Effect of the Phenol Antioxidant Katavidan on Autooxidation of Microsomes Exposed to Visible Light

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A study is performed of the effect of the phenol antioxidant katavidan on autooxidation of microsomes from rat liver exposed to visible light. It is shown that katavidan in a concentration of 10^{-3} M inhibits but in concentrations of 10^{-5}-10^{-7} M stimulates autooxidation of microsomes. No stimulation is observed under conditions of dark incubation.

Key Words: *phenol antioxidant; autooxidation of microsomes; light exposure*

Phenol antioxidants are known to interrupt the chain of free-radical oxidation (FRO) through formation of phenoxyl radical, whose transformation pathways have been described at length [1]. The main way of irreversible death of the radicals is the reaction of disproportionation. However, phenoxyl radicals are also able to form unstable ketodimers, yeilding phenoxyl radical upon decomposition. Certain concentrations of radicals ($PhO^{\bullet}$, $ROO^{\bullet}$) in the medium result in the formation of asymmetric quinoline peroxides, while the interaction of phenoxyl with O_2 yields symmetric ones.

The absorption spectra of both ketodimers and quinoline peroxides are characterized by a broad band, which includes a visible wavelengths [1]. It is probable that the decomposition of peroxides is accelerated by absorbed light, the phenoxyl radical thus formed will thereupon initiate new chains of oxidation.

The objective of the present study was to examine this possibility using a simple experimental model. We chose a model of microsomal autooxidation, since intracellular membranes are readily oxidized and the low rate of the initial stages of oxidation makes it possible to detect even a relatively weak effect.

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MATERIALS AND METHODS

Microsomes were isolated from the liver of random-bred male rats as described earlier [2]. Antioxidant in a concentrated solution was added to the incubation mixture 5 min before incubation. Light exposure was performed with a block of 6 luminescent lamps (LD20W) at a distance of 15 cm. The intensity of oxidation was evaluated from accumulation of malonic dialdehyde (MDA) in the incubation medium. The concentration of MDA and reduction of other TBA-reactive substances in $SnCl_2$-containing medium were measured as described elsewhere [3]. The effect of a compound on the rate of microsomal oxidation was assesed

TABLE 1. Rate of Oxidation of Microsomal Membranes in the Presence of Antioxidant in the Dark ($M \pm m$)

Compound	Rate of oxidation, nM MDA× ×min^{-1}×mg protein	r
Control	44.1±9.0	0.94
Antioxidant, 10^{-5} M	42.0±3.3	0.96
Antioxidant, 10^{-7} M	43.3±2.0	0.97
Antioxidant, 10^{-9} M	44.0±5.3	0.94

Note. r – correlation coefficient upon linear approximation.

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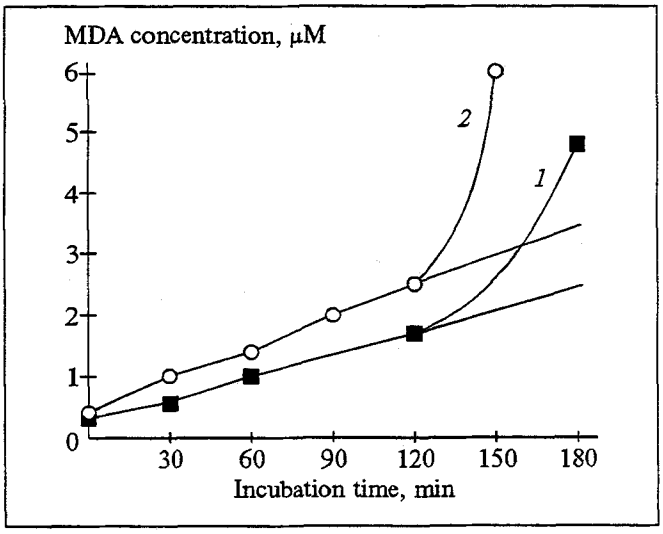

