Activity of Antioxidant Enzymes and Lipid Peroxidation in Intact and Mutagen-Treated NZW Mice

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Catalase and superoxide dismutase activities in the liver of NZW mice are 29.3 μmol H_2O_2/min×mg protein and 10.6 U/mg protein, respectively. The rate of accumulation of lipid peroxidation (LPO) products is low within the first 60 min of incubation of liver homogenates with ascorbate and then rapidly increases. A similar process is observed with Fe+ascorbate system, where LPO rate is markedly higher and lag-period lasts 10 min. Under the action of cyclophosphane the activity of catalase increases by 32%, while that of superoxide dismutase decreases by 46%, which is accompanied by a decline in the sensitivity of liver tissue to LPO induction. When LPO is induced *in vitro* by ascorbate, lag-period decreases 2-fold, while the rate of accumulation of LPO products increases by 38% and their maximum level by 35% compared with the control. Similar processes develop in the Fe+ascorbate system. Dioxydine induces no significant changes in the activities of catalase and superoxide dismutase as well as in LPO product accumulation in the ascorbate and Fe+ascorbate systems.

Key Words: *lipid peroxidation; superoxide dismutase; catalase; dioxydine; cyclophosphane, NZW mice*

Lipid peroxidation (LPO), a process normally occurring in the organism, becomes pathogenic under the action of some factors [4,5]. Lipid peroxidation products produce DNA-damaging effect and exhibit mutagenic activity [3,7,11]. There is evidence that damaging effects of some mutagens are associated with LPO activation [3].

Previously, we studied the relationships between pro- and antioxidant factors in C57Bl/6 and BALB/c mice [1]. The necessity of evaluating these factors in parallel and the difference between the activities of antioxidant enzymes and induced accumulation of LPO products as well as different changes in these parameters under the action of the mutagens cyclophosphane (CP) and dioxydine (DN) have been demonstrated.

In the present study we assessed the oxidative status of intact and mutagen-treated inbred NZW mice with genetically determined predisposition to autoimmune diseases [2].

MATERIALS AND METHODS

Experiments were performed on male NZW mice (Institute of Rheumatology, Russian Academy of Medical Sciences). The animals were maintained under the standard vivarium conditions and 12-h day light schedule. The mutagens were injected intraperitoneally: DN [1,4-di-N-oxide—2,3-bis-(oxymethyl) quinoxaline] in a dose of 300 mg/kg and CP [N'-bis-(β-chlorethyl)-N'-O-trimethylene ether of di-

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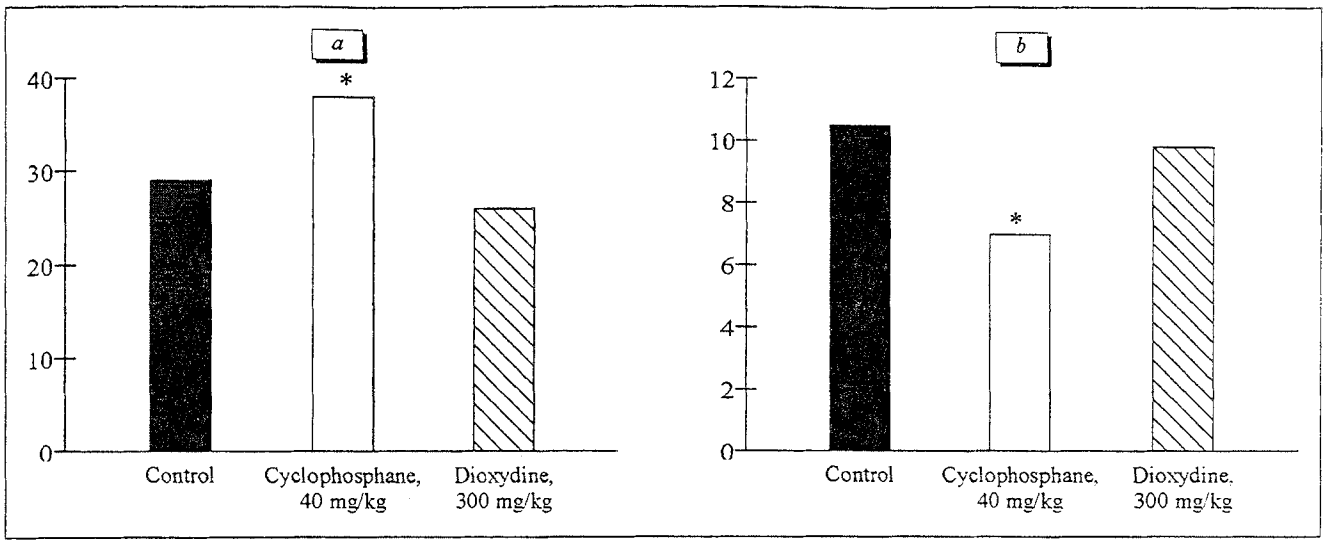

