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ORIGINAL ARTICLE

Alpha-Lipoic Acid Attenuates Oxidative Damage in Organs After Sepsis

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Abstract—Sepsis progression is linked with the imbalance between reactive oxygen species and antioxidant enzymes. Thus, the aim of this study was to evaluate the effect of alpha-lipoic acid (ALA), a powerful antioxidant, in organs of rats submitted to sepsis. Male Wistar rats were subjected to sepsis by cecal ligation puncture (CLP) and treated with ALA or vehicle. After CLP (12 and 24 h), the myeloperoxidase (MPO) activity, protein and lipid oxidative damage, and antioxidant enzymes in the liver, kidney, heart, and lung were evaluated. ALA was effective in reducing MPO activity, lipid peroxidation in the liver, and protein carbonylation only in the kidney in 12 h after CLP. In 12 h, SOD activity increased in the kidney and CAT activity in the liver and kidney with ALA treatment. Thus, ALA was able to reduce the inflammation and oxidative stress in the liver and kidney after sepsis in rats.

KEY WORDS: sepsis; alpha-lipoic acid; organ failure; oxidative damage.

INTRODUCTION

Sepsis is a complex syndrome characterized by an imbalance between pro-inflammatory and anti-inflammatory response to pathogens [1]. Sepsis and severe sepsis (sepsis accompanied by acute organ dysfunction) are leading causes of death in the USA and the most common cause of death among critically ill patients in non-coronary intensive care units (ICU) [2]. The development of organ dysfunction is strongly correlated with increased mortality, and the more organs that fail, the higher the mortality [3].

Renal and respiratory functions are the most likely organs involved in sepsis, contributing to worsening prognosis [4]. As many as a third of the patients hospitalized with community-acquired pneumonia develop acute kidney injury [5]. The hepatic system is not as frequently affected as the kidneys and lungs, but septic patients who have liver involvement have a very high mortality [2]. Furthermore, clinical studies have recognized myocardial depression as a serious manifestation in ~40 % of septic patients, with mortality ranging from 70 to 90 % in contrast with 20 % in septic patients without cardiac dysfunction [6, 7]. Therefore, to reduce the mortality in septic patients, there is an urgent need to better understand the mechanisms underlying the sepsis-caused organ dysfunction and identify new therapeutic strategies.

Some of the postulated molecular mechanisms of sepsis progression are linked to the imbalance between reactive oxygen species (ROS) production and its degradation by cellular antioxidants [8, 9]. The pro-inflammatory effects of ROS include endothelial damage, neutrophil recruitment, cytokine release, and mitochondrial impairment [1, 8, 9], all contributing to a "free radicals

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"overload" and to oxidant–antioxidant imbalance. The imbalance between antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) that are followed by oxidative damage in the major organ systems (lung, heart, liver, and kidney) was verified after sepsis induction [1, 10]. Because oxidative stress is associated with organ failure in sepsis, antioxidant therapy has been considered a potential pharmacological treatment for this condition [11].

Alpha-lipoic acid (ALA) is a phase 2-inductive nutraceutical, a naturally occurring dithiol compound that functions as an essential cofactor for mitochondrial bioenergetic enzymes [12]. ALA is recognized as a universal antioxidant capable of scavenging free radicals, chelating metals, regenerating endogenous antioxidants, and modulating various signal transduction pathways [12–15]. It is unique among antioxidants in its ability to display antioxidant properties in both oxidized and reduced forms as well as in both lipid and aqueous environments [12, 14, 15]. In preclinical and clinical studies, the therapeutic potential of ALA has been demonstrated in a variety of inflammatory disorders involving organ dysfunction, with improvement of oxidative stress parameters and inflammation [12, 13, 16–18].

The present study was established to test the possible protective effect of ALA, *via* its antioxidant properties, in different organs of rats submitted to an animal model of sepsis. The investigation was performed using the cecal ligation and puncture (CLP) model because it satisfies many of the essential attributes for an appropriate model: it has a focal infection, produces bacteremia, releases bacterial products into the circulation, and mimics the hemodynamic and inflammatory profile of human sepsis [19].

MATERIALS AND METHODS

Animals

Male Wistar rats (2–3 months, 220–310 g) were obtained from our breeding colony at UNISUL. The animals were housed in groups of five per cage with food and water available *ad libitum*, and were maintained on a 12-h light/dark cycle (lights on at 7:00 a.m.). All experimental procedures were approved by the Animal Care and Experimentation Committee of UNISUL (protocol number 13.008.4.03.IV), Brazil.

Sepsis Induction—CLP Model

Rats were subjected to CLP as previously described [20]. Briefly, they were anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg), given intraperitoneally. Under aseptic conditions, a 3-cm midline laparotomy was performed to expose the cecum and adjoining intestine. The cecum was tightly ligated with a 3.0 silk suture at its base, below the ileocecal valve, and was perforated once with a 14-gauge needle. The cecum was then squeezed gently to extrude a small amount of feces through the perforation site. The cecum was then returned to the peritoneal cavity, and the laparotomy was closed with 4.0 silk sutures. Animals were resuscitated with regular saline (50 ml/kg) subcutaneously (s.c.) immediately after and 12 h after CLP. All animals received basic support (saline at 50 ml/kg immediately after and 12 h after CLP plus antibiotics—ceftriaxone at 30 mg/kg and clindamycin 25 mg/kg) every 6 h, s.c. All animals were returned to their cages with free access to food and water. In the sham-operated group, the rats were submitted to all surgical procedures but the cecum was neither ligated nor perforated. To minimize variability between different experiments, the CLP procedure was always performed by the same investigators.

