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Article

The effect of caffeine on sepsis induced cardiovascular dysfunction

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Sepsis is characterized by inadequate microvascular tissue perfusion, leading to organ dysfunction and death. Adenosine levels increase during inflammation and tissue hypoxia, potentially influencing cardiovascular parameters through activation of four receptors coupled to the G protein (A_1, A_{2A}, A_{2B}, and A_3). Caffeine is a non-selective adenosine receptor antagonist and it might prevent the cardiovascular collapse associated with sepsis. This study aimed to assess caffeine effect on sepsis-induced cardiovascular changes in rats. Animals were submitted to cecal ligation and puncture (CLP) to induce sepsis. Two, 8, and 14 hours after the procedure, CLP and sham groups were assigned to receive caffeine (30 mg/kg, s.c.) or vehicle. Twenty-four hours later, biochemical, and hemodynamic parameters were evaluated in addition to survival rate. Sepsis resulted in hypotension, hyporesponsiveness to vasoconstrictors, reduced renal blood flow, and increased blood glucose and lactate levels. Caffeine prevented changes in glycemic levels, but not in the cardiovascular alterations induced by sepsis. Caffeine also shows no discernible impact on markers of organ dysfunction or tissue perfusion. Thus, while caffeine maintained glycemic levels, it did not mitigate sepsis-induced cardiovascular collapse, organ dysfunction, or affect mortality rates. Therefore, caffeine does not provide significant improvements during sepsis.

Keywords: Septic shock. 1,3,7-Trimethylxanthine. Hypotension. Vasoplegia. Adenosine

INTRODUCTION

Sepsis is a condition associated with high mortality rates in intensive care units (Fleischmann-Struzek *et al*., 2020) and represents a significant global health challenge. The estimated number of sepsis incidence worldwide is 19 to 49 million per year, leading to the death of 35 to 45% of patients (Rudd *et al*., 2020). Despite the substantial and alarming impact of sepsis on healthcare outcomes, a comprehensive understanding of its pathophysiology remains elusive.

Over the last few decades, several important discoveries have demonstrated that adenosine plays an essential role as an extracellular signaling molecule (Zhang, Yu-Jing, Ma, 2022). Adenosine is an endogenous nucleoside formed mainly from the breakdown of ATP and ADP nucleotides. As expected, ATP extracellular concentration increases under conditions that are associated with sepsis, such as inflammation, ischemia, and hypoxia (Schmidt *et al*., 1995). Extracellular ATP is converted to adenosine, via the intermediates ADP and AMP by the membrane-bound enzymes CD39 and CD73 (Antonioli *et al*., 2013). Extracellular adenosine exerts its actions through four distinct G protein-coupled receptors, A_1, A_{2A}, A_{2B}, and A_3. However, extracellular adenosine binds with high affinity to A_1 and A_{2A} receptors making these receptors the main mediators of adenosine response (Borea *et al*., 2018). Adenosine receptors are ubiquitous and modulate multiple physiological functions across the central, cardiovascular, peripheral, and immune nervous systems.

Interestingly, activation of A_{2A} receptor leads to vasodilation and decrease in blood pressure (Headrick

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et al., 2011). On the other hand, A_1 adenosine receptor causes vasoconstriction in the renal vasculature and controls renal vascular tone (Vallon, Mühlbauer, Osswald, 2006). Therefore, elevation of adenosine levels in sepsis (Martin *et al*., 2000) may contribute to hemodynamic disturbances through these intricate vascular effects.

Caffeine is a non-selective adenosine receptor antagonist although it demonstrates a higher affinity for A_1 and A_{2A} receptors (Chen, Eltzschig, Fredholm, 2013). Therefore, the concurrent blockade of A_1 and A_{2A} receptors by caffeine may significantly contribute to mitigating the renal ischemia and hypotension induced by the activation of these two receptors during sepsis. This dual blockade by caffeine has the potential to prevent organ dysfunction and enhance overall survival. Previous studies have already evaluated the effect of caffeine in in animals (Bauza, Remick, 2015) and humans (Ramakers *et al*., 2011). However, results of these previous reports are contradictory and part of it is due to the different doses of caffeine used (ranging from 4 to 50 mg/kg). The dose is usually based on the amount of caffeine present in popular beverages like coffee and tea. Although this approach may be suitable for studies that aim to evaluate the effects of chronic caffeine exposure, certainly it is not adequate for evaluating the consequence of acute adenosine receptor blockade. Furthermore, caffeine has been administered either after or immediately before sepsis induction (Bauza, Remick, 2015). Again, while this approach could be interesting for assessing the impact of caffeine consumption on sepsis, it may not represent a suitable protocol for evaluating the potential role of adenosine receptors in sepsis treatment. Finally, these reports used either a single dose (Bauza, Remick, 2015) or administration at 24-hours intervals (Verma *et al*., 2009). However, data in humans show that caffeine effects are brief, with a half-life about 3 and 5 hours (Tavares, Sakata, 2012), which may be even shorter in animals.

Therefore, this study aimed to assess the impact of caffeine on sepsis, using an appropriate dose and frequency of administration for the blockade of adenosine receptors, as recently established by our research group (Albino *et al*., 2023). We hypothesize that caffeine promotes a protective effect on sepsis cardiovascular changes.

