Inflammation, Vol. 42, No. 2, April 2019 (© 2018)
DOI: 10.1007/s10753-018-0919-z

ORIGINAL ARTICLE

Cannabinoid CB_1 Receptor Antagonist Rimonabant Decreases Levels of Markers of Organ Dysfunction and Alters Vascular Reactivity in Aortic Vessels in Late Sepsis in Rats

M. C. G. Leite-Avalca,[1] F. T. Staats,[1] D. Verona,[1] P. de Souza,[2] M. C. Almeida,[3] J. E. Silva-Santos,[2] and A. R. Zampronio[1,4,5]

Abstract— Sepsis is a life-threatening condition with high mortality rates that is caused by dysregulation of the host response to infection. We previously showed that treatment with the cannabinoid CB_1 receptor antagonist rimonabant reduced mortality rates in animals with sepsis that was induced by cecal ligation and puncture (CLP). This improvement in the survival rate appeared to be related to an increase in arginine vasopressin (AVP) levels 12 h after CLP. The present study investigated the effects of rimonabant on organ dysfunction, hematologic parameters, and vascular reactivity in male Wistar rats with sepsis induced by CLP. Intraperitoneal treatment with rimonabant (10 mg/kg, 4 h after CLP) abolished the increase in the plasma levels of lactate, lactate dehydrogenase, glucose, and creatinine kinase MB without altering hematological parameters (*i.e.*, leukopenia and a reduction of platelet counts). CLP increased plasma levels of nitrate/nitrite (NOx) and induced vasoconstriction in the tail artery. The treatment of CLP rats with rimonabant did not alter NOx production but reduced the vasoconstriction. Rimonabant also attenuated the hyperreactivity to AVP induced by CLP without affecting hyporesponsiveness to phenylephrine in aortic rings. These results suggest that rimonabant reduces organ dysfunction during sepsis, and this effect may be related to AVP signaling in blood vessels. This effect may have contributed to the higher survival rate in rimonabant-treated septic animals.

KEY WORDS: CB_1 receptor antagonist; sepsis; rimonabant; organ dysfunction.

INTRODUCTION

Sepsis is a multifaceted host response that occurs when the body's response to an infection injures its own tissues and organs. The clinical criteria for sepsis include evidence of organ dysfunction [1]. Even a modest degree of organ dysfunction when infection is first suspected is associated with a 10% higher mortality rate in hospitals. Therefore, detecting this condition and improving organ dysfunction require an immediate and appropriate response [2].

[1] Department of Pharmacology, Biological Sciences Section, Federal University of Paraná, Curitiba, Paraná, Brazil
[2] Department of Pharmacology, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil
[3] Center for Natural and Human Sciences, Federal University of ABC, São Bernardo do Campo, São Paulo, Brazil
[4] Departamento de Farmacologia, Centro Politécnico, Universidade Federal do Paraná, Caixa Postal 19031, Curitiba, Paraná 81540-970, Brazil
[5] To whom correspondence should be addressed at Departamento de Farmacologia, Centro Politécnico, Universidade Federal do Paraná, Caixa Postal 19031, Curitiba, Paraná 81540-970, Brazil. E-mail: aleksander@ufpr.br

High blood lactate levels are common in critically ill patients. Especially in sepsis, microcirculatory disarray may lead to insufficient oxygen that is delivered to the cell, thus increasing lactate levels. Lactate levels are frequently used to diagnose inadequate tissue oxygenation in septic patients. High blood lactate levels have been associated with a higher mortality rate [3]. However, other biomarkers have been included as predictors of the severity of sepsis and its prognosis, such as lactate dehydrogenase (LDH) [4]. Additionally, some markers, such as plasma levels of creatine kinase MB (CK-MB), are used to indicate heart injury [5]. Combinations of these markers may help determine the intensity of organ dysfunction or injury and the prognosis for septic patients.

The endocannabinoid (eCB) system is an emerging topic of research on diseases that involve the immune system. Endocannabinoids are present in both central and peripheral tissues [6]. Endocannabinoids, such as anandamide and 2-arachidonoyl-glycerol, act on two types of G protein-coupled receptors, CB_1 and CB_2 [7]. Particularly anandamide, generated by endotoxin-activated platelets and macrophages, has a vasodilator effect and induces a shock state *via* cannabinoid CB_1 receptors [8].

Other diseases involving an inflammatory component can also be modulated by endocannabinoids. It seems that the endocannabinoid system can be effective by inhibiting oxidative stress and inflammatory pathways [7]. Previous studies showed that CB1 antagonist/inverse agonist rimonabant increased the blood pressure in rats subjected to hemorrhagic shock, an important target to increase the survival rate thus, demonstrating the involvement of the endocannabinoid system at hypotensive states [9]. In clinical studies of obesity and diabetes, rimonabant decreased multiple cardiovascular risk factors, including inflammatory factors [10].

We previously showed that CB_1 receptor activation can reduce excitatory presynaptic currents in vasopressinergic cells in the supraoptic nucleus [11]. This system may be important during sepsis. A previous study by our group showed that CB_1 receptor blockade that was induced by systemic or intracerebroventricular rimonabant administration increased survival rates and peripheral levels of arginine vasopressin (AVP) in late sepsis (*i.e.*, 12 h after cecal ligation and puncture [CLP]) [12]. The present study investigated the effects of CB_1 receptor antagonism on biomarkers of organ injury (*i.e.*, lactate, lactate dehydrogenase [LDH], glucose, and CK-MB), hematologic parameters, nitrate/nitrite (NOx) levels, tail temperature, and vascular reactivity in aortic vessels in rats that were subjected to CLP-induced sepsis.

MATERIALS AND METHODS

Experimental Animals

The experiments were conducted in male Wistar rats (180–200 g) that were obtained from the animal facility of the Federal University of Paraná. The animals were housed five per cage in a temperature-controlled room at 22 °C ± 1 °C under a 12 h/12 h light/dark cycle (lights on at 7:00 AM) with food and water available *ad libitum*.