Treatments and Sample Obtention

ALA obtained from Sigma (St. Louis, MO, USA) was dissolved in saline, prepared immediately before use, and protected from light during the experiments. Immediately after the procedure, rats received 200 mg/kg of ALA or the same volume of vehicle saline [21]. Four groups ($n=7$) were used for oxidative stress experiments; they were randomly divided into four groups: (1) sham+vehicle group; (2) sham+ALA group, (3) CLP+vehicle group, and (4) CLP+ALA group.

Twelve and twenty-four hours after the surgery procedure (CLP or sham), all rats were euthanized by decapitation. The kidney, lung, heart, and liver were isolated, and samples were stored at −80 °C for subsequent analysis.

Biochemical Analyses

Myeloperoxidase Activity

Neutrophil infiltrate in tissues was measured by myeloperoxidase (MPO) activity [22]. Brain tissues were homogenized (50 mg/ml) in 0.5 % hexadecyltrimethylammonium bromide and centrifuged at $150,009 \times g$ for 40 min. The suspension was then sonicated three times for 30 s. An aliquot of supernatant was mixed with a solution of 1.6 mM tetramethylbenzidine and 1 mM H_2O_2. Activity was measured spectrophotometrically as the change in absorbance at 650 nm at 37 °C. Data were expressed as milliunits per milligram of protein.

TBARS

Lipid peroxidation was measured by formation of thiobarbituric acid reactive substances (TBARS) [23]. Samples were washed with PBS, harvested, and lysed. Thiobarbituric reactive species, obtained by acid hydrolysis of 1,1,3,3-tetraethoxy-propane (TEP), was used as the standard for the quantification of TBARS. Thiobarbituric acid (TBA) 0.67 % was added to each tube and vortexed. The reaction mixture was incubated at 90 °C for 20 min, and the reaction was stopped by placing samples on ice. The optical density of each solution was measured in a spectrophotometer at 535 nm. Data were expressed as nanomoles of malondialdehyde (MDA) equivalents per milligram of protein.

Protein Carbonyl

Protein carbonyl content was measured in organs studied using 2,4-dinitrophenylhydrazine (DNPH) in a spectrophotometric assay [24]. Briefly, sample tissues were sonicated in ice-cold homogenization buffer containing phosphatase and protease inhibitors (200 nM calyculin, 10 μg/ml leupeptin, 2 μg/ml aprotinin, 1 mM sodium orthovanadate, and 1 μM microcystin-LR) and centrifuged at $1000 \times g$ for 15 min to sediment insoluble material. Three-hundred-microliter aliquots of the supernatant containing 0.7–1.5 mg of protein were treated with 300 μl of 10 mM DNPH, dissolved in 2 M HCl, and compared with 2 M HCl alone (reagent blank). Samples then were incubated for 1 h at room temperature in the dark and stirred every 10 min. Samples were precipitated with trichloroacetic acid (final concentration of 20 %) and centrifuged at $16,000 \times g$ at 4 °C for 15 min. The pellet was washed three times with 1 ml of ethanol/ethyl acetate (1:1 v/v). Each time, the pellet was lightly vortexed and left exposed to the washing solution for 10 min before centrifugation ($16,000 \times g$ for 5 min). The final pellet was dissolved in 1 ml of 6 M guanidine in 10 mM phosphate buffer–trifluoroacetic acid, pH 2.3, and the insoluble material
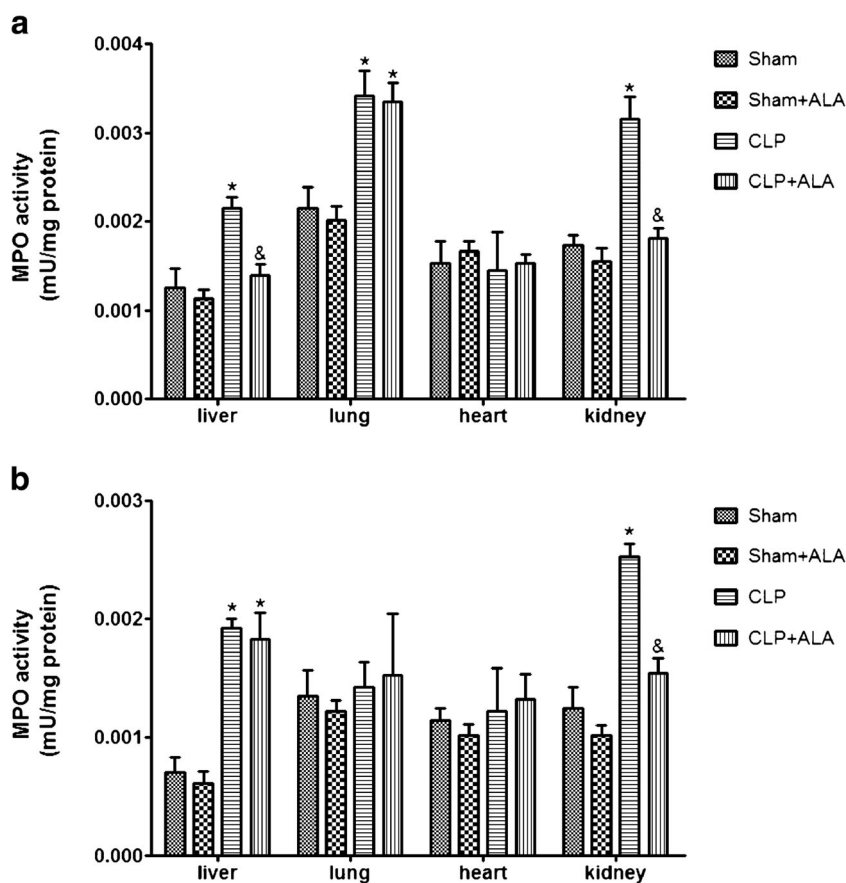

