Prooxidant-Antioxidant State of the Organism during Oxidative Stress and Correction of the L-Arginine-NO System

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The prooxidant-antioxidant balance in rats with oxidative stress was studied during correction of the L-arginine-NO system. Oxidative stress was induced by intravenous injection of *E. coli* lipopolysaccharide. Under conditions of oxidative stress the prooxidant-antioxidant imbalance was least pronounced during selective correction of the L-arginine-NO system. L-Arginine and nonselective NO synthase inhibitor had little protective effect.

Key Words: *lipid peroxidation; antioxidant system; lipopolysaccharide; nitric oxide*

The formation of reactive oxygen metabolites ($O_2^{\bullet}$, H_2O_2, OH radical, singlet oxygen, NO radical, and peroxide radicals) plays a key role in the molecular and cellular mechanisms of oxidative stress [1]. Free radical NO is synthesized in the organism and provides various cell functions. However, NO in high concentrations interacts with other oxidizers forming reactive nitrogen species that can damage target cells. Peroxynitrite formed from NO and $O_2^{\bullet}$ is a potent oxidizer reacting with biological molecules. The complex relationship between reactive nitrogen and oxygen species provides their synergistic and antagonistic effects, which depends on the rate of formation of NO and $O_2^{\bullet}$ [4].

Here we studied the prooxidant-antioxidant balance under conditions of oxidative stress accompanied by correction of the L-arginine-NO system.

MATERIALS AND METHODS

Experiments were performed on 69 male laboratory rats weighing 190-230 g. The animals were maintained in a vivarium at 20°C. Oxidative stress was induced by intravenous injection of *E. coli* lipopolysaccharide (LPS, Sigma) in a dose of 5 mg/kg (common doses of LPS vary from 2 to 20 mg/kg [8]), which led to the development of moderate oxidative stress.

The L-arginine-NO system was corrected by intravenous injection of L-arginine (300 mg/kg) 10 min before LPS administration. N^{ω}-nitro-L-arginine methyl ester (L-NAME, Sigma) was injected in a dose of 20 mg/kg. A selective NO synthase inhibitor L-lysine-N^{ω}-acetamide (L-NIL, Sigma) in a dose of 2 mg/kg was injected 45 min after LPS administration. Tissue samples were taken from the heart, lungs, liver, kidneys, and muscles 180 min after LPS administration. The doses and route of treatment with the test preparations provided appreciable correction of NO synthesis in the organism [6,9].

The concentration of conjugated dienes (CD) was estimated by measuring the amount of conjugated diene structures in hydroperoxides of polyunsaturated fatty acids [7]. The concentration of Schiff bases was determined by fluorescence of a chloroform extract at excitation and emission wavelengths of 344 and 440 nm, respectively. The measurements were performed on an F-4010 spectrofluorometer (Hitachi) [7]. Catalase activity in bio-

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logical materials was evaluated by utilization of H_2O_2, which forms a stable colored complex with molybdenum salts, on a SF-46 spectrophotometer at 410 nm [2]. α-Tocopherol was assayed by fluorescence of a heptane extract at excitation and emission wavelengths of 292 and 325 nm, respectively. The measurements were performed on an F-4010 spectrofluorometer (Hitachi) [2].

The results were analyzed by Student's *t* test.

RESULTS

Oxidative stress was accompanied by activation of lipid peroxidation (LPO) in tissues (Figs. 1 and 2). The concentration of CD in the heart, lungs, liver, and kidneys increased by 137.6, 184.1, 101.2, and 90.4%, respectively. The concentration of Schiff bases in the heart, lungs, liver, and kidneys increased by 99.2, 111.3, 81.8, and 121.3%, respectively ($p<0.001$). Prooxidant-antioxidant imbalance in tissues was least pronounced during selective correction of the L-arginine-NO system. Accumulation of CD and Schiff bases was less significant under these conditions. The concentration of CD in the heart, lungs, liver, and kidneys increased by 27.6 ($p<0.05$), 32.6 ($p<0.01$), 27.4 ($p<0.02$), and 25.1% ($p<0.02$), respectively. The concentration of Schiff bases in the heart, lungs, liver, and kidneys increased by 13.8 ($p<0.01$), 26.1, 19.6, and 16.8% ($p<0.001$), respectively. We studied the effect of LPS on the main factors of the antioxidant defence system (Tables 1 and 2). Oxidative stress was accompanied by a decrease in catalase activity and α-tocopherol concentration in tissues. Catalase activity decreased most significantly in the heart, lungs, liver and kidneys (49.6, 51.7, 50.9, and 57.2%, respectively, $p<0.001$). α-Tocopherol concentration decreased most significantly in the lungs (54.9%). L-NIL administration under conditions of oxidative stress improved the antioxidant protection. L-Arginine and nonselective NO synthase inhibitor L-NAME produced no protective effect.

There are ambiguous data on the inhibition of NO synthase. It is probably associated with differences in the models of septic shock, doses of NO synthesis inhibitors, stage of treatment with corrective drugs, and local concentration of NO [5]. NO can produce a protective or depressive effect depending on the severity of oxidative stress. Overproduction of radicals (relative to NO synthesis) is accompanied by the induction of damage [3].