Experimental Design

Animal Groups

The rats were randomly divided into three groups: (i) rats that underwent sham surgery and were treated with vehicle, (ii) rats that were subjected to CLP and treated with vehicle, and (iii) rats that were subjected to CLP and treated with rimonabant. Rimonabant was purchased from Cayman Chemical Company (Ann Arbor, MI, USA) and dissolved in carboxymethyl cellulose (CMC; 10% *w/v*, 10 mg/ml) immediately before use. Either rimonabant solution or an equal volume of vehicle (10% CMC, *w/v*) was administered by oral gavage 4 h after surgery. The dose of rimonabant that was used in this study was based on a previous study from our group [12].

Sepsis Model: Cecal Ligation and Puncture

Sepsis was induced by CLP as described previously [13]. Briefly, the rats were anesthetized with ketamine (90 mg/kg, i.p.; Vetnil Veterinary Products, Louveira, SP, Brazil) plus xylazine (10 mg/kg, i.p.; Syntec Laboratory, Cotia, SP, Brazil), and a 2-cm midline incision was made on the anterior abdomen. The cecum was exposed, ligated just distal to the ileocecal valve to avoid intestinal obstruction, punctured three times with a 16-gauge needle, squeezed to allow its contents to be extracted through the punctures, and returned to the abdominal cavity. The incision was then closed in layers. Sham-operated control animals were subjected to identical laparotomy without cecal puncture. All of the animals received 3 ml of saline subcutaneously immediately after surgery. The animals were allowed to recover in their homecages with free access to food and water.

Evaluation of Markers of Organ Failure and Hematological Parameters

Under the same anesthesia described above, blood samples were collected by cardiac puncture 6 or 24 h after

surgery using heparinized syringes and transferred to chilled plastic tubes. The blood samples were immediately subjected to analysis using a Sysmex XE-2100TM analyzer (São Paulo, Brazil) by flow cytometry with a semiconductor laser, focus, and hydrodynamic impedance method for blood cell counting. The results are expressed as the number of cells/ml. The blood samples were then centrifuged at 1000×*g* for 10 min at 4 °C, and the plasma samples were kept at − 80 °C for further analysis. Lactate, LDH, glucose, and CK-MB levels were measured in the Municipal Laboratory of Curitiba (Paraná, Brazil). The methods used for the analysis were as follows: colorimetry for lactate dosage, UV-kinetic (pyruvate-lactate) for LDH dosage, enzymatic-colorimetric using glucose oxidase for glucose dosing, and chemiluminescence for the CK-MB dosage. Plasma samples (24 h) were also used to evaluate NOx activity as described below.

Nitrate/Nitrite Assay

Nitrate and nitrite content in plasma samples was evaluated according to a previous study [14]. Briefly, zinc sulfate-deproteinized plasma samples were subjected to nitrate conversion to nitrite using *Escherichia coli* nitrate reductase. The samples were incubated with bacteria for 2 h at 37 °C and then centrifuged. The bacterial pellet was removed, and 100 μl of each sample was added to 100 μl of Griess reagent (1% sulfanilamide in 10% phosphoric acid/0.1% naphthyl-ethylenediamine in Milli-Q water) in a 96-well plate and read at 540 nm in a plate reader. Standard curves of nitrite and nitrate (0–150 μM) were run simultaneously. Because nitrate conversion was always greater than 90% under these conditions, no corrections were made. The values are expressed as micromoles of NOx (nitrate/nitrite).

Tail Vasomotor Tone Response

The animals were habituated to an ambient temperature of 28 °C (thermoneutral zone for rats) [15] on the day of the experiment. The next day, images of the animals were taken for 10 min before surgery using an infrared camera (Flir, A300 Series). The CLP or sham surgeries were performed at the same ambient temperature. Four hours after surgery, the rats were orally treated with rimonabant (10 mg/kg) or vehicle (10% CMC) as described above. Two hours after the treatments (6 h after CLP or sham surgery), images from the animals were taken for 10 min. Tail temperature was determined as the mean of three–five measurements of the third superior part of the tail in the last 5 min of the 10-min period.

Rat Aortic Ring Preparation and Isometric Tension Measurement to Evaluate Vascular Reactivity

The descending thoracic aorta was removed from sham-operated rats that were treated with vehicle, rats that were subjected to CLP and treated with vehicle, and rats that were subjected to CLP and treated with rimonabant 6 or 24 h after surgery. Connective tissues were cleaned. Immediately after removal and once the connective tissues had been cleaned off, the proximal portion of the thoracic aorta was cut into rings (3–4 mm length). The rings were then submerged in tissue bath chambers that contained 3 ml of physiological saline solution (PSS; composition 131.25 mmol/L NaCl, 4.82 mmol/L KCl, 1.59 mmol/L $CaCl_2 \cdot 2H_2O$, 1.18 mmol/L KH_2PO_4, 1.17 $MgSO_4$, 5.55 mmol/L D-glucose, 14.89 mmol/L $NaHCO_3$, and 0.03 mmol/L ethylenediaminetetraacetic acid [EDTA], pH 7.4) that was maintained at 37 °C and bubbled with a 95% O_2 and 5% CO_2 gas mixture. The aortic rings were connected to a force transducer to measure isometric contractions. The development of force was recorded using a digital polygraph and Powerlab and LabChart v. 7.1 software (both from AD Instruments, Castle Hill, Australia). The preparations were allowed to equilibrate under a tension of 1.0 g for 60 min, and the PSS was replaced every 15 min. The aortic rings were first stimulated with modified PSS that contained 60 mM KCl (composition: 70.16 mM NaCl, 59.83 mM KCl, 1.59 mM $CaCl_2 \cdot 2H_2O$, 1.18 mM KH_2PO_4, 1.17 mM $MgSO_4$, 14.89 mM $NaHCO_3$, 0.038 mM EDTA, and 5.55 mM D-glucose), followed by a washout and stabilization period of 30 min. Endothelial integrity was then confirmed by the ability of acetylcholine (1 mol/L) to induce relaxation (at least 80%) in preparations that were contracted with phenylephrine (PE; 1 mol/L). After washing and following a new 60-min stabilization period, the responses to PE or AVP (both 1 nmol/L to 100 mol/L) were determined. The patterns of vasoconstriction that developed in response to KCl, PE, and AVP were compared among groups.

