# PROTECTIVE ACTION OF ANTIOXIDANTS AND MICROSOMAL MONO-OXYGENASE INDUCERS IN ISCHEMIC AND REOXYGENATION LIVER DAMAGE

M. V. Bilenko, V. E. Kagan,
D. M. Velikhanova, and P. G. Komarov

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The writers showed previously that endogenous products of lipid peroxidation (LPO) accumulate in the liver in acute ischemia, and this correlates with depression of microsomal mono-oxygenase activity; it has been postulated that LPO processes participate in ischemic damage to NADPH-dependent oxygenases of the endoplasmic reticulum (ER) [5, 6]. It has also been shown that antioxidants inhibit accumulation of LPO products in ischemia and reoxygenation of different organs [1, 3, 9, 10] and that inducers of microsomal mono-oxygenases are responsible for preservation of a higher concentration of cytochrome P-450 and of the rate of N-demethylation of amidopyrine (AP) in ischemia of the liver [12].

The object of this investigation was to study the effect of the synthetic antioxidant ionol, the microsomal mono-oxygenase inducer phenobarbital (PH), and a combination of both on LPO processes and on the mono-oxygenase system in the membranes of ER during ischemia and reoxygenation of the liver, to compare the resistance of induced and noninduced monooxygenase systems to ischemic and reoxygenation damage, and also to establish the role of LPO in the mechanism of their origin.

## EXPERIMENTAL METHOD

Experiments were carried out on 300 Wistar rats weighing 150-200 g. The rats were kept on a starvation diet for 12 h before the experiment. Ischemia was created under intraperitoneal hexobarbital anesthesia (70 mg/kg) by applying small clamps to the vascular pedicle of the left lateral and middle lobes of the liver for 1 h, and reoxygenation was produced by removal of the clamps for 4 h. In survival experiments the ischemized lobes (63% of the total mass of the liver) were left *in situ* in the rats and the remaining lobes were resected. The animals remained under observation for 1 month. The compounds were injected intraperitoneally: ionol (4-methyl-2,6-di-tert-butylphenol) in a single dose of 240 mg/kg 24 h before ischemia, in Tween-80; PH in a dose of 80 mg/kg was given once a day for three days, the last time 24 h before ischemia. In the series with combined administration of the compounds they were injected in the same doses. To assess the effect of the compounds on the intact liver, they were injected also into animals not undergoing operations. In each series of experiments there was a corresponding control (without injection of the compounds).

Microsomes were isolated, lipids extracted, and lipid hydroperoxides (HP), Schiff bases, and the content of cytochrome P-450 and activity of NADPH-cytochrome c reductase were determined as described previously [5]. The rate of AP demethylation was determined by the method in [2].

## EXPERIMENTAL RESULTS

As Tables 1 and 2 show, during 1 h of ischemia marked accumulation of endogenous products of LPO and a decrease in activity of the mono-oxygenase system (content of cytochrome P-450, activity of NADPH-cytochrome c reductase, and rate of AP demethylation) took place in the liver of animals not receiving the compounds. Reperfusion of the ischemic lobes led to a further significant increase in the level of LPO products and a further decrease in the

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TABLE 1. Content of LPO Products in Microsomes of Rat Liver after Its Ischemia and Reperfusion (M ± m)

| Parameter | Series of investigations | Without compounds (n = 12-18) | Ionol (n = 4-8) | PH (n = 5-6) | Ionol + PH (n = 3-4) |
|---|---|---|---|---|---|
| HP, nmoles/mg lipid | Without ischemia | 3,7±0,2 | 2,2±1,4 | 3,5±1,1 | 2,2±0,8 |
| | After 1 h of ischemia | 10,1±0,7 | 5,4±0,7* | 8,0±1,3 | 5,2±0,9* |
| | | $P_1$<0,01 | | $P_1$<0,02 | |
| | After 4 h of reperfusion | 13,8±0,5 | 5,7±1,4 | 9,3±1,0 | 7,1±0,8* |
| | | $P_1$<0,01 | | $P_1$<0,01 | $P_1$<0,01 |
| | | $P_2$<0,01 | | | |
| Schiff bases, conventional units/mg lipid | Without ischemia | 20,5±1,1 | 24,6±2,3 | 20,0±2,4 | 13,1±3,5 |
| | After 1 h of ischemia | 35,2±0,6 | 32,0±1,4 | 32,9±2,3 | 20,0±3,2* |
| | | $P_1$<0,01 | $P_1$<0,01 | $P_1$<0,01 | |
| | After 4 h of reperfusion | 49,8±2,3 | 36,7±2,3* | 37,0±4,9 | 31,8±1,7* |
| | | $P_1$<0,01 | | $P_1$<0,01 | $P_1$<0,01 |
| | | $P_2$<0,01 | | | $P_2$<0,01 |

Legend. Here and in Table 2: *) differences significant between experiments with and without compounds ($P < 0.01$), $P_1$) significance of differences between experiment with ischemia or ischemia and reperfusion, and experiments without ischemia; $P_2$) significance of differences between experiments with and without reperfusion.

