Experimental Study / Deneysel Çalışma

The effect of intraperitoneal administration of zinc aspartate on myringosclerosis in perforated tympanic membranes of rats

Sıçanlarda perfore timpanik membrandaki miringosklerozda intraperitoneal çinko aspartat uygulamasının etkisi

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Objectives: The objective of the present study was to determine the effect of zinc aspartate on myringosclerosis in perforated rat tympanic membrane.

Patients and Methods: Fifteen Sprague-Dawley rats were randomly divided into three groups each containing five rats. Automicroscopic examinations were performed and then all rats were bilaterally myringotomized. Group 1 received no treatment. Group 2 was treated with intraperitoneal injection of physiological saline and group 3 with intraperitoneal injection of zinc aspartate. Tympanic bullas were harvested after 20 days. Histopathological evaluation was carried out under the light microscope.

Results: When the groups were compared in the light of the myringosclerotic findings, while there was no significant difference between group 1 and 2 (p=1.00), it was found that there were significant differences between group 1 and 3, and between group 2 and 3 (p<0.03).

Conclusion: It appears that zinc aspartat treatment has beneficial effects on prevention or retardation of the development of myringosclerosis, but further studies are needed to clarify this effect.

Key Words: Myringosclerosis; rat tympanic membrane; zinc aspartate.

Amaç: Bu çalışmada sıçan perfore timpanik membranındaki miringosklerozda çinko aspartatın etkisi araştırıldı.

Hastalar ve Yöntemler: Sprague-Dawley cinsi 15 adet sıçan her bir grupta beş sıçan olacak şekilde rastgele üç gruba ayrıldı. Otomikroskopik muayeneleri yapıldıktan sonra sıçanlara iki taraflı miringotomi uygulandı. Grup 1'e hiçbir tedavi uygulanmadı, grup 2'ye intraperitoneal serum fizyolojik ve grup 3'e ise intraperitoneal çinko aspartat uygulandı. Timpanik bullalar 20 gün sonra çıkarıldı. Işık mikroskobu altında histopatolojik inceleme yapıldı.

Bulgular: Miringosklerotik bulgulara göre gruplar karşılaştırıldığında, grup 1 ve 2 arasında anlamlı bir farklılık bulunmadı (p=1.00), grup 1 ile 3 arasında ve grup 2 ile 3 arasında anlamlı farklılıklar bulundu (p<0.03).

Sonuç: Çinko aspartat tedavisi miringosklerozun önlenmesi veya azaltılmasında yararlı etkilere sahip gibi görünüyor, fakat bu etkinin ortaya çıkarılması için daha ileri çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: Miringoskleroz; sıçan timpanik membran; çinko aspartat.

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Myringosclerosis (MS) is a prevalent entity affecting the tympanic membrane (TM). It is associated with some conditions like myringotomy, acute and/or chronic otitis media, otitis media with effusion, and trauma. It is also frequently seen after the treatment of serious otitis media through ventilation tube insertion.[1-4] In most cases, it is generally asymptomatic or associated with mild hearing loss. On the other hand, if the sclerotic plaques influence large areas of the TM or adhere to the bony annulus, ossicles or promontorium, MS may lead to marked hearing loss.[1,5] Though some hypotheses have been proposed to clarify the pathogenesis of MS, the most prominent factor in its development is an inflammatory reaction in the collagen layer of the TM. A recent study has shown that oxygen-derived free radicals and mechanical injury may be the main causative factors in the development of MS.[2] Also, previous studies reported that experimental myringotomy caused the formation of MS in the TM of rats and this myringosclerotic formation could be reduced by application of various free radical scavengers.[2,5-9]

Metal aspartates including zinc aspartate are inhibitors of oxygen free radicals.[10] Zinc aspartate treatment has been shown to reduce ischemia-reperfusion injury by its antioxidant effects after unilateral testicular torsion-detorsion in rat.[11] Mattsson et al.[2] demonstrated that topically applied scavengers like copper zinc-superoxide dismutase plus catalase and deferoxamine inhibit or reduce the development of sclerotic lesions.

In this study, we aimed at investigating the preventative effect of parenteral administration of zinc aspartate on MS in myringotomized rats.

PATIENTS AND METHODS

Fifteen Sprague-Dawley rats weighting 250-280 gr. were used in this study. All interventions to animals were performed under sterile conditions and the study protocol was approved by the Ethics Committee of Kahramanmaraş Sütçü İmam University Faculty of Medicine.

