Effects of vitamin E and prednisolone on some oxidative stress markers in endotoxemic rabbits

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SUMMARY

Effects of prednisolone (PR) and vitamin E (VE) on oxidative stress and antioxidant systems were investigated in endotoxemic rabbits. Forty rabbits were used and divided into four equal groups. Group I served as the control group. In group II, lipopolysaccharide (LPS, 100 µg/kg/h) was infused for 6 hours, whereas rabbits of groups III and IV received prior treatments with subcutaneous injection of prednisolone (10 mg/kg) (group III) or with intraperitoneal injections of vitamin E (10 mg/kg) for 4 consecutive days (group IV). Serum, liver, heart and kidney samples were obtained at 8 hours after infusion. Malondialdehyde (MDA), glutathione (GSH) concentrations and superoxide dismutase (SOD), catalase (CAT) activities were spectrophotometrically determined in tissues plus in serum (for MDA).

LPS caused statistically significant (p<0.05) increases of MDA and antioxidants in serum and in all tissues. PR and VE significantly (p<0.05) suppressed increases of MDA, SOD, CAT and GSH. As a consequence, prednisolone and vitamin E had protective effects on oxidative stress in endotoxemic rabbits.

KEY-WORDS : Vitamin E - prednisolone - oxidative stress - endotoxemia- rabbit.

RÉSUMÉ

Effets de la vitamine E et de la prednisolone sur le stress oxydatif chez les lapins endotoxémiques. Par E. YAZAR, S. KONYALIOGLU, R.COL, Y. OSMAN BIRDANE, A. LEVENT BAS et M. ELMAS.

Les effets de la vitamine E (VE) et de la prednisolone (PR) sur le stress oxydatif et les systèmes antioxydants ont été analysés sur des lapins endotoxémiques. Quarante lapins ont été répartis en 4 groupes égaux. Le groupe I a servi de groupe contrôle. Dans le groupe II, les lapins ont été perfusés par du lipopolysaccharide (LPS, 100 µg/kg/h) pendant 6 heures tandis que dans les groupes III et IV, les lapins ont reçu un traitement antérieur : soit de la prednisolone (10 mg/kg) par voie sous-cutanée (groupe III), soit de la vitamine E (10 mg/kg) par voie intrapéritonéale pendant 4 jours consécutifs (groupe IV). Le sérum, le foie, le cœur et les reins ont été prélevés 8 heures après l’administration de LPS. Les concentrations en malondialdéhyde (MDA), glutathion (GSH) et les activités de la Superoxyde Dismutase (SOD) et de la Catalase (CAT) ont été déterminées par spectrophotométrie dans les différents tissus prélevés et dans le sérum (cas du MDA).

Le LPS a induit des augmentations significatives (p<0.05) du MDA et des différents anti-oxydants dans le sérum et dans tous les tissus examinés. La prednisolone et la vitamine E ont annulé toutes les augmentations du MDA, du GSH, de la SOD et de la CAT induites par le LPS. Par conséquent, la prednisolone et la vitamine E ont prévenu l’apparition d’un stress oxydatif chez des lapins rendus endotoxémiques.


Introduction

Endotoxin, a bacterial lipopolysaccharide (LPS), is used as a model of inflammatory response associated with injury or disease states. LPS from outer membrane of gram (-) bacteria, involves a systemic inflammatory response syndrome defined as sepsis. When hypotension plus organ dysfunction occurs, the condition is described as septic shock. Septic shock is characterized by hypotension and vascular collapse, with failure of heart, kidney, lung, liver, central nervous system and the coagulation system. Death usually occurs due to refractory shock or failure of multiple organs. Systemic sepsis is associated with cardiovascular dysfunction through its effects on the myocardium, endothelium and vascular smooth muscle [6, 7, 25, 36].