MATERIAL AND METHODS

Chemical compounds

The following substances were used in this study: sodium heparin (Eurofarma, São Paulo, SP, Brazil), ketamine hydrochloride and xylazine (Syntec do Brasil Ltda, Cotia, SP, Brazil), caffeine, phenylephrine, and angiotensin II (Sigma Chemical Co., St. Louis, MO, USA), isoflurane (Instituto BioChimico, Penedo, RJ, Brazil), tramadol hydrochloride (Laboratório Teuto, Anápolis, GO, Brazil).

Animals

Male Wistar rats (12-week-old, 358 ± 13 g) used in this study were supplied by the Universidade Federal de Santa Catarina Animal Facility, housed in a temperature and light-controlled room (23 ± 2°C; 12-hours light/dark cycle) and had free access to water and food (Biobase, Biotech line, Marmeleiro, Brazil). Rats were kept in 45 x 34 x 16 cm plastic cages (5 rats per cage). All the experiments were performed between 9:00 and 16:00 hours. The procedures were previously approved by the University Institutional Ethics Committee (protocol number 301220221) and were in accordance with the Brazilian National Council of Animal Experimentation and the National Institutes of Health Animal Care Guidelines. Animal studies are reported in compliance with the ARRIVE guidelines (Sert *et al*., 2020).

Cecal ligation and puncture (CLP)

CLP surgery was performed as previously described (Wichterman, Baue, Chaudry, 1980) with minor modifications. Five minutes before initiating the procedure, the opioid analgesic tramadol (10 mg/kg, i.p.) were administered. Animals were anesthetized by isoflurane/oxygen mix. Isoflurane 5% was used for sedation and 3% for maintainance. The cecum was exposed and partially occluded by a non-obstructing ligation right above the ileocecal valve. One transfixing puncture was made through the caecum with an 18-gauge needle and a small amount of cecal content

was extravasated through the puncture. Finally, the cecum was replaced in the peritoneal cavity, and the muscles and skin of the abdominal region were sutured. Sham-operated rats underwent a similar surgical procedure with cecal exposition, but it was neither ligated nor punctured. All the animals received 50 ml/kg of saline (NaCl 0.9%) subcutaneously immediately after the procedure. Saline solution was administered for fluid resuscitation to reproduce clinical hemodynamic support and to induce a hyperdynamic circulatory phase. Animals were accommodated in cages placed on top of a heating mat (37 ± 1 °C) and for anesthesia recovery. Twelve hours after the surgical procedure the rats were again treated with tramadol (5 mg/kg i.p.) to maintain the analgesic effect.

Mean arterial pressure

Animals were submitted to anesthesia intramuscularly with ketamine and xylazine (90 and 10 mg/kg, respectively) and supplemented, when necessary, with ketamine during the complete experimental protocol. The monitoring of anesthesia was assessed by regular respiratory rate, heart rate, and absence of withdrawal reflex upon hind toe pinching. The animals were kept on a heating mat with a controlled temperature (37 ± 1 °C) throughout the experiment. A heparinized PE-20 polyethylene catheter was inserted into the right jugular vein for drug injections. A tracheal cannula was used to allow animals to breathe. Finally, a heparinized polyethylene catheter PE-50 was inserted into the left carotid artery and connected to a pressure transducer coupled to PowerLab 8/30 (AD Instruments Pty Ltd., Castle Hill, Australia) running LabChart7® software for mean arterial pressure (in mmHg) and heart rate (in BPM) recording. After 15 minutes of stabilization, the basal values of mean arterial pressure and heart rate were recorded, and dose-response curves to angiotensin II (1, 3, 10 and 30 pmol/kg, i.v.) and phenylephrine (1, 3, 10, and 30 nmol/kg, i.v.) were obtained. The doses were injected in a total volume of 250 µL (including washing of the catheter). The change in mean arterial pressure (in mmHg) was calculated and compared between the groups.

Renal blood flow

Simultaneous to the mean arterial pressure measurement, renal blood flow (in perfusion units, PU) was determined as previously described (Kovalski *et al.*, 2017). Briefly, a transverse abdominal incision was performed to assess the posterior left subhepatic space, allowing the visualization of the left kidney. Then, a laser probe (model VP3), connected to a laser Doppler blood flow monitor (moorVMSLDF2, Moor Instruments, England) was carefully placed directly on the left kidney. The laser Doppler monitor was also coupled to the PowerLab 8/30 and the renal blood flow (RBF; in perfusion unit, PU) was recorded. The probe was kept in this position and the surgical incision was covered with gauze sponges soaked in sterile saline to protect the kidney from drying. An interval of 15 minutes was allowed before measuring the basal values of renal blood flow. Changes in renal blood flow induced by angiotensin II and phenylephrine were recorded.

Blood glucose and lactate

Glycemia was measured at baseline, 6, 12, and 24 hours after sepsis induction from a drop of blood from the tip of tail of awake rats. Glucose levels were measured using an automatic analyzer (Call on Plus II, Medlevensohn, São Paulo, Brazil). Blood lactate was measured 24 hours, also from the tail blood and using the kit Accutrend® plus (Roche, Mannheim, Germany).

