Lycopene Attenuates Oxidative Stress Induced Experimental Cataract Development: An In Vitro and In Vivo Study

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OBJECTIVE: Lycopene, a nutritional antioxidant, was evaluated for its anticataract potential to further establish its role in cataract prevention.

METHODS: The ability of lycopene to modulate the biochemical parameters was investigated by in vitro studies. Enucleated rat lenses were maintained in organ culture containing Dulbecco’s Modified Eagles Medium alone or in addition with 100 μM selenite and served as the normal and control groups, respectively. For the test group, the control medium was supplemented with 10 μM lycopene. The lenses were incubated for 24 h at 37°C. At the end of the incubation period, the lenses were examined for morphologic variation, and biochemical parameters such as reduced glutathione, the lipid peroxidation product malondialdehyde, and the antioxidant enzymes glutathione peroxidase, glutathione S-transferase, superoxide dismutase, and catalase were estimated. In vivo selenite cataract was induced in 9-d-old rats by subcutaneous injection of sodium selenite (25 μmoles/kg of body weight). The rats in the test group were injected with lycopene (200 μg/kg body weight, intraperitoneally) 4 h before the selenite challenge. The incidence of cataract was observed when the rats first opened their eyes. Galactose cataract was induced in rats by feeding 30% galactose in the diet. Rats in the test group were fed orally with 200 μg/kg of lycopene daily, and rats in the control group received only vehicle. Cataract stages were graded at regular intervals.

RESULTS: A fall (25%) in the glutathione level and a rise (32%) in the malondialdehyde content were observed in control as opposed to normal lenses. Lycopene supplementation in the medium significantly (P < 0.001) restored glutathione and malondialdehyde levels. A significant decrease in the activity of antioxidant enzymes also was observed in the control lenses. A significant restoration in the activities of superoxide dismutase (P < 0.05) and catalase and glutathione S-transferase (P < 0.01), with no effect on glutathione peroxidase, was observed in the lycopene-supplemented group. Lycopene also reduced the incidence of selenite cataract. Only 9% of the eyes in the test group developed dense nuclear opacity as opposed to 83% in the control group. A significant delay in the onset and progression of galactose cataract was observed with oral feeding of lycopene. Only 35% of the eyes developed mature cataract as opposed to 100% in the control group.

CONCLUSIONS: Lycopene protects against experimental cataract development by virtue of its antioxidant properties, and it may be useful for prophylaxis or therapy against cataracts. Nutrition 2003;19:794–799. ©Elsevier Inc. 2003

KEY WORDS: lycopene, oxidative stress, cataract, antioxidant, carotenoid

INTRODUCTION

Cataract is the leading cause of blindness worldwide. Several risk factors have been identified for the development of human cataract: aging, diabetes, diarrhea, malnutrition, poverty, sunlight, smoking, hypertension, and renal failure. Although cataract is a multifactorial disease, oxidative stress has been identified as an initiating factor for the development of maturity-onset cataract. Physiologic antioxidants such as pyruvate and nutritional antioxidants such as ascorbate, vitamin E, and carotenoids were found to delay the development of experimental cataract.

Carotenoids are natural lipid-soluble antioxidants. α-Carotene, β-cryptoxanthine, lutein, and lycopene are the most abundant carotenoid components of the human diet. Among the carotenoids, β-carotene, a precursor of vitamin A, has been investigated extensively for its role in cataract prevention. However, data from most epidemiologic studies on carotenoid intake and assessment of risk of cataract have been inconclusive.

Lycopene is a major carotenoid, available primarily from tomatoes and its products. Of all carotenoids, lycopene has been shown to exhibit the highest physical quenching rate constant with singlet oxygen (Fig. 1), and its plasma level is slightly higher than that of β-carotene. Lycopene has a high antioxidative activity and exerts a protective effect in various diseases.
leagues\textsuperscript{10,11} studied the inhibitory effect of lycopene on cataract development in galactosemic rats, and Gale et al.\textsuperscript{3} reported a positive association between plasma concentration of lycopene and risk of cortical cataract. However, the abundance of lycopene in the human food supply and its superior antioxidant function to quench singlet oxygen\textsuperscript{6} and other radical species\textsuperscript{12} have warranted studies to further establish the role of lycopene in the prevention of cataract. In the present study, the anticataract potential of lycopene in selenite- and galactose-induced cataract in vitro and in vivo was evaluated.

