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EFFECT OF ALPHA - LIPOIC ACID ON LPS-INDUCED OXIDATIVE STRESS IN THE HEART

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Lipopolysaccharide (LPS) is a gram-negative bacterial endotoxin and a major factor that contributes to multiple organ failure, including heart injury. Myocardial dysfunction in septic shock depends on the presence of proinflammatory cytokines and reactive oxygen/nitrogen species. In this study, the effect of early administration of an antioxidant, α -lipoic acid (LA) on lipid peroxidation, hydrogen peroxide (H_2O_2), sulphhydryl groups (-SH groups) and total protein concentration and the glutathione redox system was evaluated in the heart homogenates obtained from LPS-induced endotoxic shock rats (*Escherichia coli* 026:B6, 30 mg/kg, *i.v.*). The animals were treated intravenously with saline or LA (60 mg/kg or 100 mg/kg *i.v.*) 30 min after LPS injection. Five hours after LPS, LA or saline administration, the animals were sacrificed and their hearts were isolated for measurements. Injection of LPS alone resulted in the development of shock and oxidative stress that was indicated by a significant increase in thiobarbituric acid reactive substances (TBARS) and H_2O_2 concentrations, a decrease in concentration of -SH groups and reduced glutathione, and by decrease in glutathione redox ratio reduced glutathione (GSH)/oxidized glutathione (GSSG) in the heart. Administration of LA after the LPS challenge resulted in an increase in the sulphhydryl group content and a decrease in TBARS and H_2O_2 concentrations in the heart as compared with the LPS group. In addition, the treatment of LA after LPS challenge significantly decreased the level of GSSG, increased the level of GSH in heart homogenates resulting in an increase of the GSH/GSSG ratio compared with the LPS group. There was no difference in oxidative stress reduction between 60 mg/kg and 100 mg/kg doses. These results indicate that early administration of LA is highly effective in dampening endotoxin-induced oxidative stress in the heart and in improving the glutathione redox system. This study supports the idea that α -LA is a free radical scavenger and a potent antioxidant.

Key words: *lipopolysaccharide, lipoic acid, endotoxic shock*

INTRODUCTION

LPS (endotoxin) derived from Gram-negative bacteria is an important pathogenic factor in myocardial dysfunction. The main cause of myocardial dysfunction in septic shock may be appearance of myocardial depression substances in plasma or impairment of myocardial perfusion. Myocardial dysfunction in septic shock depends on

the presence of cytokines, in particular tumor necrosis factor (TNF- α), interleukin 1- β or reactive oxygen/nitrogen species (nitric oxide-NO, superoxide, peroxynitrite) (1). It has been demonstrated that TNF- α impairs contractility by disturbing intracellular Ca^{2+} homeostasis in intact animals, isolated hearts and cardiomyocytes (2, 3). The heart is an organ which has post-mitotic cells and one of the highest oxygen consumption rates in the

body, as well as slow turnover of antioxidant enzymes and is, therefore, highly susceptible to ROS (4). Increased ROS production can result in myocyte hypertrophy, apoptosis, and interstitial fibrosis which may contribute to the development of depressed cardiac function and progression of cardiac failure. The degree of oxidative stress and the severity of subsequent myocardial damage might depend on the imbalance between excess production of ROS and antioxidant defense.

The first line of defense mechanism against ROS-mediated cardiac injury involves several antioxidant enzymes such as: superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSHPx). Biological compounds with antioxidant properties may contribute to tissues protection against ROS induced by LPS. One of the natural molecules known to prevent or retard oxidative stress is LA. LA is a thiol compound found naturally in plants and animals and possessing potent antioxidant properties. It is considered as a universal antioxidant that acts in the lipid phase and the aqueous phase (5, 6, 7). LA is specified for free radical quenching and metal chelating activity. It also interacts with, and regenerates other cellular antioxidants. Moreover, LA recycles vitamin C, vitamin E, and increases intracellular glutathione concentrations by increasing cellular uptake of cysteine (6). Coombes *et al.* (8) indicated that supplementation of LA improved cardiac performance after ischemia and protected the heart against lipid peroxidation induced by superoxide radicals, hydroxyl radicals, H₂O₂ and peroxy radicals.

The aim of the present study was to assess the oxidative cardiac damage in LPS administered rats and the impact of LA on myocardial damage was evaluated.