ALT, AST, creatinine, and urea levels

Sample blood was collected from the carotid catheter used for blood pressure measurement in tubes containing 5 IU heparin for each 1 mL of blood. The blood was centrifuged (1.500 x g; 10 minutes; 4 ºC). Plasma levels of urea, creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured using commercially available clinical assay kits (Labtest Diagnóstica S.A. Lagoa Santa, MG, Brazil).

NOx (nitrite + nitrate) assay

Nitric oxide (NO) production was estimated by measuring the plasma concentration of nitrate + nitrite, products of NO oxidation (Sordi *et al.*, 2011). Initially, plasma samples were deproteinized by the addition of zinc sulfate (1 hour, 0°C). In deproteinized samples obtained by centrifugation nitrate was converted to nitrite using nitrate reductase from *Escherichia coli*. After centrifugation (1.530 x g; 15 minutes) to remove bacteria, the supernatant was mixed with Griess reagent (1% sulphanilamide in 10% phosphoric acid; 0.1% naphtyl ethylenediamine). The plate was read at 540 nm in a micro plate reader (ELx800®, BioTek, USA). Standard curves of nitrite and nitrate (0–150 µM) were run simultaneously. Values are expressed as µM of NOx (nitrate + nitrite).

Experimental protocol

Rats were randomized in 2 groups and were subjected to either sham or CLP procedures. The CLP group was randomized to receive caffeine or vehicle. Caffeine (30 mg/kg, s.c.) or vehicle (saline 1 mL/kg, s.c.) were administered 2, 8, and 14 hours after the surgical procedure. Sham group was treated only with vehicle (saline 1 mL/kg, s.c.) at the same time as the CLP group. Twenty-four hours after animals were prepared for mean arterial pressure and renal blood flow measurements as described. At the end of the experiment, a blood sample was collected to measure AST, ALT, creatinine, urea, and NOx levels (Figure 1A). Animals were then killed by anesthetic overdose. The experiments were not done blindly, and the experimenter was aware of the treatments. In addition, no adopted criteria were used for inclusion or exclusion of animals.

In another set experiment, it was assessed the effect of caffeine on the sepsis survival rate. Rats were randomly distributed and injected with caffeine (30 mg/kg, s.c.) or vehicle (saline 1 mL/kg, s.c.) as above. To reproduce a clinical scenario, the CLP group also received a single dose of a broad-spectrum antibiotic (24.000 IU/kg; Veterinary Pentabiotic, Zoetis) 6 hours after surgical procedures. Survival was observed every 12 hours until 96 hours (Figure 1B).

The dose of caffeine and the frequency of administration were based on pharmacokinetic, and toxicologic tests (Wikoff *et al.*, 2017), and mainly in our previous study (Albino *et al.*, 2023).

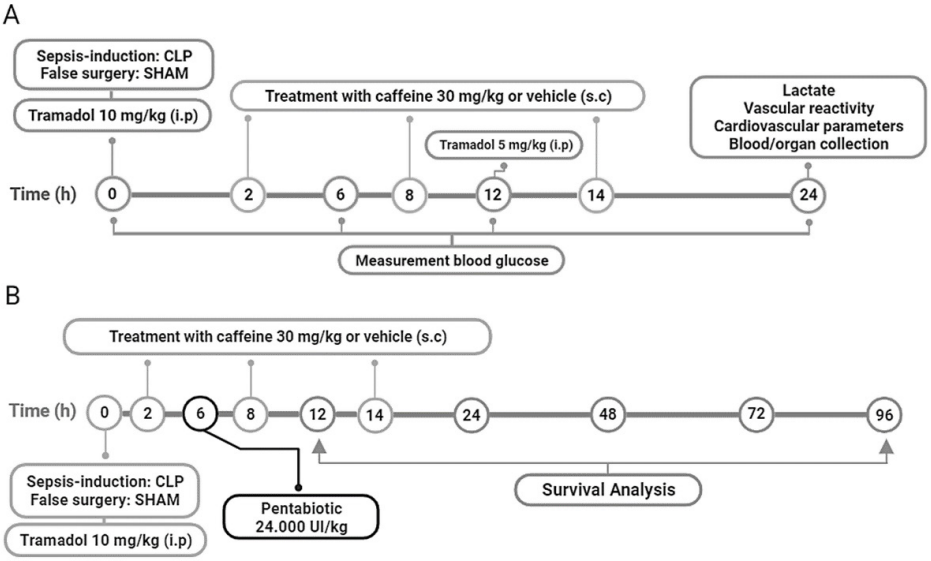

FIGURE 1 - Experimental protocol to study the effect of caffeine on sepsis. (A) Two, 8, and 14 hours after cecal ligation and puncture (CLP) or sham procedures, rats were assigned to receive either caffeine (30 mg/kg, s.c.) or vehicle (saline, 1 ml/kg, s.c.). Twenty-four hours after the CLP or sham procedures the analyses were performed. (B) For survival analysis, animals were submitted to CLP or sham surgery. Six hours after the procedure, animals received Pentabiotic (24.000 UI/kg, i.m.). Animals were monitored every 12 hours for 96 hours.