\textbf{METHODS AND MATERIALS}

\textbf{Chemicals}

Lycopene was kindly provided by Jagsonpal Pharmaceuticals (New Delhi, India). The approximate purity was 98.7\%. Dulbecco’s Modified Eagles Medium (DMEM) was obtained from Hi Media Laboratories (Mumbai, India). Galactose was procured from Sigma Laboratories (St. Louis, MO, USA). Selenite was purchased from E. Merck (Mumbai, India). Streptomycin and penicillin were obtained from Hindustan Antibiotics Ltd. (Pune, India). All other chemicals were of analytical grade.

\textbf{In Vitro Studies}

Wistar rats of either sex in the weight range of 80 to 100 g were used for the study. The animals were maintained and treated in accordance with the Association for Research in Vision and Ophthalmology statement for the use of animals in research. The animals comprised the normal group (without treatment of any type) for other cardiovascular studies being conducted in our laboratory. When these rats were killed to remove the heart tissue, the eyes were simultaneously enucleated without any delay. The lenses were carefully dissected out from a posterior approach to avoid damage. Visibly transparent lenses were incubated in DMEM containing sodium bicarbonate (0.9 g/L), streptomycin (100 \(\mu\)g/mL), and penicillin (100 IU/mL) at 37°C in an incubator with 95\% air and 5\% CO\(_2\). The lenses were incubated initially for 24 h. After incubation, lenses were processed for the estimation of biochemical parameters.

\textbf{REDUCED GLUTATHIONE (GSH).} The GSH content was estimated by the method of Moron et al.\textsuperscript{14} Half of the lenses from each group were weighed and homogenized in 1 mL of 5\% trichloroacetic acid (TCA), and a clear supernatant was obtained by centrifugation at 5000 rpm for 15 min. To 0.5 mL of this supernatant, 4.0 mL of 0.3 M Na\(_2\)HPO\(_4\) and 0.5 mL of 0.6 mM 5,5'-dithiobis-2-nitrobenzoic acid in 1\% trisodium citrate were added in succession. The intensity of the resulting yellow color was read spectrophotometrically at 410 nm. Reduced GSH was used as a standard.

\textbf{ENZYME ASSAYS.} A separate set of experiments was conducted under the same conditions as described above. After 24 h of incubation, 10\% (w/v) homogenate of lenses from each group was prepared in 50 mM phosphate buffer (pH 7.0). The enzyme activities were measured in the supernatant obtained by the centrifugation of the homogenate at 5000 rpm for 15 min at 4°C. Monitoring spectrophotometrically at 480 nm, the ability of the enzyme to inhibit the oxidation of epinephrine\textsuperscript{10} was used to assess the activity of superoxide dismutase (SOD). One unit of SOD activity was defined as the amount of enzyme inhibiting 50\% of the rate of auto-oxidation of epinephrine under the defined assay conditions. The enzyme activity of catalase (CAT) was measured spectrophotometrically at 240 nm by following the decomposition of H\(_2\)O\(_2\).\textsuperscript{17} One unit of CAT activity represented the amount of enzyme required to decompose 1 \(\mu\)mole of H\(_2\)O\(_2\)/min. Glutathione peroxidase (GPx) activity was monitored at 340 nm.\textsuperscript{18} One unit of enzyme activity was defined as the amount of GPx required to use 1 \(\mu\)mole of nicotinamide adenine dinucleotide phosphate per minute. The conjugation of GSH with 1-chloro-2,4-dinitrobenzene (CDNB), a hydrophilic substrate, was examined spectrophotometrically at 340 nm\textsuperscript{19} to measure glutathione S-transferase (GST) activity. One unit of GST was defined as the amount of enzyme required to conjugate 1 \(\mu\)mole of CDNB with GSH per minute.

To calculate the specific enzyme activity, protein in each sample was estimated by the method of Lowry et al.\textsuperscript{20}

\textbf{In Vivo Studies}

Lycopene was weighed out in aliquots and stored in dark glass vials at 20°C. Each day a fresh vial of lycopene was removed from the freezer and immediately dissolved in corn oil, using a magnetic stirrer to obtain a final concentration of 0.1 mM. The lycopene solution thus obtained was used for oral administration in rats in a dose of 200 \(\mu\)g/kg of body weight. For intraperitoneal administration in rat pups, the 10-mM stock of lycopene prepared for the in vitro studies was injected intraperitoneally, using a 27G needle.
vitro studies was further diluted with distilled water to get a final concentration of 0.1 μmole. The solution was vigorously shaken before administration.

