Effect of Lycopine on the Resistance of Rat Liver Microsomes to *In Vitro* Induced LPO

L. V. Kravchenko, S. V. Morozov, and V. A. Tutel'yan

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 136, No. 7, pp. 76-79, July, 2003
Original article submitted May 29, 2003

Lycopine in concentrations of 0.5-50 μM suppressed LPO in microsomes induced by NADPH-Fe^{2+} and by ascorbic acid-Fe^{2+}. Lycopine in a concentration of 20 μM completely prevented the decrease in the rate of benz[a]pyrene hydroxylation and activation of p-nitrophenyl-UDP-glucuronosyl transferase caused by LPO induction in microsomes.

Key Words: *lycopine; microsomes; lipid peroxidation; benz[a]pyrene hydroxylation; UDP-glucuronosyl transferase*

Oxidative damage of lipids, nucleic acids, and proteins is pathogenetic mechanism underlying the development of various chronic diseases (cancer, atherosclerosis, coronary disease, some age-associated degenerative diseases) and aging. This hypothesis is based on the results of wide-scale epidemiological studies, which detected an inverse relationship between the risk of these diseases and concentrations of bioactive compounds with antioxidant activities (for example, flavonoids and carotenoids) in food.

Alimentary dye lycopine was only recently proposed as a bioactive food additive. Lycopine is an acyclic carotene without A-provitamin activity, with a high antioxidant potential due to the presence of numerous conjugated double bonds in its molecule. Numerous *in vitro* studies on different models of AOF generation showed that by its capacity to quench singlet oxygen lycopine is superior to β-carotene and other carotenoids and that it more actively reacts with organic radicals than β-carotene [1,5,10].

The results of studies of antioxidant effects of lycopine *in vivo* are less clear. Increased consumption of lycopine-rich foodstuffs led to an increase of lycopine level in the blood, most of all in LDL, but was not always paralleled by changes in LDL resistance to *ex vivo* oxidative modification [7-9]. We showed that the treatment of rats with lycopine led to a dose-dependent increase of its concentration in the liver tissue and microsomes, but did not modify the sensitivity of microsomes to LPO induced *ex vivo* [2].

Here we investigated the effect of lycopine on microsome sensitivity to LPO *in vitro*.

MATERIALS AND METHODS

LPO of microsomal membranes was induced by NADPH-Fe^{2+} or ascorbic acid-Fe^{2+} systems. Oxidative modification of lipids was evaluated by accumulation of TBA-reactive LPO products and by changes in activities of enzymes differing by topology in the endoplasmic membrane (microsomes) [3,13].

Microsomes were isolated from the liver of male Wistar rats. The effect of lycopine on the rate of induced LPO was studied as follows: microsome suspension (1 mg protein/ml) in 40 mM Tris-HCl buffer (pH 7.4) was incubated at 37°C for 20 min with constant shaking in the presence of increasing concentrations of lycopine (0, 0.5, 1, 5, 10, 20, and 50 nmol/ml), after which NADPH in a final concentration of 0.05 mM or ascorbic acid in a final concentration of 0.8 mM, 12 μM $Fe(NH_4)_2(SO_4)_2 \times 6H_2O$ and 0.2 mM $Na_4P_2O_7 \times H_2O$ were added to the incubation medium and incubated at 37°C for 10 min with constant shaking. The reaction was stopped by 30% trichloroacetic acid, and the content of TBA-reactive compounds was measured.

Institute of Nutrition, Russian Academy of Medical Sciences, Moscow

0007-4888/03/1361-066$25.00

In order to evaluate the effect of LPO on activity of microsomal enzymes, the reaction medium containing 40 mM Tris-HCl buffer (pH 7.4), suspension of microsomes (1 mg protein/ml), NADPH or ascorbic acid in final concentrations of 0-1 mM, 12 μM $Fe(NH_4)_2(SO_4)_2 \times 6H_2O$ and 0.2 mM $Na_4P_2O_7 \times H_2O$ was incubated at 37°C for 10 min with constant shaking. The reaction was stopped by 30% TCA for measuring TBA-reactive compounds or 5 mM EDTA for measuring enzymes activities.

For evaluation of the effect of lycopine on changes in the enzyme activities induced by the microsomal membrane lipid oxidation, microsome suspension (1 mg protein/ml) in 40 mM Tris-HCl buffer (pH 7.4) was incubated at 37°C for 20 min with constant shaking with aqueous solution of lycopine (20 nmol/ml) or with the solvent, after which oxidation was carried out in a system containing 0.050 mM NADPH. After stopping the reaction with 5 mM EDTA aliquots for measuring enzyme activities were collected.

