# PHARMACOLOGY AND TOXICOLOGY

# Antioxidant Status of Rats Receiving Lycopene in Different Doses

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Oral treatment with lycopene (*per os*) in doses of 10 or 50 mg/kg for 2 weeks led to accumulation of lycopene in the liver, liver microsomes, and blood plasma, increased total plasma antioxidant activity, inhibited LPO in the liver, and decreased solubilization of lysosomal enzymes. Lycopene had no effect on *ex vivo* resistance of liver microsomes to LPO and activities of antioxidant enzymes in the liver.

**Key Words:** *lycopene; antioxidant activity; lipid peroxidation*

Lycopene is one of 600 known natural carotenoids and one of the few carotenoids present in human blood and tissues. Lycopene is the most abundant carotenoid in human tissues (21-43% of all carotenoids), but its biological role is little studied. Lycopene is used as a food dye, but as bioactive component of food it attracted attention in 1995, when wide-scale epidemiological studies revealed a relationship between high level of lycopene in the ration and low risk of some cancers [10]. Some studies showed that addition of lycopene-rich products (tomato and tomato products) to the rations decreased the severity of cardiovascular diseases [12].

Lycopene is not produced in animal organism. The main source of lycopene in human nutrition is tomato containing about 30 mg lycopene per kg wet tissue. Lycopene content in tomato products is even higher: up to 150 mg/liter in tomato juice and 100 mg/kg in tomato puree. Water melons, pink grapefruit, and pink guava also contain much lycopene [15]. Lycopene is produced by some microorganisms, for example *Choanephora*.

Structurally, lycopene is an acyclic carotene with 11 conjugated double bonds arranged linearly, usually in trans-configuration. This substance exhibits no provitamin A activity. The long chromophore in the polyenic chain determines antioxidant activity of this substance (highest antioxidant capacity in trolox equivalents among carotenoids [13]).

Some studies showed that lycopene quenches singlet oxygen and binds organic radicals in various *in vitro* model systems of AOF generation [3,9]. There are just few proofs supporting the viewpoint that lycopene really functions as an antioxidant *in vivo*. However, possible anticarcinogenic and antiatherogenic effects of lycopene are explained primarily by its antioxidant activity and capacity to protect the major biomolecules (DNA, proteins, lipids, and LDL) from oxidative modification.

Here we investigated the effect of lycopene on some parameters of the rat antioxidant status.

## MATERIALS AND METHODS

The study was carried out male Wistar rats initially weighing about 175 g. The rats were divided into 3 groups (6 animals per group). The rats were kept on semisynthetic full-value ration. Group 1 animals received 10 mg/kg lycopene solution in sunflower oil

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(though a gastric tube); group 2 received 50 mg/kg lycopene for 14 days (via the same route). Controls received the same volume of the solvent. Crystal lycopene (>80% pure) was isolated from *Blakeslea trispora* fungus belonging to *Choanephora* genus (Uralbiofarm Firm).

The antioxidant status of rats was evaluated by total blood plasma antioxidant activity (AOA) measured by the chemiluminescent method using a hemoglobin-hydrogen peroxide-luminol test system [4]. The method is based on inhibition of chemiluminescence (CL) induced by luminol oxidation in a free-radical generation system (Hb-$H_2O_2$) in the presence of blood plasma. The kinetics of CL was evaluated by two parameters: CL latency and maximum CL intensity. The concentrations of TBA-reactive LPO products and reduced glutathione were measured in liver homogenate and activities of antioxidant defense enzymes (catalase, glutathione peroxidase, glutathione reductase, glutathione reductase, and quinone reductase) in the cytosol [2,8].

The effect of lycopene on the resistance of liver microsomes to LPO *ex vivo* was evaluated by measuring the rates of spontaneous, non-induced, and induced LPO in isolated microsomes. NADPH (0.025 mM) and ascorbate in low concentrations (0.8 mM) were used as prooxidants [2]. The rate of LPO was evaluated by accumulation of TBA-reactive products over 10 min per mg microsomal protein.