SELENITE CATARACT. Cataract was induced in 9-d-old Wistar rat pups. The control and test groups had equal numbers of pups. Pups in both groups were injected subcutaneously with 0.25 μmole of sodium selenite per kilogram of body weight. Four hours before the selenite challenge, the pups in the test group were injected intraperitoneally a single dose of 200 μg of lycopene per kilogram of body weight, and those in the control group were injected intraperitoneally with vehicle only. Cataracts were observed in both groups on postnatal day 16, when the eyes of the pups first opened.

GALACTOSE CATARACT. Wistar rats of either sex in the weight range of 80 to 100 g were used for induction of cataract. They were divided into test and control groups. Both groups were fed 30% galactose in diet and water ad libitum. Lycopene, 200 μg/kg of body weight, was administered orally to the test group, and control group was fed only vehicle. The treatment was started 1 wk before galactose challenge and was continued until the end of the experiment. At regular intervals, cataract stages in both groups were observed with a slit-lamp biomicroscope after dilatation of the pupil. Different stages of cataract were graded: stage I, faint peripheral opacity; stage II, irregular peripheral opacity and slight involvement of the lens at the center; stage III, irregular opacity involving the entire lens; and stage IV, pronounced opacity readily visible as a white spot (Fig. 2).

**RESULTS**

**Effect on Morphology**

One hundred percent of the lenses in the control group developed cortical opacity after 24 h of incubation. In contrast, only 20% of the lenses in the test group developed opacity, and the remaining 80% of the lenses were clear.

**Effect on GSH and MDA Levels**

The mean GSH value in the normal lenses was 4.92 ± 0.26 μmoles/g of fresh weight of lens. A significant decrease (25%) in GSH level was observed in the presence of sodium selenite in the control as opposed to the normal group (P < 0.001). In the presence of lycopene, there was a significant restoration of GSH level in the treated lenses (P < 0.01) as opposed to the control lenses. The mean GSH values in the control and test groups were

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**Statistical Analysis**

All data are expressed as mean ± standard deviation. Student’s t test (unpaired) and chi-square test were applied, and P < 0.05 was regarded as statistically significant.
TABLE I.

**EFFECT OF Lycopene ON ANTIOXIDANT ENZYME ACTIVITY**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Normal†</th>
<th>Control‡</th>
<th>Test§</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPx</td>
<td></td>
<td>12.46 ± 0.58</td>
<td>11.06 ± 0.51†‡</td>
</tr>
<tr>
<td>SOD</td>
<td></td>
<td>0.57 ± 0.03</td>
<td>0.03 ± 0.002$§</td>
</tr>
<tr>
<td>CAT#</td>
<td>2.05 ± 0.25</td>
<td>0.87 ± 0.30‡‡</td>
<td>2.55 ± 0.41¶¶</td>
</tr>
<tr>
<td>GST**</td>
<td>6.01 ± 0.27</td>
<td>1.1 ± 0.13§§</td>
<td>7.2 ± 1.25¶¶</td>
</tr>
</tbody>
</table>

* Values represent the mean ± standard deviation of six samples.
† Lens maintained in Dulbecco’s Modified Eagle’s Medium.
‡ Lens maintained in Dulbecco’s Modified Eagle’s Medium and 100 μM sodium selenite.
§ Lens maintained in Dulbecco’s Modified Eagle’s Medium, 100 μM sodium selenite, and 10 μM lycopene.
∥ One unit of activity is defined as the amount of enzyme required to use 1 nmole of nicotinamide adenine dinucleotide phosphate per minute at 37°C.
¶ One unit of activity is defined as the amount of enzyme that inhibits 50% of the rate of auto-oxidation of epinephrine under the defined assay conditions.
# One unit of activity represents the amount of enzyme required to decompose 1 μmole of H₂O₂ per minute.
** One unit of activity is defined as the amount of enzyme required to conjugate 1 μmole of 1-chloro,2,4-dinitrobenzene with GSH per minute.
†† P < 0.05 versus normal.
‡‡ P < 0.01 versus normal.
§§ P < 0.001 versus normal.
|| P < 0.05 versus control.
¶¶ P < 0.01 versus control.
CAT, catalase; GPx, glutathione peroxidase; GST, glutathione S-transferase; SOD, superoxide dismutase.

3.69 ± 0.21 and 4.88 ± 0.48 μmolesM/g of fresh weight of lens, respectively (Fig. 3).