For measuring the content of TBA-reactive compounds, the incubation mixture after stopping the reaction with TCA was centrifuged at 6000 rpm for 10 min, 0.2 ml 0.6 n HCl and 0.8 ml 0.12 M TBA were added to 1 ml of the supernatant and heated at 100°C for 10 min. After cooling MDA content was measured at λ=532 and estimated using the molar extinction coefficient 1.56×10^5 $M^{-1}cm^{-1}$. Activity of UDP-glucuronosyl transferase (GlT) was measured with p-nitrophenol as the substrate [4] without detergent or with 0.1% Triton X-100 and the rate of benz[a]pyrene hydroxylation was evaluated as described previously [6].

Water-dispersed lycopine preparation (Hoffman—La Roche), TBA, NADPH, ascorbic acid, benz[a]pyrene (Sigma), and other reagents (extrapure for analysis) from Russian manufacturers were used in the study.

The results are presented as the means of 3-4 independent experiments.

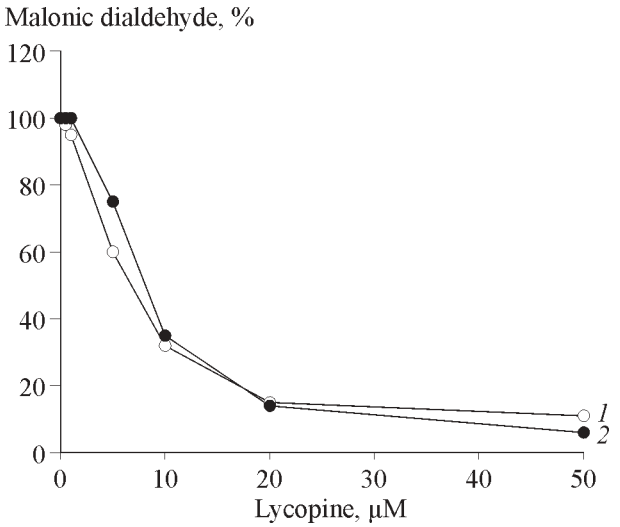

Fig. 1. Effect of lycopine on microsomal lipid peroxidation induced by NADPH (*1*) and ascorbate (*2*).

RESULTS

Incubation of microsomes with lycopine led to an increase of their resistance to LPO induced by both NADPH-Fe^{2+} and by ascorbate-Fe^{2+}. The degree of LPO inhibition did not depend on the prooxidant used in the experiment and directly depended on lycopine concentration (Fig. 1). The minimum lycopine concentration at which accumulation of LPO products notably decreased was 5 μM. The formation of MDA decreased by 32-35% in the presence of lycopine in a concentration of 20 μM and was almost completely suppressed at a concentration of 50 μM.

Induction of LPO in microsomes led to deceleration of benz[a]pyrene hydroxylation and increase in native (determined without detergent treatment) GlT activity (Table 1). Both processes directly depended on the concentrations of the resultant LPO products (MDA). Benz[a]pyrene hydroxylation was suppressed

TABLE 1. Effect of Induced LPO in Rat Liver Microsomes on the Rate of Benz[a]pyrene Hydroxylation and Activity of UDP-Glucuronosyl Transferase (GlT)

MDA, μM	Benz[a]pyrene hydroxylation, arb. Units/min/mg protein	UDP-glucuronosyl transferase, nmol/min/mg protein (native)
0.31±0.04 (spontaneous LPO)	5.20±0.49 (spontaneous LPO)	4.05±0.55 (spontaneous LPO)
7.17±0.29	2.28±0.17*	12.00±0.70*
13.20±0.37	1.17±0.04*	21.30±1.90*
18.24±1.02	0.92±0.04*	26.13±1.80*
23.80±0.34	0.68±0.03*	26.50±1.50*
25.80±0.00	0.80±0.08*	22.60±1.30*
27.40±0.26	0.70±0.04*	20.00±2.19*

Note. Spontaneous LPO: LPO values without prooxidants. *$p<0.05$ compared to spontaneous LPO.

TABLE 2. Effects of Lycopine on Changes in the Rate of Benz[a]pyrene Hydroxylation and Activity of UDP-Glucuronosyl Transferase Caused by Stimulated LPO of Rat Liver Microsomes (*M*±*m*)

Treatment	MDA, μM	Benz[a]pyrene hydroxylation, arb. units/min/mg protein	UDP-glucuronosyl transferase, nmol/min/mg protein	
			native	activated
Control (no prooxidant)	0.31±0.01	4.00±0.27	8.16±0.33	16.5±0.3
Lycopine (20 μM)	0.62±0.04*	3.80±0.20	7.53±0.72	16.6±0.3
NADPH (50 μM)	13.26±0.60*	1.02±0.02*	18.33±1.45*	17.3±1.2
NADPH (50 μM)+lycopine (20 μM)	3.22±0.12*+	3.44±0.03+	7.23±0.26+	15.7±1.6

Note. *$p<0.05$ compared to the control (without prooxidants); +$p<0.05$ compared to the effect of prooxidant without lycopine treatment.

by almost 90% in the presence of MDA concentration of 23.8 μM and did not change with subsequent increase of MDA level, while activity of GlT notably decreased at MDA concentrations >23.8 μM.

