Effect of Dioxydine and Cyclophosphane on Lipid Peroxidation and Superoxide Dismutase and Catalase Activities in C57Bl/6 and BALB/c Mice

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Twenty-four hours after intraperitoneal injection of cyclophosphane (40 mg/kg) and dioxydine (300 mg/kg) to C57Bl/6 mice, liver catalase activity dropped by 29 and 23%, respectively. In BALB/c mice, dioxydine (but not cyclophosphane) reduced catalase activity by 24%. Superoxide dismutase activity was lowered by cyclophosphane (but not dioxydine) in BALB/c mice, and by both dioxydine and cyclophosphane in C57Bl/6 mice (by 24 and 86%, respectively). The level of 2-thiobarbituric acid (TBA)-reactive lipid peroxidation (LPO) products in the liver of BALB/c mice treated with cyclophosphane and dioxydine increased 1.4- and 2.1-fold, respectively, while in C57Bl/6 mice it did not differ from the control. The initial rate of *in vitro*-induced LPO in BALB/c mice receiving cyclophosphane and dioxydine increased 1.5- and 4-fold, respectively. In C57Bl/6 mice both cyclophosphane and dioxydine inhibited the accumulation of TBA-reactive LPO products. On the whole, animals of the C57Bl/6 strain are more resistant to the LPO-inducing action of mutagens than BALB/c mice, despite the fact that the latter are characterized by a higher activity of antioxidant enzymes.

Key Words: *dioxydine; cyclophosphane; lipid peroxidation; superoxide dismutase; catalase; C57Bl/6 and BALB/c mice*

Lipid radicals as well as relatively stable products such as hydrogen- and lipoperoxides and malonic dialdehyde arising during free radical oxidation are capable of damaging cell and genetic structures [5,15], in light of which, lipid peroxidation (LPO) is considered to be of crucial pathogenetic importance [9,10]. For this reason, the elucidation of possible inductors and the study of the mechanisms of LPO activation and the antioxidant defense system assume great importance. A modern approach in such investigations is to study the role of LPO and the antioxidant systems in the mutagenic effects of xenobiotics [5].

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The individual and interstrain differences now demonstrated in the activity of antioxidant enzymes and LPO [4,5,12] make it possible to approach this problem with the use of pharmacogenetic methods based on comparative studies of different inbred strains.

Antitumor (cyclophosphane) and antibacterial (dioxydine) drugs have been shown to possess mutagenic properties [5,6]. The present study was aimed at investigating the effect of these compounds on LPO intensity and the activity of antioxidant enzymes in the liver of C57Bl/6 and BALB/c mice.

MATERIALS AND METHODS

Male mice of the strains C57Bl/6 and BALB/c aged from 6 to 8 weeks (*Stolbovaya* Nursery, Russian Academy of Medical Sciences) were used in the experi-

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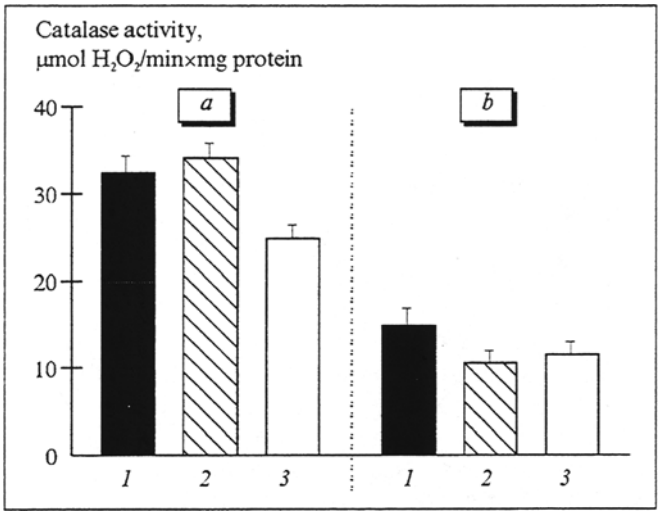

Fig. 1. Liver catalase activity in BALB/c (*a*) and C57Bl/6 (*b*) mice. Here and in Figs. 2–4: *1*) control; *2*) cyclophosphane (40 mg/kg), *3*) dioxydine (300 mg/kg); each series comprised 5 animals.