Fig. 1. Kinetics of MDA accumulation in incubation medium during autooxidation of microsomal membranes exposed to visible light. *1*) control; *2*) in the presence of 10^{-5} M katavidan.

within a linear part of the MDA accumulation curve. The concentration of iron was measured by the optical density of complexes with phenanthroline [4]. We used the water-soluble phenol antioxidant katavidan, synthesized by A. A. Volod'kin (Institute of Chemical Physics, Russian Academy of Sciences). The following reagents were used: Tris-HCl, thiobarbituric acid (TBA), phenanthroline from Serva, and other reagents (chemically pure grade) from Reakhim. The results of different series were compared using the paired nonparametric Mann-Whitney test.

Since the rate of oxidation varied in different experiments, the data are presented as a percentage of the control.

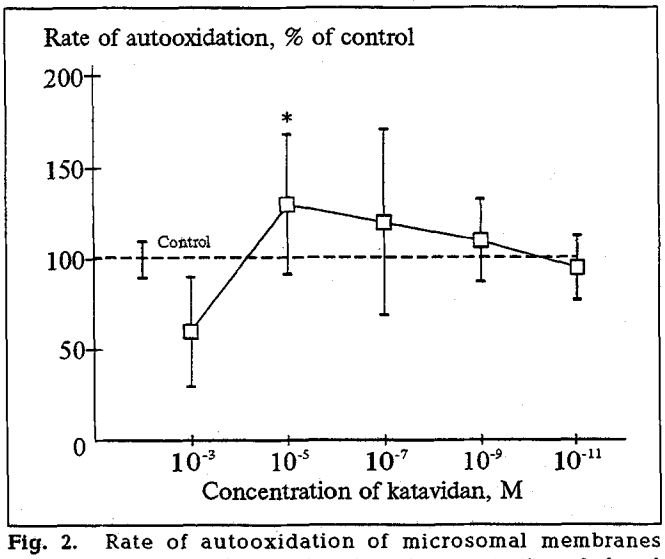

Fig. 2. Rate of autooxidation of microsomal membranes exposed to visible light as a function of concentration of phenol antioxidant katavidan. The asterisk indicates a reliable difference in comparison with the control.

RESULTS

The synthetic phenol antioxidant added to the illuminated microsome suspension in a concentration of 10^{-5} M accelerated the formation of MDA (Fig. 1).

Figure 2 demonstrates the concentration dependence of MDA accumulation in the suspension of rat liver microsomes during illumination. As is seen from the figure, the preparation (10^{-3} M) inhibited autooxidation of the microsomes, which is consistent with its antioxidative properties. In a concentration of 10^{-5} M katavidan stimulated autooxidation of the microsomes and reliably accelerate the accumulation of MDA ($p<0.04$).

We also studied the concentration dependence of the accumulation of MDA under conditions of dark incubation. No acceleration of autooxidation in the presence of antioxidant was noted. On the contrary, a certain decrease of the rate of oxidation in the presence of 10^{-5} M antioxidant is to be noted (Table 1).

Thus, we found a stimulated lipid autooxidation of liver microsomes exposed to visible light in the presence of 10^{-5} M phenol antioxidant. Apart from the above hypothesis, the following explanations of the observed effect may be proposed. It may be assumed that UV of the main band of mercury (254 nm) used in the luminescent lamp partially penetrates the reaction mixture. However, the transmission spectrum of the lamp glass below 370 nm is characterized by 100% absorption (data not shown). The observed effect may also be attributed to an admixture of free iron. Measurements reveal that the incubation mixture contains less than 1 μmol iron. Back in the 1970s it was shown that hemic iron of hemoglobin is capable of inducing FRO during illumination. Cytochrome P-450 of microsomal membranes possesses an analogous property. However, in this case as well as in the two above, the observed effect should be undependent of the concentration of phenol antioxidant.

Thus, phenol antioxidant in certain concentrations is able to accelerate lipid peroxidation during illumination with visible light. Despite its low magnitude (20-30%), this effect should be taken into account in experimental studies.

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