Sample calculation

Sample calculation was based on the standard deviation (S.D.) and the magnitude of difference between the groups obtained in the analysis of blood pressure (in mmHg) from our previous studies (Kovalski *et al*., 2017). Thus, considering 3 experimental groups, $\alpha = 0.05$, a power of 80%, and an effect size of 0.52 (f), 12 animals in each group are required for statistical significance. This sample size maintains the power of at least 80% also for the other cardiovascular parameters. To account for the mortality rate of ~25 % of CLP model in 24 hours or a potential technical loss, 15 additional animals were included in CLP group. The final number (n) in each group is indicated in figure legends. The Gpower 3.1.1 software was used to sample size calculation (Faul, Erdfelder, Lang, 2007). For the survival analysis, a sample size of 30 animals per group was calculated based on the expected rate of mortality reduction of 35%, with a power of 80% and $\alpha = 0.05$. The Primer of Statistics version 7 software was used to sample size calculation of survival experiments.

Statistical analysis

Data were expressed as the mean ± S.D. Dose response curves were analyzed by two-way ANOVA (factors: treatment and dose), followed by Sidak post hoc test. For other data, one-way or two-way ANOVA was employed, followed by Sidak post hoc test. Normality and homogeneity of variance were verified through Shapiro-Wilk and Bartlett tests, respectively. The survival was expressed as percentage of survival over time and differences were determined with a log-rank (Mantel-Cox). The tests used are indicated in the figure legends. Agonist concentration-response curves were fitted using nonlinear regression.

RESULTS

Effect of caffeine on CLP-induced cardiovascular changes

Twenty-four hours after sepsis induction, animals exhibited a reduction in the mean arterial pressure by 36 mmHg (Figure 2A, $p < 0.05$). Caffeine treatment failed to restore sepsis-induced hypotension. On the other hand, caffeine led to a significant elevation in heart rate, with a mean increase of 55 BPM (Figure 2B, $p < 0.05$). No significant changes in the basal renal blood flow were found (Figure 2C). CLP animals presented an impairment in angiotensin II response that remained unaltered by caffeine administration (Figure 3A, $p < 0.05$). CLP vehicle group did not present a hyporeactive response to phenylephrine (Figure 3B). Nevertheless, CLP caffeine group exhibited a diminished response to phenylephrine in comparison to both the CLP vehicle and sham groups (Figure 3B, $p<0.05$). Angiotensin II and phenylephrine induced dose-response reductions in renal blood flow (Figure 3C and D). Animals from CLP groups treated or not with caffeine present impairment in angiotensin II and phenylephrine response when compared to the sham group (Figure 3C and D, $p < 0.05$), respectively.

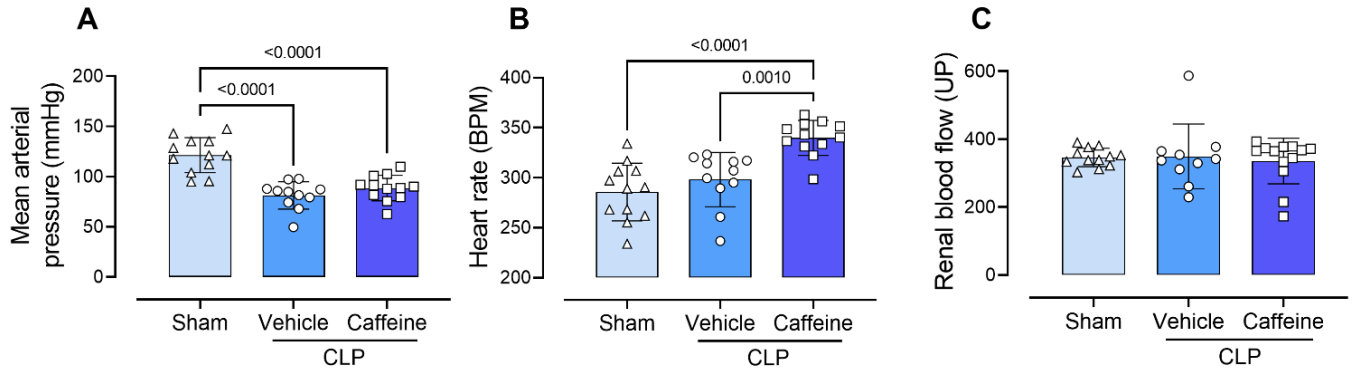

FIGURE 2 - Effect of caffeine on blood pressure, renal blood flow, and heart rate of septic rats. Two, 8, and 14 hours after the cecal ligation and puncture (CLP) or sham procedures rats were assigned to receive vehicle (saline 1 mL/kg, s.c.), or caffeine (30 mg/kg, s.c.). Twenty-four after surgery the following parameters were recorded: (A) mean arterial pressure; (B) heart rate, and (C) renal blood flow. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by the Sidak post-hoc test. Data are expressed as dot plots for each animal and the mean ± S.D., n= 10-13 animals as indicated by the dots in each group.