Fig 1. The effects of ALA on MPO activity in liver, lung, heart, and kidney, assessed in 12 h (**a**) and 24 h (**b**) after sepsis induced by CLP in rats. *Bars* represent means±SD. *$p < 0.05$ *vs.* sham, #$p < 0.05$ *vs.* sham+ALA, and &$p < 0.05$ *vs.* CLP. *ALA* (alpha-lipoic acid), *CLP* (cecal ligation and puncture), *MPO* (myeloperoxidase).

was removed by centrifugation at 16,000×*g* for 5 min. Absorbance was recorded in a spectrophotometer at 370 nm for both DNPH-treated and HCl-treated samples. Protein carbonyl levels were expressed as nanomoles of carbonyl per milligram of protein.

CAT and SOD Activities

The SOD estimation was performed based on its ability to spontaneously inhibit oxidation of adrenaline to adrenochrome [25]. 2.78 ml of sodium carbonate buffer (0.05 mM; pH 10.2), 100 μl of EDTA (1.0 mM), and 20 μl of the supernatant or sucrose (blank) were incubated at 30 °C for 45 min. Thereafter, the reaction was initiated by adding 100 μl of adrenaline solution (9.0 mM). The change in the absorbance was recorded at 480 nm for 8 min. Temperature was maintained at 30 °C throughout the assay procedure. One unit of SOD produced

approximately 50 % of auto-oxidation of adrenaline. Results were expressed as units/milligram of protein.

The CAT activity was measured by the method that employs hydrogen peroxide (H_2O_2) to generate H_2O and O_2 [26]. Samples were sonicated in 50 mmol/l phosphate buffer (pH 7.0), and the resulting suspension was centrifuged at 3000×*g* for 10 min. The sample aliquot (20 μl) was added to 980 μl of the substrate mixture. The substrate mixture contained 0.3 ml of hydrogen peroxide in 50 ml of 0.05 M phosphate buffer (pH 7.0). Initial and final absorbance was recorded at 240 nm after 1 and 6 min, respectively. A standard curve was established using purified catalase (Sigma, MO, USA) under identical conditions.

Protein Determination

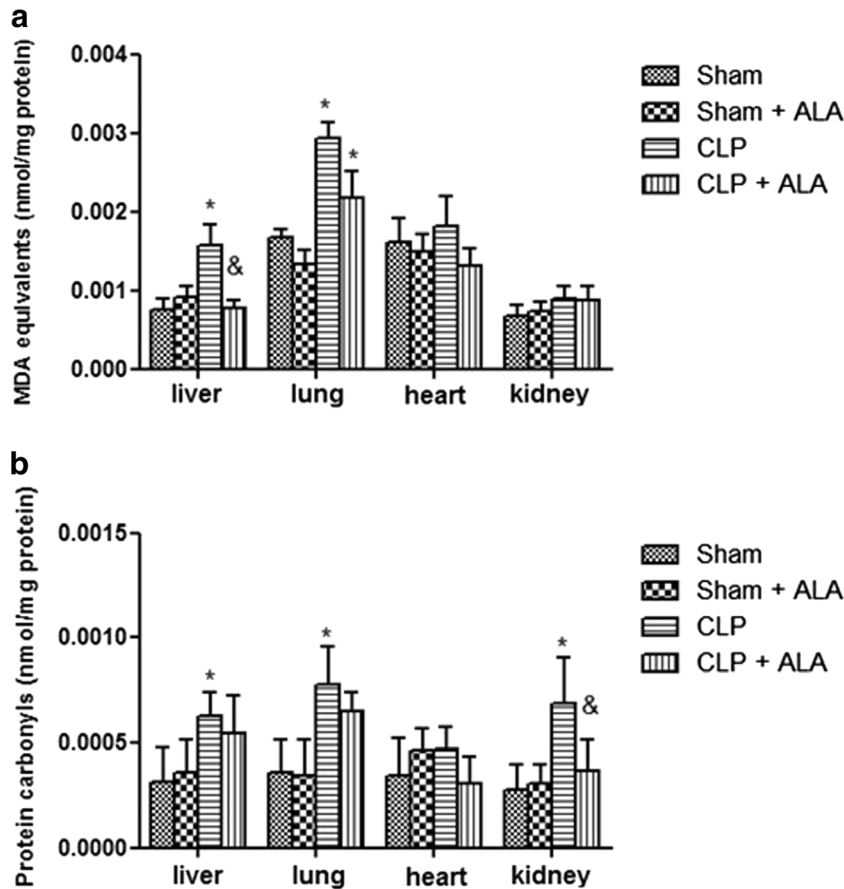All results are normalized with proteins measured by the Lowry method [27].