MATERIAL AND METHODS

Chemicals

Lipopolysaccharide (*Escherichia coli* LPS 026: B6; lyophilized powder chromatographically purified by gel filtration; protein content <1%), α -lipoic acid, thiobarbituric acid (TBA), butylated hydroxytoluene, sodium acetate trihydrate, triethanolamine hydrochloride (TEA), 5-sulfosalicylic acid hydrate (5-SSA), 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB), β -NADPH (β -nicotinamide adenine dinucleotide phosphate), glutathion reductase (GR), GSH, GSSG, 2-vinylpyridine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were obtained from POCH (Gliwice, Poland) and were of analytical grade. Shortly before the use,

LPS was dissolved in sterile pyrogen-free normal saline. DL- α -LA was mixed with sterile normal saline in a dark bottle, and NaOH was added until the solid had been dissolved. The TBA solution was prepared by dissolving 0.67 g TBA in 100 ml deionized water and then diluted 1:1 with glacial acetic acid. Phosphate buffer with EDTA (pH 7.4). Sterile, deionized water (resistance >18 M Ω cm, HPLC Water Purification System USF ELGA, England) was used throughout the study.

Animals

The experiments were performed on 63 male Wistar rats weighing 220-260 g, aged 2-3 months. The animals were housed 6 per cage under standard laboratory conditions in 12/12 h light-dark cycle (light on at 7.00 a.m.) at 20 \pm 2°C ambient temperature and air humidity of 55 \pm 5%. All animals received a standard laboratory diet and water ad libitum. All animals were given a one-week acclimation period before the onset of the experiment. The experimental procedures followed the guidelines for the care and use of laboratory animals, and were approved by the Medical University of Lodz Ethics Committee.

Experimental protocol

Animals were randomly divided into six groups as follows: Group I (control group, n= 10) received two doses of 0.2 ml of saline, half an hour apart. Group II (LPS group, n=12 (including 2 animals dead during the experiment) received 0.2 ml of saline, and half an hour later endotoxic shock was induced by injection of *Escherichia coli* lipopolysaccharide (LPS) at a dose of 30 mg/kg. Groups III and IV (LA groups, n=10 each) were given 0.2 ml of saline, and half an hour later rats were injected with a single dose of DL- α -lipoic acid, 60 mg/kg and 100 mg/kg, respectively). Groups V and VI (LPS/LA groups, n=10 each) received a single dose of LPS (30 mg/kg) and after half an hour a single dose of DL- α -LA (60 mg/kg or 100 mg/kg, respectively).

All agents were injected intravenously into the tail vein between 8.00 a. m. and 9.00 a. m. After the administration of α -LA or LPS, each group of animals was observed for a period of 5 h.

Because changes in body temperature (fever or hypothermia) are considered as an important component of the systemic response in defense against LPS and inflammation (9) we measured the body temperature of rats in the rectum using a thermistor thermometer (type PU 391/1 Czechoslovakia) before the first injection and then 1, 2, 3 and 5 h later (7, 10).

Tissue preparation

At the end of the experiment, the rats were killed with an overdose of *i. p.* pentobarbital (100 mg/kg). The hearts were excised immediately, rinsed in cold saline and weighed in order to estimate cardiac edema. The ratios of the heart weights to the body weights (HW/BW ratios) were then calculated and used as an index of cardiac edema. Then the hearts were frozen at -80°C for measurement of oxidative stress parameters.

Determination of lipid peroxidation

To determine the degree of oxidative damage in the heart we measured lipid peroxidation in heart homogenates. The lipid peroxidation products content in heart homogenates was assayed as TBARS and TBA-reactive substances in the butanol layer and were measured spectrofluorometrically using Perkin Elmer Luminescence Spectrometer LS-50 (Norwalk, CT, U. S. A.). Excitation was set at 515 nm and emission was measured at 546 nm. Sample TBARS concentration were calculated by the use of the regression equation as follows: $Y=0.39(X-X_0) - 1.32$, where Y = TBARS concentration (μM); X , X_0 = fluorescence intensity of the samples and control, respectively (arbitrary units; AU). The regression equation was prepared from triplicate assays of six increasing concentrations of tetramethoxypropane (range 0.01- 50 μM) as a standard for TBARS. Finally, the results were calculated for 50 mg of the heart tissue.

