STABILIZING ACTION OF HYDROXYBENZIMIDAZOLE AND ITS DERIVATIVES ON BIOLOGICAL MEMBRANES DURING ACTIVATION OF LIPID PEROXIDATION

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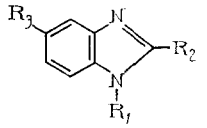
Activation of lipid peroxidation (LPO) is a universal mechanism of modification and damage to biological membranes, which lies at the basis of the development of several pathological states [1]. In this connection, during recent years compounds possessing the ability to inhibit LPO in biomembranes have been screened. In the search for such compounds it has to be taken into account that they must possess the following combination of properties: 1) their partition coefficient between polar and nonpolar phases must correspond to optimal interaction with water-soluble and lipophilic free radicals; 2) they must interact effectively with radicals without the formation of radical states of the inhibitor, which are capable of prolonging LPO effectively; 3) they must have sufficiently high mobility in the biolayer to enable interaction between molecules of antioxidants and centers of radical formation; 4) they must not have a modifying action on the structure of the lipid bilayer or on protein–lipid interactions.

Synthetic antioxidants of the phenolic type with a screened hydroxyl group are known to damage biological membranes, whereas analogs of α-tocopherol (TP), containing a long chain, do not damage membranes [2, 3]. With this in mind, it was decided to compare the action of hydroxybenzimidazole (HBI) and its derivatives on liver microsomal and brain synaptosomal membranes of rats during activation of LPO.

EXPERIMENTAL METHOD

Experiments were carried out on subcellular fractions isolated from male Wistar rats weighing 150–180 g. Microsomes were isolated from the liver by the method in [6]. Synaptosomes were obtained from the gray matter of the brain by the method in [5]. The concentration of membrane protein was determined as described previously [5].

LPO was induced in the liver microsomes in medium containing: 1 mg/ml of microsomal protein, 100 mM phosphate buffer (pH 7.4), 20 μM Fe^{++}, 0.5 mM NADPH or ascorbate. The suspension was incubated for 5 min at 37°C with constant mixing. LPO was induced in synaptosomes in medium of the following composition: 300 μg/ml of synaptosomal protein, 20 mM HEPES (pH



$R_1 = C_2H_5$	$R_1 = H$	$R_1 = CH_3$
$R_2 = CH_3$	$R_2 = C_{17}H_{35}$	$R_2 = C_{17}H_{35}$
$R_3 = OH$	$R_3 = O,C_2H_5$	$R_3 = OH$
HBI	AEOBI	AHBI

Fig. 1. Structure of HBI and its derivatives.

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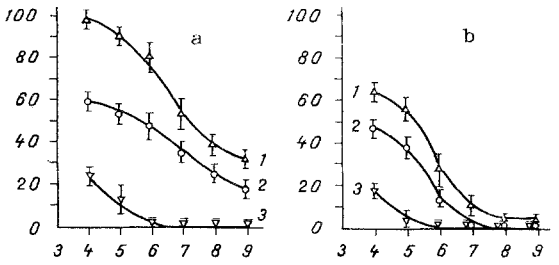


Fig. 2. Dependence of inhibition of LPO by AHBI (1), HBI (2), and AEOBI (3) in microsomal membranes of rat liver in the presence of Fe^{++} + ascorbate (a) and of Fe^{++} + NADPH (b) on concentration. Here and in Fig. 3: abscissa, negative logarithm of concentration (in M) 1; ordinate, effectiveness of inhibition (in %).

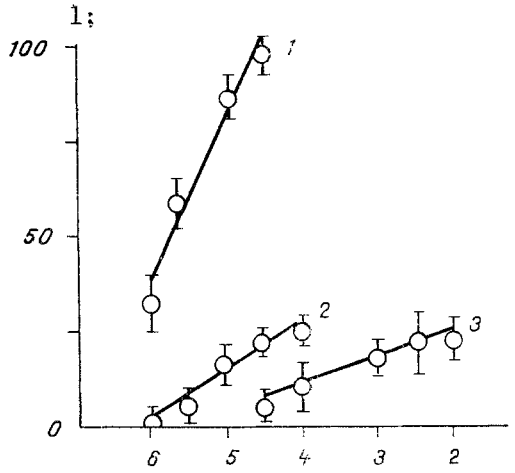


Fig. 3. Dependence of inhibition of ascorbate-dependent LPO in rat brain synaptosomes by TP (1), AHBI (2), and HBI (b) on concentration.

