BIOPHYSICS AND BIOCHEMISTRY

Antioxidant Enzymes and Lipid Peroxidation in C57Bl/6 and BALB/c Mice

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Superoxide dismutase and catalase activities and levels of thiobarbituric acid-reactive lipid peroxidation (LPO) products were estimated in the liver of C57Bl/6 and BALB/c mice. The results indicate that although antioxidant enzymes are more active in BALB/c mice, compensation of oxidation processes in this strain is possible only if LPO-inducing agents are absent or present at low levels, and that these agents, including exogenous ones, may be expected to activate lipid oxidation in this strain to a greater extent than in C57Bl/6 mice.

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

According to current concepts, cells and tissues of living organisms maintain an oxidation-reduction homeostasis whose shift toward the pro-oxidant status under the action of exogenous or endogenous factors results in "oxidative stress" with consequent damage to cellular and genetic structures [4,10]. Key players in the development of this stress are reactive oxygen species (superoxide anion, hydrogen peroxide, and hydroxyl radical), which are capable of inducing, directly or indirectly, lipid peroxidation (LPO), whose products, along with free oxygen radicals, are mainly responsible for the damage to cellular structures and to DNA and possess mutagenic properties [4]. The major constituents of the antioxidant defense system in the cell are the enzymes superoxide dismutase (SOD), which converts the superoxide anion to hydrogen peroxide, and catalase, which converts hydrogen peroxide to water and oxygen [11].

So far, virtually no comparative genotypic analyses of LPO and antioxidative enzymes in different animal strains have been published. In the present study we compared spontaneous and *in vitro*-induced LPO and SOD and catalase activities in liver tissues from C57Bl/6 and BALB/c mice.

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MATERIALS AND METHODS

C57Bl/6 and BALB/c livers were frozen in liquid nitrogen and 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 was assayed by a method previously described [12] and adapted for use with homogenates [13]. H_2O_2 breakdown was recorded with a Hitachi-557 spectrophotometer (at 240 nm) after adding aliquots of the liver homogenate. Catalase activity was calculated from the initial rate of H_2O_2 breakdown (molar extinction coefficient $E=39.4\ M^{-1}\times cm^{-1}$) and expressed in μmol H_2O_2/min×mg protein. 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 the added homogenate, using a method largely based on the approach proposed by Padros *et al.* [15].

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SOD activity was assayed spectrophotometrically (at 560 nm) by a method [9] which records the difference between the rates of superoxide radical formation in a xanthine-xanthine oxidase system before and after the addition of homogenate. Superoxide generation was estimated by the rate of formazan production from tetranitro blue tetrazolium (TNBT). In the control samples, this rate equaled 0.024 optical density unit/min. Before the assays, hemoglobin was removed from the supernatant by extraction with a chloroform:methanol mixture (3:5 by volume) in a 1:1 ratio, after which the mixture was thoroughly agitated and centrifuged at 2300 g for 10 min. SOD was determined in the top fraction by adding 300-500-μl homogenate aliquots in 3 ml of the incubation medium containing 17 mM pyrophosphate buffer ($Na_4P_2O_7 \times 10H_2O$, pH 8.3, 25°C), 0.1 mM xanthine, 0.1 mM EDTA, 0.05 mM TNBT, 1% Triton X-100, and 0.1 μM xanthine oxidase. One unit of SOD activity was taken to be the amount of enzyme required for 50% inhibition of the reaction of TNBT reduction to formazan.

The initial level of LPO products accumulated *in vivo* was estimated from the maximum of the absorption spectrum obtained for thiobarbituric acid(TBA)-reactive products at 532 nm [14]. The rate of *in vitro*-induced oxidation was recorded in two systems, one of which contained ascorbate (0.75 mM) and the other ascorbate (0.75 mM)+Fe (5 μM). Homogenate aliquots were incubated, at a 1:5 dilution, in a medium containing 30 mM Tris-KCl (pH 7.4, 37°C), the incubation time being 100 min for the first system and 40 min for the second. In control samples, auto-oxidation was recorded.