Data Analysis

The results are expressed as the mean ± standard error of the mean (SEM). The markers of multiorgan failure, hematologic parameters, NOx levels, tail skin temperature, and KCl-induced contractions were analyzed using one-way analysis of variance followed by Bonferroni's *post hoc* test. Vascular contractions that were induced by PE and AVP were analyzed using two-way analysis of variance followed by Bonferroni's *post hoc* test. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Effect of CB_1 Receptor Blockade on Markers of Multiorgan Injury

Six hours after the induction of sepsis by CLP, significant increases in plasma levels of lactate (76%; Fig. 1a), glucose (36%; Fig. 1c), and CK-MB (380%; Fig. 1d) were observed compared with the sham-operated group. LDH levels in vehicle-treated CLP animals did not significantly increase at this time point (Fig. 1b). The blockade of CB_1 receptors by rimonabant significantly reduced plasma levels of lactate (Fig. 1a), glucose (Fig. 1c), and CK-MB (Fig. 1d). Twenty-four hours after CLP, lactate levels were still significantly elevated (67%; Fig. 1a). At this time point, LDH levels were also significantly elevated (106%; Fig. 1b). Glucose levels were significantly reduced (27%; Fig. 1c), and CK-MB levels already returned to normal levels. Treatment with rimonabant 4 h after the induction of CLP also significantly reduced lactate (Fig. 1a) and LDH (Fig. 1b) levels and normalized glucose levels (Fig. 1c) 24 h after the induction of sepsis.

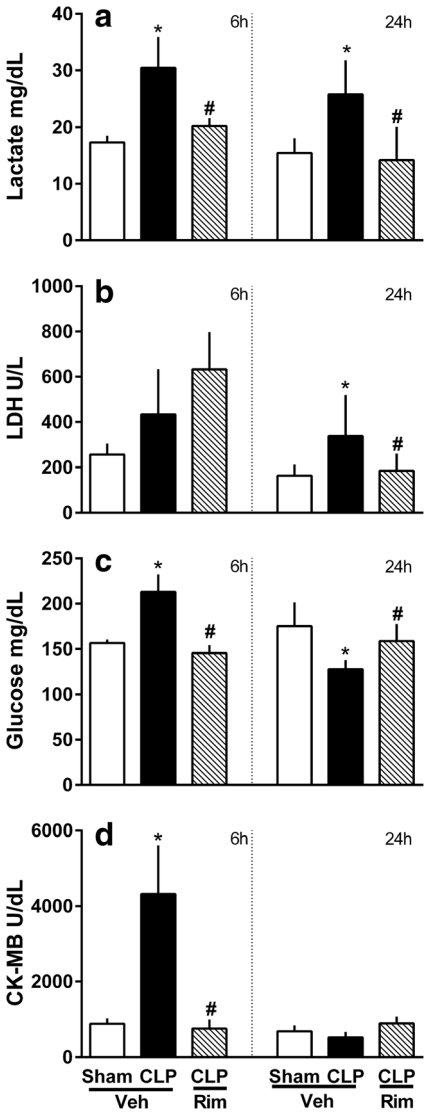

Fig. 1. Effect of CB_1 receptor blockade on biomarkers of multiorgan failure. Rats were subjected to CLP or sham surgery. Four hours after surgery, they were orally treated with vehicle (veh; 10% CMC) or rimonabant (rim; 10 mg/kg). Blood was collected 6 or 24 h after surgery to evaluate plasma levels of lactate (**a**), LDH (**b**), glucose (**c**), and CK-MB (**d**). The data are expressed as the mean ± SEM of biomarker levels in mg/dl or U/dl ($n = 6$–13). *$p < 0.05$, compared with sham/veh group; #$p < 0.05$, compared with CLP/veh group.

Effects of CB_1 Receptor Blockade on Hematologic Parameters

Six hours after CLP, sepsis caused a reduction of white blood cell counts (Fig. 2a) through reductions of lymphocytes (Fig. 2b) and monocytes (Fig. 2c) compared with the sham-operated group. The number of platelets also significantly decreased at this time point (Fig. 2d). Oral treatment with rimonabant did not influence these changes. No differences were found in cell counts 24 h after CLP (Fig. 2a–d).

Effects of CB_1 receptor blockade on plasma nitrate/nitrite levels

Twenty-four hours after CLP, plasma levels of NOx were still significantly elevated, and treatment with rimonabant did not influence this response (Fig. 3).

Effects of CB_1 Receptor Blockade on Tail Skin Temperature

Tail skin temperature was severely reduced 6 h after the induction of CLP, thus demonstrating that tail vasoconstriction (*i.e.*, a heat conservation response that is usually observed during fever) occurred (Fig. 4). Treatment with rimonabant completely abolished this response.

