It can be postulated on the basis of our findings that a relatively broad spectrum of nucleases is involved in the process of glucocorticoid-induced death of lymphoid cells. Poly(ADP-ribosylation) has no effect on activity of the identified nucleases.

LITERATURE CITED

1. I. I. Votrin, N. N. Khodarev, and A. G. Baskan'yan. Vestn. Akad. Med. Nauk SSSR, No. 3, 88 (1983).
2. V. A. Pospelev, S. B. Svetlikova, and V. I. Vorob'ev, Mol. Biol., 11, No. 4, 781 (1977).
3. J. J. Cohen and R. C. Duke, J. Immunol., 132, No. 1, 38 (1984).
4. M. M. Compton and L. A. Cidlowski, J. Biol. Chem., 262, 8288 (1988).
5. S. A. Coughlin and D. M. Green, Anal. Biochem., 133, No. 2, 322 (1983).
6. W. M. Hunter and F. C. Greenwood, Nature, 194, 93 (1962).
7. D. A. Johnson and J. W. Gautsch, Gene Anal. Tech., 1, 3 (1984).
8. J. Kyhle-Andersen, J. Biochem. Biophys. Meth., 10, No. 3/4, 203 (1984).
9. U. K. Laemmli, Nature, 277, 680 (1970).
10. D. J. McConcey, P. Hartzell, and S. K. Duddy, Science, 242, 256 (1988).
11. D. J. McConcey, P. Hartzell, P. Nicotera, et al., FASEB J., 3, No. 3, 1843 (1989).
12. R. J. Roccia and P. Doty, J. Mol. Biol., 61, No. 2, 445 (1971).
13. A. L. Rosenthal and S. A. Lack, Analyt. Biochem., 80, No. 1, 76 (1977).
14. A. H. Wyllie, Nature, 284, 555 (1980).
15. K. Yoshihara, Y. Tanaka, and T. Kamiya, ADP-Ribosylation, DNA Repair, and Cancer, M. Miwa et al. (eds.), Tokyo and Utrecht (1983), pp. 183-193.

MECHANISMS OF THE ANTIOXIDATIVE ACTION OF SCREENED PHENOLS IN BIOMEMBRANES: EFFECTS OF IONOL AND ITS DERIVATIVES

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The development of oxidative stress induces a deficiency of natural antioxidants in the body, with the results that lipid peroxiation (LPO) becomes uncontrollable [9, 15]. To maintain the balance between pro- and antioxidant systems, an additional quantity of antioxidants must be introduced. Emergency replenishment of deficient antioxidants with the aid of vitamin E, a natural inhibitor of free-radical oxidation, is limited by the mechanisms of its transport (tocopherol-carrying proteins) [5, 6], and as a result, it is insufficiently effective. This difficulty can be overcome with the aid of synthetic antioxidants, for which specific transport mechanisms do not exist in the body [2].

One of the most widely used synthetic antioxidants is ionol (4-methyl-2,6-di-tert-butylphenol), permitted in the USSR and many other countries as a food preservative [17]. However, it has recently been shown that ionol possesses a direct membrane-destabilizing action in experiments in vitro, causing a sharp increase in membrane permeability [7]. The toxicity of ionol in doses in excess of 500 mg/kg has been demonstrated in vivo, and is manifested as hyperplasia of the lungs and changes in the cell components in rats [10, 16]. It is considered that these toxic effects of ionol are due to the action of intermediates of its metabolic activation in the cytochrome P-450 system [11, 12].

The study of the characteristics of screened phenols (and, in particular, of ionol derivatives) have been included in the program of the joint Bulgarian–Soviet "Biostab" Project with the ultimate aim of selecting homologs: 1) possessing sufficiently high antioxidative activity in biomembranes, 2) not exhibiting direct membrane-disturbing effects, and 3) not

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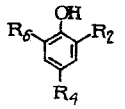
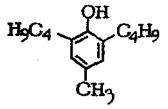