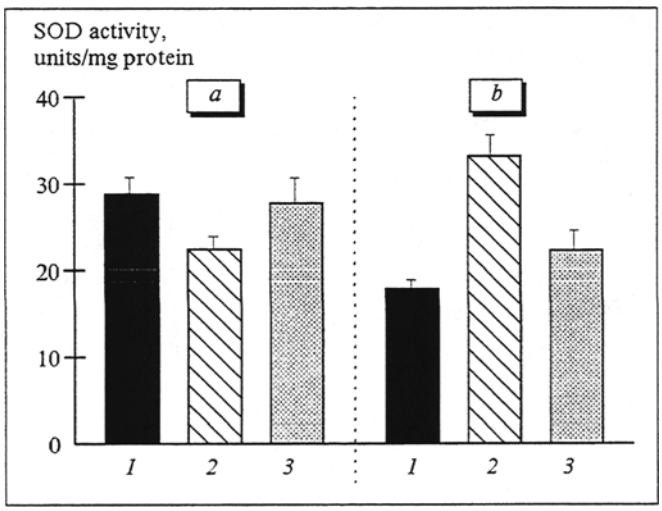

Fig. 2. Liver superoxide dismutase (SOD) activity in BALB/c (*a*) and C57Bl/6 (*b*) mice.

ments. The animals were kept under standard vivarium conditions with a 12-hour illumination regime. The mutagens were injected intraperitoneally, dioxydine [1,4-di-N-oxide of 2,3-bis-(hydroxymethyl)quinoxaline] in a dose of 300 mg/kg and cyclophosphane (N'-bis-(β-chloroethyl)-N'-O-trimethyl ester of phosphoric acid diamide) in a dose of 40 mg/kg. The animals were sacrificed 24 hours postinjection and the liver was removed and stored in liquid nitrogen until use. The tissue was homogenized in a Teflon-glass homogenizer at a 1:4 ratio in a medium containing 20 mM Tris-HCl and 100 mM KCl (pH 7.4) at 0°C.

Catalase activity was assayed by a previously described method [11] on a Hitachi-577 spectrophotometer (at 240 nm) and expressed in μmol H_2O_2/min×mg protein (molar extinction coefficient E= 39.4 $M^{-1}cm^{-1}$). The protein concentration was determined from the fourth derivative of the absorption spectrum at 240-320 nm in a medium containing 20 mM histidine, 50 mM NaCl (pH 7.2), 8.1% sodium dodecyl sulfate, and an aliquot of homogenate.

Superoxide dismutase (SOD) activity was assayed spectrophotometrically (at 560 nm) as described elsewhere [7]. Hemoglobin was eliminated from the supernatant by extraction with a chloroform:methanol (3:5 v/v) mixture in a ratio of 1:1.

The initial level of *in vivo*-accumulated LPO products was determined on a Hitachi-557 spectrophotometer from the maximum of the absorption spectrum for 2-thiobarbituric acid (TBA)-reactive products [13]. The rate of *in vitro*-induced oxidation was measured in the following systems: first, 0.75 mM ascorbate and, second, 0.75 μM ascorbate+5 mM Fe^{2+}. The samples were incubated at 37°C in a medium containing 30 mM Tris-HCl (pH 7.4) at a dilution of 1:5 during 100 and 40 min, respectively, for the above-described systems. In control samples, auto-oxidation was assayed.

RESULTS

The data on the effect of the mutagens on catalase activity in C57Bl/6 mice are presented in Fig. 1, *b*. Both cyclophosphane and dioxydine are seen to reduce hepatic catalase activity by 29 and 23%, respectively. In BALB/c mice dioxydine produces a 24% inhibition of catalase, while cyclophosphane has no reliable effect on the activity of this enzyme (Fig. 1, *a*).

