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The effect of lycopene on experimental myringosclerosis

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ABSTRACT

Objectives: The aim of this study was to investigate the effect of lycopene on myringosclerosis development using histopathological and immunohistochemical analyses.
Methods: Fifty-six intact tympanic membranes of 28 guinea pigs were included in the study. Subjects were randomly divided into four groups ($n = 7$/group). Group I (control group) did not receive any treatment after myringotomy. Group II (lycopene treatment after myringotomy) received oral lycopene (once daily at the same time, 10 mg/kg, dissolved in water, administered with a catheter). The treatment lasted seven days. Group III (lycopene treatment before and after myringotomy), received lycopene treatment (same dose and route of administration) for seven days. Myringotomy was performed on day 8, and lycopene treatment was initiated immediately, and continued for seven days (same dose and route of administration). Group IV (lycopene treatment before myringotomy) received lycopene treatment one week before myringotomy using the same method and dose for seven days. Myringotomy was performed on day 8. Lycopene was not administered after myringotomy.

Fourteen days after myringotomy, myringosclerosis was evaluated automicroscopically and scored. Following decapitation, bulla were removed and immersed in a 10% formaldehyde solution. Sections were cut for histopathological and immunohistochemical examination, and thickness, sclerosis, inflammation, and collagen-IV accumulation were scored semi-quantitatively.
Results: In the present study, the level of myringosclerosis was significantly lower in lycopene-treated groups compared to the control group ($p < 0.05$). In addition, thickness, inflammation, sclerosis, and collagen-IV accumulation were significantly lower in the lycopene-treated groups compared to the control group ($p < 0.05$). The timing of lycopene administration – i.e. before and/or after surgery – did not cause any difference with respect to myringosclerosis development.
Conclusion: Lycopene, a strong antioxidant, may represent a good alternative treatment to prevent the development of myringosclerosis.

Introduction

Myringosclerosis is a disease characterized by the hyaline degeneration and calcification of the collagen structure present in lamina propria of the tympanic membrane [1]. While this condition, which could present itself after myringotomy or ventilation tube application as a part of effusion otitis media (EOM) treatment, is usually asymptomatic; in particular, the formation of large sclerotic plaques may lead to hearing loss [2,3].

The human middle ear cavity has an oxygen level of 5–10% under normal conditions. Paracentesis or ventilation tube application in EOM treatment has been shown to increase the oxygen concentration in middle ear, and cause free oxygen radical formation, which in turn accelerates the inflammatory process, and leads to tissue damage, and myringosclerosis development [4–6].

Lycopene, which is found in fruits, vegetables, and green plants, is a member of the carotenoid family [7–9]. Acting as an antioxidant, it scavenges the reactive oxygen species (ROS), and regulates the immune system by reducing the oxidative stress damage [10]. In vitro studies on the antioxidant effect of lycopene have shown that it inactivates the free oxygen radicals hydrogen

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peroxide (H_2O_2) and nitric oxide (NO), and protects lymphocytes against the membrane damage and cell death brought by NO radicals [11]. The plasma depletion half-life of lycopene was between 2 and 3 days [12,13].

The aim of this study was to evaluate the effect of lycopene, an antioxidant molecule, on myringosclerosis development in an experimental guinea pig model, using automicroscopic, histopathological, and immunohistochemical methods.

Material and methods

Approval for the experimental animal study was obtained from Fırat University Animal Experiments Ethics Committee (date: 22.11.2011, no: 129). Twenty-eight adult guinea pigs weighing between 400 and 800 g, with intact external auditory canal and tympanic membrane (TM) (Veterinary Control and Research Institute) were included in the study. The animals were divided into four groups ($n = 7$ animals/group, 14 ears/group) (Table 1):

Group I (Control group, n = 14): Fourteen TMs from seven guinea pigs did not receive any treatment after myringotomy.

Group II (Lycopene treatment after myringotomy, n = 14): Following myringotomy, 14 TMs of seven guinea pigs received 10 mg/kg of lycopene (dissolved in water, orally administered via orogastric canula, once daily at the same time) starting from day 1. The treatment lasted for seven days.

Group III (Lycopene treatment before and after myringotomy, n = 14): Prior to myringotomy, 14 TMs of seven guinea pigs received 10 mg/kg of lycopene (dissolved in water, orally administered via orogastric canula, once daily at the same time). The treatment lasted for seven days. Myringotomy was performed on day 8, and lycopene administration was continued as described above.

Group IV (Lycopene treatment before myringotomy, n = 14): One week before myringotomy, 14 TMs of seven guinea pigs received 10 mg/kg of lycopene (dissolved in water, orally administered via orogastric canula, once daily at the same time). The treatment lasted for seven days, and myringotomy was performed on day 8. Lycopene treatment was discontinued after myringotomy.