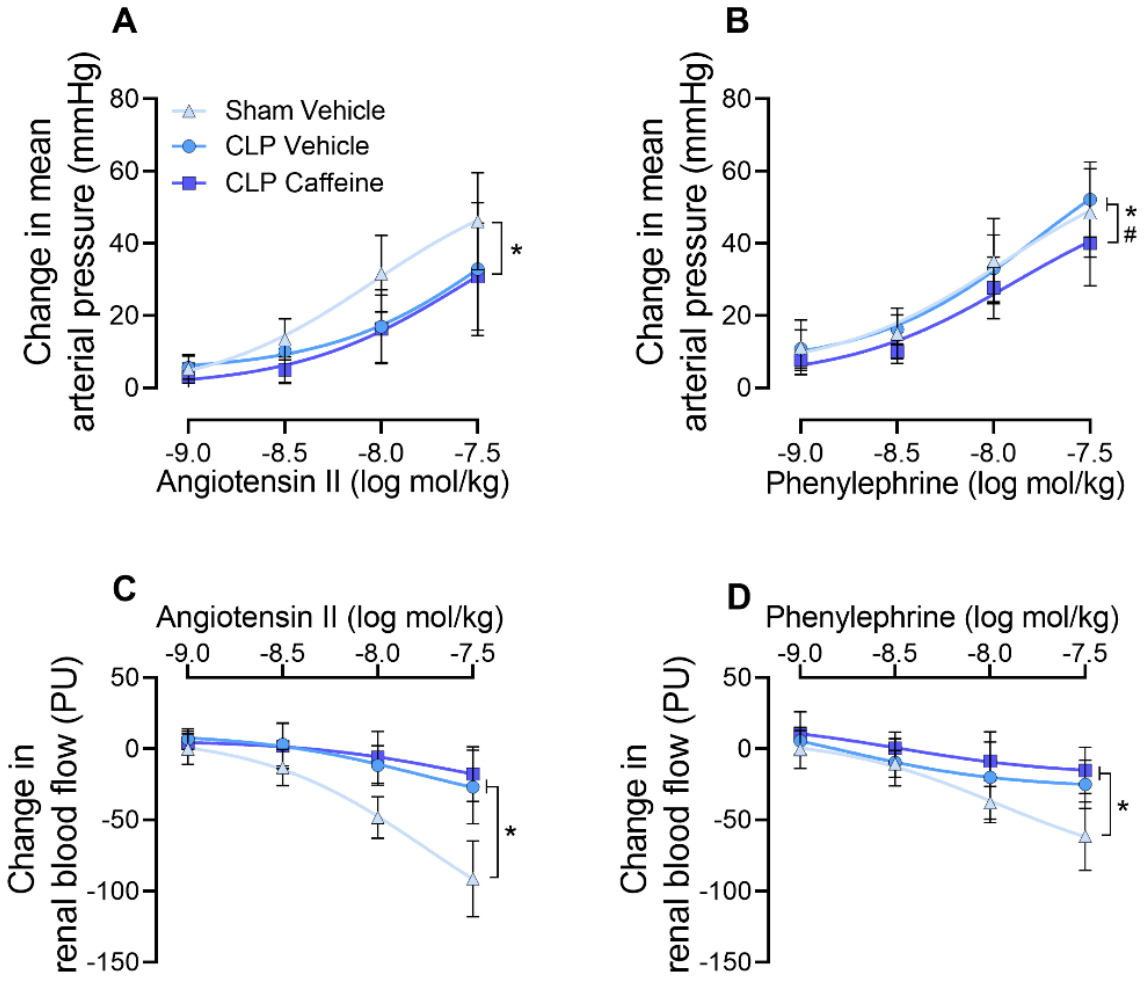

FIGURE 3 - Effect of caffeine on the response to vasopressors on blood pressure and renal blood flow of septic rats. Twenty-four hours after cecal ligation and puncture (CLP) or sham procedures, animals were anesthetized and prepared for in vivo vascular reactivity assessment with non-cumulative and increasing doses of angiotensin II (A-C) and phenylephrine (B-D). The results represent the mean ± S.D., n= 9-12 animals. Statistical analyses were performed using a two-way analysis of variance (ANOVA) followed by Sidak post-hoc test. *$p < 0.05$, CLP compared with sham group. $^{\#}p < 0.05$ CLP+caffeine compared to CLP vehicle.

Effect of caffeine on organ dysfunction and systemic inflammation

CLP group exhibited an increase in plasma levels of ALT, NOx and lactate when compared to sham animals (Figure 4B, E and F, respectively, $p < 0.05$) and these changes were not affected by caffeine treatment. Animals treated with caffeine presented increased plasma levels of AST, when compared to the sham group (Figure 4A, $p < 0.05$). Creatinine was not changed by CLP procedure nor by caffeine treatment (Figure 4C). CLP group exhibited a decrease in plasma levels of plasma urea compared to sham group, but the treatment with caffeine did not affect urea levels (Figure 4D, $p < 0.05$).

