Studying the Photoprotective Activity of a New Class of Heteroaromatic Antioxidants

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Photoprotective activity of heteroaromatic compounds (derivatives of 3-hydroxypyridine, amino-6-hydroxybenzothiazole, and 5-hydroxybenzimidazole) was studied in the system of UV-induced cardiolipin peroxidation. Although all three compounds had the antioxidant effect during free radical oxidation of luminol, only derivatives of amino-6-hydroxybenzothiazole and 5-hydroxybenzimidazole inhibited the process of UV-induced lipid peroxidation. The 3-hydroxypyridine derivative did not inhibit UV-induced cardiolipin peroxidation, which was probably related to degradation of this compound under the influence of UV light and formation of degradation products that cannot inhibit free radical processes.

Key Words: *antioxidant activity; peroxidation; free radical oxidation*

One of the promising approaches to the development of new products to modulate intracellular free radical processes suggests the synthesis of hydrophilic compounds that are structurally similar to natural substances with antioxidant properties.

This class of synthetic antioxidants includes several derivatives of 3- hydroxypyridine (structural analogues of vitamin B_6), 5-hydroxybenzimidazole (fragment similarity to vitamin B_6), and 6-hydroxybenzothiazole. Some compounds of this class are used in practice. The medicinal products emoxypin and mexidol were synthesized from 3-hydroxypyridine derivatives. A plant growth regulator Ambiol was developed from 5-hydroxybenzimidazole derivatives [3].

The search for new antioxidant compounds with photoprotective properties is an urgent problem of medicine and cosmetology. These products not only inhibit free radical processes, but are also resistant to photoinduced damage.

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The photoprotective properties of new compounds belonging to a class of heteroaromatic antixidants were studied in the system of UV-induced peroxidation of cardiolipin liposomes. Experiments were performed with derivatives of 3-hydroxypyridine (substance I), amino-6-hydroxybenzothiazole (substance II), and 5-hydroxybenzimidazole (substance III) that belong to various groups and exhibit the same antioxidant activity.

MATERIALS AND METHODS

Substances I-III were synthesized as described elsewhere [4]. The chemical structure of these substances is shown in Table 1 and Fig. 1. Aqueous solutions of study substances were used in an initial concentration of 10^{-3} M.

Cardiolipin liposomes were obtained by suspension of the cardiolipin membrane in phosphate buffered saline [6]. The kinetics of UV-induced cardiolipin peroxidation was estimated from the accumulation of thiobarbituric acid-reactive products (TBA-reactive products) [1]. UV irradiation was performed using a DRK-120 mercury lamp (120 W).

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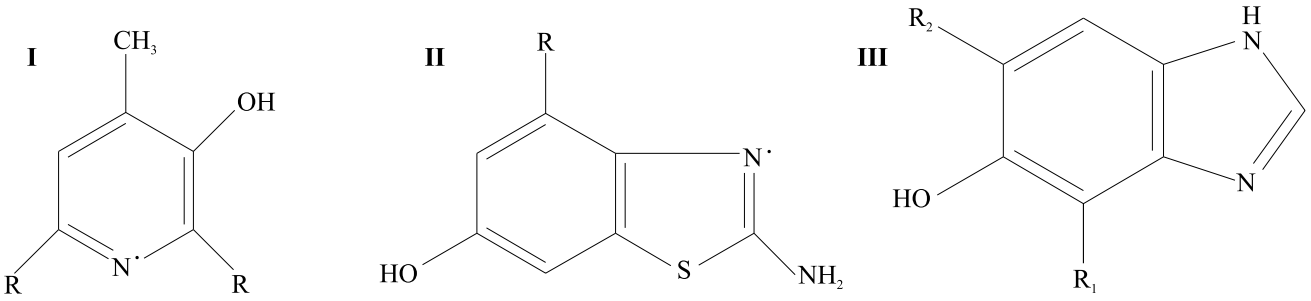

Fig. 1. Chemical structure of heteroaromatic antioxidants.

The antioxidant properties of substances were evaluated in a model oxidation system consisting of hemoglobin, H_2O_2, and luminol [2]. We measured the maximum luminescence (amplitude of chemiluminescence) and time between the addition of an inducing agent (H_2O_2) and the induction of luminescence (latency). The kinetics of chemiluminescence was recorded on a Shimadzu RF 5301PC spectrofluorometer. The ability of inhibitors to interact with aqueous phase radicals in the model system was evaluated quantitatively. The results of chemiluminescence quenching were expressed in coordinates of the Stern-Volmer equation as follows [5]: $I_0/I_i=1+K_iC$, where I_0 and I_i are the amplitudes of model system chemiluminescence in the absence and presence of inhibitor; K_i is the constant of luminescence quenching (radical trapping by inhibitor in the model system); and C is the molar concentration of inhibitor.

RESULTS

The antioxidant properties of substances I, II, and III were evaluated by the chemiluminescence method (Table 1).

The constants for these substances were of the same order (relatively high). However, the relative antioxidant activity of substance II was 4-fold higher than that of substance I. It could be suggested that all three substances inhibit UV-induced free radical processes. However, the inhibitory properties of substances II and III should be greater than those of substance I.