Determination of H_2O_2

Briefly, 50 mg of the heart tissue fragments were homogenized with 2 ml of 1.15% potassium chloride. Then, 10 μl aliquot of tissue homogenate was mixed with 90 μl of PBS (pH 7.0) and 100 μl of horseradish peroxidase (1 U/ml) containing 400- μmol homovanilic acid (HRP +HVA assay) or with 90 μl of PBS and 100 μl of 1 U/ml horseradish peroxidase only (HRP assay). Both homogenates were incubated for 60 min at 37°C . Subsequently, 300 μl of PBS and 125 μl of 0.1 M glycine-NaOH buffer (pH 12.0) with 25 mM EDTA were added to each homogenate sample. Excitation was set at 312 nm and emission was measured at 420 nm (Perkin Elmer Luminescence Spectrometer, Beaconsfield UK). Readings were converted into H_2O_2 concentration using the regression equation: $Y=0.012X - 0.0007$, where Y = H_2O_2 concentration in homogenate (μM); X =intensity of light emission at 420 nm for HRP + HVA assay reduced by HRP assay emission (arbitrary units, AU). The regression equation was prepared from three series of

calibration experiments with 10 increasing H_2O_2 concentrations (range 10-1000 μM). The lowest H_2O_2 detection was 0.1 nM, with intraassay variability not exceeding 2%.

Measurement of total sulfhydryl groups in heart homogenates

Total -SH group content in heart homogenates was determined according to the 2,2-dithio-bis-nitrobenzoic acid (DTNB) assay as described by Ellman (11). The absorbance of the samples were measured at 412 nm using spectrophotometer Pharmacia LKB-Ultrospect III. Readings were converted into sulfhydryl group content (μM) by the use of the regression equation $Y=-0.2998 + 241.945X$, where Y = free sulfhydryl group content (μM), X = intensity of light emission at 420 nm (arbitrary units, AU). The regression equation was prepared from three repeats of standard experiments with increasing concentrations of glutathione (range 2-200 μM).

Determination of protein concentration

Protein was measured by the method of Lowry *et al.* (12), using bovine serum albumin as standard.

Determination of GSH levels

Total glutathione (tGSH), GSH and GSSG were measured in heart homogenates. Briefly, the hearts were homogenized in cold 5% 5-SSA (1ml of 5% 5-SSA/100 mg of tissue) using glass homogenizers. Then the homogenates were centrifuged (10 000g, 10 min, 4°C) and aliquot samples of the supernatant were frozen at -80°C for a maximum of three weeks. The total GSH determination was performed in a 1 ml cuvette containing 0.7 ml of 0.2 mM of NADPH, 0.1 ml of 0.6 mM DTNB, 0.150 ml of H_2O and a 50 μl sample. The cuvette with the mixture was incubated for 5 min at 37°C and then supplemented with 0.6 U of GR in 100 mM sodium phosphate buffer with EDTA, pH 7.5. The reaction kinetics was followed spectrophotometrically at 412 nm for 5 min by monitoring the increase in absorbance.

GSSG concentration was determined in supernatant aliquots by the same method after optimization of pH to 6-7 with 1M TEA and derivatization of endogenous GSH with 2-vinylpyridine (v:v). The reduced supernatant GSH level was calculated as the difference between total GSH and GSSG. The increments in absorbance at 412 nm were converted to GSH and GSSG concentrations using a standard curve (3.2-500 μM GSH for total GSH and 0.975-62 μM for GSSG). Duplicate GSH

