gradual alterations in ventricular function[22]. Kaszaki et al.[14] suggests that the NO produced by cNOS contributes to the myocardical depression observed in endotoxemia conditions. NO should have a negative inotropic effect on myocardical contractility; thus, by blocking its synthesis, positive intotropic mediators would prevail, increasing contractile force. These hypotheses were confirmed in a study utilizing models of endotoxemia in dogs, in which the activation of cNOS was demonstrated during the early stages of sepsis and during later stages the activation of iNOS, as well as an improvement in myocardical contractility with the use of a selective iNOS[23]. Tao and McKenna[24], also, demonstrated that the activity of NOS during endotoxemia contributes to a deterioration of the cardiac myocytes' contratility. Our main goal in the employment of combined vasoactive amines was to guarantee adequate oxygen delivery, correcting the inadequate cardiac output observed in sepsis conditions. Both dopamine and dobutamine optimize cardiac output, mainly by increasing systolic volume, in addition to contribute significantly to the increase in heart rate. Dopamine is more closely

Table 1- Definitions: G = Group; MABP = mean arterial blood pressure; H.R = heart rate; R.R = respiratory rate. Data are presented as mean ± SEM. Comparisons between average were made every 30 minutes.* = p ≤ 0,05 between groups.

time	30'	60'	90'	120'	150'	180'	210'	240'
G1-MABP	-6.67 ± 6.79	2.5 ± 4.23	4.17 ± 3.96*	3.33 ± 4.41*	-1.67 ± 5.58	-4.17 ± 6.38*	-3.33 ± 6.28*	-4.17 ± 6.38*
G2-MABP	-12.5 ± 2.14	-5 ± 4.08*	-10 ± 4.65*	-20.83 ± 6.88*	-24.17 ± 9.26	-35 ± 13.41*	-74.17±13.19*	-75 ± 12.65*
G3-MABP	-5 ± 4.28	-3.33 ± 3.57*	-4.17 ± 3.52*	-12.5 ± 2.81*	-26.67 ± 8.03	-28.33 ± 7.49*	-35 ± 7.19*	-62.5 ± 6.8*
G4-MABP	-8.33 ± 2.47	9.17 ± 5.07*	4.17 ± 5.07*	-1.67 ± 6.54*	-3.33 ± 11.23	10.83± 10.75*	17.5 ± 18.33*	3.33 ± 19.39*
G1-HR	17.67 ± 11.17	28.33 ± 16.51	28.33± 16.51*	19.5 ± 16.23*	28.33 ± 16.51	10.67 ± 14.94	10.67 ±14.94*	1.83 ± 12.32*
G2-HR	41.67 ± 13.62	71.33 ± 11.38	62.5 ± 16.5	71.33 ± 11.38	62.5 ± 16.5	-20.83 ± 71.63	-258.83±90.99*	-250 ± 96.28*
G3-HR	38.5 ± 20.28	71.33 ± 25.26	80.17 ± 21.55	104.17± 20.83	20.83 ± 90.51	-3.17 ± 86.16	-3.17 ± 86.16*	-178.67±96.33
G4-HR	36.67 ± 12.24	78.33 ± 24.15	90.33± 18.74*	95 ± 28.61*	95 ± 28.61	119 ± 19.79	114.33±14.94*	-10.67 ± 85.75
G1-R.R	-2 ± 3.05	1.33 ± 1.33	-2 ± 1.37	-2.67 ± 2.23	-3.33 ± 1.23	-4.67 ± 1.23	-4.67 ± 1.23*	-4 ± 1.79*
G2-R.R	2 ± 2.25	4 ± 4.62*	1.33 ± 5.33	4.67 ± 2.81*	4.33 ± 4.27	-11.67 ± 16.62	-50 ± 18.61*	-51.33±17.78*
G3-R.R	10 ± 4.23	8 ± 3.42*	4.67 ± 4.43	6 ± 3.54*	-11.33 ± 12.19	-12 ± 12.17	-12.67±11.93*	-49.33 ± 16.28
G4-R.R	4.67± 2.4	-4.67 ± 4.67*	-1.33 ± 3.95	-5.33 ± 4.69*	-6 ± 5.24	-7 ± 5.55	-4.67 ± 3.78	-16.67 ± 10.75

Table 2: Definition of observations: AST= aspartate aminotransferase; ALT= alanine aminotransferase; Group 1= control; Group 2= (LPS); Group 3= treatment using vasoactive amines (dopamine + dobutamine); Group 4= treatment using vasoactive amines (dopamine + dobutamine) + L-NAME. Data are presented as means ± SEM. * p ≤ 0,05 between groups.