In endotoxemia, many cytokines and reactive oxygen species (ROS) are released from leukocytes. ROS are key mediators of the multiple organ failure in endotoxic shock, and they are essential mediators of endotoxin-induced mortality. They interact with each other and mutually modulate their production and release [1, 3, 5, 9, 16, 36]. ROS cause lipid peroxidation of membrane phospholipids, which can alter membrane fluidity and impair the activities of different mitochondrial enzymes, leading to decreased intracellular energy levels, cell necrosis and organ failure. Lipid peroxides produced at the affected site are released into the circulation and metabolized in the liver [7, 10]. During lipid peroxidation, free radicals interact for producing malondialdehyde (MDA) in the termination phase. The plasma MDA concentration is frequently used as a biomarker for an overall lipid peroxidation [18]. It is increasingly recognized that ROS damage plays a key role in LPS induced septic shock [4, 21, 33, 35].

Antioxidants within the cells such as Superoxide Dismutase (SOD), Catalase (CAT) and glutathione (GSH) protect against oxidative stress. SODs, a family of enzymes that catalyze the dismutation of two superoxide radicals to hydrogen peroxide and molecular oxygen, reduce tissue concentrations of superoxide radicals and prevent the production of hydroxyl radical, but they contribute to the local formation of hydrogen peroxide [19, 31]. CAT is mainly a heme-containing enzyme. The predominant subcellular localization of enzyme is peroxisomes, in which it catalyzes the dismutation of hydrogen peroxide to water and molecular oxygen. CAT activity is highest in the liver, relatively high in kidney, and very low in heart [13, 19]. GSH is the most abundant intracellular thiol-compound antioxidant in all living aerobic cells. It serves as a substrate for GSH peroxidase and GSH-S-transferase and it is also an effective scavenger of ROS. To carry out its multiple role in cellular antioxidant defense, GSH is oxidized to GSSH, which can be reduced back to GSH by GSH reductase [8, 19]. When the antioxidant capacity is overwhelmed within the cell, lipid peroxidation occurs.

On the other hand, corticoids such as prednisolone (PR), decrease plasma inflammatory cytokine concentrations. It is well known that cytokines are released from phagocytes and play an important role in endotoxic shock as well [28, 37]. Vitamin E (VE, alpha tocopherol), a lipid soluble vitamin, plays an important role in preventing lipid peroxidation of polyunsaturated fatty acids in cell membranes. Thus it protects tissues from oxidative damage [6, 19].

Most of studies analyze the oxidative stress induction by LPS mainly in liver and only few studies have been focused on heart and kidney in LPS-induced oxidative stress. Consequently; the aims of this study were 1) to evidence the dismutations of superoxide radicals in heart and kidney in LPS-induced oxidative stress. It is important to mention that catalase (CAT) activity is highest in the liver, and relatively high in kidney, and very low in heart [13, 19]. GSH is the most abundant intracellular thiol-compound antioxidant in all living aerobic cells. It serves as a substrate for GSH peroxidase and GSH-S-transferase and it is also an effective scavenger of ROS. To carry out its multiple role in cellular antioxidant defense, GSH is oxidized to GSSH, which can be reduced back to GSH by GSH reductase [8, 19]. When the antioxidant capacity is overwhelmed within the cell, lipid peroxidation occurs.