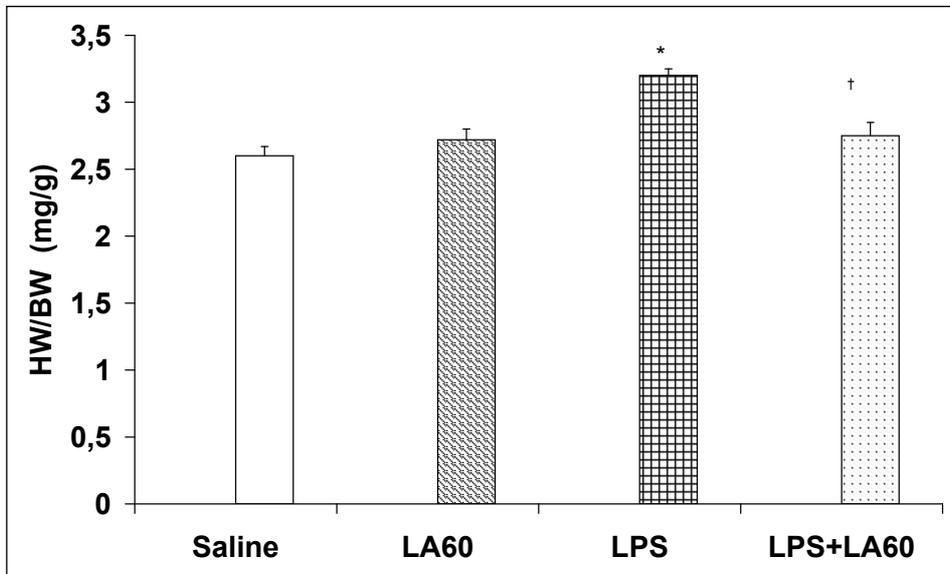


Fig.1. Changes in the ratio of HW (heart weight)/BW (body weight) in control and experimental groups of rats. Lipopolysaccharide (LPS) caused increase in HW/BW, lipoic acid at a dose of 60 mg/kg (LA60) reduced the changes in the values; however, values of HW/BW were still higher than those in the control group. LA at a dose of 100 mg/kg caused similar changes (data not shown). The data are mean \pm SE. * $P < 0.001$ compared with controls and the LA group. † $P < 0.01$ compared with the LPS group.

Table 1. Effect of administration of lipoic acid (LA) on oxidative stress parameters in heart homogenates of rats with lipopolysaccharide-induced shock. Mean \pm SE.

Parameter	Saline	LA60	LA100	LPS	LPS/LA60	LPS/LA100
TBARS (μM)	11.49 \pm 0.63	10.6 \pm 0.93	9.9 \pm 0.8	51.99 \pm 1.48#	36.47 \pm 3.47*	35.8 \pm 2.69**
H ₂ O ₂ (μM)	0.325 \pm 0.036	0.205 \pm 0.019	0.200 \pm 0.02	0.634 \pm 0.043#	0.428 \pm 0.075†	0.41 \pm 0.07‡
Free -SH groups (μM)	4.32 \pm 0.42	7.34 \pm 1.09	8.1 \pm 1.8	3.12 \pm 0.5¥	8.72 \pm 1.3*‡	8.89 \pm 1.33*‡
Total protein (μM)	263.9 \pm 12.6	319.7 \pm 16.4	324 \pm 16.17	255.3 \pm 9.3Ψ	315.5 \pm 29 †	329.1 \pm 1.9†

* $P < 0.01$ vs. LPS

** $P < 0.001$ vs. LPS

$P < 0.001$ vs. saline

† $P < 0.05$ vs. LPS

‡ $P < 0.01$ vs. saline

¥ $P < 0.05$ vs. LA100

Ψ $P < 0.01$ vs. LA60 and LA100.

and GSSG determinations were repeated three times. The results were expressed in μM .

Statistical analysis

The data are presented as mean \pm SE, if not stated otherwise, from 10 survived animals in each group. The statistical analysis was performed by ANOVA followed by Duncan's multiple range test as post-hoc. P value lower than 0.05 was considered significant.

RESULTS

The changes in rectal temperature of the experimental animals were described in our earlier papers (7, 10).

The HW/BW ratio markedly increased following LPS administration (Fig. 1). Administration of LA reduced the increases in HW/BW ratio. However, the values in the LPS+LA60 or LPS+LA100 groups were higher than those in the saline group ($p < 0.01$). The results suggest that LA attenuated cardiac edema.

Changes in heart homogenates oxidative injury indices

The animals challenged with LPS produced about 4.5-fold increase in lipid peroxidation levels in the heart homogenates, which was measured by the formation of TBARS, compared to the control group (51.99 ± 1.48 vs. $11.495 \pm 0.63 \mu\text{M}$, $p < 0.001$, $n=10$) (Table 1). The administration of LA (60 mg/kg body weight) after LPS challenge significantly reduced

Table 2. Heart status of glutathione metabolism in controls (physiological saline) and after administration of lipoic acid (LA), lipopolysaccharide (LPS) and LPS +LA60.