For evaluation of possible damaging effect of high lycopene doses on the liver [15], plasma concentrations of alkaline phosphatase and AlAT were measured using Lachema kits, total and nonsedimentable activities of lysosomal enzymes (arylsulfatases A and B, β-galactosidase, and β-glucuronidase) were measured in liver homogenates and cytosol fraction.

The content of vitamin E (α-tocopherol), the main LPO regulator, was measured in the plasma, liver tissue and microsomal fraction in parallel with lycopene measurements. Lycopene and α-tocopherol were extracted with hexane from methanol-deproteinized samples, the extract was evaporated to dryness in nitrogen stream, and the residue was re-dissolved in eluent (acetonitril-methanol-dichloromethane, 50:40:10, v/v). The concentrations of lycopene and α-tocopherol were measured by isocratic reversed phase nonaqueous HPLC with spectrophotometric detection of α-tocopherol at λ=292 nm and lycopene at λ=450 nm.

The results were statistically processed by dispersion analysis (ANOVA) using Student's *t* test and Statgraphics software.

## RESULTS

No lycopene was detected in the plasma, liver, or liver microsomes of control rats receiving semisynthetic ration (Table 1). Treatment with lycopene led to a dose-dependent accumulation of lycopene in liver tissue and microsomal fraction. The concentration of lycopene in microsomes in groups 1 and 2 was 2.2 and 4.3% of its liver content, respectively. Plasma level of lycopene was very low (68 nmol/liter in animals receiving high dose). Lycopene treatment did not modulate the content of vitamin E in the liver and microsomal fraction of group 2 rats, but considerably decreased it in liver microsomes of group 1 animals. Plasma content of vitamin E tended to decrease in groups 1 and 2 (by 20 and 23%, respectively, Table 1).

Plasma AOA expressed in ascorbate equivalents increased by 25 and 37% in groups 1 and 2, respectively (Table 2). This manifested in lengthening of CL latency (by 25 and 40%) and in a decrease in its maximum amplitude (by 15 and 26%, respectively).

No appreciable changes in activities of antioxidant enzymes (glutathione peroxidase, glutathione reductase, catalase, quinone reductase) and glutathione content in the liver were detected in rats treated with lycopene (Table 3). Only activity of glutathione transferase was higher than in the control. On the other hand, the content of LPO end-products (TBA-reactive

**TABLE 1.** Content of Lycopene and Vitamin E in the Liver, Liver Microsomes, and Blood Plasma of Rats Treated with Lycopene (*M*±*m*)

| Parameter | | Liver, μmol/kg wet tissue | Microsomes, μmol/kg wet tissue | Blood plasma, μmol/liter |
|---|---|---|---|---|
| Lycopene | control | 0 | 0 | 0 |
| | group 1 | 27.7±0.9 | 0.60±0.04 | N. d. |
| | group 2 | 40.2±1.9 | 1.73±0.47 | 0.068±0.013 |
| Vitamin E | control | 20.3±1.3 | 1.46±0.17 | 8.15±0.87 |
| | group 1 | 20.9±1.3 | 0.64±0.10* | 6.50±0.45 |
| | group 2 | 20.4±1.2 | 1.85±0.77 | 6.26±0.48 |

**Note.** N. d.: no data. Here and in Tables 2-5: *$p<0.05$ compared to the control.

**TABLE 2.** Effect of Lycopene on Total Plasma Antioxidant Activity in Rats (*M*±*m*)

| Parameters | Animal group | | |
|---|---|---|---|
| | control | 1st | 2nd |
| CL latency, sec | 70.8±7.0 | 88.4±7.0 | 99.2±10.0* |
| Maximum CL amplitude, thousands pulses/10 sec | 317±42 | 271±22 | 234±11 |
| Antioxidant capacity (ascorbic acid equivalent, μM) | 171±16 | 213±15 | 235±21* |