Fig. 2 illustrates the kinetics of UV-induced accumulation of TBA-reactive peroxidation products of cardiolipin liposomes in the presence of test substances. Substances II and III had a strong inhibitory effect on UV-induced cardiolipin peroxidation. These substances at relatively low concentration (0.3-0.5 mM) completely inhibited the photoinduced accumulation of TBA-reactive products. Substance II had a greater photoprotective effect that substance III, which correlated with the constant of chemiluminescence quenching. However, substance I did not have photoprotective activity in this system. By contrast, this substance sometimes had a stimulatory effect on cardiolipin photooxidation (Fig. 2). These results indicate that substances II and III are potent photoprotectors during UV-induced lipid peroxidation *in vitro*. Substance I had no photoprotective properties in this system.

As differentiated from substances II and III, substance I was unstable in the UV light. Degradation of this substance resulted in the formation of products that did not have antioxidant properties. Fig. 2 illustrates the differential spectra of substances I-III after 30-min UV irradiation. This treat-

TABLE 1. Constants of Luminol Chemiluminescence Quenching and Optical Absorption Maxima for Heteroaromatic Antioxidants

Substance	Absorption maxima, nm	Molar extinction coefficients	Chemiluminescence quenching constants, K_i
Substance I	217	2.3×10^4	1.2×10^5
	253	5.6×10^3	
	319	8.1×10^3	
Substance II	222	2.8×10^4	4.5×10^5
	267	1.1×10^4	
Substance III	209	2.7×10^4	3.7×10^5
	285	1.2×10^4	

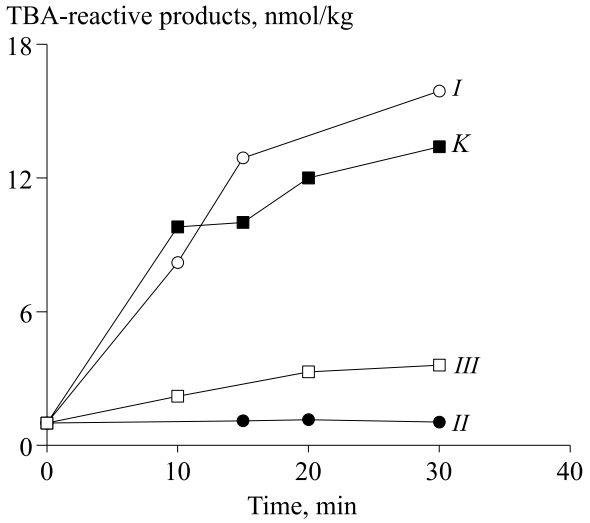

Fig. 2. Effect of substances I (3-hydroxypyridine derivative), II (amino-6-hydroxybenzothiazole derivative), and III (5-hydroxybenzimidazole derivative) on the kinetics of UV-induced peroxidation of cardiolipin liposomes. Substance I (0.48 mM, *I*); substance II (0.33 mM, *II*); substance III (0.5 mM, *III*); and *C*, control (without inhibitor). Reaction medium: 0.05 M potassium phosphate buffer (pH 7.4), 0.1 mM EDTA, 0.25-0.35 mg/ml cardiolipin liposomes, and substances I-III.

TABLE 2. Chemiluminescence Quenching Constants for Native and UV-Irradiated Heteroaromatic Antioxidants

Substance	Quenching constants	
	before irradiation	30 min after irradiation
I	1.2×10^5	0.7×10^4
II	4.5×10^5	3.8×10^5
III	3.7×10^5	2.8×10^5

ment was followed by significant changes in the spectrum of substance I (particularly at 217 nm). Optical density of the sample decreased by 1.8 U (78%). The absorption maximum was undetected at 253 nm. However, small and opposite changes were revealed in the absorption spectra of substances II and III. Therefore, substances II and III are more resistant to UV irradiation that substance I. It can be expected that the antioxidant activity of substances II and III will remain unchanged after UV irradiation (as differentiated from substance I, Table 2).

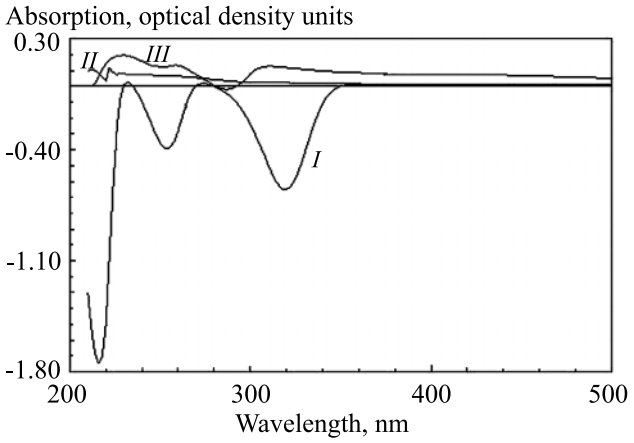

Fig. 3. Differential spectra of test substances after 30-min UV irradiation. Reaction medium: 0.05 M potassium phosphate buffer (pH 7.4) and 1 mM substances I-III. Sample volume 3.0 ml. Substance I (*I*); substance II (*II*); and substance III (*III*).

UV irradiation had little effect on the constant of luminol chemiluminescence quenching for substances I and III (15-24%, Table 2). By contrast, UV irradiation of substance I was accompanied by a significant decrease in the antioxidant activity (by 94%). These data explain the fact that substance I has no photoprotective activity in the system of UV-induced cardiolipin peroxidation. UV irradiation of substance I is accompanied by significant structural changes, which contributes to the loss of antioxidant properties. It cannot be excluded that the products formed during UV irradiation of substance I exhibit the prooxidant activity (Fig. 2).

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