2. Materials and methods

A) ANIMAL TREATMENTS

Endotoxemia was induced by E. coli LPS (Escherichia coli O111; B4, Sigma) in New Zealand white rabbits (male, 12-16 months old, 2-2.5 kg, Veterinary Research Institute, Adana). Forty rabbits were randomly divided into four equal groups. Animals were anaesthetized by intramuscular injection of ketamine-HCl (30 mg/kg) and xylazine-HCl (0.02 mg/kg) followed by intramuscular boosts of ketamine-HCl throughout the experiment. The rabbits of Group I (control group) were only perfused with 60 ml of NaCl 0.15 M (10 ml/hr by marginal ear vein for 6 hours). In group II, rabbits were perfused with LPS dissolved in 60 ml NaCl 0.15 M at a dose of 100 µg/kg/hr (10 ml/hr for 6 hours) [17]. In Group III, rabbits received a subcutaneous injection of prednisolone (10 mg/kg, injection volume: 0.8 to 1 ml, Prednisolon(r) amp, Fako, Istanbul, Turkey), 30 min before the LPS perfusion (100 µg/kg/hr for 6 hours) [37]. In group IV, vitamin E (DL-alpha tocopherol acetate, Evigen(r) amp, Aksu Farma, Istanbul, Turkey) was intraperitoneally injected (10 mg/kg, injection volume: 60 to 80 µ1) for 4 successive days, and 10 min after the vitamin final injection, LPS (100 µg/kg/hr) was perfused [39]. Animals were killed, and serum, liver, heart and kidney samples were taken at 8 hr after starting the LPS perfusion.

B) DETERMINATION OF MALONDIALDEHYDE

MDA concentrations were determined by using thiobarbituric acid dissolved in sodium solution according to SATOH’s procedure [26]. The release of lipid peroxide color reaction was performed by heating serum, and proteins of tissue homogenates precipitate with this reagent in a weak acid solution [26, 38].

C) DETERMINATION OF ANTIOXIDANTS

GSH was determined in serum and tissue homogenate by using Ellman’s reagent (DTNB). In this method, DTNB [5-5'-dithiobis-(2-nitrobenzoic acid)] is reduced by GSH to NMBA (2-nitro-5-mercaptobenzoic acid). This compound NMBA is deep yellow and the absorbance is spectrophotometrically at 412 nm [27].

CAT activity was determined using the method of AEIB [2]. The decomposition of H2O2 was directly followed by the decrease of absorbance at 240 nm. The results were expressed as U/mg protein.

SOD activity was determined using the Randox-Ransod enzyme kit : the oxidation of xanthine catalyzed by xanthine oxidase (XOD) generates superoxide radicals that react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium-chloride to form a red formazon dye. Because SOD catalyzes the dismutation of O2·-, the second reaction is limited when SOD is present in the sample, leading to decrease the absorbance at 505 nm. The results are expressed as U/mg protein.

D) STATISTICAL ANALYSES

All values are expressed as mean ± Standard errors (SE). The results were analyzed by Tukey multiple range test (SPSS for windows, release 6.0). In all cases, probability of error of less than 0.05 was selected as the criterion for statistical significance.

3. Results

The MDA and GSH concentrations and the SOD and CAT enzyme activities according to rabbit treatments are given in Table I.

In control group, the observed MDA and GSH concentrations were the lowest in the liver and the highest in the heart (p<0.01). On the contrary, the CAT activity was minimal in heart and maximal in kidney (p<0.01). The SOD activity was identical in all examined organs.

With LPS treatment, significant increases of MDA concentrations were obtained in serum and in all the studied tissues (heart, liver and kidney) (p<0.05), indicating that the
endotoxin induced a systemic and multiple organ oxidative damage. Moreover, tissue GSH concentrations and SOD, CAT activities significantly increased (p<0.05), showing that the anti-oxidant defenses were exacerbated during LPS-induced oxidative stress. The increase of MDA concentrations was maximal in the kidney and minimal in the liver (figure 1). The highest GSH concentrations were obtained in the heart, and the lowest in the liver. SOD and CAT activities markedly increased in liver and in kidney in comparison to heart (p<0.05) (Table I and figure 2). Consequently, the