Effect of CB_1 Receptor Blockade on Vascular Reactivity in Aorta Vessels After the Induction of Sepsis by CLP

Organ dysfunction is closely related to changes in vascular reactivity. We evaluated the vascular

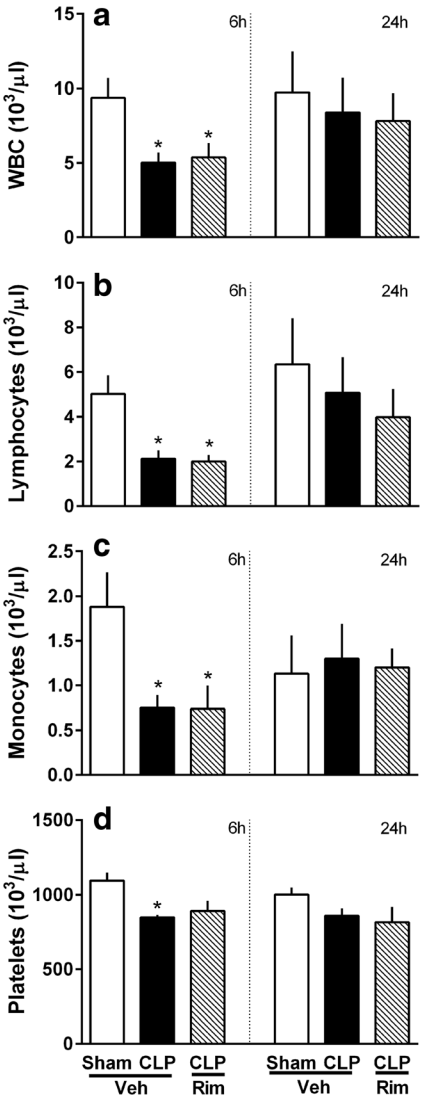

Fig. 2. Effect of CB_1 receptor blockade on hematologic parameters. Rats were subjected to CLP or sham surgery. Four hours after surgery, they were treated with vehicle (veh; 10% CMC) or rimonabant (rim; 10 mg/kg). Blood was collected 6 and 24 h after surgery to evaluate the number of white blood cells (WBC) (**a**), lymphocytes (**b**), monocytes (**c**), and platelets (**d**). The data are expressed as the mean ± SEM of the number of cells $\times 10^3/\mu l$ ($n = 5–7$). *$p < 0.05$, compared with sham/veh group.

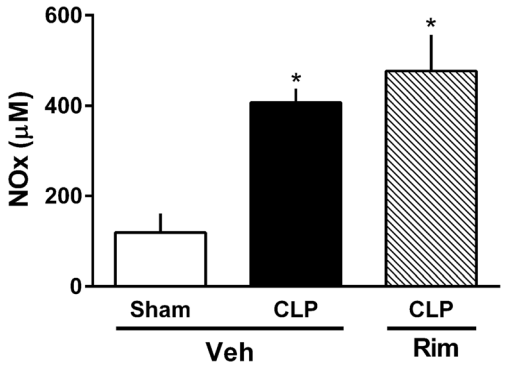

Fig. 3. Effect of CB_1 receptor blockade on NOx levels. Rats were subjected to CLP or sham surgery. Four hours after surgery, they were treated with vehicle (veh; 10% CMC) or rimonabant (rim; 10 mg/kg). Blood was collected 24 h after surgery to evaluate NOx levels. The data are expressed as the mean ± SEM of NOx levels in µM ($n = 5$). *$p < 0.05$, compared with sham/veh group.

responsiveness of aorta vessels. The maximum contractile effect (Emax) of KCl was reduced from 1.43 ± 0.10 g to 1.03 ± 0.09 g in rat aortic rings from sham-operated and CLP rats 6 h after surgery, respectively (Fig. 5a). Similarly, aortic rings that were obtained from CLP rats 6 h after surgery presented lower reactivity to PE (Fig. 5c). This pattern of loss of contractility was not observed 24 h after CLP (Fig. 5b, d), and CB1 blockade did not alter the hyporesponsiveness (Figs. 5a–d). Despite the sensitivity to AVP has not been reduced 6 h after the CLP surgery (Fig. 5e), aortic rings obtained from those animals subjected to CLP 24 h before presented augmented responses to AVP, a phenomenon that was not found when the animals received the CB1 antagonist rimonabant after CLP (Fig. 5f). The vascular reactivity of rimonabant-treated animals was statistically identical to the sham-operated group.

DISCUSSION

Septic shock represents a major complication in critical care medicine that can lead to multiple organ dysfunction and the development of organ injury is the most important clinical event during sepsis and the most common cause of death [16].

This study suggests that rimonabant may reduce organ failure following sepsis since it reduced organ failure markers. This improvement was reflected by reductions of markers of multiorgan failure, such as lactate, glucose, and CK-MB, in the first hours after CLP-induced sepsis after treatment with rimonabant. Rimonabant also reduced LDH levels and increased glucose levels 24 h after CLP. However, rimonabant did not alter leukopenia or NOx levels that were induced by CLP but reduced fever-associated vasoconstriction. Additionally, the sepsis-induced late hyperreactivity to AVP in aortic rings was abolished by rimonabant treatment.