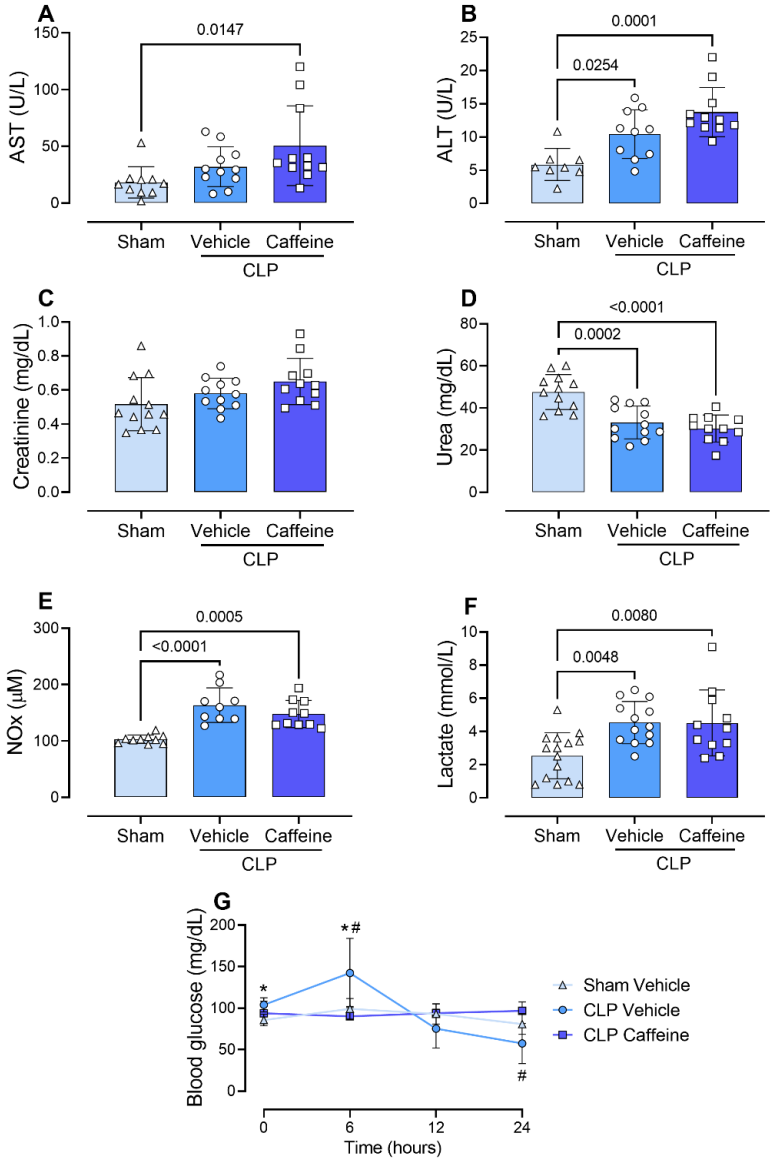

FIGURE 4 - Effect of caffeine in plasmatic markers of organ dysfunction, inflammation, lactate, and blood glucose of septic rats. Twenty-four hours after surgery, blood was obtained to assess AST (A), ALT (B), creatinine (C), urea (D), NOx (E), and lactate (F). Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by the Sidak post-hoc test. Data are expressed as dot plots for each animal and the mean ± S.D., n= 9-12. Glycemic levels (G) were evaluated 0, 6, 12, and 24 hours after surgery. Statistical analyses were performed using a two-way analysis of variance (ANOVA) followed by the Sidak post-hoc test. The results represent the mean ± S.D., n= 5-10. *$p < 0.05$, CLP compared with sham group. $^{\#}$ $p < 0.05$ CLP+caffeine compared to CLP vehicle.

Sepsis induces variation in blood glucose levels

In CLP vehicle animals, hyperglycemia was observed within 6 hours, followed by hypoglycemia at 24 hours. CLP caffeine-treated animals maintained normal glycemia levels until 24 hours after sepsis induction (Figure 4G, $p < 0.05$).

Effect of caffeine on survival rate

Survival of the CLP rats declined in the first 24 hours and remained constant up to 96 hours. Caffeine did not change the mortality when compared with the CLP control group (Figure 5).

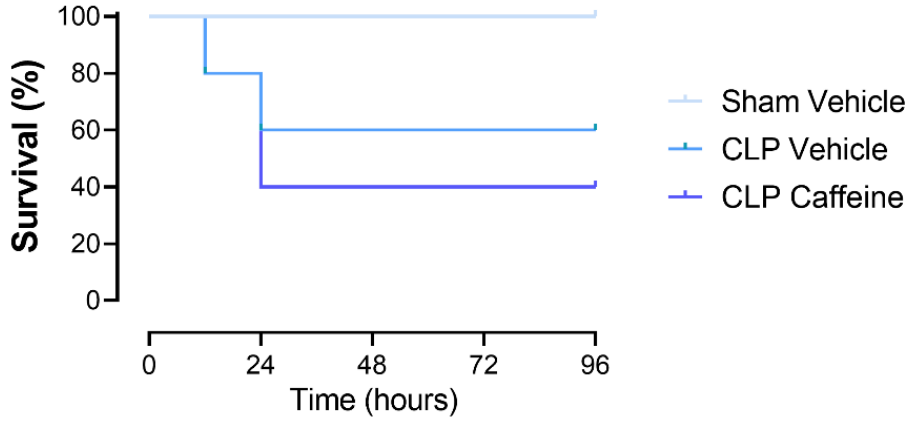

FIGURE 5 - Effect of caffeine on sepsis mortality. Cecal ligation and puncture (CLP) animals were randomly assigned to receive caffeine (30 mg/kg, s.c.) or vehicle (1 mL/kg, s.c.) at 2, 8 and 14 hours after surgical procedures. The mortality rate was monitored and recorded every 12 hours for a period of 4 days. The antibiotic was administered 6 hours after to procedure (24.000 IU/kg, i.m.). Sham n= 7 animals, CLP vehicle n= 10 and CLP caffeine n= 10 animals. Data were analyzed using the Log-rank test (Mantel-Cox).