Group	AST (mg%)	ALT (mg%)	Platelet (mm3)	Urine output (ml)	Urea (mg%)	Creatinine (mg%)
Group 1	465 ± 91.81	159.33 ± 43.06	510666.67±45878.47	1.5 ± 0.66	114.67 ± 8.51	1.39 ± 0.16
Group 2	679.33 ± 155.5	376.67 ± 162.32	506000±62685.59	0.6 ± 0.19	157.83 ± 13.13 *	1.92 ± 0.26
Group 3	1445.67 ± 521.1	927.67 ± 452.87	346000±83593.46	0.75 ± 0.36	121.83 ± 8	2.17 ± 0.37
Group 4	795.67 ± 130.04	391.67 ± 113.38	513000±35960.09	0.67 ± 0.22	154.67 ± 7.87 *	1.8 ± 0.17

related to the increase in MABP, whereas dobutamine increases cardiac output by increasing systolic volume[25]. It has been suggested that the use of NOS inhibitors in sepsis presents technical and practical advantages. When overproduction of an endogenous vasodilator is inhibited, vascular tone can be restored without causing specific constriction. In addition, some adverse effects of NOS inhibitors can be corrected by means of a combined infusion of a positive inotropic agent, such as dobutamine[26]. It was also demonstrated that positive inotropic response to dobutamine is increased by the concomitant infusion of L-NMMA[27].

Concerning respiratory rate, the present study verified that the use of L-NAME alone did not contribute to alteration in respiratory rate response, once no significant statistical differences were encountered between endotoxemic groups. Minnard et al.[28] reported that the inhibition of NO synthesis in rats results in intense hypoxemia, which is caused by the increase in neutrophile infiltration; the adhesion and liberation of secretory products, as well, as by cytotoxic mediators in the lungs. In addition, they demonstrate that the blockage of NO synthesis by means of L-NAME promotes an increase in pulmonary hypertension, which could lead to death by cardiac failure. In a study of the inhibition of NO production exhaled during septic shock conditions in rats, demonstrate that LPS increases the production of NO, and that L-NAME prevents this increase[29]. They also verified in rats treated with LPS, histologic evidence of pulmonary interstitial inflammation associated with L-NAME infusion suggesting that it might act as a enabler of lung damage in endotoxemic animals. Fox et al.[30] argued that NOS inhibitors such as L-NAME increase systemic arterial pressure and systemic vascular resistance, but do not have any effect on pulmonary arterial tone. It is known that in rats, as in human beings, acute hypoxia results in alterations in the respiratory and cardiovascular processes, known as behavioral arousal[31]. It is characterized by hyperventilation, hypocapnia, tachycardia, and an increase in systemic arterial blood pressure. In addition to the stimulation of pulmonary receptors and hypocapnia contributing to tachycardia, central collateral inspiratory neurons have an inhibitory effect on cardiac vagal neurones. Thus, every time there is a respiratory stimulus, the stability of sympathetic neurones is exacerbated, facilitating tachycardia and vasoconstriction. When hypoxia is constant and progressive, despite the increase in cerebral blood flow, the direct effect of the diminished cerebral oxygen delivery is a depression of central respiratory neurones, which causes a decrease in the respiratory rate, and, consequently, an increase in hypoxia. In the present study, the use of L-NAME associated to the infusion of vasoactive amines did not cause significant alterations in respiratory rate in relation to Groups 2 and 3. We observed that the group which received L-NAME, dopamine, and dobutamine, presented, at the end of the observation period, a greater survival rate than Groups 2 and 3. Four deaths were registered in Group 2, three in Group 3, and only one in Group 4, which demonstrates that behavioral arousal, related to LPS, was not exacerbated by the use of a NOS inhibitor.