Parameters	Saline	LA60	LPS	LPS + LA60
Total GSH (μM)	92.2 \pm 5.48	83.1 \pm 17.3	15.43 \pm 2.4 $\ddagger\text{¥}$	131.58 \pm 17.6*
GSH (μM)	74.4 \pm 7.1	58.4 \pm 6	6.32 \pm 2.3 $\ddagger\text{†}$	102.2 \pm 6**
GSSG (μM)	23.1 \pm 4.3	7.86 \pm 0.8	21.73 \pm 2.4 ††	21.05 \pm 2
GSH/GSSG (redox status)	3.8 \pm 1.1	7.44 \pm 0.7	0.32 \pm 0.13 $\#\text{†}$	4.95 \pm 0.4**

LA60 - lipoic acid 60 mg/kg of body weight. GSH: reduced glutathione; GSSG: glutathione disulfide. Values are expressed as mean \pm SE, of 8 animals per group.

* P<0.01 vs. LPS

** P<0.001 vs. LPS

\ddagger P<0.001 vs. saline

$\#$ P<0.05 vs. saline

¥ P<0.02 vs. LA60

† P<0.001 vs. LA

†† P<0.01 vs. LA

the extent of lipid peroxidation in the heart homogenates as compared to the LPS group (36.47 \pm 3.4 μM vs. 51.99 \pm 1.48, $p < 0.01$, $n = 10$). A similar decrease was obtained with LA at the dose of 100 mg/kg body weight (35.8 \pm 269 μM , $p < 0.01$, $n = 10$).

The mean H_2O_2 concentration in the animals challenged with LPS was 1.9 -fold elevated as compared to the control group (0.634 \pm 0.043 μM vs. 0.326 \pm 0.036 μM , $p < 0.001$, $n = 10$). This increase in hydrogen peroxide generation in the heart in response to LPS was significantly attenuated by LA in a dose of 60 mg/kg or 100 mg/kg (0.428 \pm 0.075 μM ; 0.41 \pm 0.07, $p < 0.05$, $n = 10$, respectively) (Table 1).

Infusion of LPS resulted in a significant decrease in the -SH group content in comparison to the LA100 group values (3.12 \pm 0.5 μM vs. 8.1 \pm 1.8 μM , $p < 0.05$, $n = 10$). Treatment of LPS-challenged animals with either dose of LA restored the -SH group content in the heart homogenates as compared to LA group [8.72 \pm 1.3 μM , (60 mg/kg) and 8.89 \pm 1.3 μM , (100 mg/kg), $p < 0.01$, $n = 10$] (Table 1).

There was a significant decrease in total protein in the hearts of endotoxic rats as compared to rats receiving LA 60 and LA 100 ($p < 0.05$). Treatment with LA60 or LA 100 after LPS challenge increased total protein in endotoxic rats ($p < 0.05$).

Since the oxidative stress in the tissue generally involves the GSH system, we measured the level of total GSH (tGSH), oxidized GSH (GSSG), reduced GSH and the ratio of GSH/GSSG in each group. In the LPS-treated group, myocardial GSH level (an indicator of antioxidant capacity) was significantly decreased as compared to the saline ($p < 0.01$) or the LA group ($p < 0.02$) and was associated with an increase in GSSG ($p < 0.01$). The GSH/GSSG ratio, which reflects

intracellular glutathione redox balance, was also significantly decreased 5h after the injection of LPS as compared to the saline ($p < 0.05$) or the LA group ($p < 0.02$). In the LPS/LA group, both the reduced GSH level and the GSH/GSSG ratio were increased as compared to the LPS group ($p < 0.001$) (Table 2).

DISCUSSION

In the present study, administration of LPS to rats resulted in development of oxidative stress damage in heart tissue. This effect was indicated by an increase in the concentration of TBARS, lipid peroxidation indices and H_2O_2 concentration, decrease in the concentration of the total sulfhydryl group and GSH and by increase in heart weight/body weight ratio. The increase in TBARS concentration in our study was a result of increased production of ROS. Ritter *et al.* (13) indicated the increase in TBARS content in the myocardium of septic rats and the imbalance between SOD and CAT activities. Azevedo *et al.* (14) revealed small loci of subendocardial coagulative necrosis with discrete inflammatory infiltrate in the heart sample obtained from endotoxic pigs.