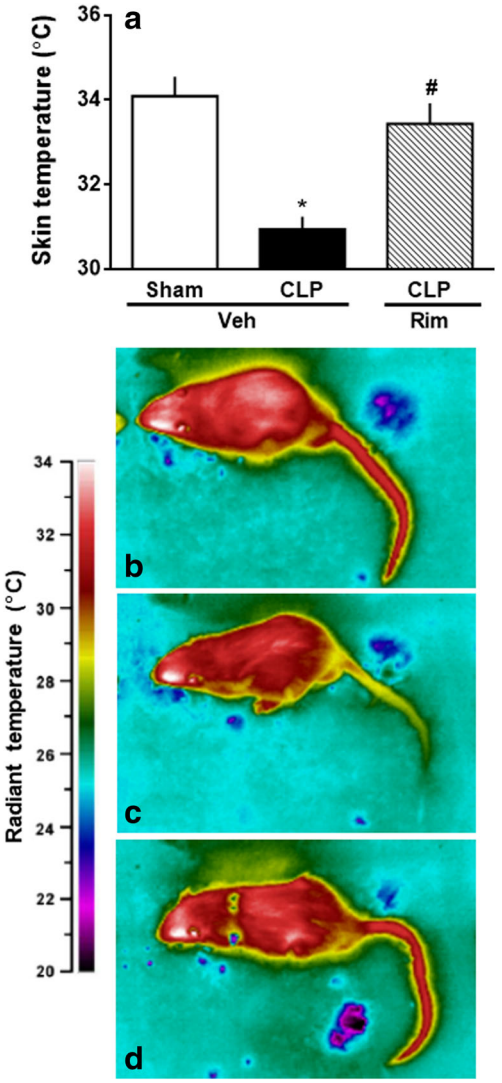

Fig. 4. Effect of CB_1 receptor blockade on tail skin temperature. Rats were subjected to CLP or sham surgery. Four hours after surgery, they were treated with vehicle (veh; 10% CMC) or rimonabant (rim; 10 mg/kg). Skin temperature was evaluated 6 h after CLP using a thermocamera. A Skin temperature measured on the superior third of the tail. B–D Representative images of animals in the groups that are presented in A, respectively, sham/veh (B), CLP/veh (C), and CLP/rim (D). The data are expressed as the mean ± SEM of skin temperature in °C ($n = 5–6$). *$p < 0.05$, compared with sham/veh group; #$p < 0.05$, compared with CLP/veh group.

Effect of CB_1 Receptor Blockade on Markers of Multiorgan Injury

Lactate normally increases in patients who experience sepsis or septic shock. High lactate levels are the first predictor of mortality in this syndrome. This increase in lactate levels may derive from glycolysis and the higher turnover of glucose [17]. Increases in plasma lactate levels are frequently used to diagnose inadequate tissue oxygenation in septic patients and are related to multiorgan failure [6]. In the present study, an increase in lactate levels 6 and 24 h after sepsis induction was observed, corroborating previous observations by Brooks et al. and confirming organ failure [17]. Other plasma markers also confirmed this condition. Yang et al. reported that elevated LDH levels in old rats, in addition to elevated lactate levels, is also related to multiorgan failure when present in the blood circulation, and this increase occurred 20 h after CLP [18]. Similar results were observed in the present study. However, Zhou et al. observed this increase in LDH levels as early as 6 h after CLP. This discrepancy may be related to the severity of sepsis [19].

The present results also revealed that blood glucose levels increased 6 h after CLP. At 24 h, a significant reduction of glucose levels was observed. This two-phase glycemic response has been previously reported [20]. The initial hyperglycemic phase seems to be mediated by the increase in stress hormones such as noradrenaline and glucagon which stimulate the hepatic glycogenolysis and gluconeogenesis, whereas hypoglycemia occurs later through organ hypoperfusion [20].

Zhang et al. showed that sepsis increased plasma CK-MB levels, an indicator of myocardial lesions [5]. Another study showed that CK-MB levels increased 6 h after CLP and reached a peak after 24 h [19]. Our results showed that CLP significantly increased CK-MB levels after 6 h, but no significant changes were observed after 24 h. Again, this may be related to the severity of septic shock.

Altogether, these data suggest that sepsis that was induced by CLP in the present study was consistent with previous observations and sufficient to induce some degree of multiorgan failure. Nonetheless, the duration and intensity of this response may vary among different studies, likely because of the intensity of the septic state.

We previously performed electrophysiological experiments in rat brain slices and found that endothelin-1 (ET-1) inhibited excitatory postsynaptic currents in vasopressinergic magnocellular cells, an effect that depended on the release of eCBs that act on presynaptic CB_1 receptors [11]. Central levels of ET-1 were shown to increase 4–8 h after CLP-induced sepsis [21]. Therefore, we hypothesize that this increase in ET-1 levels would consequently release eCBs that in turn would reduce the release of AVP during sepsis, thus contributing to vasodilation and hypotension that in turn contribute to organ failure. The blockade of CB_1 receptors by rimonabant in