Our results show that NO modulates the development of oxidative stress. High-specificity markers of oxidative stress (isoprostane concentration,

TABLE 1. Catalase Activity in Tissues of Rats during Oxidative Stress Accompanied by Correction of the L-Arginine-NO System (mmol H_2O_2/sec×g protein, *M±m*)

Tissue	Control	LPS	L-NAME	LPS+L-NAME	L-Arginine	L-Arginine+LPS	L-NIL	LPS+L-NIL
Heart	41.28±1.79	20.79±2.32*	42.40±1.71+	26.31±2.63*	42.94±1.10+	27.83±3.65*	44.51±2.26+	31.19±2.14*+
Lungs	35.28±1.69	17.04±3.18*	34.51±2.07+	21.87±2.77*	36.78±2.06+	19.29±3.97*	37.72±2.14+	25.99±2.18*+
Liver	93.69±1.14	46.02±2.73*	98.86±3.13+	47.20±4.16*	90.60±3.61+	52.41±3.55*	89.96±2.67+	62.16±3.59*+
Kidneys	66.04±2.99	28.24±4.82*	68.04±3.13+	30.40±3.30*	63.14±4.09+	34.17±3.52*	69.91±1.09+	46.83±4.41*+

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

TABLE 2. α-Tocopherol Concentration in Tissues of Rats during Oxidative Stress Accompanied by Correction of the L-Arginine-NO System (nmol/g, *M±m*)

Tissue	Control	LPS	L-NAME	LPS+L-NAME	L-Arginine	L-Arginine+LPS	L-NIL	LPS+L-NIL
Heart	191.9±3.57	123.4±7.66*	199.5±2.60+	131.8±6.40*	186.2±2.21+	133.2±3.52*	189.8±5.18+	148.6±5.89*+
Lungs	280.1±5.90	126.3±9.94*	279.5±6.36+	142.4±5.84*	270.5±7.06+	138.8±9.27*	269.8±5.13+	191.3±7.37*+
Liver	135.6±3.76	94.3±4.38*	140.7±2.61+	94.5±5.25*	131.7±2.45+	97.5±5.57*	129.7±3.72+	113.5±3.91*+
Kidneys	181.1±4.59	141.8±5.69*	195.1±5.88+	147.1±5.56*	186.7±4.09+	148.2±6.64*	185.0±3.33+	159.7±4.10*+

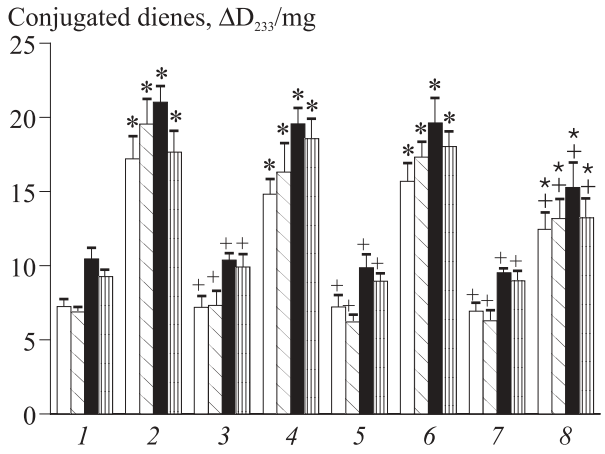

Fig. 1. Concentration of conjugated dienes in rats during oxidative stress under conditions of correction of the L-arginine-NO system. Here and in Fig. 2: light bars, heart; dark bars, liver; slant shading, lungs; vertical shading, kidneys. Control (*1*), LPS (*2*); L-NAME (*3*); LPS+L-NAME (*4*); L-arginine (*5*); L-arginine+LPS (*6*); L-NIL (*7*); LPS+L-NIL (*8*). $p<0.05$: *compared to the control; +compared to LPS.

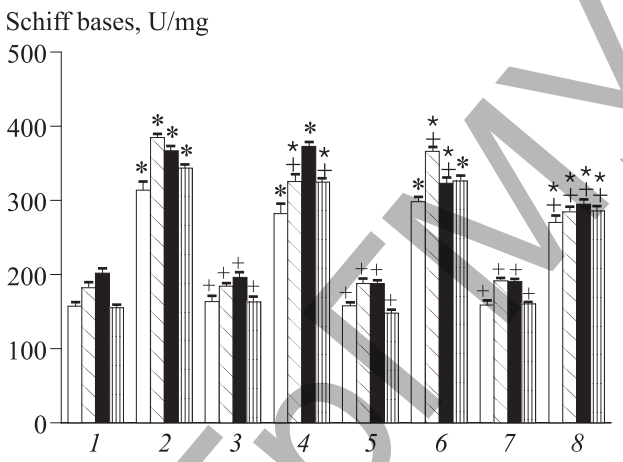

Fig. 2. Concentration of Schiff bases in rats during oxidative stress under conditions of correction of the L-arginine-NO system.

degree of DNA damage, *etc*.) hold much promise for evaluation of the role of NO in its pathogenesis.

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