Fig. 2. The effects of ALA on TBARS (**a**) and protein carbonyl levels (**b**) in liver, lung, heart, and kidney, assessed in 12 h after sepsis induced by CLP in rats. *Bars* represent means±SD. *$p<0.05$ *vs.* sham, $p<0.05$ *vs.* sham+ALA, and &$p<0.05$ *vs.* CLP. *ALA* (alpha-lipoic acid), *CLP* (cecal ligation and puncture), TBARS (thiobarbituric acid reactive species).

Statistical Analysis

Results were analyzed by Statistical Package for the Social Sciences software (SPSS, Chicago, IL, USA). Data are expressed as mean±SD, and each value reflects seven animals per group. Differences among experimental groups were determined by one-way analysis of variance (ANOVA), followed by Tukey post hoc test. In all comparisons, statistical significance was set at $p < 0.05$.

RESULTS

Previous studies showed that the CLP model has an overall 28-day mortality rate of 50 % [28, 29], and most of the deaths in the early phase (days 1–5) occur within the first 3 days. Therefore, samples collected at the 12- and 24-h time point from an animal predicted to die will likely be within 2 days of that outcome. Thus, in the present study, we measured neutrophil infiltrate, lipid peroxidation, protein carbonylation, and SOD and CAT activities in the liver, lung, kidney, and heart of rats 12 and 24 h after CLP. The animals also received saline solution and ALA.

Figure 1 illustrates the MPO activity as a neutrophil infiltrate marker in the liver, lung, heart, and kidney of the rats 12 and 24 h after CLP and after being treated with ALA. At 12 h after CLP, we verified an increase in MPO activity in the liver, lung, and kidney and ALA was effective in decreasing these increases in the liver and kidney as showed in Fig. 1a. When we evaluated in the 24 h after CLP surgery, we observed an increase of MPO in CLP compared with sham groups in the liver and kidney and ALA reverted only in the kidney (Fig. 1b).
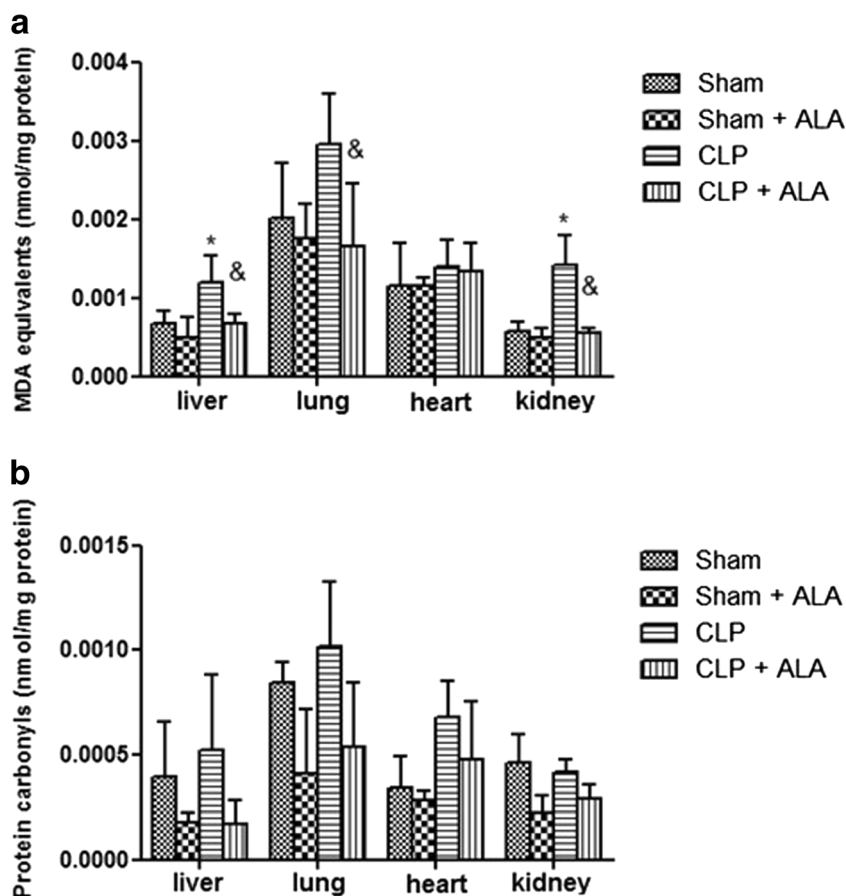

Fig. 3. The effects of ALA on TBARS (**a**) and protein carbonyl levels (**b**) in liver, lung, heart, and kidney, assessed in 24 h after sepsis induced by CLP in rats. *Bars* represent means±SD. *$p < 0.05$ *vs.* sham, $p < 0.05$ *vs.* sham+ALA, and &$p < 0.05$ *vs.* CLP. *ALA* (alpha-lipoic acid), *CLP* (cecal ligation and puncture), TBARS (thiobarbituric acid reactive species).

As seen in Fig. 2a, in the 12 h after CLP, the sepsis was associated with an increase in the TBARS in the liver and lung. Sepsis caused a significant increase in these levels when compared to the sham-operated group. However, ALA was able to reverse this effect on hepatic tissue. At the same time, the protein carbonylation levels were increased when observed in the CLP group in the liver, lung, and kidney (Fig. 2b) and the ALA administration decreased significantly these levels only in the kidney.

When evaluating the lipid and protein oxidative damage in 24 h (panels a and b, respectively, of Fig. 3), we observe an increase in lipid peroxidation in the liver and kidney in the CLP group and ALA treatment reverts these levels in both organs. On the other hand, we do not find significant differences for protein carbonylation in 24 h after CLP.