Fig. 1. Catalase (*a*) and superoxide dismutase (*b*) activities in liver homogenate of NZW mice. Ordinate: *a*) catalase activity, μmol H_2O_2/min×mg protein ; *b*) superoxide dismutase activity, U/mg protein. Each group consisted of 5 mice. *$p<0.05$ compared with the control.

amide phosphate] in a dose of 40 mg/kg. The animals were sacrificed 24 h after injection, the liver was excised and frozen in liquid nitrogen. It was then homogenized (1:4) at 0°C in a Teflon-glass homogenizer in a buffer containing 20 mM Tris-HCl and 100 mM KCl, pH 7.4.

The catalase activity was assayed by the method [9] with modifications [8] in a Hitachi-557 spectrophotomer (240 nm) and expressed in μmol H_2O_2/min×mg protein with the molar extinction coefficient equal to 39.4 $M^{-1}cm^{-1}$. The protein concentration was determined from the fourth derivative of the light absorbance spectrum at 240-320 nm in medium containing 20 mM histidine, 50 mM NaCl (pH 7.2), and 8.1% SDS, which was added to the homogenate.

The superoxide dismutase (SOD) activity was determined spectrophotometrically (560 nm) [6] in medium containing pyrophosphate buffer ($Na_4P_2O_7$× $10H_2O$, pH 8.3 at 25°C), 0.1 μM xanthine, 0.1 mM EDTA, 0.05 mM tetranitro blue tetrazolium, 1% Triton, and 0.1 mM xanthine oxidase. Hemoglobin was extracted from the supernatant with chloroform: methanol (3:5, v/v) added to the 1:1 ratio. A unit of SOD activity was defined as the amount of the enzyme providing a 50% inhibition of the reduction of tetranitro blue tetrazolium to formazan.

The initial content of LPO products accumulates *in vivo* was determined by the maximum light absorbance (532 nm) of 2-thiobarbituric acid reactive substances (TBARS) [10]. The rate of *in vitro* induced LPO was determined in two systems: ascorbate (0.75 mM) and ascorbate (0.75 mM)+Fe (5 μM). Incubation was carried out at 37°C and 5-fold dilution in medium containing 30 mM Tris-HCl (pH 7.4) for 100 and 40 min, respectively, with a parallel control for auto-oxidation.

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

RESULTS

The initial catalase activity in male NZW mice is 2-fold higher than in C57Bl/6 (liver resistant to LPO) and is practically the same as that in BALB/c mice (liver sensitive to LPO). The activity of SOD in NZW mice (10.6 U/mg protein) is 1.7- and 2.7-fold lower that in C57Bl/6 and BALB/c mice, respectively. In order to find out whether the activity of the antioxidant enzymes in NZW mice is sufficiently high to compensate mutagen-induced oxidative processes, we measured the rate of LPO product accumulation in liver homogenates in the presence of ascorbate and Fe+ascorbate. Ascorbate induces low-intensity LPO and allows one to record the maximum difference in the rate of LPO product accumulation, while the maximum level of TBARS resulting from high-intensify LPO can be measured in a more potent Fe+ascorbate system.

In the presence of ascorbate, the rate of LPO product accumulation in liver homogenates of NZW mice remains low during the first 60 min of incubation and then rapidly increases. A similar process was observed in the Fe+ascorbate system. However, in this case the LPO rate is much higher and lag period lasts 10 min. It is noteworthy that in comparison with C57Bl/6 and BALB/c mice [1], in NZW mice both the rate of LPO product accumulation and the maximum level of LPO products were the highest in the ascorbate system, while in the Fe+ascorbate system both parameters were higher