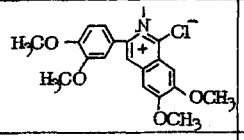
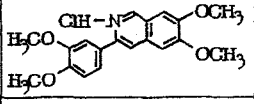
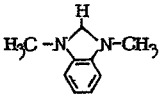
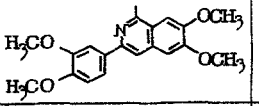
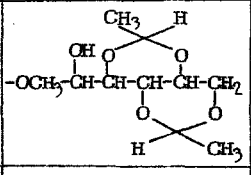
	R_2	R_4	R_6
I	-OH	$-C_4H_9$	$-C_4H_9$
II	$-OC_{16}H_{33}$	$-C_4H_9$	$-C_4H_9$
III	$-OCH_2COONa$	$-C_4H_9$	$-C_4H_9$
IV	$-OCH_2COOH$	$-C_4H_9$	$-C_4H_9$
V	$-C_4H_9$	$-CH_2-CH(NH_2)-COOH$	$-C_4H_9$
VI	$-OC_{12}H_{25}$	$-C_4H_9$	$-C_4H_9$
VII	$-C_4H_9$	$-CH_2-CH(COOC_2H_5)_2$ C_2H_5	$-C_4H_9$
VIII	$-C_4H_9$		$-C_4H_9$
IX	$-O-CH_2-CH_2-N$ 	$-C_4H_9$	$-C_4H_9$
X	$-C_4H_9$		$-C_4H_9$
XI	$-C_4H_9$		$-C_4H_9$
XII	$-C_4H_9$		$-C_4H_9$
XIII	$-C_4H_9$	$-CH_2-C(NHCOCH_3)-(COOC_2H_5)_2$	$-C_4H_9$
XIV		$-C_4H_9$	$-C_4H_9$
XV	$-C_4H_9$	$-CH_3$	$-C_4H_9$

Fig. 1. Partition coefficients of ionol derivatives in a two-phase system of heptane:water.

active substrates of cytochrome P-450 (i.e., not undergoing metabolic toxification). In the investigation described below the antiradical and antioxidative activity of various ionol derivatives were determined in model chemical systems and in membrane fractions of natural origin.

EXPERIMENTAL METHOD

Microsomal membranes were obtained by differential centrifugation [4]. The protein concentration was determined by the biuret method. LPO was induced by a system of Fe^{2+} + ascorbate/NADPH in 0.1M buffer, pH 7.4. The final concentrations of Fe and ascorbate were 40 mM and 0.5 mM, respectively. Accumulation of malonic dialdehyde (MDA), a secondary product of LPO, was determined by the reaction with 2-thiobarbituric acid [14]. The ionol derivatives were added to the incubation medium as alcoholic solutions, the alcohol concentration not exceeding 0.5%. The value of the constant K_7 was determined by a manometric method, involving oxidation of cumene in the presence of isopropylnitrylbenzene as initiator [3].

TABLE 1. Values of 50% Inhibition Constants (K_{50}) of Luminol-Dependent Chemiluminescence in the Presence of Fe^{2+} + NADPH or Fe^{2+} + Ascorbate and Inhibition of KO_2-Induced Luminol-Dependent Chemiluminescence by Ionol and Its Derivatives

Compound	10^{-5} M (%)	K_{50} (Fe^{2+} + NADPH)	K_{50} (Fe^{2+} + ascorbate)
I	30,7	$3,16 \cdot 10^{-6}$	$2,75 \cdot 10^{-6}$
II	0,0	$>10^{-3}$	$>10^{-3}$
III	26,4	$1,23 \cdot 10^{-4}$	$1,55 \cdot 10^{-4}$
IV	20,9	$9,77 \cdot 10^{-5}$	$1,32 \cdot 10^{-4}$
V	0,0	$2,75 \cdot 10^{-4}$	$3,09 \cdot 10^{-4}$
VI	3,3	$>10^{-3}$	$>10^{-3}$
VII	0,0	$6,03 \cdot 10^{-6}$	$2,0 \cdot 10^{-5}$
VIII	0,0	$2,0 \cdot 10^{-5}$	$2,51 \cdot 10^{-5}$
IX	33,8	$7,24 \cdot 10^{-6}$	$1,26 \cdot 10^{-5}$
X	13,6	$2,69 \cdot 10^{-6}$	$1,00 \cdot 10^{-5}$
XI	23,0	$1,82 \cdot 10^{-5}$	$1,70 \cdot 10^{-5}$
XII	0,0	$1,02 \cdot 10^{-4}$	$9,55 \cdot 10^{-5}$
XIII	21,9	$8,32 \cdot 10^{-6}$	$5,89 \cdot 10^{-6}$
XIV	2,7	$1,58 \cdot 10^{-5}$	$2,19 \cdot 10^{-5}$
XV	0,0	$9,55 \cdot 10^{-6}$	$1,07 \cdot 10^{-5}$
		$9,12 \cdot 10^{-6}$	$1,00 \cdot 10^{-5}$
XI	23,0	$1,82 \cdot 10^{-5}$	$1,70 \cdot 10^{-5}$