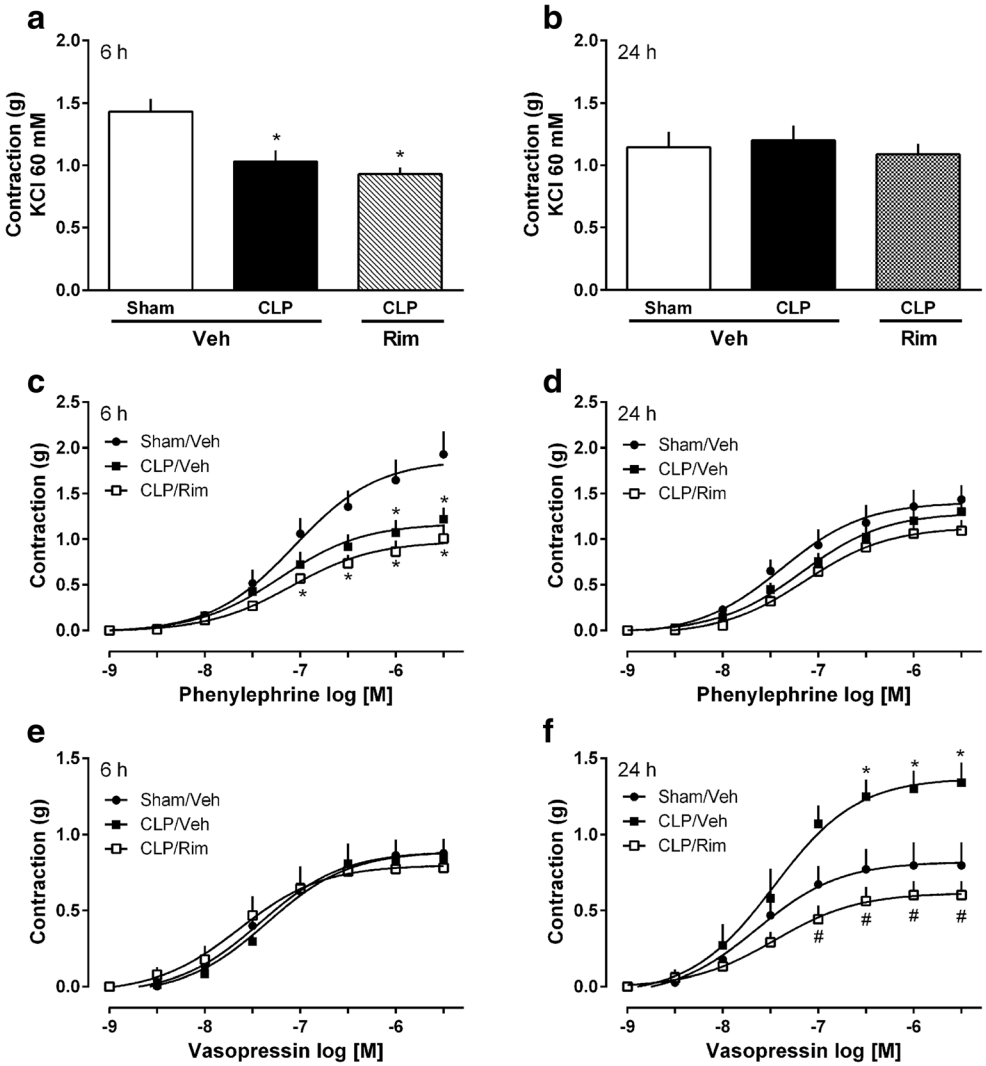

Fig. 5. Effect of CB_1 receptor blockade on vascular reactivity in aortic rings. Rats were subjected to CLP or sham surgery. Four hours after surgery, they received vehicle (veh; 10% CMC) or rimonabant (rim; 10 mg/kg). Aortic rings were collected 6 h (**a**–**c**) and 24 h (**d**–**f**) after surgery to evaluate the vascular reactivity to a high-KCl solution (**a**, **b**) and cumulative concentrations of phenylephrine (**c**, **d**) and vasopressin (**e**, **f**). The data are expressed as the mean ± SEM of contractions in g ($n = 6$/group). *$p < 0.05$, compared with sham/veh group; #$p < 0.05$, compared with CLP/veh group.

the central nervous system should increase AVP release and improve multiorgan failure. In fact, we previously found that treatment with rimonabant 4 h after CLP increased survival rates and the release of AVP 12 h after CLP [12]. The present study further supports this hypothesis because all of the plasma markers of multiorgan failure improved to levels that were similar to sham-operated animals both 6 and 24 h after CLP. This protective effect of rimonabant against multiorgan failure should thus improve the survival rate. Rimonabant is probably acting as a CB1 receptor antagonist since our previous study showed that rimonabant and the CB1 receptor antagonist AM251 had similar effects [12].

Effects of CB_1 Receptor Blockade on Hematologic Parameters

Our previous studies suggested that the beneficial effects of rimonabant on increasing survival rates were attributable to an effect on the central nervous system. Intracerebroventricular rimonabant administration reduced mortality in animals that were subjected to CLP, and

peripheral signs of sepsis (*e.g.*, the number of bacterial colony-forming units in peritoneal exudates, cellular migration to the peritoneal cavity, leukopenia, and plasma interleukin-6 levels) remained unchanged [12]. Leukopenia, particularly lymphocytopenia, is one of the most frequent manifestations of experimental sepsis [22] and clinical sepsis [23] and may be related to an increase in lymphocyte apoptosis or leukocyte migration. Corroborating our previous study [12], we found that leukopenia and the reduction of platelet counts were unchanged after rimonabant treatment.

Both CB_1 and CB_2 receptors are found peripherally, although CB_1 receptor expression is more widespread in the central nervous system [24]. CB_2 receptors are involved in immunomodulation and are expressed in immune system cells. Corroborating our previous study, the present study found that leukopenia and the reduction of platelets counts were unchanged after rimonabant treatment. Rimonabant did not alter leukopenia after CLP; therefore, the improvement in multiorgan failure does not appear to be related to alterations of the peripheral inflammatory response.

Effects of CB_1 receptor blockade on plasma NOx levels and tail skin temperature

To further substantiate that the improvement in multiorgan failure that was promoted by rimonabant was unrelated to a reduction of the severity of the inflammatory response *per se*, we evaluated the effects of rimonabant on the dynamics of blood vessel contractions and dilation during sepsis.

Septic shock is a sub-condition of sepsis that is accompanied by profound circulatory and metabolic abnormalities [2]. Among such abnormalities are a reduction of arterial blood pressure and changes in body temperature (*e.g.*, fever or hypothermia). The reduction of arterial blood pressure results from a reduction of the sensitivity to vasoconstrictors (*e.g.*, norepinephrine, angiotensin II, and AVP) and an increase in the production of vasodilators, such as nitric oxide. An increase in nitric oxide production is achieved through the induction of vasoactive enzymes by cytokines, such as nitric oxide synthase. Peripheral vasodilation also plays a pivotal role in hypothermia because an increase in heat loss occurs. Conversely, the febrile response is caused by an effect of cytokines in the central nervous system, particularly in the hypothalamus, which induces heat production (*e.g.*, shivering and non-shivering thermogenesis) and the activation of heat conservation mechanisms (*e.g.*, peripheral vasoconstriction).

In the present study, we found that NOx, stable products of nitric oxide oxidation [25], was higher in CLP rats compared with sham-operated animals, corroborating previous results [26, 27]. The blockade of CB_1 receptors by rimonabant did not alter NOx levels in blood. We also found that CLP induced vasoconstriction in the tail, which is consistent with the development of a febrile response in the early hours after CLP. Rimonabant reversed this vasoconstriction. This finding is consistent with previous results [12], in which we observed a febrile response in animals 6–8 h after CLP. Additionally, previous studies showed that the response of the vascular system during sepsis varies according to the system that is evaluated (*e.g.*, tail artery *vs.* mesenteric artery) and also according to the time point and stimulus [28]. Different from the superior mesenteric and carotid arteries, the tail artery was hyperresponsive to the vasoconstrictors norepinephrine and AVP 6 and 18 h after CLP [28]. These results suggest that rimonabant alters the dynamics of blood vessel contractility rather than alters the production of vasodilators (*e.g.*, nitric oxide), which would improve multiorgan failure.