To antioxidant enzymes, CAT activity (Fig. 4a) at 12 h showed a significant decrease in antioxidant protection exerted by this enzyme in CLP *versus* sham in the liver,

heart, and kidney and ALA increased these levels only in the liver and kidney. The SOD activity at the same time showed significant decrease after sepsis (Fig. 4b) in the liver and kidney, and ALA was effective in increasing SOD activity levels in the kidney.

Twenty-four hours after sepsis induction, the CAT activity was significantly decreased only in the liver (Fig. 5a) and ALA did not reestablish these levels. In contrast, after CLP surgery, we observed a decrease in the SOD activity in the majority of organs (Fig. 5b). However, in none of these organs was ALA effective in reversing these levels.

DISCUSSION

In this study, the administration of ALA (200 mg/kg) in CLP-induced sepsis rats (i) reduced the neutrophil infiltrate and lipid peroxidation in the liver and kidney, (ii)
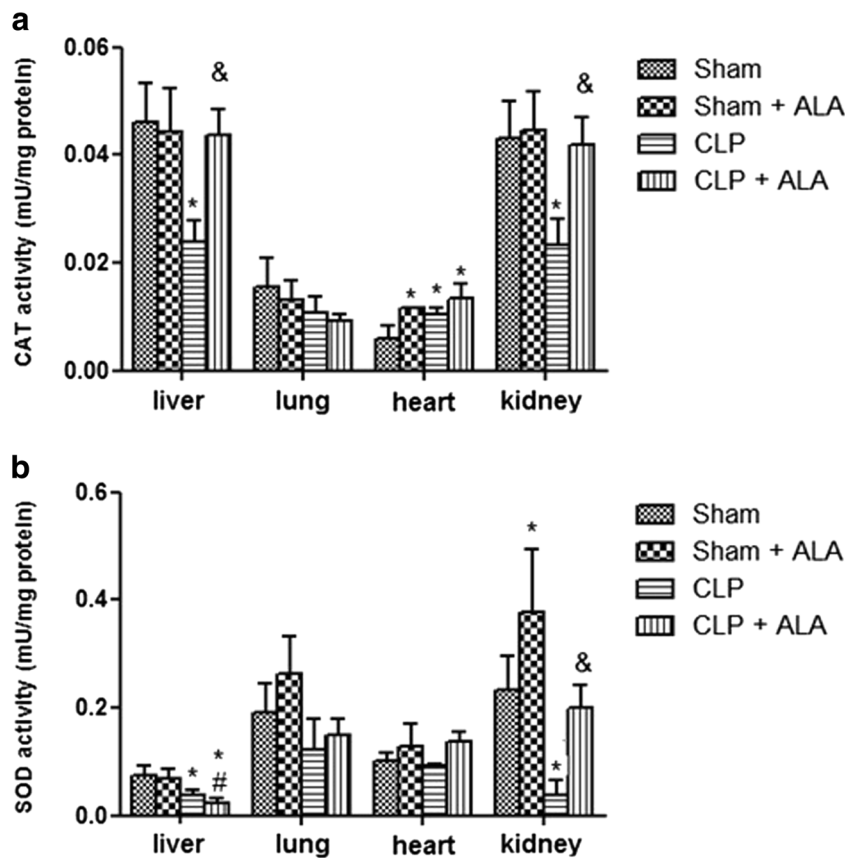

Fig. 4. The effects of ALA on CAT activity (**a**) and SOD activity (**b**) in liver, lung, heart, and kidney, assessed in 12 h after sepsis induced by CLP in rats. *Bars* represent means±SD. *$p<0.05$ *vs.* sham, #$p<0.05$ *vs.* sham+ALA, and &$p<0.05$ *vs.* CLP. *ALA* (alpha-lipoic acid), *CLP* (cecal ligation and puncture), *CAT* (catalase), *SOD* (superoxide dismutase).
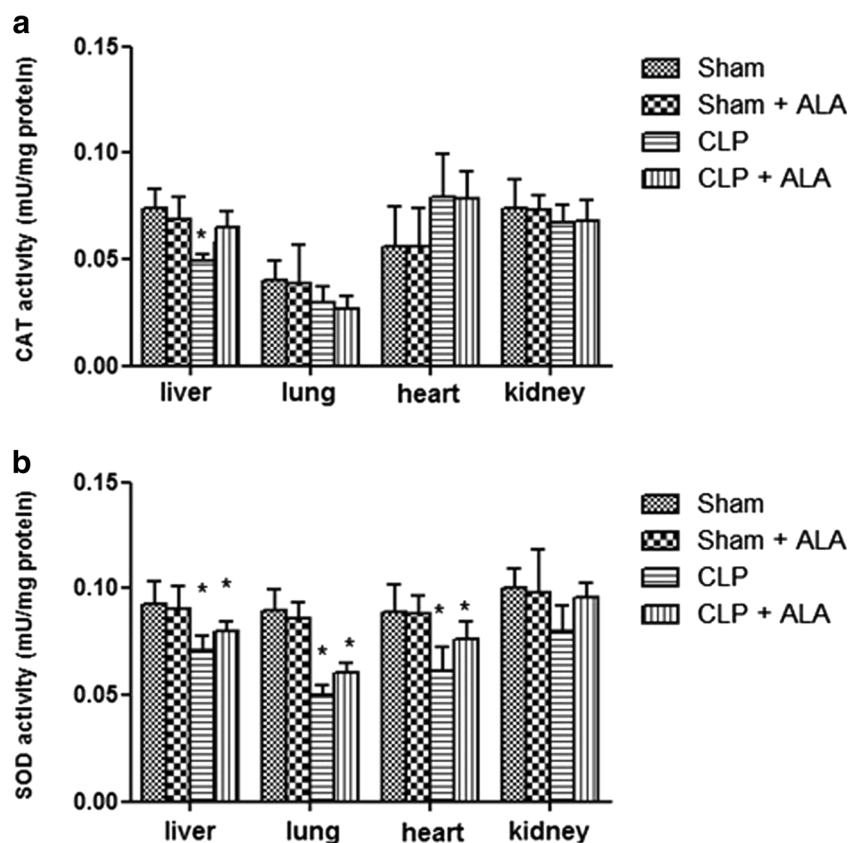
Fig. 5. The effects of ALA on CAT activity (**a**) and SOD activity (**b**) in liver, lung, heart, and kidney, assessed in 24 h after sepsis induced by CLP in rats. *Bars* represent means±SD. *$p < 0.05$ vs.* sham, $p < 0.05$ *vs.* sham+ALA, and $p < 0.05$ *vs.* CLP. *ALA* (alpha-lipoic acid), *CLP* (cecal ligation and puncture), *CAT* (catalase), *SOD* (superoxide dismutase).