Cyclophosphane, but not dioxydine, inhibits SOD in BALB/c mice (Fig. 2, *a*), whereas in C57Bl/6 mice dioxydine and, especially, cyclophosphane increase SOD activity by 24 and 86%, respectively, in comparison with the control (Fig. 2, *b*).

Thus, in C57Bl/6 mice both mutagens reduce catalase activity, while in BALB/c only dioxydine does so. Both these compounds activate SOD in C57Bl/6 mice, while in BALB/c only cyclophosphane inhibits this enzyme.

The reduced activity of the antioxidant enzymes may result from either suppression of LPO [2] or its activation and hyperproduction of LPO products which inhibit their activity [3,8]. On the other hand, activation of the antioxidant enzymes may be indicative of either suppressed, unchanged or even activated LPO [1,14,16]. Therefore, a physiological evaluation of the mutagen-induced alteration in the activity of the antioxidant enzymes requires a study of the state of LPO.

Cyclophosphane and dioxydine are found to increase the level of TBA-reactive products in BALB/c mice 1.4- and 2.1-fold, respectively, but do not affect it in C57Bl/6 mice. The initial level of LPO products is very stable and has been previous-

ly shown not to differ in intact mice of the C57Bl/6 and BALB/c strains [4]. Consequently, the observed rise of this parameter in BALB/c mice suggests a sharp difference in the level of LPO under the action of the mutagens in these animals.

Investigating further, we assessed *in vitro*-induced LPO in the liver homogenates of mutagen-treated animals. As is seen from Fig. 3, *a*, when LPO is activated *in vitro* with ascorbate, the initial rate of accumulation of TBA-reactive LPO products rises 1.5-fold in cyclophosphane-treated and more than 4-fold in dioxydine-treated BALB/c mice. In an evaluation of the kinetics of accumulation of LPO products, the initial latency should be taken into account: a shorter latency corresponds to more active oxidation. The latency decreases in the following order: BALB/c→cyclophosphane-treated animals→dioxydine-treated animals. However, as follows from the data presented in Fig. 3, *a*, in such a mild oxidation system the curve of the accumulation of LPO products does not attain a plateau for a long time, i.e., the maximal level of oxidation cannot be assessed. This dictated the use of a more effective oxidation system, namely Fe^{2+}-ascorbate. The maximal level of oxidized products in cyclophosphane-treated BALB/c mice does not differ from the control, but the curve more rapidly reaches a plateau, and correspondingly the latency is shortened (Fig. 4, *a*), while in animals of the same strain injected with dioxydine LPO is enhanced, the maximal level of oxidized products being 1.5-fold higher than in the control.

It is important to note that in BALB/c mice the LPO-stimulating effect of dioxydine is more pronounced than that of cyclophosphane and is accompanied by inhibition of catalase. Cyclophosphane activates LPO to a lesser extent and has no effect on catalase activity, but markedly inhibits SOD. Thus, there are some differences in the action of cyclophosphane and dioxydine on the liver of BALB/c mice.

A study of the antioxidant enzymes and LPO level in C57Bl/6 mice showed that both cyclophosphane and dioxydine inhibit catalase in this strain, and therefore both mutagens may be anticipated to activate LPO. This, however, does not follow directly from our experimental data, since the initial levels of LPO products in animals treated with mutagens do not differ from the control values and the accumulation of oxidized products in the *in vitro* system of ascorbate-induced LPO in C57Bl/6 mice proceeds slowly and does not attain a plateau even after the maximal time of incubation. In this strain cyclophosphane and dioxydine not only do not activate LPO but even reduce the rate of accumulation of TBA-reactive LPO products in comparison with the control (Fig. 3, *b*).