DISCUSSION

In the present study, we conducted experiments to investigate the effects of caffeine, a non-selective inhibitor of adenosine receptors on septic animals. Our findings revealed that caffeine administration successfully maintained normal blood glucose levels and led to an increase in heart rate. However, we did not observe significant changes in hemodynamic parameters or survival rates in septic animals following caffeine treatment. These results suggest that while caffeine may help preserve glycemic levels during sepsis, it may have adverse effects by increasing heart rate. Consequently, in our experimental conditions, we have demonstrated that caffeine does not provide substantial improvement in the treatment of sepsis.

We employed the cecal ligation and puncture (CLP) model of sepsis. CLP is the most widely utilized model for investigating sepsis, since it accurately reproduces the course and characteristics of human sepsis, including the hemodynamic and metabolic phases (Buras, Holzmann, Sitkovsky, 2005). In fact, we have demonstrated that CLP induces cardiovascular changes and organ dysfunction effectively reproduced critical aspects of clinical sepsis.

Previous studies have already evaluated the effects of caffeine on experimental sepsis. Verma and colleagues (2009) have demonstrated that caffeine stimulated oxidative phosphorylation and increasing the extraction and consumption of oxygen by the myocardium during sepsis induced by CLP model. In another study, caffeine increased the left ventricular pressure (Bedet *et al*., 2020) but these authors used a low dose of caffeine (7.5 mg/kg, the equivalent of 1-1.5 cups of coffee) administrated late on sepsis (24 and 48 hours after CLP procedure). However, according to our previous data, this dose and

frequency of administration of caffeine are not suitable for efficiently blocking adenosine receptors (Albino *et al*., 2023). Furthermore, the delayed assessment of sepsis parameters makes the comparison with our data difficult. In another study conducted by Bauza and Remick (2015), mice were administered a single dose of 20 mg/kg (s.c.) caffeine immediately after the CLP procedure. Also, an osmotic pump was subcutaneously placed the day before CLP, providing a continuous infusion of caffeine at a rate of 10 mg/kg/hour for a total duration of 24 hours. Notably, this continuous caffeine infusion appears to effectively block caffeine receptors, ensuring adequate receptor blockade in this experimental context (Bauza, Remick, 2015). However, apart from heart rate, the study lacks a comprehensive evaluation of cardiovascular parameters.

In sepsis, cardiovascular dysfunction is characterized by hypotension that often necessitates the use of vasopressor therapy. In CLP model, animals exhibit significant hypotension due to various cardiovascular abnormalities (Zaky *et al*., 2014). Although we observed hypotension in the animals, caffeine treatment did not successfully prevent this hypotension. Although sepsis is typically linked to a reduction in renal blood flow, contributing to renal dysfunction and impaired perfusion leading to necrosis (Schrier, Wang, 2004), we did not observe any changes in renal blood flow or an increase in plasma markers of kidney function in septic animals. However, we assessed these parameters in a single moment. Therefore, it is conceivable that at later stages of sepsis there may be a potential to observe a decline in renal flow and a deterioration in renal function. It is interesting to note that no significant difference in plasma urea levels was observed in the CLP group in our study. However, previous research has shown that elevated urea levels are associated with increased severity of neonatal sepsis (Li *et al*., 2021). NOx is an inflammatory index associated with cardiovascular changes in sepsis (Fernandes, Assreuy, 2008). Nevertheless, caffeine proved ineffective in reducing NO levels, aligning with its observed lack of impact on the cardiovascular changes induced by sepsis.

It is well known that patients with sepsis often experience persistent tachycardia, which is associated with an increased risk of mortality (Hasegawa *et al*., 2021). In our study, animals treated with caffeine exhibited an increase in heart rate. This effect of caffeine on heart rate can be attributed to its ability to stimulate adrenal chromaffin cells, leading to increased secretion of catecholamines by raising intracellular calcium levels in these cells. Several studies have documented elevated levels of catecholamines in both septic patients and animal models (Hahn *et al*., 1995). Therefore, caffeine may indirectly contribute to the positive inotropic and chronotropic effects through the activation of β-adrenergic receptors by increasing the release of catecholamines. Additionally, adenosine is involved in the reduction of heart rate through activation of the A_1 receptors on the sinus and atrioventricular node, slowing impulse conduction (Funakoshi *et al*., 2007). Regardless of the underlying mechanism, the elevation in heart rate precipitates a rise in tissue oxygen consumption. This heightened demand may predispose to ischemia and cardiac dysfunction.

In sepsis, vasopressors are employed to restore the blood pressure in order to maintain a mean arterial pressure of ≥ 65 mmHg (Singer *et al*., 2016). However, there are patients who fail to respond adequately to vasoconstrictors, a condition known as vasoplegia (Burgdorff, Bucher, Schumann, 2018). Consistent with this observation, we noted a decrease in the pressure response to angiotensin II. Despite some studies suggesting that caffeine can mitigate inflammation leading to tissue alterations (Surma, Sahebkar, Banach, 2023), it has not demonstrated efficacy in reversing sepsis-associated vasoplegia. In contrast to that observed with angiotensin II, sepsis did not diminish the response to phenylephrine, an adrenergic agonist. Despite the unexpected outcome, this finding provides valuable insights, revealing that caffeine has the potential to impar the response to phenylephrine in septic animals. This highlights the potential negative impact of caffeine on maintaining an adequate perfusion pressure in septic animals.

