Effects of lycopene on the model of oleic acid-induced acute lung injury

Suat TÜRKOĞLU¹, Mehmet Hamdi MÜZ², Reşat ÖZERCAN³, Ferit GÜRSU⁴, Gamze KIRKIL²

¹ SB Batman Bölge Devlet Hastanesi, Göğüs Hastalıkları Kliniği, Batman, Turkey,
² Fırat Üniversitesi Tıp Fakültesi, Göğüs Hastalıkları Anabilim Dalı, Elazığ, Turkey,
³ Fırat Üniversitesi Tıp Fakültesi, Patoloji Anabilim Dalı, Elazığ, Turkey,
⁴ Fırat Üniversitesi Tıp Fakültesi, Biyokimya Anabilim Dalı, Elazığ, Turkey.

ÖZET
Oleik asit ile oluşturulan akut akciğer hasarı modelinde likopenin etkileri

Giriş: Bu çalışmada, akciğer hasarı modelinde likopenin koruyucu etkisinin araştırılması amaçlandı.

Materyal ve Metod: Çalışmaya 28 adet Wistar rat alındı. Kontrol grubuna (n= 7) serum fizyolojik + etanol (9/1) infüzyonu uygulandı. Oleik asit (OA) grubuna (n= 7), OA (100 mg/kg) tek doz intravenöz olarak uygulandı. Mısır yağı + OA grubuna (n= 7), beş hafta mısır yağı (1 mL/gün) gavajla verildi. Likopen + OA grubuna (n= 7), beş hafta likopen gavajla verildi ve beşinci haftanın sonunda OA (100 mg/kg) uygulandı. OA verildikten dört saat sonra kan ve akciğer doku örnekleri alındı. Malondialdehid, süperoksit dismutaz, glutatyon peroksidaz ve doku katalaz enzim aktivite düzeyleri ölçüldü.

Bulgular: Kontrole göre OA ile mısır yağı + OA gruplarında artmış olan serum ve akciğer doku malondialdehid düzeyi, likopen + OA grubunda kontrol değeri düzeyinde idi (p< 0.05). Serum ve doku süperoksit dismutaz ve glutatyon peroksidaz enzim aktiviteleri kontrolde aynı değişiklikle, lıkopen + OA grubunda diğer gruplara göre belirgin artış mevcuttu (p< 0.05). Kontrol grubunun histopatolojik değerlendirmesi normalken, OA ve mısır yağı + OA gruplarında perivasküler, alveoler ödem, hemoraji, belirgin nötrofil infiltrasyonu, alveoler yapılıarda destrüksiyon saptandı. Likopen + OA grubunda daha az nötrofilik infiltrasyon, perivasküler ve alveoler ödem izlendi.

Sonuç: Lıkopenen zengin diyet akciğer hasarının önlenmesinde önemli role sahip olabilir.

Anahtar Kelimeler: Akut akciğer hasarı, rat model, likopen.

SUMMARY
Effects of lycopene on the model of oleic acid-induced acute lung injury

Suat TÜRKOĞLU¹, Mehmet Hamdi MÜZ², Reşat ÖZERCAN³, Ferit GÜRSU⁴, Gamze KIRKIL²

¹ Clinic of Chest Diseases, Batman Region State Hospital, Batman, Turkey,
² Department of Chest Diseases, Faculty of Medicine, Firat University, Elazığ, Turkey,
³ Department of Pathology, Faculty of Medicine, Firat University, Elazığ, Turkey,
⁴ Department of Biochemistry, Faculty of Medicine, Firat University, Elazığ, Turkey.

Yazılaşma Adresi (Address for Correspondence):
Dr. Gamze KIRKIL, Firat Üniversitesi Tıp Fakültesi, Göğüs Hastalıkları Anabilim Dalı, ELAZIĞ - TURKEY
e-mail: gamkirkil@yahoo.com
Introduction: This study, we aimed to investigate the protective effect of lycopene in lung injury rat model.

Materials and Methods: Twenty eight Wistar rats were enrolled into the study. Control group (n= 7) were applied PBS + ethanol (9/1). A single dose of 100 mg/kg oleic acid (OA) intravenously was administrated to OA group (n= 7). One mL of corn oil was given daily to corn oil + OA group (n= 7) by gavage for five weeks. Lycopene was given by gavage to lycopene + OA group (n= 7) for five weeks. At the end of the 5th weeks, OA were given. Four hour after OA administration, lung tissue, blood samples were taken. Malondialdehyde, superoxide dismutase, glutathione-peroxidase, catalase levels were determined.