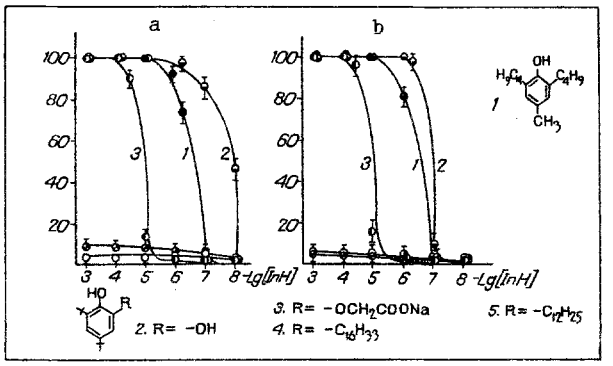


Fig. 2. Action of several ionol derivatives on enzymic (Fe^{2+} + NADPH)-dependent (a) and nonenzymic (Fe^{2+} + ascorbate)-dependent (b) LPO in microsomal fraction from rat liver as a function of concentration.

Homologs of ionol were synthesized in the Rostov Institute of Organic and Physical Chemistry [1, 13]. All compounds were characterized by the results of elementary analysis.

EXPERIMENTAL RESULTS

Even the simplest homogeneous systems during liquid-phase oxidation of hydrocarbons, antioxidative activity is a complex value which depends on the set of kinetic characteristics, the reactivity of the inhibitor, and the conditions of oxidation [2]. However, the value of the constant K_7 (the velocity constant of the reaction: $InH + RO_2 \rightarrow In + RO_2H$) is held to characterize this activity completely. Table 1 gives the results of measurements of the values of K_7 for several ionol derivatives during interaction with peroxide radicals of cumene (at 60°C). For the whole group of compounds values of K_7 can be seen not to differ significantly, in agreement with data in the literature [2]. The two main factors affecting K_7, namely the distribution of electron density in the molecule of the phenols and the steric factor, depending only on O-alkyl substitution, act independently. K_7 increases with an increase in the electron-donating ability of the substituent pair (which is probably connected with the higher value for compound 11). For different variants of o-substitution, the value of the constant K_7 changes comparatively little.

Dependence of the antioxidative action of inhibitors of free-radical reactions on the conditions of oxidation ought probably to be exceptionally strong in heterophasic systems and, in particular, in biological membranes. The partition coefficient between the polar (aqueous) phase and the hydrophobic (liquid) regions of the membrane, in which lipid alkoxy- and peroxy-radicals are probably located, is evidently important. For that reason, in a separate series of experiments partition coefficients of ionol derivatives were measured in a two-phase system of heptane:water. The results show that, with the exception of two acetylated derivatives (compounds II and III), the ionol homologs used were sufficiently hydrophobic and were located mainly in the hydrocarbon phase (Fig. 1). This means that on their addition to membrane suspensions, they ought to be inserted into the hydrophobic regions of the biomembranes.