Anesthesia was performed using intraperitoneal ketamine hydrochloride (Ketalar®, Pfizer Warner Lambert, USA; 50 mg/kg) and xylazine hydrochloride (Ksilazol, PROVET Veterinary Products Industry Istanbul, Turkiye; 5 mg/kg) injection, and then standard myringotomy (1 mm in diameter) was performed in the lower front quadrant of both TMs under automicroscopic view, using an ear speculum and a sterile myringotomy blade (D-481, D-242, Aygun Surgical Instruments, Samsun, Turkiye) in accordance with the timing of lycopene treatment (Fig. 1).

Myringosclerosis evaluation was performed 14 days after myringotomy (Fig. 2). Accordingly, specimens were assigned a score of (0) if there was no visible sclerotic lesion under automicroscopic view, a score of (+) if only a sclerotic lesion was localized in a neighboring position to the malleus arm, a score of (++) if there was a lesion on the upper front quadrant in addition to the sclerotic lesions neighboring the malleus arm, and a score of (+++) if there were wide, sclerotic lesions extending from the malleus arm to the annulus, and transversing along the annulus [14] (Fig. 3).

Table 1
Distribution of groups.

Groups	Lycopene application
Group I ($n = 7$, 14 ears)	No
Group II ($n = 7$, 14 ears)	After myringotomy (during 7 days)
Group III ($n = 7$, 14 ears)	Before (during 7 days) and after myringotomy (during 7 days)
Group IV ($n = 7$, 14 ears)	Before myringotomy (during 7 days)

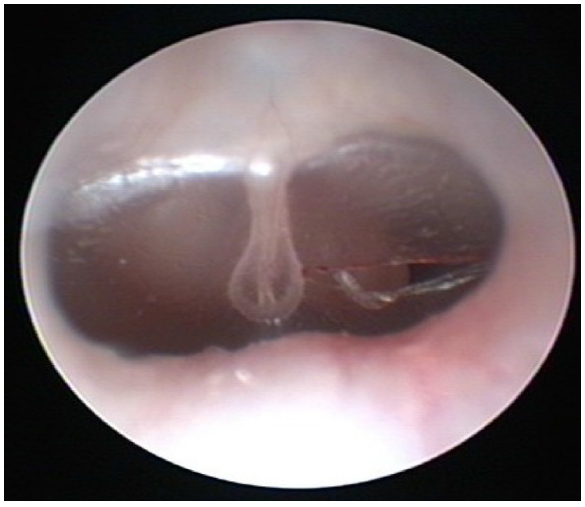

Fig. 1. Myringotomy under automicroscopic view (right tympanic membrane).

Following automicroscopic evaluation, subjects were injected with high-dose intraperitoneal pentothal (Pental® Sodium, I.E. Ulagay Ilac Sanayi, Istanbul, Turkiye), and decapitated. The bullae were then removed, and bulla specimens were immersed in 10% formaldehyde solution, and stored for histopathological examination.

Histopathological and immunohistochemical evaluation

Histopathological preparations and evaluations were performed in Department of Pathology at Fırat University Medical Faculty by the same pathologist. Following formol fixation, specimens were incubated in formic acid for decalcification, and were divided into two so that they would lay perpendicular to the manubrium mallei, and pass through the myringotomy area. Paraffin blocks were prepared by tilting two pieces toward the section surface. Five micrometer-thick sections were histopathologically and immunohistochemically stained with hematoxylin–eosin (H&E), Masson's trichrome (MT), Ki-67, collagen-IV, and CD-34. Immunohistochemical stainings were performed on an automated instrument (Benchmark-XT, Ventana Medical Systems, California, USA).

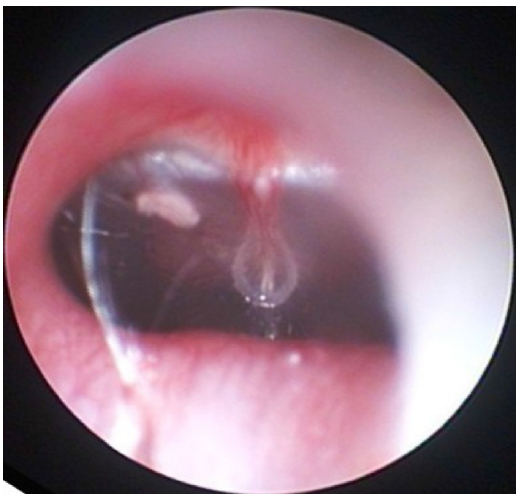

Fig. 2. Myringosclerotic focus during automicroscopic evaluation (left tympanic membrane).