7.4), 145 mM NaCl, 5 mM KCl, 5 mM $NaHCO_3$, 1.3 mM $MgCl_2$, 0.3 μM Fe^{++}, and 10 μM ascorbate. The suspension of synaptosomes was incubated for 30 min at 37°C with constant mixing.

The development of the LPO reaction was judged from accumulation of malonic dialdehyde, the concentration of which was determined by its reaction with 2-thiobarbituric acid [1]. The antioxidants were added to the suspension of microsomes and synaptosomes in alcoholic solutions, so that the final volume of ethanol did not exceed 1% of the original volume.

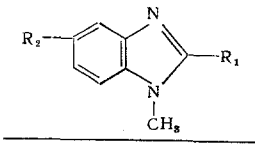
HBI and its derivatives alkylhydroxybenzimidazole (AHBI) and alkylethoxybenzimidazole (AEOBI) (Fig. 1) were synthesized at the Institute of Pharmacology, Academy of Medical Sciences of the USSR. The purity of the compounds was not below 99.5%. The HEPES, sucrose, glucose, and sodium ascorbate used in the work were obtained from "Serva" (West Germany). The remaining reagents were of Soviet origin and of the chemically pure grade.

EXPERIMENTAL RESULTS

In the experiments of series I the effectiveness of the inhibitory action of HBI and its derivatives on development of LPO reactions in the microsomes was compared after different methods of initiation. Dependence of the degree of inhibition of NADPH- and ascorbate-dependent microsomal LPO by HBI and its derivatives on concentration is shown in Fig. 2. HBI and its derivative AHBI had an inhibitory action on both the ascorbate- and the NADPH-dependent method of induction of LPO (Fig. 2). With both methods of LPO induction, the HBI derivative AEOBI, which does not possess the OH-group essential for the inhibitory action of phenolic inhibitors [7], had virtually no inhibitory action.

It will be noted that with both methods of LPO induction, the more hydrophobic AHBI was also a much more effective inhibitor of LPO than HBI. It was found that 50% inhibition of

TABLE 1. Values of 50% Inhibition of LPO Constants of HBI, AHBI, and AEOBI in Microsomal Membranes of Rat Liver

Inhibitor	Method of induction	
	Fe^{++} + ascorbate	Fe^{++} + NADPH
R_1-CH_3, R_2-OH	$2{,}5 \cdot 10^{-6}$ M	$1{,}0 \cdot 10^{-4}$ M
R_1-$C_{17}H_{35}$, R_2-OH	$6{,}0 \cdot 10^{-8}$ M	$5{,}0 \cdot 10^{-4}$ M
R_1-$C_{17}H_{35}$, R_2-OCH_3	10^{-3} M	10^{-3} M

LPO took place with AHBI in concentrations 20-30 times lower than the concentration of HBI (Table 1). The difference in the effectiveness of the inhibitory action of HBI and AHBI can evidently be explained on the grounds that HBI, being readily soluble in water, has high affinity for the lipid phase of the microsomes and it is therefore relatively ineffective as an inhibitor of the radicals generated in it.

The facts will be noted that both HBI and AHBI are more effective inhibitors of LPO when induced by the nonenzymic method. It will be clear from Table 1 that although the LPO levels were comparable with both methods of induction, 50% inhibition of ascorbate-dependent LPO was achieved by HBI and AHBI in concentrations two orders of magnitude lower than the concentrations of these compounds that correspond to 50% inhibition of NADPH-dependent LPO. This difference in their inhibitory action can probably be explained by the different location of the centers of pre-radical generation in the case of NADPH- and of ascorbate-dependent microsomal LPO.