reduced the oxidative damage in proteins, in the kidney, and (iii) increased SOD activity in the kidney and increased CAT activity in the liver and kidney. This study provides novel evidence that the application of ALA seems to improve injury of multiple organs in the CLP-induced sepsis rats as a consequence of oxidative damage. This could be due to antioxidant activity by ALA in animals with CLP-induced sepsis.

The CLP-induced sepsis model is characterized by a biphasic process and mimics many of the pathophysiologic features of clinically relevant polymicrobial sepsis [29, 30]. An early phase results from a surge of the unbridled ROS and reactive nitrogen species (RNS) and pro-inflammatory cytokines mediated primarily by neutrophils, macrophages, and monocytes, whereas a late phase is marked by a sustained immunosuppressive response induced primarily by apoptosis of immune, epithelial, and/or endothelial cells [31–34].

Although multiple ROS are produced by neutrophils and macrophages for killing invading bacteria in the body,

these species can also damage host tissues when they are produced superfluously. In this way, ROS induces irreversible cellular damage to lipids and proteins, causing mitochondrial dysfunction and organ failure [35].

Our present study demonstrated that neutrophil infiltration was increased in the organs studied in CLP-induced septic rats, which was attenuated by ALA administration in the liver and kidney accompanied by lipid peroxidation and protein carbonylation. The liver and kidney are thought to be major organs responsible for the initiation of organ dysfunction during sepsis [36, 37]. A recent study showed that ALA treatment attenuates kidney injury induced by endotoxins, such as renal tubular dysfunction, by suppression of apoptosis and inflammation [37]. Besides these results, it was verified that the oxidative stress and the mitochondrial dysfunction caused by endotoxemia are prevented by ALA [38].

Here, our observations related with kidney oxidative damage are consistent with preclinical studies that showed the ALA effect on kidney protection from oxidative stress

associated to inflammatory conditions [39] and prolonged survival correlated with increased kidney function in CLP-induced septic rats [40]. Similarly, a clinical study suggests that ALA is a beneficial and recommended supplement, especially for dialysis patients [21].

Once the ALA treatment presented reduction in the oxidative damage, we determined the SOD and CAT activities. SOD is an enzyme that uses the superoxide anion $(O_2^{\bullet-})$ as substrate and produces hydrogen peroxide (H_2O_2). This molecule is a substrate to the peroxidases, the CAT being the most important peroxidase in the organs studied in this work [41]. Indeed, we showed in our experiment that sepsis provoked a tendency to decrease SOD and CAT activities 12 h after CLP in the kidney and liver, which were reversed by treatment with ALA. Several studies showed that dietary supplementation with ALA induced a decrease in oxidative stress, while restoring diminished levels of the antioxidants [42, 43]. However, in 24 h, it was effective in decreasing SOD and CAT activities in different organs but it was not reversed with ALA treatment. The normalization of oxidative damage levels by ALA in 24 h after CLP may be indicative that it can decompose $O_2^{\bullet-}$ and H_2O_2. Chemical studies indicated that ALA and your dithiol—dihydro-lipoic acid (DHLA) reduced form—scavenge hydroxyl radicals, hypochlorous acid, and singlet oxygen [44] and were able to reduce the $O_2^{\bullet-}$-driven oxidation of a sensitive spin probe, in a manner comparable to that of SOD [45].

In conclusion, we used the most clinically relevant sepsis model to monitor sepsis-induced multiple organ dysfunction, and our findings support the hypothesis that ALA decreased sepsis-induced oxidative damage in organs by its antioxidative properties. This was based on the attenuation of neutrophil infiltrate, lipid peroxidation, and protein carbonylation as well as the SOD and CAT activity increase mainly in the liver and kidney by ALA in animals with sepsis. Thus, we suggest that ALA could be a potential adjuvant for protecting tissues from oxidative stress and preventing organ dysfunction caused by CLP-induced sepsis.