TABLE 2. Activity of Mono-oxygenase System in Microsomes of Liver after Its Ischemia and Reperfusion (M ± m)

| Parameter | Series of investigations | Without compounds (n = 12-21) | Ionol (n = 3-9) | DH (n = 5-7) | Ionol + PH (n = 3-4) |
|---|---|---|---|---|---|
| Cytochrome P-450, nmoles/mg protein | Without ischemia | 0,82±0,02 | 0,72±0,03* | 2,14±0,20* | 2,05±0,21* |
| | After 1 h of ischemia | 0,52±0,02 | 0,48±0,04* | 1,23±0,08* | 1,50±0,09* |
| | | $P_1$<0,01 | $P_1$<0,01 | $P_1$<0,01 | $P_1$<0,01 |
| | After 4 h of reperfusion | 0,38±0,03 | 0,57±0,04* | 0,78±0,12* | 0,93±0,03* |
| | | $P_1$<0,01 | $P_1$<0,02 | $P_1$<0,01 | $P_1$<0,01 |
| | | $P_2$<0,01 | | $P_2$<0,01 | $P_2$<0,01 |
| NADPH-cytochrome c reductase, nmoles cytochrome c/min/mg protein | Without ischemia | 13,6±0,5 | 12,9±0,5 | 16,7±0,7* | 20,3±0,5* |
| | After 1 h of ischemia | 9,7±0,3 | 10,3±1,0 | 15,4±1,5* | 14,4±2,2* |
| | | $P_1$<0,01 | $P_1$<0,05 | | $P_1$<0,01 |
| | After 4 h of reperfusion | 7,2±0,3 | 11,0±1,3* | 13,8±1,1* | 8,3±0,1* |
| | | $P_1$<0,01 | | | $P_1$<0,01 |
| | | $P_2$<0,01 | | | $P_2$<0,01 |
| Amidopyrine N-demethylase, nmoles CHOH/min/mg protein | Without ischemia | 5,7±0,4 | 4,3±0,3* | 13,7±1,9* | 15,7±2,5* |
| | After 1 h of ischemia | 2,1±0,04 | 2,5±0,6 | 5,7±0,5* | 7,0±2,6* |
| | | $P_1$<0,01 | $P_1$<0,02 | $P_1$<0,01 | $P_1$<0,01 |
| | After 4 h of reperfusion | 2,1±0,2 | 2,7±0,5 | 3,9±0,2* | 6,3±0,7* |
| | | $P_1$<0,01 | $P_1$<0,05 | $P_1$<0,01 | $P_1$<0,01 |

cytochrome P-450 content and activity of NADPH-cytochrome c reductase, evidence of marked reoxygenation damage to the mono-oxygenase system after 1 h of ischemia of the liver.

Injection of ionol and combination of ionol with PH had a significant effect on the level of LPO products in the liver of the intact rats, but significantly lowered accumulation of HP and Schiff bases during ischemia and reperfusion of the ischemic liver. A tendency for accumulation of LPO products to decrease during ischemia and reoxygenation of the liver also occurred when PH alone was given, in agreement with the reciprocal relations observed between mono-oxygenase activity and the level of endogenous LPO products in ER of the liver [7, 8].

The study of the state of the mono-oxygenase system (Table 2) showed that injection of ionol into the intact animals did not induce a mono-oxygenase system such as was found previously by workers who used the compound in larger doses or repeatedly [11, 12], and it even brought about a small decrease in the cytochrome P-450 content and in the rate of AP demethylation. During ischemia of the liver ionol had no protective action on components of the mono-oxygenase system, but, by completely inhibiting the accumulation of LPO products during reoxygenation, it prevented the fall in the cytochrome P-450 content and activity of NADPH-cytochrome c reductase in the early postischemic period. A marked protective effect of the mono-oxygenase system in reoxygenation damage also was obtained when reperfusion activation of LPO was inhibited by another method, namely by inhalation of a hypoxic gas mixture by the animals during 4 h of reperfusion, leading to a state of temporary moderate hypoxemia.

Injection of PH and a combination of PH with ionol into intact rats caused an increase (by 2.5 times) in the content of cytochrome P-450, an increase in NADPH-cytochrome c reductase

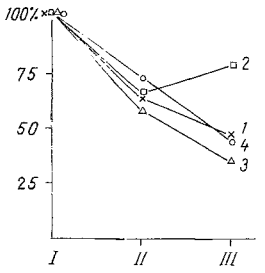


Fig. 1

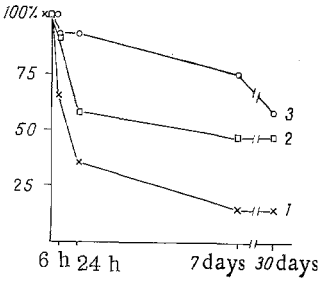


Fig. 2

Fig. 1. Dynamics of changes in cytochrome P-450 content in microsomes of rat liver after its ischemia and reperfusion (in percent of initial level): I) without ischemia, II) after 1 h of ischemia, III) after 4 h of reperfusion. 1) Without compounds, 2) with ionol, 3) with PH, 4) with ionol + PH.