Animals were anesthetized with intramuscular injections of ketamine hydrochloride (40 mg/kg) and xylazine (5 mg/kg). Under the otomicroscope and through an aural speculum, the upper posterior quadrant of the TMs in both ears of rats was perforated with a myringotomy knife. The animals were later randomly divided into three groups, each containing five animals. Group 1 received no treatment. Group 2 were treated with intraperitoneal injections of physiological saline and group 3 with intraperitoneal injection of zinc aspartate at a dose of 30 mg/kg for 10 days [DL-hydrogen aspartate; zinc aspartate (ZA) 30 mg/kg, UNIZINC, Köhler Pharma, Germany] starting on the day of surgery. Twenty days after the myringotomy, the rats were again anaesthetized and TM examinations were blindly performed by an ear-nose-throat specialist using an otomicroscope. Then the rats were sacrificed through intraperitoneal thiopental sodium injections and decapitated. The bullas were removed under otomicroscopy, fixed with a 10% formaldehyde solution and then decalcified with 7% nitric acid solution. After the routine processing, 4 mm thick sections were stained with hematoxylin-eosin. On the light microscopic examination, the degree of sclerosis and the intensity of fibroblastic proliferation in the lamina propria of TMs were semiquantitatively evaluated as follows: none (no visible MS), occasional (a few lesions in the lamina propria), moderate (single and confluent lesions in the lamina propria), and severe (extensive confluent lesions in the lamina propria).

The Fisher's exact test was used for comparisons between the groups for sclerosis. $P<0.05$ values were accepted as significant.

RESULTS

On comparison of the groups based on the histopathologic findings, while there was a more intense fibroplastic proliferation and sclerosis in the lamina propria of rat TM in both control and saline groups (Fig. 1, 2), there was only a mild or moderate fibroplastic proliferation and sclerosis in the lamina propria of the rats' TM in the zinc aspartate group. Here, the normal tympanic membrane without fibroplastic proliferation and sclerosis is given for purposes of comparison (Fig. 3).

When the groups were compared based on the myringosclerotic findings, while there was no statistically significant difference between the groups 1 and 2 ($p=1.00$), there were significant differences both between the groups 1 and 3, and 2 and 3 ($p<0.03$).

The histopathological findings of myringosclerosis in the zinc, control and saline groups are shown in Table 1.

DISCUSSION

Several hypotheses have been proposed to clarify the pathogenesis of MS, and the most prominent

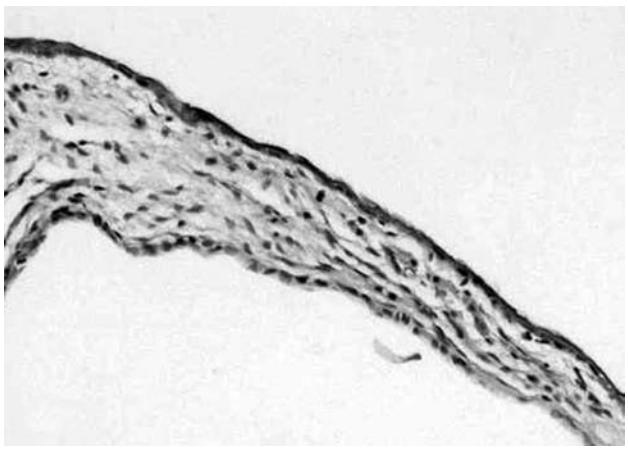

Fig. 1. There was more intense fibroplastic proliferation and sclerosis in lamina propria of rat in control group (H-E x 200).

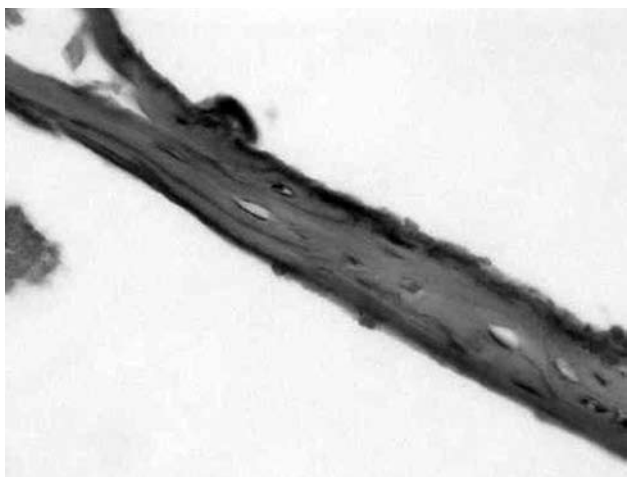

Fig. 2. There was more intense fibroplastic proliferation and sclerosis in lamina propria of rat in saline group (H-E x 400).

factor in its development is an inflammatory reaction in the collagen layer of the TM.[12] Insertion of a ventilation tube through the tympanic membrane admits ambient air into the middle ear cavity, causing relative hyperoxia.[13] A previous study claimed that hyperoxia causes formation of free oxygen radicals, which may exacerbate an inflammatory process.[14] In a study by Mattsson et al.,[15] three groups of rats with myringotomized tympanic membranes were exposed to different oxygen concentrations of