Results: Malondialdehyde levels of serum, lung tissues were increased in OA, corn oil + OA groups than control, where as decreased to controls levels in lycopene + OA group (p< 0.05). Superoxide dismutase, glutathione-peroxidase activities of serum, tissue increased moderately or they were closed with control values. There was significant increase in lycopene + OA group values. Histopathological examination of control group was normal. OA, cornoil + OA groups had perivascular, alveolar edema, hemorrhage, prominent neutrophil infiltration, destruction in alveolar structure. Lycopene + OA group had less neutrophilic infiltration, perivascular, alveolar edema.

Conclusion: Lycopene rich diet may have an important role preventing damages in lungs.

Key Words: Acute lung injury, rat model, lycopene, oxidant, antioxidant.
OA group (n= 7): ALI was performed by a single intravenous injection of 100 mg/kg OA (cis-9-octadecenoic acid; Sigma-Aldrich Germany) on the last day of examination after feeding in standard rat chow for five weeks. The suspension comprised 25 mg/mL pure OA suspended in ethanol, then 0.9% NaCl added to the suspension (ethanol/NaCl= 1/9) (6).

OA + corn oil group (n= 7): One mL of corn oil was given daily to this group by gavage for five weeks. At the end of the 5th week, a single dose of 100 mg/kg OA was administered.

Lycopene + OA group (n= 7): Twenty mg/kg/day Lycopene (Lycopene 10% FS; Roche redivivo) in the corn oil was given by gavage to this group for five weeks. At the end of the 5th week, a single dose of 100 mg/kg OA was administered.

Biochemical Analysis

Four hours after OA infusion, rats were decapitated under intramuscular 80 mg/kg ketamine anesthesia, according to the ethics guidelines, and blood samples were collected for biochemical analysis. Blood samples were centrifuged at 3500 rpm for 10 minutes, separated serum samples transferred to eppendorf tubes and stored at -80°C until analysis. Right lungs were removed, wrapped separately in aluminum foil, frozen in dry ice and stored at -80°C until the preparation of tissue homogenate samples for the measurement of malondialdehyde (MDA) levels and catalase (CAT), glutathione-peroxidase (GSH-Px), superoxide dismutase (SOD) activities.

Frozen lung tissues dissolved, washed with isotonic NaCl solution and dried in room temperature with absorbent paper. Also the wet weights of tissues were determined. Tissues were kept cold and sliced into small pieces with a bistoury and transferred to the glass tubes. 2 mL cold Tris-HCl buffer solution (pH 7.4; 0.2 M Tris-HCl buffer) was added to the tissues and this buffer used for all studies. Tissues were homogenized in 16,000 rpm for two minutes using Ultra Turrax T25 Basic (Germany) Homogenizer. The homogenization completed to three minutes by adding 4 mL buffer else. A portion of the homogenates were vortexed and transferred to eppendorf tubes. The homogenates centrifuged at 3500 g for 45 minutes at +4°C for preparing the supernatants.

Measurement of MDA levels: Serum MDA levels were measured by the thiobarbituric acid (TBA) method, which was modified from methods of Satoh and Yagi (11,12). Peroxidation was measured as the production of MDA, which in combination with TBA forms a pink chromogen compound whose absorbance was measured spectrophotometrically at 532 nm. Serum MDA results were expressed as nmol/mL. Lung tissue MDA levels were analyzed by the method of Ohkawa and expressed as nmol/mg protein (13).

Measurement of SOD activity levels: SOD activity in lung tissue and serum samples was measured according to the method of Sun et al. and modification of Durak et al. by determining the reduction of nitro blue tetrazolium (NBT) by superoxide anion produced with xanthine/xanthine oxidase system (14,15). One unit for SOD activity was expressed as the amount of protein that causes 50% inhibition in NBT reduction rate. Results were defined as units per milligram protein (U/mg protein).

Measurement of GSH-Px activity levels: GSH-Px activity in lung tissue and serum samples were measured according to the method of Paglia and Valentine, by monitoring the oxidation of reduced nicotinamide adenine dinucleotid phosphate (NADPH) at 340 nm (16). Enzyme units were defined as the number of micromoles of NADPH oxidised per minute. Results were defined as units per milligram protein (U/mg protein).

Measurement of CAT activity levels: CAT activity in lung tissue samples were determined according to the method of Aebi by measuring the decomposition of hydrogen peroxide at 240 nm (17). And results were expressed as rate constant per second per milligram protein (k/mg protein).

Histologic Analysis of the Lung

Left lungs were fixed in 10% formaldehyde. After embedding in paraffin, the tissues were cut into 3 µm sections and stained with hematoxylin-eosin methods and assessed by light microscope (Olympus BX-50, Japan).

Histological apperance of groups were graded as follows (18);

Grade 1: Normal histopathology.
Grade 2: Mild neutrophil infiltration.
Grade 3: Moderate neutrophil infiltration, perivascular edema, alveolar edema, partially destruction in alveolar structure.
Grade 4: Severe neutrophil infiltration, abscess formation, destruction in alveolar structure.