Caffeine was found ineffective in reduce liver failure. In fact, its administration resulted in elevated AST levels in septic animals, potentially exacerbating organ dysfunction. This effect could be attributed to the potential hepatotoxic effect of caffeine (Sato *et al*., 1985). It is noteworthy that control animals treated with

caffeine did not demonstrate an elevation in plasma AST levels (Figure S1). Consequently, the adverse effects of caffeine appear to be discernible only in the presence of a diseased condition. This outcome was unexpected, and our experimental design was not specifically tailored to investigate the interplay between treatment and sepsis. Additional studies are needed to explore this unforeseen interaction.

Lactate is a widely used clinical parameter as a marker of tissue perfusion and plays a crucial role as a predictor of mortality in patients with sepsis (Liu *et al*., 2019). CLP group in our study exhibited increased lactate levels. However, caffeine treatment did not have an impact on lactate levels. The inability of caffeine to influence lactate levels implies that it does not directly affect tissue perfusion or metabolic processes associated with lactate production and clearance. While caffeine may have other effects, such as modulating heart rate or blood glucose levels, its influence on lactate levels appears to be limited.

Sepsis can induce a metabolic derangement leading to hyper and /or hypoglycemia (Neyens, Gaskill, Chalela, 2018). Interestingly, hyperglycemia during sepsis is associated with an increased risk of mortality, mainly in patients with early-stage sepsis (Dungan, Braithwaite, Preiser, 2009). CLP + caffeine group maintained normal glycemic levels for up to 24 hours. This finding is consistent with previous studies that have demonstrated the ability of caffeine ingestion to decrease glycemia levels. This effect may be attributed to increased expression of GLUT4 in skeletal muscle and adipocytes, resulting from elevated intracellular calcium levels and enhanced expression of the enzyme MAPK (Park *et al*., 2009). In addition, the hypoglycemic state observed in the late phase of sepsis is also associated with a higher mortality rate (Wang *et al*., 2021). On the other hand, caffeine administration had a protective effect on hypoglycemia observed in septic animals. This effect may be due to stimulation in the release of hypoglycemia counter-regulatory hormones (adrenaline and cortisol) by caffeine (Debrah *et al*., 1996). Hence, caffeine shows promise in its potential to regulate blood glucose levels over an extended period. However, further studies are required to fully explore this effect.

In our study, it was found that repeated administration of caffeine does not have an impact on survival rates during the acute phase of sepsis. These findings are consistent with a previous report that utilized CLP model and demonstrated that both a single dose of caffeine and continuous infusion did not affect survival rates (Bauza, Remick, 2015). Therefore, while caffeine may enhance glucose blood levels, it does not alter the response to vasoconstrictors, markers of organ dysfunction, blood pressure, or survival rate.

It is important to emphasize that our study has certain limitations. Caffeine acts as a non-selective antagonist and affects various molecular targets, which complicates the understanding of its mechanism of action. Therefore, the use of selective antagonists of adenosine receptors could be considered as a future option to further investigate its effects. Additionally, we did not evaluate the inflammatory profile of the animals in our study. Furthermore, the survival analysis was conducted with a small number of replicates, which may limit the general applicability of the results. Therefore, further experiments are necessary to assess other important parameters related to sepsis.

Caffeine prevented changes in glycemic levels, but it did not mitigate sepsis-induced cardiovascular collapse, organ dysfunction, or affect mortality rates. The unforeseen impact of caffeine on glycemia during sepsis presents an intriguing avenue insufficiently explored in this study. Nonetheless, it invites future investigation to discern if this caffeine-induced effect depends on adenosine receptor activation. There are previous studies showing that activation of A_1 receptors inhibits the release of insulin in beta cells, decreasing plasma insulin concentrations by up to 33% (Johansson, Salehi, Sandstro, 2007). This emphasizes the imperative for employing selective antagonists to unravel the intricacies of this phenomenon. Additionally, an unresolved question persists regarding whether the caffeine effect is directly mediated through pancreatic action. There is compelling evidence indicating that adenosine has the potential to augment glucose uptake within tissues through insulin-independent mechanisms (Thong, Graham, 2002). Employing pancreatic cells or the isolated organ could offer valuable insights to elucidate this aspect,

contributing to a more comprehensive understanding of the mechanism of caffeine on glycemic levels. Lastly, exploring insulin levels alongside tissue-specific insulin responses would add depth to our understanding and provide valuable insights into the broader physiological implications of the observed effects.

CONCLUSION

In summary, our study demonstrated that repeated injections of caffeine, a non-selective antagonist of adenosine receptors, effectively regulate glycemic levels during sepsis. However, no change was observed after caffeine administration on vascular response or renal blood flow induced by vasoconstrictors. Thus, while caffeine administration does not elicit significant changes during sepsis, it does impact cardiovascular function to some extent, highlighting the need for further investigation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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