A significant increase (32%) in MDA level was found in the control opposed to the normal lenses (28.2 ± 1.56 nmols/g of fresh weight of lens; P < 0.01). Lycopene supplementation significantly protected (P < 0.001) the test group lenses from lipid peroxidation; the MDA content was 25.5 ± 3.9 nmols/g of wet weight of lens (Fig. 3).

**Effect on Enzyme Activity**

The activities of GPx, SOD, CAT, and GST decreased significantly in the control lenses incubated with sodium selenite. Incorporation of 10 μM of lycopene in the medium resulted in significant restoration of the activities of SOD, CAT, and GST. The level of significance for SOD was P < 0.05, and that for CAT and GST was P < 0.01 in the lycopene-supplemented versus control lenses (Table I). However the activity of GPx was not significantly restored after lycopene supplementation.

**Effect on Selenite Cataract In Vivo**

Subcutaneous injection of micromolar concentrations of sodium selenite led to the development of 100% nuclear opacities in the eyes of the control group on the postnatal day 16. Of these, 17% of the eyes developed pinpoint opacity and 83% developed dense nuclear opacity. In contrast, in the test group, 18% of the eyes were clear, 73% of the eyes developed pinpoint opacity, and only 9% of the eyes developed dense nuclear opacity (Fig. 4).

**Effect on Galactose Cataract In Vivo**

The onset of cataract in the galactose-fed rats of the control group was observed on day 7. All 20 eyes showed cataractogenic lenticular changes. In contrast, in the lycopene-fed animals, 30% of the eyes were normal on day 7. By day 30, 100% of the control lenses developed mature cataract, whereas only 35% of the test lenses had mature cataract. The different stages of cataract in both groups on various days are shown in Figure 5.

**DISCUSSION**

Age-related cataract is a significant problem worldwide. Oxidative stress has been suggested as a common underlying mechanism of cataractogenesis, and augmentation of the antioxidant defenses of the lens has been shown to prevent or delay cataract. Different agents with diverse chemical structures have shown antioxidant properties in different systems, and their beneficial effects have been demonstrated in various pathologic conditions including cataract. During the past two decades, great emphasis has been placed on the possible roles of physiologic and nutritional antioxidants in cataract, because cataract is a slowly progressing disease, and a person might have to be on life-long treatment once the
Cataract has developed. Most of these agents have one or another drawback such as short half-life, adverse effects, thermalability, and poor bioavailability. However, efforts are ongoing to find an antioxidant that forms a part of our diet and can be consumed daily.

A diet rich in carotenoid-containing food is associated with a number of health benefits. Lycopene is one of the major carotenoids in the diets of people in developed and developing countries. Lycopene may protect the eye against oxidative damage and play a critical role in visual function. The identification of a diverse range of carotenoids including lycopene in ocular tissues, their protective role in age-related macular degeneration, significantly higher bioavailability of lycopene than of β-carotene, and above all its high oxygen-quenching capacity makes lycopene a potential candidate to be screened as an anticataract agent and formed the basis of the present study. Recent evidence that glucose-intolerant states are the result of increased oxidative stress and that lycopene may play a role in reducing the pathogenesis of diabetes through its antioxidant capacity further inspired us to evaluate the anticataract potential of lycopene.

Cataract is a multifactorial disease associated with several risk factors. Various experimental models have been developed to delineate the mechanisms of cataractogenesis and focus the identification of the crucial targets. Selenite-induced cataractogenesis in young rats has been shown to mimic human senile cataract with respect to several morphologic and biochemical changes in the lens. This model, being reproducible, has been used extensively to evaluate the anticataract potential of different test agents. A single dose of selenite administration leads to impaired oxidative defense, membrane damage, and cataract formation. Oxidation of the critical sulfhydryl groups of Ca2+-ATPase on lens epithelial membrane, influx of calcium from the aqueous humor, activation of calpain, cleavage of N-terminal extensions of β-crystallins of the lens, interaction between exposed charged groups, and the formation of insoluble protein aggregates are some of the steps leading to the development of opacification. Selenite-induced opacification of the lens in vitro also has been demonstrated.