The data obtained on C57Bl/6 mice with the use of the Fe^{2+}-ascorbate system are presented in Fig. 4, *b*. This oxidation system provides a high level of LPO and the curve characterizing the accumulation of oxidized products in the control reaches a plateau. During the first 20 min the rate of accumulation of LPO products is higher in the control. This is in conformity with the data obtained in ascorbate-induced LPO (Fig. 3, *b*). However, when the time of incubation in the Fe^{2+}-ascorbate system is increased to 40 min, the accumulation of LPO products in the control bottoms out, whereas in liver homogenates from the dioxydine-treated mice it continues to rise.

Under conditions of slow oxidation (ascorbate) or at the initial stages of intense oxidation (Fe-ascorbate) in C57Bl/6 mice a certain protective effect against the induction of LPO appears even af-

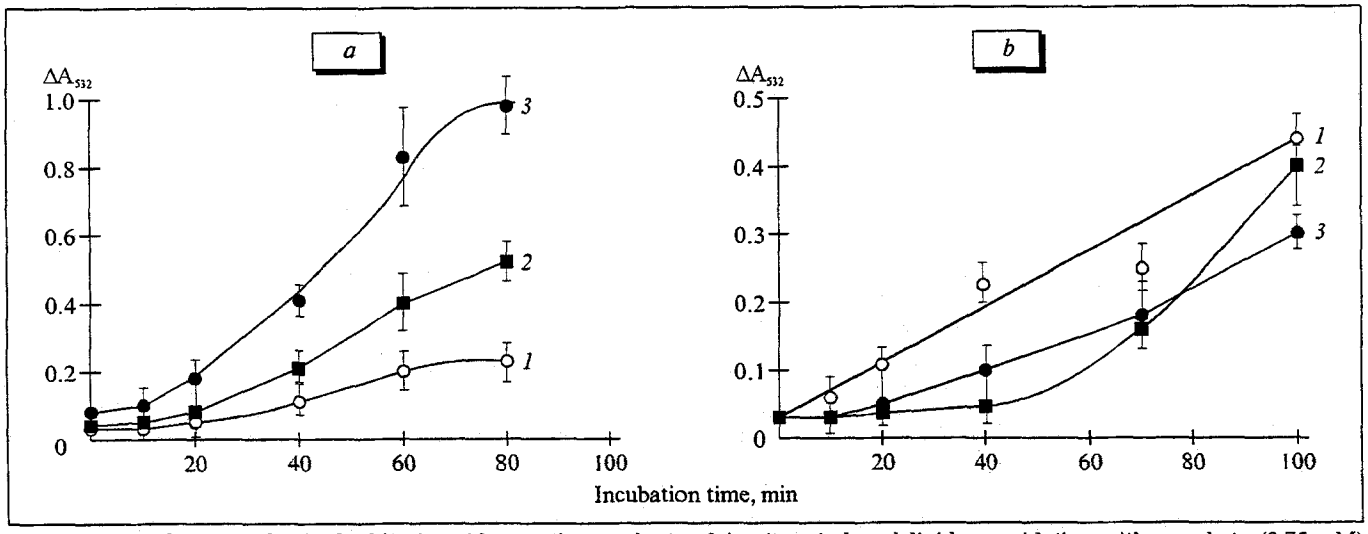

Fig. 3. Accumulation of 2-thiobarbituric acid-reactive products of *in vitro*-induced lipid peroxidation with ascorbate (0.75 mM) in the liver of BALB/c (*a*) and C57Bl/6 (*b*) mice.