REFERENCES

1. Hotchkiss, R.S., and I.E. Karl. 2003. The pathophysiology and treatment of sepsis. *The New England Journal of Medicine*. 348: 138.
2. Angus, D.C., W.T. Linde-Zwirble, J. Lidicker, G. Clermont, J. Carcillo, and M.R. Pinsky. 2001. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Critical Care Medicine* 29: 1303–10.
3. Marshall, J.C., D.J. Cook, N.V. Christou, G.R. Bernard, C.L. Sprung, and W.J. Sibbald. 1995. Multiple organ dysfunction score: a reliable descriptor of a complex clinical outcome. *Critical Care Medicine* 23: 1638–52.
4. Martin, G.S., D.M. Mannino, S. Eaton, and M. Moss. 2003. The epidemiology of sepsis in the United States from 1979 through 2000. *The New England Journal of Medicine* 348: 1546–54.
5. Murugan, R., V. Karajala-Subramanyam, M. Lee, S. Yende, L. Kong, M. Carter, C.D. Angus, and J.A. Kellum. 2010. Acute kidney injury in non-severe pneumonia is associated with an increased immune response and lower survival. *Kidney International* 77: 527–35.
6. Romero-Bermejo, F.J., M. Ruiz-Bailen, J. Gil-Cebrian, and M.J. Huertos-Ranchal. 2011. Sepsis-induced cardiomyopathy. *Current Cardiology Reviews* 7: 163–183.
7. Hochstadt, A., Y. Meroz, and G. Landesberg. 2011. Myocardial dysfunction in severe sepsis and septic shock: more questions than answers? *Journal of cardiothoracic and vascular anesthesia* 25: 526–35.
8. Andrades, M.E., C. Ritter, and F. Dal-Pizzol. 2009. The role of free radicals in sepsis development. *Frontiers in Bioscience* 1: 277–87.
9. Barichello, T., J.J. Fortunato, A.M. Vitali, G. Feier, A. Reinke, J.C. Moreira, J. Quevedo, and F. Dal-Pizzol. 2006. Oxidative variables in the rat brain after sepsis induced by cecal ligation and perforation. *Critical Care Medicine* 34: 886–9.
10. Matsukawa, A., M.H. Kaplan, C.M. Hogaboam, N.W. Lukacs, and S.L. Kunkel. 2001. Pivotal role of signal transducer and activator of transcription (Stat)4 and Stat6 in the innate immune response during sepsis. *Journal of experimental medicine* 193: 679–688.
11. Andrades, M., C. Ritter, M.R. de Oliveira, E.L. Streck, J.C.F. Moreira, and F. Dal-Pizzol. 2011. Antioxidant treatment reverses organ failure in rat model of sepsis: role of antioxidant enzymes imbalance, neutrophil infiltration, and oxidative stress. *Journal of surgical research* 167: e307–13.
12. Goraca, A., H. Huk-Kolega, A. Piechota, P. Kleniewska, E. Ciejka, and B. Skibska. 2011. Lipoic acid—biological activity and therapeutic potential. *Pharmacological Reports* 63: 849–858.
13. Abdel-Zaher, A.O., R.H. Abdel-Hady, M.M. Mahmoud, and M.M.Y. Farrag. 2008. The potential protective role of alpha-lipoic acid against acetaminophen-induced hepatic and renal damage. *Toxicology* 243: 261–270.
14. Bilska, A., and L. Włodek. 2005. Lipoic acid—the drug of the future? *Pharmacological Reports* 57: 570–7.
15. Singh, U., and I. Jialal. 2008. Alpha-lipoic acid supplementation and diabetes. *Nutrition Reviews* 66: 646–657.
16. Khabbazi, T., R. Mahdavi, J. Safa, and P. Pour-Abdollahi. 2012. Effects of alpha-lipoic acid supplementation on inflammation, oxidative stress, and serum lipid profile levels in patients with end-stage renal disease on hemodialysis. *Journal of Renal Nutrition* 22(2): 244–250.
17. Kang, K.P., D.H. Kim, Y.J. Jung, A.S. Lee, S. Lee, S.Y. Lee, K.Y. Jang, M.J. Sung, S.K. Park, and W. Kim. 2009. Alpha-lipoic acid attenuates cisplatin-induced acute kidney injury in mice by suppressing renal inflammation. *Nephrology Dialysis Transplant* 24: 3012–3020.
18. Gianturco, V., A. Bellomo, E. D'Ottavio, V. Formosa, A. Iori, M. Mancinella, G. Troisi, and V. Marigliano. 2009. Impact of therapy with alpha-lipoic acid (ALA) on the oxidative stress in the controlled NIDDM: a possible preventive way against the organ dysfunction? *Archives of Gerontorology and Geriatrics* 49: 129–133.
19. Wichterman, K.A., A.E. Baue, and I.H. Chaudry. 1980. Sepsis and septic shock—a review of laboratory models and a proposal. *Journal of Surgical Research* 29: 189–201.
20. Fink, M.P., and S.O. Heard. 1990. Laboratory models of sepsis and septic shock. *Journal of Surgical Research* 49: 186–196.

21. Li, G., L. Gao, J. Jia, X. Gong, B. Zang, and W. Chen. 2014. α-Lipoic acid prolongs survival and attenuates acute kidney injury in a rat model of sepsis. *Clinical and Experimental Pharmacology and Physiology* 41: 459–68.