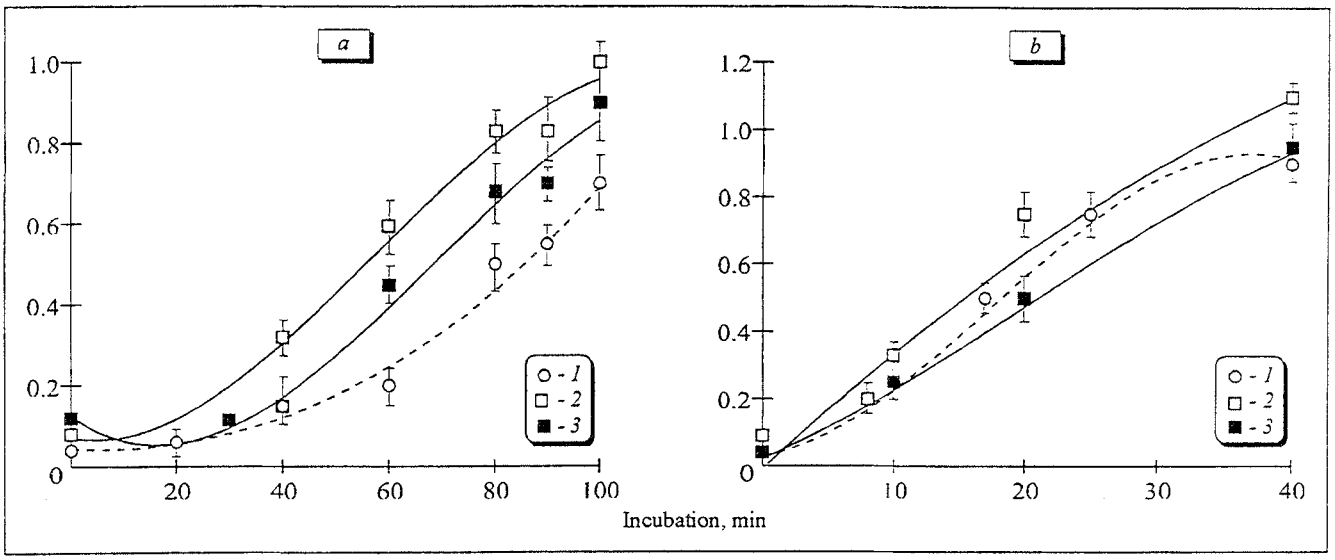

Fig. 2. Accumulation of TBARS in liver homogenates of NZW mice upon LPO induced with ascorbate (0.75 mM, *a*) or Fe (5 μM)+ascorbate (0.75 mM, *b*). *1*) control; *2*) cyclophosphane (40 mg/kg); *3*) dioxydine (300 mg/kg). Ordinate: light absorbance at 532 nm.

than in C57Bl/6 mice and did not differ from those of BALB/c mice.

Thus, liver antioxidant levels in NZW mice do not compensate for oxidation *in vitro* and presumably *in vivo* after the treatment with LPO-inducing mutagens.

In order to test this hypothesis, the activities of antioxidant enzymes and accumulation of LPO products were studied in NZW mice treated with CP and DN.

The alkylating promutagen CP [3] increased catalase activity by 32% and decreased SOD activity by 46% (Fig. 1).

In NZW mice, CP markedly lowered liver sensitivity to induced LPO (Fig. 2). In the presence of ascorbate (low-intensity LPO), lag-period decreased 2-fold, the rate of LPO product accumulation increased by 38%, and the maximum content increased by 35% compared with the control (Fig. 2, *a*). A similar situation developed in Fe+ascorbate system: LPO increased considerably after treatment with CP (Fig. 2, *b*).

Irrespective of high catalase activity in NZW mice, CP stimulated LPO in these animals as it did *in vitro*.

Treatment of NZW mice with the pro-oxidant mutagen DN [3] induced no significant changes in catalase and SOD activities (Fig. 1) and the rate of LPO product accumulation in the Fe+ascorbate system (Fig. 2).

However, comparison of the parameters of LPO induced by different oxidants in liver homogenates of NZW mice treated with CP or DN with those of C57Bl/6 and BALB/c mice [1] showed that the rate of LPO product accumulation is considerably higher in NZW mice. Bearing in mind the mutagenic activity of LPO products and the evidence that mutagenic effects of CP and DN can be mediated by free-radical oxidation [3], it can be supposed that NZW mice are more sensitive to these mutagens than C57Bl/6 and BALB/c mice.

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