**TABLE 3.** Changes in Antioxidant Status of Rats Treated with Lycopene (*M*±*m*)

| Parameters | Animal group | | |
|---|---|---|---|
| | control | 1st | 2nd |
| TBA-reactive substances, nmol MDA/g liver tissue | 142.8±11.4 | 88.5±8.3* | 66.8±5.7* |
| SH-glutathione, μmol/g liver tissue | 4.60±0.55 | 4.89±0.73 | 5.58±0.61 |
| Glutathione peroxidase, nmol/min/mg protein | 40.9±2.9 | 47.6±2.8 | 38.0±3.7 |
| Glutathione reductase, nmol/min/mg protein | 74.1±8.0 | 78.8±13.0 | 85.8±6.4 |
| Catalase, mmol/min/mg protein | 0.22±0.01 | 0.21±0.01 | 0.19±0.01 |
| Glutathione transferase, μmol/min/mg protein | 0.86±0.03 | 1.05±0.05* | 1.07±0.07* |
| Quinone reductase, μmol/mg protein | 0.45±0.01 | 0.44±0.07 | 0.36±0.07 |

**TABLE 4.** Lycopene Effect on LPO Resistance of Liver Microsomes *Ex Vivo* (*M*±*m*)

| Parameters | | Animal group | | |
|---|---|---|---|---|
| | | control | 1st | 2nd |
| Non-induced LPO, NADPH-dependent LPO | nmol MDA/mg protein/10 min | 0.27±0.01 | 0.34±0.02* | 0.33±0.02* |
| | nmol MDA/mg protein/10 min | 8.28±0.22 | 8.45±0.18 | 8.68±0.43 |
| | degree of induction (times)° | 30.7 (100) | 24.8 (-20) | 26.3 (-14) |
| Ascorbate-dependent LPO | | | | |
| | nmol MDA/mg protein/10 min | 3.56±0.29 | 4.22±0.09 | 4.63±0.07* |
| | degree of induction (times)° | 13.2 (100) | 12.4 (-6) | 14.0 (6) |

**Note.** °in comparison with non-induced LPO in the same group. Changes in the degree of induction in experimental groups in comparison with the control are shown in parentheses (%).

substances) decreased significantly in experimental groups 1 (by 38%) and 2 (by 53%).

Interestingly, despite accumulation of lycopene, the rate of non-induced LPO in liver microsomes of experimental rats was higher than in liver microsomes from control rats (Table 4). Lycopene did not modify the rate of *ex vivo* induced NADPH-dependent LPO in microsomes, but the rate of ascorbate-dependent LPO was higher than in the control. On the other hand, comparison of the rates of induced and spontaneous LPO revealed virtually no differences between the groups in ascorbate-dependent LPO and less pronounced induction of NADPH-dependent LPO (to 20%).

Treatment with lycopene for 2 weeks did not affect daily weight gain, relative weight of the viscera, serum enzyme activities, and total activity lysosomal enzymes. At the same time, nonsedimentable activity of arylsulfatases A and B, β-galactosidase and β-glucuronidase decreased, especially in group 2 animals (by 27, 48, and 60%, respectively, in comparison with the control (Table 5).

Lycopene was not detected in the plasma, liver tissue, and microsomal fraction of rats receiving a semisynthetic ration. Lycopene was detected in appreciable amounts in the liver and liver microsomes of rats treated with lycopene for 2 weeks, while its concentration in the plasma was much lower. Similar results were obtained in previous studies [5,6] demonstrating predominant accumulation of lycopene in the liver in rats and humans. After intragastric adminis-

tration of lycopene in a dose of 50 mg/kg for 2 or 8 weeks its concentration in the liver increased to 16 μM, but its plasma concentration did not exceed 82 nmol/liter. Increasing the dose of lycopene did not increased its blood level [5]. It should be noted that in humans receiving only 0.78-2.30 mg daily plasma concentrations of lycopene varied from 70 to 1790 nmol/liter [11]. These data attest to lower bioavailability of lycopene in rats in comparison with humans.

The detected changes in vitamin E content in rat liver microsomes after administration of lycopene in a dose of 10 mg/kg in the absence of changes in its concentration in the liver are difficult to explain. The decrease in plasma concentration of vitamin E in animals treated with lycopene can be explained by lycopene-vitamin E competition for gastrointestinal absorption or for binding with lipoproteins.