The action of several ionol derivatives on enzymic (Fe^{2+} + NADPH)-dependent and nonenzymic (Fe^{2+} + ascorbate)-dependent LPO in the microsomal fraction from rat liver, as a function of concentration, is shown in Fig. 2. Although antiradical activities of the ionol derivatives in a homogeneous system (K_7) are sufficiently close, their antioxidative activities in microsomal membranes differ very sharply. For instance, compounds with long-chain alkyl substituents virtually do not inhibit LPO either in enzymic or in nonenzymic systems, in concentrations up to 10^{-3} M. Ionol itself is an effective LPO inhibitor in microsomes in concentrations of $3.3 \cdot 10^{-7}$ M, and the hydroxylated derivative of ionol is even more

effective, K_{50} corresponding to a concentration of $1.2 \cdot 10^{-8}$ M for enzymic LPO. The remaining ionol derivatives, in their half-maximal efficacy of antioxidative action in liver microsomes, occupy the concentration range from 10^{-5} to 10^{-7} M (Table 1). This character of the antioxiative action of ionol derivatives is not a specific features of liver microsomal membranes, but is recorded in other membranes also and, in particlar, in rat brain synaptomal membranes (Table 1).

The results indicate that correlation does not exist between the degree of inhibition of LPO in biomembranes and the value of K_7 for these compounds. It can be tentatively suggested that interaction of the reagents in isomembranes, which are microheterogeneous ordered systems, is largely determined by their orientation, their mutual accessibility, and their mobility [8]. Consequently, when choosing membrane antioxidants, guidance must be taken not only from their chemical characteristics, but also from their behavior in biomembranes.

LITERATURE CITED

1. A. A. Volodkin and V. V. Ershov, Izv. Akad. Nauk SSSR, Ser. Khim., No. 342 (1962).
2. V. A. Roginski, Phenolic Antioxidants [in Russian], Moscow (1988).
3. N. M. Émanuél, E. T. Denisov, and Z. K. Maizus, Chain Reactions of Hydrocarbon Oxidation in a Liquid Phase [in Russian], Moscow (1965).
4. A. Archakov, G. Bachmanova, and I. Karusina, Acta Biol. Med. Germ., 38, 229 (1979).
5. C. Guarnieri, F. Flamingui, and M. Caldarera, Ital. J. Biochem., 184, 222 (1979).
6. C. Guarnieri, F. Flamingui, and M. Caldarera, Biochem. J., 190 469 (1980).
7. V. E. Kagan, E. A. Serbinova, K. N. Novikov, et al., Arch. Toxicol., Suppl., Suppl., 302 (1986).
8. V. E. Kagan, E. A. Serbinova, R. A. Bakalova, and Ts. S. Stoytchev, Free Radicals, Oxidant Stress and Drug Action, London (1987), pp. 425-441.
9. V. E. Kagan, Lipid Peroxidation in Biomembranes, Boca Raton, Florida (1988).
10. K. Ley and E. Muller, Chem. Ber., 89, 1402 (1956).
11. A.A . Marino and J. T. Mitchell, Proc. Soc. Exp. Biol. (New York), 40, 122 (1972).
12. Y. Nakagawa, K. Hiraga, and T. Suga, Chem. Pharm. Bull., 27, 442 (1979).
13. Y. Nakagawa, K. Hiraga, and T. Suga, Chem. Pharm. Bull., 27, 480 (1979).
14. N. A. Porter, Y. Nolon, and I. Ramdas, Biochim. Biophys. Acta, 441, 506 (1976).
15. S. A. Serbinova, M. B. Kadiiska, R. A. Bakalova, et al., Toxicol. Lett., 47, 119 (1989).
16. O. Takanashi and K. Hiraga, Toxicol. Appl. Pharmacol., 43, 399 (1978).
17. WHO Food Addit. Ser. (eds. Joint WHO/FAO Expert Committee on Food Additives), No. 15, 106 (1980).

DETECTION OF NEUTROPHILIC MYELOPEROXIDASE IN RAT SKELETAL MUSCLES AFTER MUSCULAR ACTIVITY

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KEY WORDS: myeloperoxidase, neutrophil, skeletal muscles, muscular activity

Intensive muscular activity (MA) leads to the release of cytoplasmic and structural proteins of skeletal muscles into the blood stream, evidently as a result of disturbance of tissue integrity [6, 12]. Histologic and histochemical studies have revealed foci of necrosis of skeletal muscles after MA [3, 11]. Since an essential role in the development of tissue injury is ascribed to neutrophils [2, 14], it was decided to study the problem of the appearance of these cells in skeletal muscles after MA, by measuring the neutrophilic marker enzyme myeloperoxidase [10, 13] and its distribution in subcellular fractions.

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