Effect of Endotoxemia on Skin Antioxidant Enzymes under Experimental Conditions

A. V. Ivanova, E. V. Mikhal'chik, E. A. Miroshnikova*, E. V. Lukasheva*, and L. G. Korkina

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 142, No. 10, pp. 393-395, October, 2006
Original article submitted March 3, 2006

Intraperitoneal injection of bacterial lipopolysaccharide in a dose of 1 mg/kg was followed by prestimulation of whole blood leukocytes in rats. Activities of peroxide- and lipoperoxide-utilizing antioxidant enzymes glutathione peroxidase, glutathione S-transferase, and catalase increased 1 day after lipopolysaccharide administration, while the content of malonic dialdehyde in the skin remained unchanged.

Key Words: *endotoxemia; antioxidant enzymes; skin*

Recent experiments showed that endotoxin (LPS from gram-negative bacteria) induces oxidative damage to the vascular endothelium due to activation of superoxide radical generation by endothelial cells [3] and neutrophils [13]. LPS stimulates adhesion of neutrophils to the endothelium [10] and increases the respiratory burst in neutrophils under conditions of exogenous stimulation (priming effect) [8]. Experimental endotoxemia induced by administration of purified LPS is accompanied by activation of lipid peroxidation in target tissues (lungs and liver). High risk of oxidative tissue damage during endotoxemia is associated with not only increased generation of reactive oxygen species (ROS) and nitrogen by cells, but also inhibition of antioxidant enzymes (*e.g.*, lung catalase) [5]. Increasing interest to the role of LPS and neutrophils in the inflammatory response of the skin is related to infectious complications of surgical treatment [12]. Published data show that wound healing is decelerated after local administration of LPS [9]. Intradermal injection of LPS increases albumin release from the plasma to the skin [7]. Experimental and clinical data suggest that the imbalance in the prooxidant/antioxidant system of intact skin and wound modulates wound healing [14]. However, activity of antioxidant enzymes in the skin during systemic endotoxemia remains unknown.

Changes in activity of tissue antioxidant enzymes during endotoxemia result from increased superoxide generation by endothelial cells, activation of nuclear translocation of transcription factor (NF-κB), intensive synthesis of proinflammatory cytokines (adhesion molecules), phosphorylation of NADH oxidase in neutrophils, stimulation of local production of the superoxide radical and H_2O_2, activation of genes for the synthesis of protective antioxidant enzymes [3], and direct inactivation of enzymes under the influence of ROS [6].

Here we studied the effect of experimental endotoxemia induced by intraperitoneal injection of LPS on blood leukocytes and skin antioxidant enzymes.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 300-400 g. The animals were maintained in a vivarium under standard conditions. Each experimental group consisted of 6 rats. Endotoxemia in treated animals was induced by intraperitoneal injection of *E. coli* O111:B4 LPS (1 ml, Sigma) in 0.9% NaCl (1 mg/kg). The study was performed under ether anesthesia. The sample (venous blood

Russian State Medical University; *Russian People's Friendship University, Moscow

0007-4888/06/1424-0413

and skin biopsy specimens) were taken 24 h after LPS administration. Control animals received intraperitoneal injection of 0.9% NaCl.

The blood was stabilized with anticoagulant heparin and aliquots were taken to study spontaneous and phorbol ester-induced (0.15 μM) chemiluminescence (CL) activated with luminol (0.2 mM). The measurements were performed on a Wallah 1251 luminometer (LKV). Leukocytes were counted in a Goryaev chamber. For isolation of plasma and erythrocyte pellet the blood was centrifuged at 400*g*. The content of reduced glutathione in erythrocytes was measured as described elsewhere [1].

Skin samples were homogenized on ice in a Potter homogenizer (10-fold volume of 0.1 M potassium phosphate buffer, pH 7.4, for 5-8 min). The samples were centrifuged at 800*g* and 10°C for 30 min. The supernatant was used for biochemical studies.

Spectrophotometry data were calculated per protein concentration (method of Lowry). Myeloperoxidase (MPO) activity was measured in the reaction with *o*-dianisidine at 560 nm. Glutathione peroxidase activity was estimated by the ability of samples to catalyze the reaction of reduced glutathione with tert-butyl hydroperoxide (t-BHP). Reduced glutathione content in samples was measured before and after incubation with t-BHP using 5,5'-dithio-bis-(2-nitro)-benzoic acid [1]. Glutathione S-transferase (GT) activity was determined by the rate of enzyme-catalyzed formation of reduced glutathione with 1-chloro-2,4-dinitrobenzene (maximum absorption at 340 nm) [1]. Catalase activity was estimated by the decrease in H_2O_2 absorption at 240 nm [1]. Superoxide dismutase (SOD) activity in samples was measured by inhibition of epinephrine autooxidation in carbonate buffer at pH 10.3 [12]. The intensity of lipid peroxidation was estimated from the concentration of malonic dialdehyde (MDA).

The results were analyzed by means of Statistica software. The data are presented as means and standard deviations. The significance of differences was evaluated by Student's *t* test.

RESULTS

Spontaneous CL of the blood and total leukocyte count in the blood from treated animals tended to increase after intraperitoneal injection of LPS (Table 1). Whole blood CL depends on leukocyte count and functional activity. Spontaneous CL was calculated per million leukocytes (specific CL). The estimated values were similar in control and treated rats (4.2±1.7 and 5.1±1.3 mV/million, respectively). Therefore, LPS does not directly initiate the respiratory burst in blood leukocytes.