Spontaneous (without induction) LPO slightly increased in microsomes preincubated with 20 μM lycopine and accumulation of MDA in a concentration negligible for enzyme activity was observed (Table 2). Microsomal lipid oxidation induced by 50 μM NADPH without lycopine pretreatment resulted in an essential increase of MDA content in the incubation medium, 4-fold suppression of benz[a]pyrene hydroxylation rate, and a 2.3 times increase in native GlT activity. NADPH-induced lipid oxidation was 4-fold suppressed in microsomes preincubated with lycopine, while enzyme activity virtually did not differ from the control. Total activity of GlT measured in the presence of Triton X-100 did not change after LPO induction or lycopine treatment.

Hence, our data indicate that lycopine inhibits NADPH-dependent and ascorbate-dependent membranous LPO in the microsomal fraction *in vitro*. Although the former process is enzymatic and the latter nonenzymatic, they have a common mechanism of chain free-radical reaction. Similar results were obtained in the study of antioxidant activities of other carotenoids *in vitro*. It was shown [11,12] that astaxanthine, cantaxanthine, and far less so, β-carotene suppressed NADPH-induced LPO in microsomes isolated from rat liver, and their effect is similar to that of α-tocopherol (interruption of the lipid oxidation chain).

Studies of the effect of lycopine on the development of free-radical LPO reactions in liposomes [1] showed that similarly to β-carotene and α-tocopherol, lycopine inhibited LPO mainly by capturing free radicals and, presumably, by reacting with AOF in water phase [1].

We regarded changes in activities of microsomal enzymes during induced LPO along with accumulation of LPO products as an adequate indicator of the structural and functional status of microsomes. It is known that long-chain PUFA in phosphatidylethanolamine and phosphatidylcholine are first oxidized during free-radical lipid oxidation in microsomes *in vitro*, which leads to destruction of the membrane hydrophobic core and modification of its characteristics, including its permeability. Cytochrome P-450 and hydroxylation reaction are most sensitive to these changes because of disordered hydrophobic microenvironment of the hem and, presumably, its oxidation. The rate of cytochrome P-450 inactivation reflects the status of its phospholipid microenvironment [3]. As for GlT and primarily 1A6 isoform (p-nitrophenol is the specific substrate), it is transformed from the latent (native) form into active if the membrane permeability increases. This is explained by specific topology of the enzyme in the membrane: the catalytic center of the enzyme is situated on the internal surface of the microsomal vesicle, that is, behind the lipophilic "barrier", and impairment of the lipid bilayer structure facilitates the delivery of the substrate and co-substrate, thus activating the enzyme [13]. This explains why LPO induction does not modify GlT form "activated" by Triton X-100.

Hence, incubation of microsomes with lycopine leads to inhibition of microsomal lipids oxidation and suppresses accumulation of MDA correlating with essentially less marked changes in the microsomal enzymes activities. Comparison of our findings with published data suggests that antioxidant effect of lycopine *in vitro* is explained by its capacity to capture lipid radicals and interrupt the oxidative chain, as a result of which the structural and functional characteristics of microsomes are retained.

REFERENCES

1. G. I. Klebanov, A. B. Kapitanov, Yu. O. Teselkin, *et al.*, *Biologicheskie Membrany*, No. 2, 227-237 (1998).
2. L. V. Kravchenko, S. V. Morozov, N. A. Beketova, *et al.*, *Byull. Eksp. Biol. Med.*, **135**, No. 4, 414-418 (2003).

3. A. I. Archakov and G. I. Bachmanova, *Cytochrome P-450 and Active Oxygen*, London (1990).
4. B. Burchell and P. Weatherill, *Methods Enzymol.*, **77**, 169-176 (1981).
5. A. Cantrell, D. J. McGarvey, G. T. Truscott, *et al.*, *Arch. Biochem. Biophys.*, **412**, No. 1, 47-54 (2003).
6. W. Dehnen, R. Tomingas, and J. A. Roos, *Anal. Biochem.*, **53**, 373-383 (1973).
7. T. R. Dugas, D. W. Morel, and E. H. Harrison, *J. Lipid Res.*, **39**, 999-1007 (1998).
8. C. W. Hadley, S. K. Clinton, and S. J. Schwartz, *J. Nutrition*, **133**, No. 3, 727-732 (2003).
9. I. A. Hininger, A. Meyer-Wenger, U. Moser, *et al.*, *J. Am. Coll. Nutr.*, **20**, No. 3, 232-238 (2001).
10. Y. M. Naguib, *J. Agric. Food Chem.*, **48**, No. 4, 1150-1154 (2000).
11. P. Palozza and N. I. Krinsky, *Free Rad. Biol. Med.*, **11**, No. 4, 407-414 (1991).
12. P. Palozza and N. I. Krinski, *Arch. Biochem. Biophys.*, **297**, No. 2, 291-295 (1992).
13. A. Radominska-Pandya, P. J. Czernik, and J. M. Little, *Drug. Metab. Rev.*, **31**, No. 4, 817-899 (1999).