Fig. 2. Survival rate of rats (in percent) after ischemia of liver for 1 h. 1) Without compounds, 2) with ionol, 3) with ionol + PH. Abscissa, period of observation.

activity (by 1.5 times), and acceleration of AP demethylation (by 2.4-2.8 times). In the course of ischemia, parameters of the mono-oxygenase system in animals induced by PH and by a combination of compounds fell during reperfusion of the liver, but their absolute value remained significantly higher than in experiments without the compounds (PH), or they even moved closer to their level in intact animals (combination of compounds).

Meanwhile the relative content of cytochrome P-450 (Fig. 1), calculated as a percentage of its initial level, like the relative rate of AP demethylation and activity of NADPH-cytochrome c reductase, changed about equally during 1 h of ischemia in all series and after 4 h of reperfusion in all series except that with ionol, a result which differs from those in the literature [12] and which points to absence of any significant differences in the resistance of PH-induced and noninduced mono-oxygenase systems to ischemic and reoxygenation damage.

Calculation of the ratio between the increase in HP (Table 1) and the decrease in cytochrome P-450 content (Table 2) during ischemia (6.4:0.3) and during reoxygenation (3.7:0.14) in experiments without the compounds showed that for every molecule of cytochrome P-450 destroyed there were 21 and 26 molecules of HP, respectively, which agrees roughly with the quantity of HP (15-20 molecules) required to destroy one molecule of cytochrome P-450 *in vitro* [7]. In the series with ionol, loss of one molecule cytochrome P-450 during ischemia occurred against the background of an increase of 13 molecules of HP, whereas during reperfusion the increase in HP was extremely small and was not accompanied by destruction of cytochrome P-450. By contrast with this, in the experiments with PH and a combination of compounds, despite the smaller increase in HP, the loss of cytochrome P-450 increased, due to destruction of one molecule of cytochrome P-450 in connection with an increase of 2.5-5.5 HP molecules. This evidently points to greater sensitivity of the induced cytochrome P-450 to LPO products than of the noninduced form.

To conclude, the compounds which were used not only inhibited LPO and led to stabilization or a higher level of components of the mono-oxygenase system of ER in the liver, but also significantly increased the survival rate of animals with ischemic lobes of the liver (Fig. 2), i.e., they gave rise to a broader hepatoprotective effect.

Intensification of LPO processes in ischemia and reoxygenation of the liver and the protective effect of the antioxidant ionol thus point to an essential role of LPO in the mechanism of reoxygenation damage to the mono-oxygenase system of ER in the liver. The use of PH alone or in combination with ionol does not protect the mono-oxygenase system against ischemia and reoxygenation damage, but facilitates preservation of high activity of this system. The use of ionol and of a combination of ionol with PH also leads to a significant increase in the survival rate of animals with an ischemic liver, so that these compounds can be recommended as agents for the anti-ischemic protection of the mono-oxygenase system of ER and of the liver as a whole.

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# STABILIZING ACTION OF α-TOCOPHEROL IN POSTISCHEMIC DAMAGE TO THE MEMBRANE HYDROXYLATING SYSTEM OF THE ENDOPLASMIC RETICULUM OF RAT LIVER

G. G. Voronov and P. I. Lukienko

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KEY WORDS: ischemia of the liver; hydroxylation of xenobiotics; microsomes; lipid peroxidation; α-tocopherol.

One cause of injury to membrane structures and cell enzyme systems connected with them during the period of reoxygenation of organs after ischemia may be activation of free-radical lipid peroxidation [2].

Accordingly, in the investigation described below, the effect of the antioxidant α-tocopherol on activity of the hydroxylating system of enzymes in the endoplasmic reticulum of the liver was studied in the postischemic period.

## EXPERIMENT METHOD

Experiments were carried out on 80 noninbred male albino rats weighing 180-210 g, kept on the standard animal house diet. α-Tocopherol was injected into the stomach of the animals of one group in the form of an oily emulsion in a dose of 50 mg/kg every 12 h for 48 h before and 3 days after ischemia of the liver. The dose, therapeutic form, and interval between injections were chosen on the basis of data on its optimal action as antioxidant in the corresponding doses [15], its better resorption from the intestine in emulsion form [10], and its maximal tissue level 12 h after peroral administration [5].

Rats of the other group also received the oily emulsion for the same period of time, but without α-tocopherol.

Laboratory of Biochemical Pharmacology, Department of Regulation of Metabolism, Academy of Sciences of the Belorussian SSR, Grodno. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 4, pp. 33-34, April, 1983. Original article submitted July 1, 1982.

  