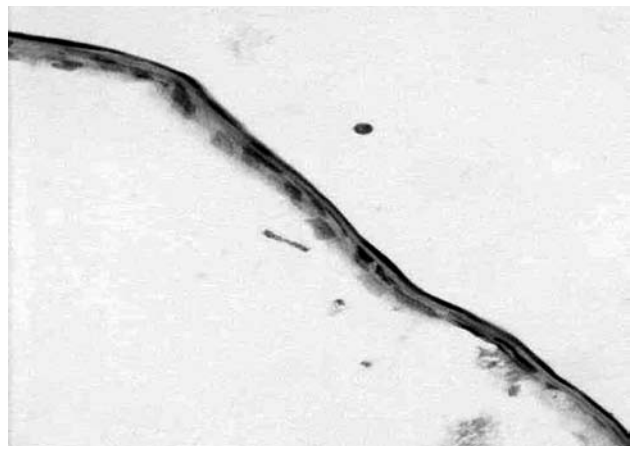

Fig. 3. Normal tympanic membrane without fiproplastic proliferation and sclerosis (H-E x 200).

10%, 15%, and 40% for one week. A fourth group was kept in ambient air. All hyperoxic animals had more myringosclerotic lesions compared with the ambient air group and the hypoxic animals showed less pronounced myringosclerotic lesions. They concluded that increased oxygen concentrations in the middle ear cavity will increase the likelihood of myringosclerotic deposits. They also suggested that the mechanisms involved could be related to the formation of oxygen radicals. Hyperoxia in the tympanic cavity may lead to excessive production of oxygen free radicals, which may initiate the process involved in the development of sclerotic plaques.[16] In an experimental rat model with N-acetylcystein (NAC), Özcan et al.[17] found that the topical treatment of N-acetylcystein reduced the levels of malondialdehyde (MDA) and nitric oxide (NO) production, and these results suggest that topical NAC application may be useful for the prevention of MS.

The use of certain antioxidant substances inhibited the development of MS by their oxygen free radical scavenger effects. Mattsson et al.[2] demonstrated that topically applied scavengers like copper zinc-superoxide dismutase plus catalase and deferoxamine inhibit or reduce the development of sclerotic lesions. In a study about topical ascorbic

Table 1. Histopathological findings of myringosclerosis in the zinc, control and salin groups

Group	n*	Seriously MS	Moderate MS	Mild MS	No MS
Control (group 1)	10	3	5	2	–
Salin (group 2)	10	2	6	2	–
Zinc (group 3)	10	–	2	3	5

n*: Number of subject; MS: Myringosclerosis; No MS: No visible MS.

acid application, myringosclerosis developed in all of the ears in the saline group while it developed only in two out of nine ears where ascorbic acid was used.[6] In rats, Özcan et al.[8] reported that MS development was statistically significantly decreased in the NAC group compared to the control and saline groups. Görür et al.,[7] on the other hand, showed that intraperitoneal selenium in rats decreased the myringosclerosis development in comparison to saline. In an experimental rat model with intraperitoneal L-carnitine and saline, Akbaş et al.[5] found that the L-carnitine group had 35% sclerosis while the control group had 80%. In the present study, the intraperitoneal administration of zinc aspartate significantly reduced MS in myringotomized rats.

Metal aspartates, including the zinc aspartate, are inhibitors of reactive oxygen species.[10] Their inhibitory activities are the consequence of both the scavenging of free radicals and the inhibition of xanthine oxidase and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activities.[10,11] Özkan et al.[11] have shown that parenteral zinc aspartate treatment reduces ischemia-reperfusion injury by its antioxidant effects after unilateral testicular torsion-detorsion in rat. In a study, it was suggested that immediate administration of zinc aspartate and its continuation for some period could prevent the progression of the injury and improve the healing process.[18] After the free radical damage is reduced in many organs such as the stomach, intestines, brain, and the retina by the zinc complex, the interest was drawn to zinc compounds, which traditionally are used for the treatment of skin ailments, suppresing inflammation and accelerating wound healing.[19-22] Another study showed that the treatment with copper zinc-superoxide dismutase plus catalase and deferoxamine inhibited or reduced the development of myringosclerosis, whereas the ears treated with copper sulfate plus iron chloride appeared unaffected.[2] In the present study, less MS formation was observed in the group treated with parenteral zinc aspartate than in the control and physiological saline groups. These findings suggest that zinc aspartate has a beneficial effect on the prevention of MS formation, an effect that may depend on the antioxidant effects of zinc aspartate.

In conclusion, it appears that zinc aspartate used parenterally in this study has beneficial effects on the prevention or retardation of myringosclerotic formations. Further studies in larger groups are needed to elucidate the potential role of zinc aspartate in the prevention of myringosclerotic formation.

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