Effect of CB_1 Receptor Blockade on Vascular Reactivity in Aorta Vessels after the Induction of Sepsis by CLP

Hypotension and low tissue blood flow that are caused by systemic vasodilation are likely the most important causes of organ injury and hence organ failure. This is associated with an inability to regularly maintain tone in response to endogenous or exogenous vasoactive agents [29]. However, the vascular system may present impairments in the ability to maintain tone, which may be either decreased or increased, depending on the specific vasoconstrictor, experimental model of sepsis, and period of evaluation [28, 30].

Bernardelli et al. reported a decrease in carotid artery reactivity to PE 6 and 18 h after CLP and to AVP 18 h after CLP. In the present study, we observed a reduction of PE-induced contractions in the aorta 6 h after CLP, and this response returned to normal levels 24 h after CLP. Notably, we observed hyperresponsiveness to AVP 24 h after CLP. This greater response to AVP has been observed in other vascular beds, such as the tail artery [28] and small mesenteric arteries [31]. Indeed, this phenomenon has also been observed in septic patients. Septic patients presented impairments in the vasoconstriction response to PE and angiotensin II in the forearm, and the vasoconstrictor response to AVP was exaggerated [32]. The precise reasons for these changes in AVP sensitivity during sepsis (*e.g.*,

refractory, no changes, or hypersensitivity) are currently unknown, but some studies suggest that the explanation may be found at the level of vascular receptors [31]. Based on the available data, vascular beds appear to develop hypersensitivity at later times (*i.e.*, late sepsis), but this may also depend on the severity of sepsis.

Importantly, the present study found that rimonabant did not influence the hyporesponsiveness to PE but normalized the hyperreactivity to AVP in aortic rings from rats that were subjected to CLP. This result indicates that the blockade of CB_1 receptors by rimonabant affects the AVP response. We previously showed that the treatment of septic animals with rimonabant increased blood levels of AVP 12 h after CLP [12]. The higher levels of ET-1 in the central nervous system during sepsis may increase the release of eCBs that in turn act on vasopressinergic neurons to reduce the release of AVP. Therefore, the blockade of CB_1 receptors by rimonabant would increase the release of AVP, and this effect would contribute to improvements in multiorgan dysfunction.

CONCLUSION

The main finding of the present study was that oral treatment with rimonabant normalized the levels of biomarkers of organ dysfunction, such as lactate, LDH, glucose, and CK-MK, in rats with sepsis that was induced by CLP, although some signs of the severity of sepsis (leukopenia, lower platelet counts, and higher NOx levels) remained unchanged. Oral treatment with rimonabant also attenuated hypercontractility that was induced by vasopressin but did not affect hypocontratility that was induced by PE. This improvement in multiorgan failure may be related to a better response of the vessels to AVP. Although more studies are necessary, rimonabant should be considered as a supplementary therapy for sepsis.

ACKNOWLEDGMENTS

The authors thank MSc. Tatiane M.B.B. Telles for the help with the analysis in the Municipal Laboratory of Curitiba.

FUNDING

This study was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Grant # 473194/2012-0.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that they have no conflict of interest.

Ethical Approval. All procedures performed in studies involving animals were approved by the institution's Ethical Committee for Animal Use and were in accordance with Brazilian and EU Directive 2010/63/EU Guidelines for Animal Care. All efforts were made to minimize the number of animals used and their suffering.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES

1. Wiersinga, W.J., S.J. Leopold, D.R. Cranendonk, and T. van der Poll. 2014. Host innate immune responses to sepsis. *Virulence* 5 (1): 36–44.
2. Singer, M., C.S. Deutschman, C.W. Seymour, M. Shankar-Hari, D. Annane, M. Bauer, R. Bellomo, G.R. Bernard, J.D. Chiche, C.M. Coopersmith, R.S. Hotchkiss, M.M. Levy, J.C. Marshall, G.S. Martin, S.M. Opal, G.D. Rubenfeld, T. van der Poll, J.L. Vincent, and D.C. Angus. 2016. The third international consensus definitions for sepsis and septic shock (sepsis-3). *JAMA* 315 (8): 801–810.
3. Bakker, J., M.W. Nijsten, and T.C. Jansen. 2013. Clinical use of lactate monitoring in critically ill patients. *Annals of Intensive Care* 3 (1): 12.
4. Duman, A., A. Akoz, M. Kapci, M. Ture, S. Orun, K. Karaman, and K.A. Turkdogan. 2016. Prognostic value of neglected biomarker in sepsis patients with the old and new criteria: predictive role of lactate dehydrogenase. *The American Journal of Emergency Medicine* 34 (11): 2167–2171.
5. Zhang, L., et al. 2015. Poly (ADP-ribose) synthetase inhibitor has a heart protective effect in a rat model of experimental sepsis. *International Journal of Clinical and Experimental Pathology* 8 (9): 9824–9835.
6. Lafreniere, J.D., and C. Lehmann. 2017. Parameters of the endocannabinoid system as novel biomarkers in sepsis and septic shock. *Metabolites* 7 (4): pii: E55.
7. Maccarrone, M., I. Bab, T. Bíró, G.A. Cabral, S.K. Dey, V. di Marzo, J.C. Konje, G. Kunos, R. Mechoulam, P. Pacher, K.A. Sharkey, and A. Zimmer. 2015. Endocannabinoid signaling at the periphery: 50 years after THC. *Trends in Pharmacological Sciences* 36 (5): 277–296.
8. Sarker, K.P., S. Obara, M. Nakata, I. Kitajima, and I. Maruyama. 2000. Anandamide induces apoptosis of PC-12 cells: involvement of superoxide and caspase-3. *FEBS Letters* 472 (1): 39–44.
9. Villanueva, A., S.M. Yilmaz, W.R. Millington, R.A. Cutrera, D.G. Stouffer, L.H. Parsons, J.F. Cheer, and C. Feleder. 2009. Central cannabinoid 1 receptor antagonist administration prevents endotoxic