The analysis of hepatic function indicated no statistically significant differences concerning serum levels of hepatic transaminases. This indicates that the use of a non-selective NOS inhibitor in association with vasoactive amines did not act as a enabler of liver damage. One should not discard, however, the possibility that the demonstration of the experiment was insufficient for these effects to be felt. Chamulitrat et al.[32], demonstrated that the use of NOS inhibitors alone does not increase liver damage. The liver contains a large pool of inflammatory cells (such as Kuppfer cells) which, when stimulated by the endotoxin, produce several cytokines, and other mediators. Two separate mechanisms are involved in cellular destruction: necrosis and apoptosis. The endotoxin at first induces apoptosis, which is followed by the appearance of necrosis, the liver being the most affected organ. TNFα and NO can be effective molecules in the induction of

apoptosis in the liver, in endotoxic shock conditions[33]. Gundersen et al.[34], in a study of hepatic function in experimental models of endotoxic shock with pigs, argued that endotoxin infusion induced marked morphological alterations the liver, mainly in centrilobular areas, the most noticeable of which were inside the sinusoids. Kupfer cells were edematous containing platelets and electron-dense materials in cytoplasmic vacuoles. These alterations increased as time progressed, causing the appearance of fibrin deposits, and the destruction of hepatocytes. When aminoethyl-isotiourea (AE-ITU), a selective inhibitor of iNOS, was infused, histological alterations became much milder, and hepatocytes were well preserved. Hepatic transaminases AST and ALT did not suffer significant alterations in comparison to the control group, indicating that a the use of a selective iNOS inhibitor did not have harmful consequences on morphology, as well as on hepatic function. In mice, the endotoxin infusion produced a significant decrease in mesenteric blood flow (MBF), and in hepatic and splenic congestion. Moreover, it established in mice a high degree of inflammatory damage. The infusion of aminoguanidine blocked the endotoxin's effects on MBF and on the liver, but had no effect on the spleen. Where as, the infusion of L-canavanine blocked the endotoxin's effects on the liver, but had no effect on MBF or on the spleen[35].

No significant statistical difference between groups was verified in an analysis of serum levels of platelets. The LPS, in rats, besides inducing alterations in intrinsic and extrinsic coagulation pathways, also induced thrombocytopenia. The latter can not be directly measured through the platelet-activating factor (PAF), but can be measured through the production of TNFα[36]. It is also known that other components of the vascular wall besides the endothelium can modulate platelet function via NO liberation, thus playing an important role in coagulation[37]. In the present study, no induction of thrombocytopenia was observed. This indicates that, during the period of observation, the inhibition of NO synthesis, throught about by the use of a non selective NOS inhibitor (L-NAME), was not the sole factor in inducing thrombocytopenia.

The analysis of renal function was accomplished by means of the measurement of urine output and serum levels of creatinine and urea. In the present study, no significant statistical differences concerning urine output or creatinine serum levels were found; however, these were found in the serum levels of urea. In the group which received LPS alone, the level of plasmatic urea was significantly superior to that of the control group. This dysfunction was improved by the infusion of dopamine and dobutamine, but not by their association to L-NAME. This association, however, did not have a detrimental effect on renal dysfunction already established by the endotoxin, which demonstrates that the association does not contribute to an increase in kidney damage in endotoxemia conditions, probably due to the employment of vasoactive amines. It is known that, in sepsis conditions, the administration of dopamine and dobutamine increase the regional blood flow, as well as oxygen delivery[38]. Sener and Smith[39] demonstrated, in sheep, that NO plays an important role in systemic and renal hemodynamic regulation. They verified that the administration of acetylcholine caused an increase in renal blood flow, a response which was attenuated by the infusion of L-NAME. They also showed that in administering L-NAME a dose-dependent decrease in renal blood flow ocurred, as well as a dose-dependent increase in renal vascular resistence. Gardiner et al.[40] demonstrated that in septic rats NO exerts an opposition to the vasoconstrictor influences, appropriately increasing regional vascular conductance, which improves regional perfusion. Treatment with L-NAME resulted in vasoconstriction of variable magnitude.

In conclusion, we use an experimental model of endotoxic shock on rats. Hemodynamic and biochemical responses were verified, consistent with the condition of endotoxemia and its repercussions while under with a combination of treatment with vasoactive amines dopamine and dobutamine, associated to a non-selective NOS inhibitor (L-NAME). Our study had as its aim the treatment of the endotoxic shock, and not its pre-treatment. The use of non-selective NOS inhibitors was shown to promote recovery from the state of hypotension established by endotoxemia, and that the association of the vasoactive amines (dopamine and dobutamine) promoted recovery from hypotension, not causing bradycardia or altering the already established respiratory rate, increasing survival rate of tested animals. The use of a non-selective NOS inhibitor for endotoxic shock did not increase the hepatic injury already established by endotoxemia, nor provoke thrombocytopenia or aggravate kidney dysfunction. Thus we raise the hypothesis that a combined therapy of vasoactive amines dopamine and dobutamine with a non-selective NOS inhibitor may be beneficial in the treatment of septic shock.

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