Statistical Analysis

Data are expressed as mean ± SE. For statistical analysis, the non-parametric Kruskal-Wallis test was used.
Comparisons between groups were performed using the Mann-Whitney Rank Sum test. p value < 0.05 denotes the presence of a significant statistical difference.

RESULTS

Serum MDA levels of OA group was statistically higher than control group (p< 0.05), when we compared lycopene + OA group with OA group, we saw that serum MDA levels was significantly lower in lycopene + OA group (p< 0.01). Tissue MDA levels in control and lycopene + OA groups was similar, levels of control and lycopene + OA group was significantly lower than cor- noil + OA group (p< 0.05).

Serum SOD levels was significantly higher in lycopene + OA group than control group and OA group (for both p< 0.01). Tissue SOD levels of OA group was significantly higher than control group (p< 0.01). Moreover, lycopene + OA group levels was significantly higher than other three groups (p< 0.01, p< 0.05, p< 0.01, respectively).

When evaluated serum GSH-Px levels of groups, although lycopene and cornoil + OA group levels was higher than control group, no significant difference was seen between groups. Tissue GSH-Px levels of lycopene + OA group was significantly higher than other three groups (p< 0.01, p< 0.01, p< 0.05, respectively).

Tissue CAT levels of OA, cornoil + OA, and lycopene + OA groups was significantly higher than control group (p< 0.01, p< 0.05, p< 0.01, respectively). Lycopene + OA group levels was also significantly higher than OA, and cornoil + OA groups (p< 0.01, p< 0.05, respectively).

Serum and tissue levels of MDA, SOD, GSH-Px, and tissue levels of CAT of all groups is shown on Table 1.

Histopathological examination of lung tissue were as follows; control group had a normal appearance (Figure 1), OA, cornoil + OA groups had perivascular edema, alveolar edema, hemorrhage, prominent neutrophil infiltration, and destruction in alveolar structure (Figure 2,3). Lycopene + OA group had less neutrophilic infiltration, perivascular and alveolar edema, and alveolar structure was prevented (Figure 4).

DISCUSSION

Histopathological changes in acute lung injury begins with increase in neutrophile leucocytes, and the formation of free oxygen radicals in lung cells. Lipid peroxidation is the main cause of the damage caused by free oxygen radicals in cells and tissues. Lipid peroxidation completed with converting of lipid peroxides to active aldehyde and other carbonyl compounds. MDA, alcohol, etane, pentanes were some of the compounds pro-

Table 1. Serum and lung tissue levels of MDA, SOD, GSH-Px, and tissue levels of CAT of all groups.

<table>
<thead>
<tr>
<th></th>
<th>Group I (control)</th>
<th>Group II (OA)</th>
<th>Group III (cornoil + OA)</th>
<th>Group IV (lycopene + OA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum MDA levels (nmol/mL)</td>
<td>5.59 ± 2.11*</td>
<td>9.01 ± 1.62†</td>
<td>7.12 ± 1.47</td>
<td>5.13 ± 1.96</td>
</tr>
<tr>
<td>Tissue MDA levels (nmol/mL)</td>
<td>41.62 ± 6.34⁸</td>
<td>51.48 ± 13.07</td>
<td>66.62 ± 15.28‡</td>
<td>41.11 ± 3.75</td>
</tr>
<tr>
<td>Serum SOD levels (U/mg)</td>
<td>10.60 ± 1.46</td>
<td>10.83 ± 2.54†</td>
<td>15.52 ± 5.88</td>
<td>18.51 ± 4.26§</td>
</tr>
<tr>
<td>Tissue SOD levels (U/mg)</td>
<td>7.09 ± 0.50*</td>
<td>8.71 ± 1.17†</td>
<td>8.25 ± 1.63‡</td>
<td>11.46 ± 2.71§</td>
</tr>
<tr>
<td>Serum GSH-Px levels (U/mg)</td>
<td>1.90 ± 0.25</td>
<td>1.95 ± 0.33</td>
<td>3.07 ± 1.07</td>
<td>3.02 ± 1.35</td>
</tr>
<tr>
<td>Tissue GSH-Px levels (U/mg)</td>
<td>2.04 ± 0.30</td>
<td>1.98 ± 0.32‡</td>
<td>2.10 ± 0.76‡</td>
<td>3.29 ± 0.74§</td>
</tr>
<tr>
<td>Tissue CAT levels (U/mg)</td>
<td>0.08 ± 0.02*</td>
<td>0.12 ± 0.01†</td>
<td>0.12 ± 0.04‡</td>
<td>0.20 ± 0.05§</td>
</tr>
</tbody>
</table>

* p< 0.05 when compared group I vs. group II.
# p< 0.05 when compared group I vs. group III.
§ p< 0.05 when compared group I vs. group IV.
† p< 0.05 when compared group II vs. group IV.
‡ p< 0.05 when compared group III vs. group IV.
OA: Oleic acid, MDA: Malondialdehyde, SOD: Superoxide dismutase, GSH-Px: Glutathione-peroxidase, CAT: Catalase.
duced at the end of the reaction. So, MDA is used as an indirect indicator of lipid peroxidation (19,20).