In the present study, anticataract potential of lycopene was evaluated in vitro and in vivo. In vitro, lenses were exposed to a high selenium concentration in the presence and absence of lycopene in the culture medium. Lycopene, a lipophilic compound, posed the problem of solubility and stability in aqueous culture medium. However, by occasional shaking, it could be suspended as microcrystals, and the solution appeared clear to the naked eye. In our previous studies on human lens epithelial cells, lycopene degraded by 41.2% within 4 h. Based on present results, the medium was changed every 6 h during the lens organ culture experiments. Under these experimental conditions, 100% of the lenses incubated in selenite-containing medium developed cortical opacities within 24 h of incubation. In contrast, the presence of lycopene significantly reduced the opacification, and 80% of lenses remained absolutely clear. Chemical analysis of these lenses clearly demonstrated a significant depletion of GSH and increased membrane damage as indicated by the levels of MDA, the product of membrane lipid peroxidation. Such changes in GSH and MDA levels in presence of selenite have been reported. Restoration of GSH and MDA levels, protection against aggregation and insolubilization of lens proteins, and maintenance of lens clarity without doubt establish the protective action of lycopene. However, the present study did not show whether lycopene enters the lens as microcrystals or whether its presence in the medium maintains lens membrane integrity by preventing the oxidation of the critical sulfhydryl groups on the lens epithelial cell membrane and the subsequent cascade of deleterious changes mentioned above. It can be hypothesized that lycopene microcrystals settle from the suspension on the surface of the lens membrane and, being lipid soluble, exerts its action on the surface or gains access to the lens epithelial cells. However, detection of lycopene levels in the lens can only lead to one conclusion. That lycopene significantly impairs antioxidant enzymes in the presence of selenite is an interesting finding. The levels of SOD, CAT, and GST were significantly hampered with selenite and positively modulated in the presence of lycopene, whereas GPx activity was unaffected by lycopene. Interestingly, the levels of SOD, CAT, and GST were higher than normal in the presence of lycopene. The data clearly demonstrated that lycopene significantly improves the antioxidant defense mechanisms of the normal lens. Routine consumption of lycopene in the form of food may offer a prophylactic measure against not only the onset and progression of cataract but also other diseases. Significantly raised SOD activity in the presence of lycopene in the present study is in accordance with previous findings and further confirms its higher oxygen quenching activity.

Fig. 5. Effect of lycopene on the progression of galactose cataract. Control: Rats were fed orally with 30% galactose via diet and vehicle. Test: Rats were fed lycopene (200 μg/kg body weight) orally with galactose in the diet. Figure shows the percentage of eyes with different grades of opacity on 7, 14, 21, and 30 d.
as demonstrated by Di Mascio and colleagues. Significantly raised CAT activity in the presence of lycopene offers additional protection not only by the efficient removal of hydrogen peroxide formed in situ in the presence of selenite but also by the action of activated SOD. The results of the present study indicated a decrease in GSH level with no effect on GPx activity. From the levels of GST, it can be concluded that GSH leaks out, thus making the lens vulnerable to oxidative damage, or is diverted for the detoxification of an electrophilic moiety generated in the system. An additional function of lycopene as a detoxifying agent is clearly indicated from the present study.

Based on our findings of in vitro studies that lycopene acts as an antioxidant, lycopene was evaluated against selenite-induced cataracts in young rats. Lycopene significantly protected the lens morphology and clarity: 18% of the eyes were absolutely clear, and only 9% of the eyes developed dense nuclear opacity in the lycopene-treated group; in contrast, 83% of the control eyes developed dense nuclear opacity and none of the eyes was clear.

The antiacataract effect of lycopene in selenite cataract in vivo encouraged us to evaluate its potential in galactose-induced cataract. Lycopene offered significant protection in the galactose-induced cataract in rats. There was a decrease in onset and a delay in progression of cataract in rats fed lycopene orally. This result is similar to that obtained by Pollack and colleagues, These published works provided no information on levels of lycopene in the aqueous humor or the lens but clearly suggests its bioavailability. The anticataract effect of lycopene in selenite cataract in vivo significantly protected the lens against oxidative stress followed by membrane damage and a weakened antioxidant defense mechanism may be responsible for some of the early changes in diabetes. From the current study, it is evident that lycopene also protects the lens against oxidative stress. Our results on selenite- and galactose-induced cataracts in vivo and in vitro not only demonstrate the protective effect of lycopene but also indicate that lycopene prevents cataractogenesis by virtue of its antioxidant properties. Lycopene, therefore, may be useful for prophylaxis or therapy against cataracts.

SUMMARY

The study on the evaluation of the antiacataract potential of lycopene in experimental animals indicated that it modulates antioxidant parameters in the enucleated eye lenses. It also attenuates selenite-induced cataract and delays the onset and progression of galactose cataract in vivo, so it may be useful for cataract therapy.