In the experiments of series II the effectiveness of the inhibitory action of HBI and AHBI on the development of ascorbate-dependent LPO reactions in brain synaptosomes was compared. The experiments showed that, starting with a concentration of 10^{-5} M, HBI inhibits synaptosomal LPO (Fig. 3). This inhibitory effect was weak, and did not exceed 25% when AHBI was present in a concentration of 10^{-4} M. Effects of higher concentrations of AHBI could not be studied because of its low solubility in ethanol. HBI was even less effective as an inhibitor of synaptosomal ascorbate-dependent LPO. It can be concluded from a comparison of the data in Figs. 2 and 3 that AHBI and HBI inhibit synaptosomal ascorbate-dependent LPO in much higher concentrations than liver microsomal membranes. The accessibility of centers of radical formation in synaptosomal ascorbate-dependent LPO is evidently much lower for HBI and AHBI than in the case of ascorbate-dependent microsomal LPO. However, considering the different conditions of LPO induction in the case of synaptosomes and microsomes, it cannot be asserted unequivocally that HBI and AHBI have a lower inhibitory action in the case of synaptosomes. To resolve this problem the effectiveness of the inhibitory action of HBI and AHBI in synaptosomes and microsomes was compared with the action of the natural antioxidant TP. In the case of microsomal ascorbate-dependent LPO the action of AHBI was comparable with that of TP. In synaptosomes AHBI inhibited LPO much less effectively than TP (Fig. 3).

The results as a whole thus indicate that AHBI differs from HBI in being a fairly effective antioxidant during activation of LPO in microsomal membranes, but has no such effect in the case of activation of LPO in brain synaptosomes. Nevertheless, since AHBI possesses at least some of the essential requirements for antioxidant screening listed above, the study of its possible use to stabilize the membranous structures of the liver in pathological states based on activation of LPO would seem to be promising.

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PHOSPHATASE ACTIVITY OF BLOOD AND WOUND EXUDATE LEUKOCYTES DURING HEALING OF AN EXPERIMENTAL ASEPTIC WOUND

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Cytochemical determination of alkaline phosphatase (AlP) and acid phosphatase (AcP) activity in some cases can be a reliable laboratory test of the character and course of the pathological process in surgical patients [2, 4, 8]. However, the dynamics of changes in the phosphatase activity of blood and wound exudate cells during healing of aseptic wounds has not been adequately studied [1, 8].

In this investigation the phosphatase activity of blood and wound exudate leukocytes was studied during healing of an aseptic experimental wound.

EXPERIMENTAL METHOD

Altogether 110 male Wistar rats weighing 190-200 g were used. Models of skin wounds were created by the method described previously [3]. Films of peripheral blood taken from the caudal vein and squash preparations of wound exudate were used as the test objects. Blood-and wound-exudate leukocytes were studied daily from the 1st through the 10th days, and again on the 12th and 15th days of the experiment; besides the times mentioned above, the cells also were studied before the operation (background). At each point of time 8-10 rats were used, and were decapitated after specimen taking. The leukocyte formula was determined on films stained by the Romanovsky–Giemsa method. AlP activity was detected by the method in [7] and AcP activity as in [10].

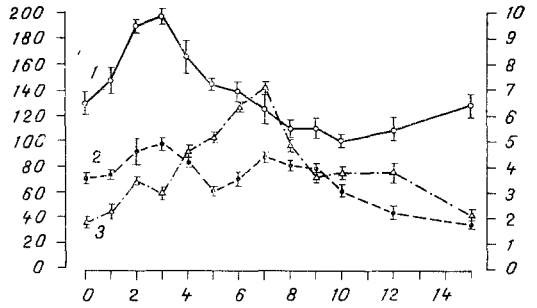

Fig. 1. Changes in phosphatase activity of peripheral blood neutrophils during healing of aseptic wounds in rats. Abscissa, time after wounding (in days); ordinate, AlP (in optical density units) and AcP (in conventional units [11]) levels; scale on right shows absolute number of neutrophils in 1 μl blood × 10^3). 1) AlP, 2) AcP, 3) number of neutrophils.

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