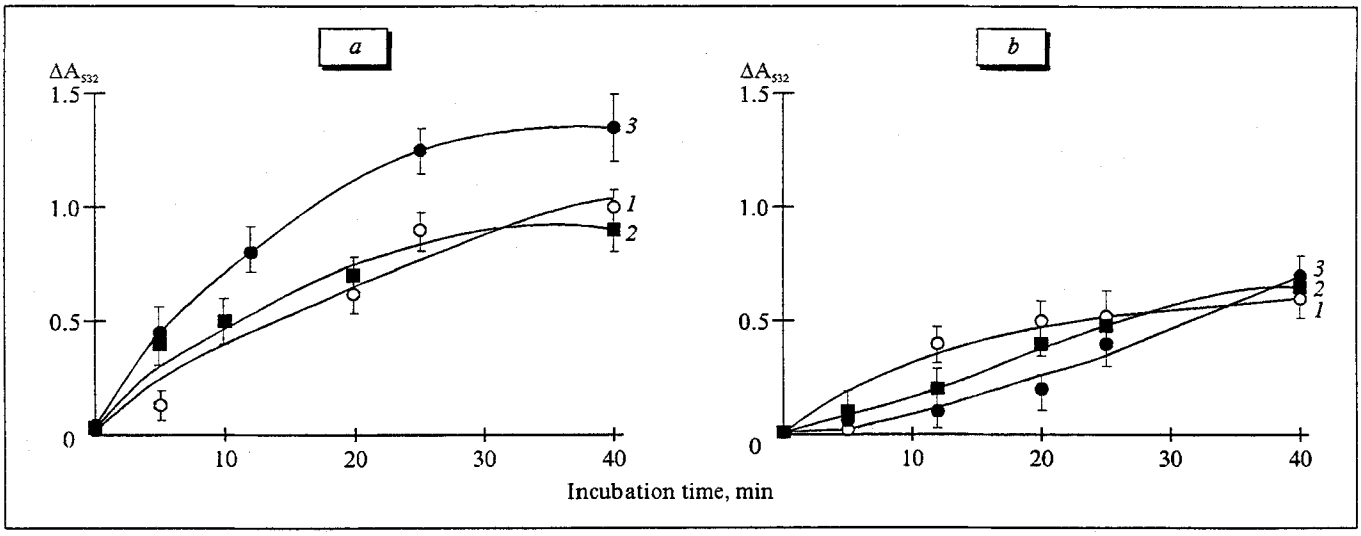

Fig. 4. Accumulation of 2-thiobarbituric acid-reactive products of *in vitro*-induced lipid peroxidation in the Fe^{2+} (5 μM) + ascorbate (0.75 mM) system in the liver of BALB/c (*a*) and C57Bl/6 (*b*) mice.

ter the injection of mutagens. Under the action of mutagens catalase activity drops (Fig. 1, *a*), but against the background of cyclophosphane administration we observed a marked increase of SOD activity (85%), which may be considered to be a compensatory activation of the enzyme of the antioxidant defense system in response to the LPO-inducing agent. The compensatory effect is so potent that it not only prevents the activation of LPO but even inhibits it. Dioxydine also reliably, albeit less markedly, activates SOD in C57Bl/6 mice (Fig. 2, *b*). This is probably the reason for the certain resistance of the liver tissue to *in vitro*-induced LPO. On the whole, the experiments on C57Bl/6 mice demonstrate that in this strain, similarly to BALB/c mice, superoxide anion radical plays a crucial role under the action of cyclophosphane, but not dioxydine.

Our findings attest, first, to the difference in the LPO-inducing capacity of cyclophosphane and dioxydine. In animals of both strains, and especially in BALB/c mice, dioxydine treatment leads to a higher initial level of TBA-reactive products and to a more rapid inducible accumulation of these products *in vitro*. On the other hand, cyclophosphane modulates mainly SOD activity, while dioxydine affects primarily catalase activity. Second, the relationship between the pro- and antioxidant systems is different in mice of the studied strains. C57Bl/6 mice are more resistant to the prooxidant action of cyclophosphane and dioxydine and are able to compensate for the endogenous or *in vitro*-induced LPO to the baseline level, probably due to activation of SOD and catalase. In BALB/c mice, the initial activity of the enzymes of the antioxidant defense system is higher, but it drops after cyclophosphane or dioxydine treatment, and the intensity of *in vitro*-induced LPO sharply increases, the level of TBA-reactive products rising several tens of times in comparison with the initial level and surpassing the control values many times.

On the whole, C57Bl/6 mice are more resistant to the LPO-inducing action of mutagens in comparison with BALB/c mice, even though the latter are characterized by a higher initial activity of the antioxidant enzymes.

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