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

RESULTS

As shown in Figs. 1 and 2, the initial levels of hepatic SOD and catalase activities in BALB/c mice were 1.6 and >2 times higher, respectively, than in C57Bl/6 mice ($p<0.01$), which indicates that wide differences exist between the two strains in the states of their pro- and antioxidant systems. This conclusion is based on the observations, made in physiological and pathophysiological studies [1,3,5,16-18], that changes in catalase and SOD activities in animals of one species do not generally exceed 30-50% and 30-35%, respectively, in a variety of situations including stress, adaptation to stress, vitamin E excess or deficiency, and continuous or intermittent hypoxia.

Even slight elevations of catalase activity are known to be associated with physiologically compensated increases in the activity of free-radical processes

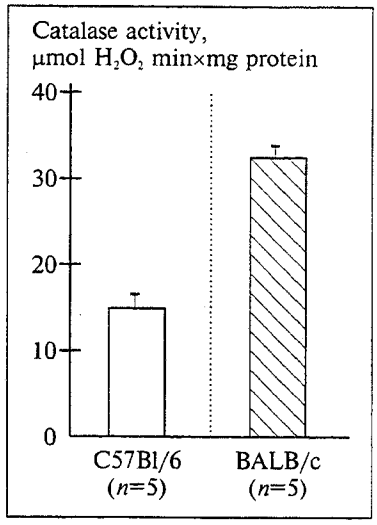

Fig. 1. Catalase activity in C57Bl/6 and BALB/c livers.

generating hydrogen peroxide. However, excessive LPO activation is not necessarily compensated for through a rise in catalase activity. Indeed, this enzyme is often inhibited [7,8,11]. On the other hand, the activity of antioxidant enzymes may be reduced even when LPO products are present at low levels [6]. This complicates analysis of the results characterizing alterations in the activity of antioxidant enzymes and makes it necessary to evaluate the intensity of free-radical processes. It is common practice to estimate the initial level of LPO products, although the usefulness of this index is very limited because it changes little under various influences [1-3,5-8] and from one animal species to another (see below). It seems that under ordinary conditions the initial levels of LPO products can be effectively kept constant by compensatory cellular mechanisms. However, the operation of these mechanisms can easily be disrupted through stimulation of oxidation processes with endogenous or exogenous oxidizing agents, so that marked differences between test series can be registered by measuring the rates at which induced accumulation of LPO products

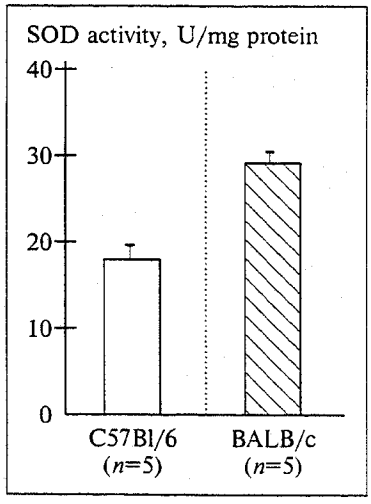

Fig. 2. SOD activity in C57Bl/6 and BALB/c livers.