hypotension affecting norepinephrine release in the preoptic anterior hypothalamic area. *Shock* 32 (6): 614–620.
10. Pacher, P., and G. Kunos. 2013. Modulating the endocannabinoid system in human health and disease–successes and failures. *The FEBS Journal* 280 (9): 1918–1943.
11. Zampronio, A.R., J.B. Kuzmiski, C.M. Florence, S.J. Mulligan, and Q.J. Pittman. 2010. Opposing actions of endothelin-1 on glutamatergic transmission onto vasopressin and oxytocin neurons in the supraoptic nucleus. *The Journal of Neuroscience* 30 (50): 16855–16863.
12. Leite-Avalca, M.C., et al. 2016. Involvement of central endothelin ETA and cannabinoid CB1 receptors and arginine vasopressin release in sepsis induced by cecal ligation and puncture in rats. *Shock* 46 (3): 290–296.
13. Rattmann, Y.D., S.M. Malquevicz-Paiva, M. Iacomini, and L.M.C. Cordeiro. 2013. Galactofuranose-rich polysaccharides from Trebouxia sp. induce inflammation and exacerbate lethality by sepsis in mice. *Phytochemistry* 94: 206–210.
14. Fernandes, D., et al. 2006. Nitric oxide-dependent reduction in soluble guanylate cyclase functionality accounts for early lipopolysaccharide-induced changes in vascular reactivity. *Molecular Pharmacology* 69 (3): 983–990.
15. Gordon, C.J. 1990. Thermal biology of the laboratory rat. *Physiology & Behavior* 47 (5): 963–991.
16. Karnatovskaia, L.V., and E. Festic. 2012. Sepsis: a review for the neurohospitalist. *Neurohospitalist* 2 (4): 144–153.
17. Brooks, H.F., C.K. Osabutey, R.F. Moss, P.L.R. Andrews, and D.C. Davies. 2007. Caecal ligation and puncture in the rat mimics the pathophysiological changes in human sepsis and causes multi-organ dysfunction. *Metabolic Brain Disease* 22 (3–4): 353–373.
18. Yang, W.L., and G. Ma. 2016. Combined administration of human ghrelin and human growth hormone attenuates organ injury and improves survival in aged septic rats. *Molecular Medicine* 22: 1.
19. Zhou, L., M. Gao, Z. Xiao, J. Zhang, X. Li, and A. Wang. 2015. Protective effect of astaxanthin against multiple organ injury in a rat model of sepsis. *The Journal of Surgical Research* 195 (2): 559–567.
20. Maitra, S.R., M.M. Wojnar, and C.H. Lang. 2000. Alterations in tissue glucose uptake during the hyperglycemic and hypoglycemic phases of sepsis. *Shock* 13 (5): 379–385.
21. Sharma, A.C., S.J. Motew, S. Farias, K.J. Alden, H.B. Bosmann, W.R. Law, and J.L. Ferguson. 1997. Sepsis alters myocardial and plasma concentrations of endothelin and nitric oxide in rats. *Journal of Molecular and Cellular Cardiology* 29 (5): 1469–1477.
22. Osuchowski, M.F., J. Connett, K. Welch, J. Granger, and D.G. Remick. 2009. Stratification is the key: inflammatory biomarkers accurately direct immunomodulatory therapy in experimental sepsis. *Critical Care Medicine* 37 (5): 1567–1573.
23. Cohen, J. 2002. The immunopathogenesis of sepsis. *Nature* 420 (6917): 885–891.
24. Katchan, V., P. David, and Y. Shoenfeld. 2016. Cannabinoids and autoimmune diseases: A systematic review. *Autoimmunity Reviews* 15 (6): 513–528.
25. Ho, J.T., M.J. Chapman, S. O'Connor, S. Lam, J. Edwards, G. Ludbrook, J.G. Lewis, and D.J. Torpy. 2010. Characteristics of plasma NOx levels in severe sepsis: high interindividual variability and correlation with illness severity, but lack of correlation with cortisol levels. *Clinical Endocrinology* 73 (3): 413–420.
26. Vincent, J.L. 1998. Cardiovascular alterations in septic shock. *The Journal of Antimicrobial Chemotherapy* 41 (Suppl A): 9–15.
27. Lorente, J.A., et al. 1993. L-arginine pathway in the sepsis syndrome. *Critical Care Medicine* 21 (9): 1287–1295.
28. Bernardelli, A.K., et al. 2016. Vasoplegia in sepsis depends on the vascular system, vasopressor, and time-point: a comparative evaluation in vessels from rats subjected to the cecal ligation puncture model. *Canadian Journal of Physiology and Pharmacology* 94 (11): 1227–1236.
29. OBrien, J.M., Jr., et al. 2007. Sepsis. *The American Journal of Medicine* 120 (12): 1012–1022.
30. Bennett, T., R.P. Mahajan, J.E. March, P.A. Kemp, and S.M. Gardiner. 2004. Regional and temporal changes in cardiovascular responses to norepinephrine and vasopressin during continuous infusion of lipopolysaccharide in conscious rats. *British Journal of Anaesthesia* 93 (3): 400–407.
31. Barrett, L.K., N.N. Orie, V. Taylor, R.P. Stidwill, L.H. Clapp, and M. Singer. 2007. Differential effects of vasopressin and norepinephrine on vascular reactivity in a long-term rodent model of sepsis. *Critical Care Medicine* 35 (10): 2337–2343.
32. Kienbaum, P., C. Prante, N. Lehmann, A. Sander, A. Jalowy, and J. Peters. 2008. Alterations in forearm vascular reactivity in patients with septic shock. *Anaesthesia* 63 (2): 121–128.