Plasma AOA is determined mainly by low molecular-weight antioxidants of alimentary origin, such as vitamin E, ascorbic acid, and can be regarded as an indicator of balance between AOF and antioxidant levels, including those received with food. In our study the appearance of lycopene in the plasma was paralleled by a significant increase in its antioxidant activity (capacity). The changes in CL kinetics suggest that the increase in plasma lycopene concentration is associated with an increase of plasma capacity to capture AOF and to react with luminol radicals [3,4].

Lycopene accumulation in rat liver tissue correlated with pronounced suppression of generation of LPO products in the liver, but this was not associated with changes in activities of antioxidant enzymes. A similar study on male Wistar rats receiving lycopene orally in a dose of 50 mg/kg for 2 weeks also showed no changes in activities of antioxidant enzymes, and only a higher dose of lycopene led to a moderate increase in glutathione transferase activity [6]. It seems that the effect of lycopene on LPO process in the liver is associated with its antiradical effects, rather that modulation of enzyme activities. The increase of glutathione transferase activity in rats treated with lycopene, though it is moderate (by 22-24%), can considerably contribute to utilization of LPO products. Similarly to glutathione peroxidase, glutathione transferase effectively reduces lipid hydroperoxides and, in contrast to peroxidase, can reduce phospholipid hydroperoxides in membranes [1]. Since glutathione transferases constitute up to 10% liver cytosol protein, their role in the regulation of LPO processes in cells seems to be very important.

We believe that changes in the resistance of macromolecules (lipids, proteins, DNA) to *ex vivo* oxidative modification can be used for evaluation of antioxidant characteristics of food components *in vivo* as a marker of antioxidant effect. Incorporation of lycopene in rat liver microsomes in our experiments did not improve their resistance to *ex vivo* peroxidation. However, in previous *in vitro* experiments incubation of microsomes with lycopene in a concentration >1 μM (*i.e.* >1.5 nmol/mg protein) considerably inhibited induced LPO. Presumably, the protective effect of lycopene depends on its concentration in microsomes: lycopene concentration in the microsomes of groups 1 and 2 rats after its intragastric administration was 0.03 and 0.09 nmol/mg protein, respectively. Moderate changes in biophysical parameters and rigidity of membranes were observed in the presence of lycopene incorporation in swine liver microsomes in concentrations of 1-6 nmol/mg protein [14].

The data on the dose-dependent inhibition of nonsedimentable activities of lysosomal enzymes characterizing mechanical stability of lysosomal membranes are of particular interest. Hydrophobic properties of lycopene suggest that *in vivo* it can be incorporated into the hydrophobic core of the membrane, where it becomes an integral component of its structure and can modify membrane rigidity, thickness, and mechanical strength [7]. The decrease in nonsedimentable lysosomal enzyme activities detected in our experiments can be regarded as an indirect proof of lycopene incorporation into the lysosomal membrane improving its resistance. It is also possible that decreased solubilization of lysosomal enzymes can be associated with reduced formation of LPO products in the liver. This hypothesis is based on experimental findings, showing that although the rate of peroxide formation in isolated lysosomes is much lower (10-fold) than in microsomes and mitochondria (3-fold), lyso-

**TABLE 5.** Nonsedimentable Activity of Liver Lysosomal Enzymes (% of Total) in Rats Treated with Lycopene (*M*±*m*)

| Parameters | Animal group | | |
|---|---|---|---|
| | control | 1st | 2nd |
| Arylsulfatases A and B | 3.73±0.01 | 3.31±0.08* | 2.35±0.14*+ |
| β-Galactosidase | 5.94±0.28 | 5.90±0.29 | 3.11±0.20* |
| β-Glucuronidase | 5.68±0.20 | 5.39±0.18 | 2.17±0.12* |

**Note.** *$p$<0.05 compared to group 1.

somal membrane is highly sensitive to exogenous and endogenous lipid hydroperoxides, *i. e.* foreign hydroperoxides [2].

Hence, our findings indicate that despite low bioavailability of lycopene in rats in comparison with humans, it modulates the antioxidant status even at its plasma concentrations corresponding to the mean low level in human blood: increases total antioxidant activity of the plasma and suppresses the formation of LPO products in the liver. Lycopene capacity to improve the resistance of lysosomal membrane and decrease solubilization of lysosomal enzymes *in vivo* was demonstrated for the first time.

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