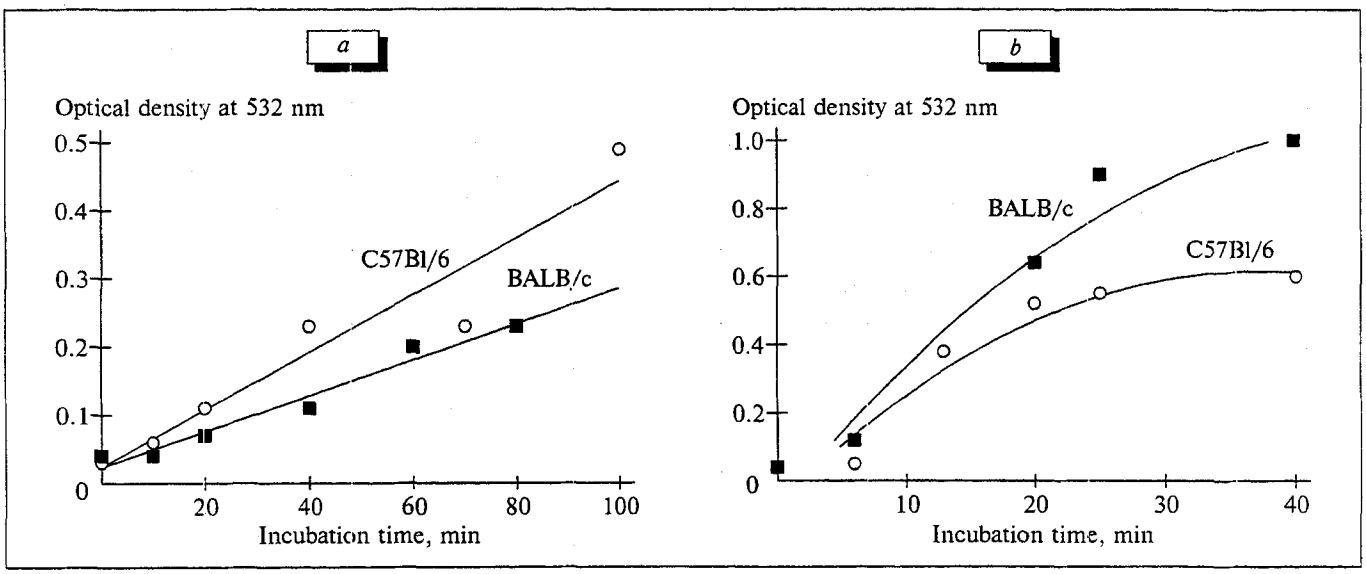

Fig. 3. Accumulation of TBA-reactive LPO products after *in vitro* LPO induction in C57Bl/6 and BALB/c liver homogenates using (*a*) ascorbate (0.75 mM) and (*b*) Fe (5 mM)+ascorbate (0.75 mM).

occurs *in vitro*. Using correlation analysis, we found the initial rate of such induced accumulation to be the most meaningful parameter for judging how the oxidation process develops.

As has been shown in several studies [2,5,7], recording of the initial levels of LPO products and of their accumulation after LPO induction in relation to antioxidant enzyme activity can yield definitive information on whether reduced activity of a particular enzyme signifies damage or compensated adaptation and whether or not activation of the enzyme will be beneficial to the organism. Taking advantage of this approach, we undertook a comparative analysis of antioxidant enzyme activities and LPO levels in the mouse strains under study.

The interstrain difference in the initial level of LPO products was slight and insignificant (Fig. 3, *a*). Added ascorbate activated oxidation *in vitro* at a low rate and the content of LPO products did not reach a plateau, so that their maximum possible level could not be recorded in the time interval used. The highest level of LPO products and the highest LPO rate recorded for C57Bl/6 mice amounted to 140% and 134%, respectively, of the corresponding values for BALB/c mice.

At first glance, these results suggest that BALB/c mice are more resistant to LPO because catalase and SOD activities are much higher (Figs. 1 and 2) and fewer oxidized products accumulate after LPO induction by ascorbate *in vitro* (Fig. 3). This is only partly true, however. Indeed, in the absence of exogenous oxidation activators, the considerably higher activities of these enzymes ensure nearly the same initial level of TBA-reactive products as in BALB/c and, in the presence of slight LPO activation by ascorbate *in vitro*, can not only compensate for the activation, but even slow the LPO process. However, when an iron-ascorbate oxidation system capable of producing a high degree of oxidation was used and the concentrations of its components were so selected (Fe=1.2 nmol/mg protein, ascorbate=0.75 mM) as to achieve the highest possible initial oxidation rate, it was found that the initial oxidation rate was 1.5 times higher and the maximal accumulation of TBA-reactive products 1.6 times higher in BALB/c (Fig. 3, *b*). In other words, LPO cannot be effectively inhibited in this strain in the presence of strong activators, despite the higher catalase and SOD activities; in C57Bl/6, by contrast, effective protection against LPO induction can be afforded with strong activators, even though the antioxidant enzymes are less active.

It may therefore be concluded that the higher activities of antioxidant enzymes in BALB/c mice will compensate for the activation of oxidation processes only if LPO activators are absent or weak. Otherwise, i.e., in the presence of potent activators, to which certain mutagens with pro-oxidant activity may belong, a greater LPO activation and, consequently, worse damage by LPO products may be expected in this strain as compared to C57Bl/6.