It is well documented that LPS causes oxidative stress by intensification of proinflammatory cytokines production and by inducing the generation of ROS by different mechanisms (15, 16). Lipid peroxidation is known to cause cellular injury by inactivation of membrane enzymes and receptors, depolymerization of polysaccharides as well as protein cross-linking and fragmentation (15, 17). This results in severe metabolic dysfunction and loss of cell integrity as well as genomic stability. Heart tissue is rich in

polyunsaturated fatty acids and is known for its high oxygen consumption. Therefore, it is more susceptible to oxidative stress than other tissues (18). In addition, the heart has relatively lower levels of antioxidant enzyme activity than the majority of other tissues.

Increasing formation of ROS in our study is indicated by increased H_2O_2 concentration in heart homogenates of LPS-challenged rats. H_2O_2 is formed during superoxide anion dismutation and is one of the most stable of toxic oxygen metabolites. H_2O_2 takes part in many biochemical and physiological processes, but, in excessive amount, exerts harmful effects. It is also volatile and, due to the lack of charge, increases membrane permeability. It also allows promotion of radical reactions at a great distance from its origin. H_2O_2 may, in the presence of iron (Fenton reaction leading to hydroxyl radical generation), oxidise lipids to their hydroperoxy products, thus leading to heart damage. The basic enzymes regulating the intracellular H_2O_2 concentration are catalase and glutathione peroxidase. Decline in the activity of these enzymes during excess ROS production (19) results in the intense H_2O_2 conversion to toxic hydroxyl radicals which may contribute to the LPS-induced oxidative stress. The role of H_2O_2 in ROS generation was shown in many studies (20). Recently, Viola *et al.* (20) indicated that H_2O_2 caused activation of I_{Ca-L} channel in ventricular myocytes, increased mitochondrial uptake of calcium and increased superoxide anion production by the mitochondria.

In our study, the decrease in the content of -SH groups in LPS-treated rats also suggests increased formation of ROS or nitrogen species in the heart tissue. Similarly, Radi *et al.* (21) revealed that the reduced content of sulfhydryl group after endotoxemia may be attributed to overproduction of oxidants and NO, since high levels of NO were associated with oxidation of the sulfhydryl group. The reduction in the content of -SH groups may be a result of a decrease in the synthesis of protein containing -SH groups, a decrease in GSH synthesis and in the activity of glutathione reductase (22), transferase, or an increase of GSH peroxidase (23). The reduced protein concentration in heart homogenates observed in our study may result from LPS-induced enhancement of oxidative processes (20).

Glutathione is known to be a major low molecular weight scavenger of free radicals in the cytoplasm. In the present study, decreased intracellular ratio of GSH/GSSG after LPS administration indicates that LPS altered antioxidant capacity and thiol redox state in heart tissue. GSH is essential for the protection of thiol and other nucleophilic groups in proteins from the toxic metabolites and lower GSH levels under conditions of intracellular stress lead to oxidation and damages

of lipids, proteins and DNA by ROS (24). Similarly, Tsiotou *et al.* (25) observed a decrease in the tissue levels of GSH and increase in GSSG concentration during the initial phase of septic shock.

In our experimental model, a beneficial role of LA (60 mg/kg) during endotoxemia was demonstrated by inhibition of heart lipid peroxidation, as manifested by lowering TBARS level, decreased H_2O_2 concentration, increased concentration of free sulfhydryl groups and glutathione in the heart homogenates. Administration of LA at the dose of 100 mg/kg caused a similar effect. All protective effects of LA are attributable to its dithiol group. In mammalian cells, exogenously supplied LA is rapidly reduced to its dithiol form, the dihydrolipoic acid (DHLA) (24). Thiol groups of LA and DHLA make them capable of scavenging a variety of ROS (singlet oxygen, H_2O_2 , hydroxyl radicals) and nitrogen species in comparable or higher levels than those observed for GSH (26). In addition, LA recycles other cellular antioxidants, including CoQ, vitamins C and E, glutathione and chelates iron and copper which are involved in production of hydroxyl radical (27, 28). LA is also a redox regulator of proteins such as myoglobin, thioredoxin and transcription factor NF- κ B (29).

Increased concentration of free -SH groups in the LPS/LA animals indicates that this antioxidant is potent to reduce heart damage by inhibiting oxidation of -SH groups in proteins, or to enhance synthesis of protein containing sulfhydryl groups. In our study, LA was effective even when administered 0.5 h after LPS challenge.

