PROTECTIVE ACTION OF LIPID- AND WATER-SOLUBLE ANTIOXIDANTS ON THE CYTOCHROME P-450 SYSTEM DURING LIPID PEROXIDATION IN LIVER MICROSOMES

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UDC 612.351.11:577.152.112].
015.6:577.161.3

KEY WORDS: microsomes; cytochrome P-450; α-tocopherol; carnosine

Both components of the microsomal monooxygenase system, namely NADPH-cytochrome P-450 reductase and cytochrome P-450 itself, are integral membrane proteins [2, 3]. Certain domains of these proteins may be exposed into the polar region of the membrane [10]. It can be tentatively suggested that as a result of attack by active forms of oxygen the resulting damage is due to oxidative modification not only of hydrophobic, but also of polar domains of the molecules of this polyenzyme system. In that case not only lipid-soluble antioxidants, interacting with lipid radicals, but also water-soluble antioxidants, interacting with oxygen radicals in water, ought to have a protective action against injury by active forms of oxygen.

The aim of this investigation was to compare the efficacy of the protective action of the lipid-soluble antioxidant α-tocopherol and the water-soluble antioxidant carnosine, and also a combination of both, on the monooxygenase system of rat liver microsomes during induction of nonenzymic lipid peroxidation (LPO).

EXPERIMENTAL METHOD

Membranes were obtained from liver microsomes of Wistar rats by the method in [1]. Activity of ethylmorphine-N-dimethylase was determined as in [7] and activity of aniline hydroxylase as in [6]. Activity of NADPH-cytochrome c-reductase was estimated by the method described in [9]. Cytochrome P-450 was determined quantitatively by the method in [8]. The protein concentration was determined by a modified method of Lowry et al. [5]. Values used for specific activities were 3.6 ± 0.3 nmole/mg protein/min for ethylmorphine-N-dimethylase, 0.32 ± 0.04 nmole/mg protein/min for aniline hydroxylase, 45 μmoles/mg protein/min for NADPH-cytochrome c-reductase, and the concentration of cytochrome P-450 in control preparations of liver microsomes was 0.66 nmole/mg protein. LPO was induced by addition of Fe^{2+} to 10 μM and ascorbate to 0.5 mM in 0.1 M K,Na-phosphate buffer, pH 7.4. The reaction was stopped by addition of ionol (10^{-4} M). Accumulation of LPO products was recorded by the reaction with 2-thiobarbituric acid (TBA) [14]. The α-tocopherol and carnosine were obtained from "Fluka," the cytochrome c and NADPH from "Boehringer," and TBA from "Serva." Other reagents were of Soviet manufacture and of the chemically pure grade.

EXPERIMENTAL RESULTS

Incubation of liver microsomes in the presence of inducers of a nonenzymic LPO system (Fe^{2+} + ascorbate) led to marked accumulation of TBA-active LPO products (8.5 nmoles malonic dialdehyde – MDA – per milligram protein; Table 1). Taking previous experiments into account, the concentrations of α-tocopherol and carnosine were chosen (10^{-7} and 10^{-4} M, respectively) in the presence of which only partial inhibition of LPO took place (Table 1). A combination of water- and lipid-soluble antioxidants gave additional, though not significant, inhibition of LPO through their combined action. This probably indicates that the mechanisms of action of the two antioxidants are not completely identical. Under the conditions used, induction of LPO in microsomal membranes was accompanied by inactivation of NADPH-cytochrome c-reductase (72.5%) and of cytochrome P-450 (67.0%), and also by reduction

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 2, pp. 187-188, February, 1989. Original article submitted May 30, 1988.

 0007-4888/89/0002-0198$12.50

TABLE 1. Protective Action of α-Tocopherol and Carnosine on the Cytochrome P-450 System during Induction of LPO (M ± m)

Parameter	Control	Induction of LPO			
		α-tocopherol		carnosine	α-tocopherol + carnosine
MDA, nmoles/mg protein	1,5±0,1	8,5±0,5	7,1±0,3	8,0±0,4	6,6±0,3
Cytochrome P-450 concentration, %	100,0	67,0±3,7	86,5±4,3	73,5±3,7	107,5±6,4
NADPH-cytochrome c-reductase activity, %	100,0	72,5±3,6	83,0±3,3	73,0±3,8	102,5±5,1
Ethylmorphine-N-dimethylase activity, %	100,0	86,0±4,8	88,0±4,0	83,0±4,9	119,0±9,5
Aniline hydroxylase activity, %	100,0	73,8±3,8	80,6±4,0	84,5±4,2	92,2±4,6

of cytochrome P-450-dependent activities of ethylmorphine-N-dimethylase (86.0%) and aniline hydroxylase (73.8%; Table 1). During induction of LPO in the presence of each antioxidant separately a protective effect was observed, and was greater for α-tocopherol than for carnosine (Table 1). In the case of the combined action of the antioxidants their protective action on the monooxygenase system was considerable greater, and became virtually complete. This suggests that protection of the two enzymes is realized not only in the hydrophobic, but also in the polar region of the membrane.

Considering that accumulation of oxidative degradation products of hydrophobic fatty-acid residues of lipid when a combination of antioxidants was used was only a little greater than the effect of each of them separately, the protective action of the two antioxidants obtained against enzymes of the monooxygenase system can be interpreted on the basis of the following hypothesis on the essential role of oxidative modification of polar domains of the enzymes. In this context it is worth mentioning results showing that accumulation of LPO products up to a concentration of 5 nmoles MDA/mg protein is not accompanied by degradation of cytochrome P-450 into a catalytically inactive form. It can be tentatively suggested that oxidative modification of some hydrophobic lipids is ineffective with respect to such conversion of cytochrome P-450. Further accumulation of LPO products is accompanied by a fall in the P-450 concentration.

It can be concluded from the results as a whole that optimization of the protective action of antioxidants on the monooxygenase system in liver microsomes requires the use of both water- and lipid-soluble scavengers of free radicals.

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