22. De Young, L.M., J.B. Kheifets, S.J. Ballaron, and J.M. Young. 1989. Edema and cell infiltration in the phorbol ester-treated mouse ear are temporally separate and can be differentially modulated by pharmacologic agents. *Agents Action* 26: 335–341.

23. Esterbauer, H., and K.H. Cheeseman. 1990. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. *Methods in enzymology* 186: 407–421.

24. Levine, R.L., D. Garland, and C.N. Oliver. 1990. Determination of carbonyl content in oxidatively modified proteins. *Methods in Enzymology* 186: 464–478.

25. Bannister, J.V., and L. Calabrese. 1987. Assays for superoxide dismutase. *Methods of Biochemical Analysis* 32: 279–312.

26. Aebi, H. 1984. Catalase in vitro. *Methods in Enzymology* 105: 121–6.

27. Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry* 193: 265–275.

28. Craciun, F.L., E.R. Schuller, and D.G. Remick. 2010. Early enhanced local neutrophil recruitment in peritonitis-induced sepsis improves bacterial clearance and survival. *Journal of Immunology* 185: 6930–8.

29. Osuchowski, M.F., F. Craciun, K.M. Weixelbaumer, E.R. Duffy, and D.G. Remick. 2012. Sepsis chronically in MARS: systemic cytokine responses are always mixed regardless of the outcome, magnitude, or phase of sepsis. *Journal of Immunology* 189: 4648–4656.

30. Oberholzer, A., C. Oberholzer, and L.L. Moldawer. 2001. Sepsis syndromes: understanding the role of innate and acquired immunity. *Shock* 16: 83–96.

31. Yang, S., C.S. Chung, A. Ayala, I.H. Chaudry, and P. Wang. 2002. Differential alterations in cardiovascular responses during the progression of polymicrobial sepsis in the mouse. *Shock* 17: 55–60.

32. Oberholzer, C., A. Oberholzer, M. Clare-Salzler, and L.L. Moldawer. 2001. Apoptosis in sepsis: a new target for therapeutic exploration. *The FASEB Journal* 15: 879–892.

33. Hattori, Y., K. Takano, H. Teramae, S. Yamamoto, H. Yokoo, and N. Matsuda. 2010. Insights into sepsis therapeutic design based on the apoptotic death pathway. *Journal of Pharmacological Sciences* 114: 354–365.

34. Olguner, C.G., U. Koca, E. Altekin, B.U. Ergür, S. Duru, P. Girgin, A. Taşdöğen, K. Gündüz, S. Güzeldağ, M. Akkuş M, et al. 2013. Ischemic preconditioning attenuates lipid peroxidation and apoptosis in the cecal ligation and puncture model of sepsis. *Experimental and Therapeutic Medicine* 5: 1581–1588.

35. Tsai, K.L., H.J. Liang, Z.D. Yang, S.I. Lue, S.L. Yang, and C. Hsu. 2014. Early inactivation of PKCε associates with late mitochondrial translocation of Bad and apoptosis in ventricle of septic rat. *Journal of surgical research* 186: 278–286.

36. Alvarez, S., and P.A. Evelson. 2007. Nitric oxide and oxygen metabolism in inflammatory conditions: sepsis and exposition to polluted ambients. *Frontiers in Bioscience* 12: 964–974.

37. Wang, P., and I.H. Chaudry. 1996. Mechanism of hepatocellular dysfunction during hyperdynamic sepsis. *American Journal of Physiology* 270: R927–938.

38. Suh, S.H., K.E. Lee, I.J. Kim, O. Kim, C.S. Kim, J.S. Choi, H.I. Choi, E.H. Bae, S.K. Ma, J.U. Lee, et al. 2014. Alpha-lipoic acid attenuates lipopolysaccharide-induced kidney injury. *Clinical and Experimental Nephrology* 19: 82–91.

39. Vanasco, V., M.C. Cimolai, P. Evelson, and S. Alvarez. 2008. The oxidative stress and the mitochondrial dysfunction caused by endotoxemia are prevented by alpha-lipoic acid. *Free Radical Research* 42: 815–823.

40. Cimolai, M.C., V. Vanasco, T. Marchini, N.D. Magnani, P. Evelson, and S. Alvarez. 2014. α-Lipoic acid protects kidney from oxidative stress and mitochondrial dysfunction associated to inflammatory conditions. *Food & Function* 5: 3143–50.

41. Safa, J., M.R. Ardalan, M. Rezazadehsaatlou, M. Mesgari, R. Mahdavi, and M.P. Jadid. 2014. Effects of alpha lipoic acid supplementation on serum levels of IL-8 and TNF-α in patient with ESRD undergoing hemodialysis. *International Urology and Nephrology* 46: 1633–8.

42. Halliwell, B., and J.M.C. Gutteridge. 1999. *Free radicals in biology and medicine*. Oxford: Oxford Science.

43. Arivazhagan, P., and C. Panneerselvam. 2000. Effect of DL-alphalipoic acid on neural antioxidants in aged rats. *Pharmacological Research* 42: 219–222.

44. Gasic-Milenkovic, J., C. Loske, and G. Munch. 2003. Advanced glycation end products cause lipid peroxidation in the human neuronal cell line SH-SY5Y. *Journal of Alzheimer's Disease* 5: 25–30.

45. Packer, L., E.H. Witt, and H.J. Tritschler. 1995. Alpha-lipoic acid as a biological antioxidant. *Free Radical Biology & Medicine* 19: 227–250.