Plasma and BAL levels of MDA have been shown to increase significantly in experimental studies of acute lung injury (21,22). Septic lung injury models in rats showed the increase in lung tissue MDA levels which decreased after application of N-acetylsistein or methylene blue (18,23). Karahan et al. reported that lycopene decreased plasma and sera MDA levels that increased in experimental oxidative stress induced by cisplatin and gentamycine (24). Another study in which gastric carcinogenesis was induced by N-methyl-N-nitro–N-nitrosoguanidin showed the increase in lipid peroxidation, and the investigators reported that lycopene decreased the lipid peroxidation products in blood (25). In an acute lung injury model in rats, Köksel et al. reported the increase in MDA levels in lung tissue, plasma and BAL, and decrease in these levels after applying an antioxidant, caffeic acid phenethyl ester (CAPE) (26).

In our study, higher sera and tissue MDA levels of group II and III proved the lung injury formation, and serum and tissue MDA levels of lycopene given group (group IV) even below the levels found in the control group to suggest that lycopene prevents lipid peroxidation.

It is known that in order to prevent the lipid peroxidation in ARDS patients, increase in total antioxidant capacity and decrease in glutation specific antioxidants occurs and this causes a specific decrease in antioxidant defence of lung (27). Liu et al. determined a decrease in SOD enzyme activity at early stages of ARDS induced by oleic acide (28). In an other study conducted in ARDS patients, no changes were seen in SOD, GSH-Px enzyme activities but a little increase in CAT enzyme activities (3). In other studies carried out in ARDS patients and patients with sepsis found the increased serum levels of CAT, and SOD, decreased levels of glutation (29-31). It is also reported that decreases in GSH-
Effects of lycopene on the model of oleic acid-induced acute lung injury

PxB, SOD, and CAT enzyme activities in oxidative stress can be regulated by lycopene (25,32).

In our study, a little increase determined in tissue, and serum SOD, GSH-Px enzyme activities and tissue CAT activities in OA and corn oil + OA groups when compared with control group. However, significant increases were determined in lycopene + OA group when compared with other groups.

Dose of lycopene intake in the studies shows heterogeneity. While 6.5 mg/day lycopene decreases lung cancer risk in nonsmoker women, the dose in nonsmoker men is 12 mg/day (9). 30 mg lycopene per day is indicated for prevention of exercise-induced asthma (33). Although high levels of lycopene and other carotenoids in lungs provide an additional protection against to oxidative damage, no dose-dependent relationship between increased tomato consumption and reduced risk of lung cancer was found (34). Lower values of serum carotenoids were found in dead lung cancer patients and it is reported that carotenoid support slowed down the disease progression in lung cancer patients (35). The dose of lycopene as an antioxidant used in our study (20 mg/kg) may be accepted as an effective dose and there were no side effects reported for this dose.

Koksel et al. showed alveolar edema, congestion, neutrophil infiltration and damage in pulmonary structures in oleic acid lung injury model. These authors reported that lung injury was decreased by giving antioxidant CAPE and NAC (6,26). In an experimental model of sepsis, Ozdulger et al. determined interstitial edema, inflammatory cell infiltration and degeneration in pulmonary structure and also they reported edema, infiltration and pulmonary degeneration decreased with NAC therapy. Liu and his colleagues (18,28) have created an ARDS model in rats with OA, and they reported that pulmonary interstitial edema and pulmonary hemorrhage were reduced when SOD was given before OA application. Gultekin and colleagues have created an experimental acute pancreatitis in rats and showed neutrophil infiltration, alveolar edema, enlargement and wall thickening in lungs when decreased by giving leptin (36).

In our study, we determined that corn oil + OA, and OA groups had intense neutrophil infiltration, prominent perivascular and alveolar edema, hemorrhage, and impaired alveolar structure consistent with the literature. Lycopene + OA group showed mild neutrophil infiltration, perivascular and alveolar edema, and preserved alveolar structure, suggests that lycopene may prevent the progression of lung damage.

As a result, lycopene rich diet has an important role in preventing damages in lungs that is open to oxidative stress, and we can say that extensive clinical studies will better explain the subject.

CONFLICT of INTEREST

None declared.

REFERENCES