The marked differences revealed between C57Bl/6 and BALB/c mice in the activity of antioxidant enzymes and the intensity of *in vitro*-induced LPO highlight the need for a proper evaluation of the physiological significance of these enzymes.

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REFERENCES

1. V. D. Antonenkov and L. F. Panchenko, *Byull. Eksp. Biol. Med.*, **102**, № 11, 550-552 (1986).

2. Yu. V. Arkhipenko, G. G. Konovalova, L. M. Dzhaparidze, *et al.*, *Byull. Eksp. Biol. Med.*, **106**, № 12, 670-671 (1988).
3. V. A. Gusev and L. F. Panchenko, *Vopr. Med. Khimii*, **28**, № 4, 8-25 (1982).
4. A. D. Durnev and S. B. Seredenin, *Khim.-Farm. Zh.*, **24**, № 10, 7-14 (1990).
5. A. A. Kubatiev and S. V. Andreev, *Myocardial Metabolism* [in Russian], Moscow (1981).
6. F. Z. Meerson, Yu. V. Arkhipenko, I. I. Rozhitskaya, *et al.*, *Byull. Eksp. Biol. Med.*, **114**, № 7, 14-15 (1992).
7. T. G. Sazontova, Yu. V. Arkhipenko, and F. Z. Meerson, *Byull. Eksp. Biol. Med.*, **104**, № 10, 411-413 (1987).
8. M. Bernier, D. Y. Hearse, and A. S. Manning, *Circ. Res.*, **58**, № 3, 331-340 (1986).
9. C. Beauchamp and I. Fridovich, *Anal. Biochem.*, **44**, 276-287 (1971).
10. B. Halliwell and J. M. C. Gutteridge, *Free Radicals in Biology and Medicine*, Oxford (1984).
11. R. H. Julicher, L. B. Tijberg, L. Sternberg, *et al.*, *Life Sci.*, **35**, № 12, 1281-1288 (1984).
12. H. Luck, in: *Methods of Enzymatic Analysis*, New York (1963), pp. 885-894.
13. F. Leighton, B. Poole, H. Beaufay, *et al.*, *J. Cell Biol.*, **37**, 482-513 (1968).
14. H. Okhawa, N. Ohishi, and K. Yagi, *Anal. Biochem.*, **95**, № 2, 351-358 (1979).
15. E. Padros, M. Dunach, A. Morrison, *et al.*, *Trends Biochem. Sci.*, **9**, № 12, 508-510 (1984).
16. M. E. Persy, *Biochem. Cell Biol.*, **62**, № 10, 1006-1014 (1984).
17. I. M. Tolmasoff, T. Ono, and R. G. Culter, *Proc. Nat. Acad. Sci. USA*, **77**, № 5, 2777-2781 (1980).
18. P. Valk, I. Gille, A. Oostra, and H. Joenjt, in: *Oxygen Radicals in Chemistry and Biology*, New York (1989), pp. 695-697.

A Method for Monitoring Toxic Damage to the Liver, Based on Measurement of Total Water and Its Magnetic Relaxation Characteristics

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Acute poisoning of rats with the hepatotropic poison tetrachloromethane was accompanied by increases in the liver content of total water and its spin-lattice (T_1) and spin-spin (T_2) relaxation times and by a decrease in the T_1/T_2 ratio, with reversal of the correlation between T_1 and T_2. The antihypoxic agent sodium γ-oxybutyrate normalized water metabolism in the liver almost completely. It is concluded that total water content and T_1 are the more informative parameters for monitoring both toxic liver damage and the efficacy of its pharmacological correction.

Key Words: *liver; tetrachloromethane; water; magnetic relaxation time*

Since disturbances of water-electrolyte balance are known to play an important role in the pathogenesis of many diseases caused by toxic chemicals [6], we made an attempt to diagnose toxic liver disease and monitor the efficacy of its pharmacological correction by analyzing parameters of water metabolism in an animal model of acute tetrachloromethane (TCM) poisoning. This particular model was chosen because its pathogenetic mechanisms and physiological, biochemical, and morphological characteristics are well known [8,11,13,15] and because of evidence that there may be a water imbalance in the liver damaged by TCM [5,12].

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MATERIALS AND METHODS

A total of 32 random-bred male white rats weighing 180-210 g were used. Of these, 22 were injected with

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