Induced CL of whole blood in treated rats 3-fold exceeded that in control animals. MDA content in the plasma, as well as reduced glutathione concentration in erythrocytes remained unchanged under these conditions. It can be hypothesized that LPS had a prestimulation effect on circulating neutrophils, but did not induce the immediate release of ROS into the blood.

Activities of MPO and SOD in the skin of treated rats slightly surpassed the corresponding parameters in control animals. Activities of glutathione peroxidase and catalase in treated rats significantly surpassed the control. GT activity in treated rats was 3 times higher than in control animals. No intergroup differences were found in MDA content in the skin (Table 2).

Bacterial endotoxin (LPS) is an inflammatory factor present in the wall of gram-negative bacteria. Activation of systemic reaction in response to intraperitoneal injection of LPS was confirmed by the increase in radical-generating activity of leukocytes of the whole blood. This parameter was estimated by *in vitro* recording of CL upon stimulation with

TABLE 1. Blood Parameters in Experimental Animals (*M*±*m*)

Parameter	Control group (NaCl)	Treated group (LPS)
Plasma MDA, μM	2.01±0.63	2.14±0.50
Spontaneous CL, mV	71.6±24.6	106.3±58.5
Induced CL, mV	703.9±159.3	2155±696*
Reduced glutathione in erythrocytes, μg/mg hemoglobin	1.82±0.11	1.89±0.07
Leukocytes, million/ml	17±2	20±6

Note. Here and in Table 2: *$p<0.05$ compared to the control group.

TABLE 2. Skin Enzyme Activity in Experimental Animals (*M*±*m*)

Parameter (per g protein)	Control group (NaCl)	Treated group (LPS)
MPO, μmol/g	90.8±25.9	121.8±21.3
Catalase, U/mg	4.42±1.97	9.38±3.02*
SOD, U/mg	9.4±0.7	11.0±2.3
GT, μmol CDNB/g/min	3.5±1.5	10.1±3.1*
Glutathione peroxidase, μg glutathione/mg/min	1.8±0.9	3.9±0.8*
MDA, μmol/g	1.4±0.5	1.8±0.3

Note. CDNB, 1-chloro-2,4-dinitrobenzene.

phorbol ester. A similar effect of LPS was revealed previously in whole blood samples from patients with endotoxemia and underlies the CL method to estimate plasma endotoxin concentration [8].

Similarly to the lung tissue, MPO activity in the skin tended to increase due to neutrophil migration into tissues during endotoxemia. Published data show that MPO activity increases by 5 times 1 h after LPS administration [15]. The observed changes in the skin were less significant compared to lung tissue. The probability of the respiratory burst in neutrophils migrating into the skin increases after cell adhesion to extracellular matrix proteins. Even small number of neutrophils can trigger the release of ROS from neutrophils under these conditions [4].

Activities of glutathione peroxidase and GT significantly increased 1 day after intraperitoneal injection of LPS. Activities of SOD and catalase in the skin tended to increase during this period. We hypothesized that LPS increases ROS generation in the skin, which induces the synthesis of protective antioxidant enzymes. The induction of enzymes was sufficient for skin protection from the increased oxidative load. A significant increase in MDA content in the skin was not revealed under these conditions. Our results indicate that the skin is not a target tissue during endotoxemia. However, the probability of endotoxemia-induced oxidative damage to the skin increases in individuals with decreased adaptive capacities of the organism (*e.g.*, in severe traumas and age-related disorders) [9].

REFERENCES

1. *Medical Laboratory Technologies* [in Russian], Eds. A. I. Karpishchenko, St. Petersburg (1999), pp. 20-72.
2. P. C. Andrews and N. I. Krinsky, *Handbook of Methods for Oxygen Radical Research*, Florida (1986), pp. 297-302.
3. E. L. Chan and J. T. Murphy, *J. Surg. Res*., **111**, 120-126 (2003).
4. W. C. Cioffi, D. G. Burelson, and B. A. Pruitt, *Arch. Surg*., **112**, 860-865 (1992).
5. R. Daryani, C. LaLonde, D. Zhu, and R. H. Demling, *Circ. Shock*, **32**, 273-280 (1990).
6. R. Daryani, B. S. LaLonde, D. Zhu, *et al*., *J. Trauma*, **30**, 1330-1334 (1990).
7. T. Iuvaone, F. D'Acquisto, N. Van Osselaer, *et al*., *Br. J. Pharmacol*., **123**, 1325-1330 (1998).
8. J. Marshall, P. M. Walker, D. M. Foster, *et al*., *Crit. Care*, **6**, 342-348 (2002).
9. Z. Metzger, D. Nitzan, S. Pitaru, *et al*., *J. Endod*., **28**, 30-33 (2002).
10. K. Minegishi, M. Tanaka, O. Nishimura, *et al*., *Am. J. Physiol. Endocrinol. Metab.*, **283**, E1308-E1315 (2002).
11. H. P. Misra and I. Fridovich, *J. Biol. Chem*., **247**, 3170 (1972).
12. Y. Moriwaki, M. Sugiyama, and H. Katamura, *Gastroenterology (Tokyo)*, **16**, 471-477 (1992).
13. H. S. Murphy, R. L. Warner, N. Bakopoulos, *et al*., *Shock*, **12**, 111-117 (1999).
14. M. Rojkind, J. A. Dominguez-Rosales, N. Nieto, and P. Greenwel, *Cell. Mol. Life Sci*., **59**, 1872-1891 (2002).
15. R. Shencar and E. Abraham, *J. Immunol*., **163**, 954-962 (1999).