Treatment with LA after LPS challenge restored the reduced GSH level significantly and ameliorated the GSH/GSSG ratio suggesting that this antioxidant might modulate cardiac antioxidative defense by elevation of tissue glutathione redox status. The decrease of GSSG in heart tissue after LA administration may be due to the direct action of DHLA, which is a potent reducing agent and therefore converts GSSG to GSH (27). Other authors indicated that LA is able to correct deficient thiol status of the cell by increasing *de novo* synthesis of cellular GSH by improving cystine utilization (6). Moreover, GSH can react as a non-enzymatic antioxidant by direct interaction of -SH group with ROS, or can be involved in an enzymatic detoxification reaction for ROS, as a cofactor or coenzyme (30).

The increase of protein concentration in the LPS plus LA group evidences improved antioxidative capacity of the cardiac tissue and may be associated with an increased amount of antioxidative enzymes or thiol proteins. Similar results were observed in other tissues (31).

We indicated that LA abrogated also the extent of endotoxin-induced heart damage, as evidenced by

decreases in HW/BW ratio. Additionally, our and other studies indicate that LA takes part in the thermoregulation during endotoxemia (7, 10, 32).

On the basis of these studies, it is possible to conclude that lipoic acid may reduce oxidative stress in the heart by alleviating lipid peroxidation through free radical scavenging or by enhancing the synthesis of antioxidants containing -SH groups and GSH which then detoxify free radicals. The present work highlights that α -LA alleviates the indices of oxidative stress in the heart even when this antioxidant is administered after LPS challenge and improves glutathione redox system.