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Group I (Control)</th>
<th>Group II (LPS)</th>
<th>Group III (LPS+PR)</th>
<th>Group IV (LPS+VE)</th>
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<tbody>
<tr>
<td><strong>MDA</strong> (nmol/mg)</td>
<td></td>
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<tr>
<td>Liver</td>
<td>0.992 ± 0.066 a</td>
<td>1.386 ± 0.099 b</td>
<td>1.027 ± 0.051 a</td>
<td>0.918 ± 0.071 a</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.063 ± 0.009 a</td>
<td>0.570 ± 0.081 b</td>
<td>0.189 ± 0.036 a</td>
<td>0.033 ± 0.003 a</td>
</tr>
<tr>
<td>Heart</td>
<td>0.408 ± 0.038 a</td>
<td>1.304 ± 0.127 b</td>
<td>0.251 ± 0.022 ac</td>
<td>0.064 ± 0.005 c</td>
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<tr>
<td><strong>SOD</strong> (U/mg)</td>
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<tr>
<td>Liver</td>
<td>9.940 ± 0.781 a</td>
<td>56.67 ± 14.11 b</td>
<td>8.736 ± 0.786 a</td>
<td>10.76 ± 4.510 a</td>
</tr>
<tr>
<td>Kidney</td>
<td>10.76 ± 0.736 a</td>
<td>57.38 ± 21.25 b</td>
<td>9.834 ± 0.908 a</td>
<td>8.770 ± 0.539 a</td>
</tr>
<tr>
<td>Heart</td>
<td>8.409 ± 0.196 a</td>
<td>36.82 ± 8.615 b</td>
<td>9.293 ± 2.064 a</td>
<td>6.964 ± 1.585 a</td>
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<tr>
<td><strong>CAT</strong> (mU/mg)</td>
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</tr>
<tr>
<td>Liver</td>
<td>8 ± 1 a</td>
<td>49 ± 2 b</td>
<td>14 ± 2 a</td>
<td>11 ± 1 a</td>
</tr>
<tr>
<td>Kidney</td>
<td>22 ± 1 a</td>
<td>72 ± 8 b</td>
<td>18 ± 2 a</td>
<td>17 ± 1 a</td>
</tr>
<tr>
<td>Heart</td>
<td>3 ± 1 a</td>
<td>7 ± 1 b</td>
<td>4 ± 1 a</td>
<td>4 ± 1 a</td>
</tr>
<tr>
<td><strong>GSH</strong> (µg/mg)</td>
<td></td>
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<tr>
<td>Liver</td>
<td>4.92 ± 0.51 a</td>
<td>30.35 ± 4.95 b</td>
<td>7.25 ± 0.65 a</td>
<td>3.87 ± 0.40 a</td>
</tr>
<tr>
<td>Kidney</td>
<td>8.96 ± 1.01 a</td>
<td>37.36 ± 5.49 b</td>
<td>12.46 ± 1.65 a</td>
<td>10.09 ± 1.35 a</td>
</tr>
<tr>
<td>Heart</td>
<td>17.15 ± 1.03 a</td>
<td>41.58 ± 3.32 b</td>
<td>17.81 ± 1.68 a</td>
<td>13.38 ± 1.17 a</td>
</tr>
</tbody>
</table>

a,b,c; Different letters in the same row is statistically significant (p<0.05).

**TABLE I.** — Effects of vitamin E and prednisolone on malondialdehyde (MDA), glutathione (GSH) concentrations, and superoxide dismutase (SOD), catalase (CAT) activities in endotoxemic rabbits (n = 40, means ± Standard Errors). LPS : lipopolysaccharide (100 µg/kg/hr for 6 hours), PR : prednisolone (10 mg/kg subcutaneously before LPS treatment), VE : vitamin E (10 mg/kg intraperitoneally for 4 consecutive days before LPS treatment).
enhancement of anti-oxidant systems in a given tissue during LPS-induced oxidative stress was roughly proportional to the amounts of protective compounds initially present (figure 2).

When VE and PR were administrated before LPS, no significant increase of MDA, GSH concentrations and of SOD, CAT activities was recorded in serum and in all examined tissues. Moreover, significant decreases of MDA concentrations in heart and in kidney were noticed in comparison to control values (p<0.05) when rabbits were previously treated by VE (group IV).

4. Discussion and conclusion

In the present study, LPS perfusion induced multiple organ oxidative damages characterized by MDA concentration increases in serum and in examined tissues (heart, liver and kidney). These results were in good agreement with previous studies which reported increases of MDA concentrations in plasma, liver [3, 4, 24], diaphragmatic muscle [20] and in brain [1]. Indeed, ROS were released from leukocytes and were essential mediators of multiple organ failure and in endotoxemic shock.

LPS caused statistically significant increases in SOD, CAT activities and GSH concentrations in liver, heart and kidney. In many studies, similar and conflicting results associated with antioxidant systems have been reported. LPS increased GSH concentrations in serum, hepatocytes, thymus, peritoneal leukocytes, macrophages, CAT and SOD activities in heart [4, 8, 23, 29, 33], and in myocyte [34]. By contrast, it was reported that LPS decreased brain and spleen GSH concentrations, CAT and SOD activities in liver [1, 14, 23, 33, 36] and in macrophages [12, 29]. On the other hand, bacterial infections have also induced conflicting results. Salmonella typhimurium LT-2 decreased liver SOD activity during 11 days, while Salmonella typhimurium SL-1181 had no effect on liver SOD activity. In contrary, Pseudomonas aeruginosa increased liver SOD activity at the third day [30]. These discrepancies could result from the animal species, the bacterial types and the LPS structure, the tissue contents of anti-oxidant compounds and the sampling kinetic. Because measured antioxidants may be within the physiological range for a given protocol in a given tissue, anti-oxidants and MDA have to be analyzed together.

The ROS massively released from leukocytes during oxidative stress would indirectly be reduced by GSH peroxidase into alcohols, leading to the parallel oxidation of GSH. So decreases (and not increases) of GSH concentrations in tissue would be occur. However, GSH was regenerated in reduced form through the GSH reductase activity, and the observed increases of GSH concentrations emphasized the efficiency of this regeneration pathway. Because LPS was slowly perfused to rabbits and not rapidly administrated to animals in IV bolus, the release of ROS from white cells would be progressive, allowing an efficient conversion into chemical reduced forms and simultaneously the recycling of GSH. Moreover, different inflammatory mediators implicated in oxidative stress like hydroperoxides and some cyto-
kines directly induced the de novo synthesis of anti-oxidant enzymes [11, 22, 32] and contributed to the partial control of oxidative injuries by this way.

Prior treatments with VE or PR suppressed LPS-induced increases of MDA concentrations and anti-oxidant systems, indicating that tocopherol and corticoids have blocked the occurrence of oxidative stress. As PR repressed the cytokine expression by macrophages and other leukocytes, the production of ROS was reduced, limiting the intensity of oxidative stress. Vitamin E directly reduced membrane peroxides into hydroperoxides by promoting the termination phase during radical reactions, and consequently facilitated the local action of membrane GSH peroxidases on hydroperoxides. Because ROS were more rapidly and more efficiently reduced or less produced, they did not accumulate and no de novo synthesis of anti-oxidant enzymes could occur. So, no increase of anti-oxidant defenses was evidenced during our experiment. Moreover, the protective effects of VE or PR would be reinforced by the prior treatments and by the administration protocol of LPS. Indeed, the amounts of cellular anti-oxidant systems were already increased before the LPS deleterious actions and they were all more relevant since the ROS formation was gradual during LPS perfusion. Besides, since VE induced significant decreases of MDA concentrations in kidney and in heart in comparison to controls, this anti-oxidant compound would exert more protective effects than PR. Previous reports [14, 15] indicated that VE suppressed increases of MDA concentrations in brain and in peritoneal tissues. However, it would be probable that the beneficial effects of PR and VE on oxidative stress were not so efficient if these compounds were injected after LPS treatment.

As a conclusion, results obtained from this study suggested that LPS induced significant changes in SOD, CAT activities and GSH concentrations in liver, heart and kidney. Especially the kidney was affected by LPS administration as liver and heart. Prior anti-oxidant administration such as prednisolone and vitamin E could be preventive treatments for endotoxic shock.

Acknowledgement

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5. References