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REFERENCES

1. Grandel U, Fink L, Blum A *et al.* Endotoxin-induced myocardial tumor necrosis factor- α synthesis depresses contractility of isolated rat hearts. Evidence for a role of sphingosine and cyclooxygenase-2-derived thromboxane production. *Circulation* 2000; 102: 2758-2771.
2. Krishnagopalan S, Kumar A, Parrillo JE, Kumar A. Myocardial dysfunction in the patient with sepsis. *Curr Opin Crit Care* 2002; 8: 376-388.
3. Ramana KV, Willis MS, White MD *et al.* Endotoxin-induced cardiomyopathy and systemic inflammation in mice is prevented by aldose reductase inhibition. *Circulation* 2006; 114: 1838-1846.
4. Tanguy S, de Leiris J, Besse S, Boucher F. Ageing exacerbates the cardiotoxicity of hydrogen peroxide through the Fenton reaction in rats. *Mech Ageing Dev* 2003; 124: 229-235.
5. Packer L. Neuroprotection by the metabolic antioxidant alpha-lipoic acid. *Free Radic Biol Med* 1997; 22: 359-378.
6. Han D, Handelman G, Marcocci L *et al.* Lipoic acid increases de novo synthesis of cellular glutathione by improving cysteine utilization. *Biofactors* 1997; 6: 321-338.
7. Goraca A, Jozefowicz-Okonkwo G. Protective effects of early treatment with lipoic acid in LPS-induced lung injury in rats. *J Physiol Pharmacol* 2007; 58: 541-549.
8. Coombes JS, Powers SK, Hamilton KL *et al.* Improved cardiac performance after ischemia in aged rats supplemented with vitamin E and α -lipoic acid. *Am J Physiol* 2000; 279: R2149-R2155.
9. Soszyński D, Chelminiak M. Intracerebroventricular injection of neuronal and inducible nitric oxide synthase inhibitors attenuates fever due to LPS in rats. *J Physiol Pharmacol* 2007; 58: 551-561.
10. Skibska B, Jozefowicz-Okonkwo G, Goraca A. Protective effects of early administration of alpha-lipoic acid against lipopolysaccharide-induced plasma lipid peroxidation. *Pharmacol Rep* 2006; 399-404.
11. Ellman GL. SH groups determination in biological fluids. *Anal Biochem* 1970; 46: 237.
12. Lowry OH, Rosebrough AL, Farr AL, Randall R. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951; 193: 265-275.
13. Ritter C, Andrades M, Frota Junior ML *et al.* Oxidative parameters and mortality in sepsis induced by cecal ligation and perforation. *Intensive Care Med* 2003; 29: 1782-1789.
14. De Azevedo LC, Park M, Noritomi DT, Maciej AT, Brunialti MK, Salomao R. Characterization of an animal model of severe sepsis associated with respiratory dysfunction. *Clinics* 2007; 62: 491-498.
15. Zhao L, Chen YH, Wang HJ *et al.* Reactive oxygen species contribute to lipopolysaccharide-induced tetragenesis in mice. *Toxicol Sci* 2008; 103: 149-157.
16. Jaworek J, Konturek SJ, Macko M *et al.* Endotoxemia in newborn rats attenuates acute pancreatitis at adult age. *J Physiol Pharmacol* 2007; 58: 131-147.
17. Luqman S and Rizvi SJ. Protection of lipid peroxidation and carbonyl formation in proteins by capsaicin in human erythrocytes subjected to oxidative stress. *Phytother Res* 2006; 20: 303-306.
18. Rozenberg S, Besse S, Brisson H *et al.* Endotoxin-induced myocardial dysfunction in senescent rats. *Critical Care* 2006; 10: R124-132.
19. Pigeolet E, Corbisier P, Houbion A *et al.* Glutathione peroxidase, superoxide dismutase, and catalase inactivation by peroxides and oxygen derived free radicals. *Mech Ageing Dev* 1990; 51: 283-297.
20. Viola HM, Arthur PG, Hool LC. Transient exposure to hydrogen peroxide causes an increase in mitochondria-derived superoxide as a result of sustained alteration in L-type Ca²⁺ channel function in the absence of apoptosis in ventricular myocytes. *Circ Res* 2007; 100: 1036-1044.
21. Radi R, Beckman JS, Bush KM, Freeman BA. Peroxynitrite oxidation of sulfhydryls: The cytotoxic potential of superoxide and nitric oxide. *J Biol Chem* 1991; 266: 4244-4250.
22. Savitha S, Tamilselvan J, Anusuyadevi M, Panneerselvam C. Oxidative stress on mitochondrial antioxidant defense system in the aging process. Role of DL- α -lipoic acid and L-carnitine. *Clin Chim Acta* 2005; 355: 173-180.
23. Malmezat T, Breuille D, Capitan P, Mirand PP, Obled Ch. Glutathione turnover is increased during the acute phase of sepsis in rats. *J Nutr* 2000; 130: 1239-1246.
24. Nordberg J, Arner ES. Reactive oxygen species, antioxidants and the mammalian thioredoxin system. *Free Rad Biol* 2001; 31: 1287-1312.
25. Tsiotou AG, Sakorafas GH, Anagnostopoulos G, Bramis J. Septic shock; current pathogenetic concept from a clinical perspective. *Med Sci Monit* 2005; 11: RA76-RA85.
26. Biewenga GP, Haenen GR, Bast A. The pharmacology of the antioxidant lipoic acid. *Gen Pharmacol* 1997; 29: 315-331.
27. Lu SC. Regulation of glutathione synthesis. *Molecular Aspects Medicine* 2008; doi: 10.1016/j.mam.2008.05.005.
28. Trujillo M, Folkes L, Bartesaghi S, Kalyanaraman B, Wardman P, Radi R. Peroxynitrite-derived carbonate and nitrogen dioxide radicals readily react with lipoic acid and dihydroliipoic acid. *Free Rad Biol Med* 2005; 39: 279-288.
29. Novotny L, Rauko P, Cojocel C. alpha-Lipoic acid- the potential for use in cancer therapy. *Neoplasma* 2008; 55: 81-86.
30. Zhang W, Wei H, Hagen T, Fre B. α -Lipoic acid attenuates LPS-induced inflammatory responses by activating the phosphoinositide 3-kinase/Akt signaling pathway. *Proc Natl Acad Sci USA* 2007; 104: 4077-4082.

31. Li X, Liu Z, Luo C et al. Lipoamide protects retinal pigment epithelial cells from oxidative stress and mitochondrial dysfunction. *Free Radic Biol Med* 2008; 144: 1465-1474.
32. Riedel W, Lang U, Oetjen U, Schlapp U, Shibata M. Inhibition of oxygen radical formation by methylene blue, aspirin, or alpha-lipoic acid, prevents bacterial-lipopolysaccharide-induced fever. *Mol Cell Biochem* 2003; 247: 83-94.

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