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EXPERIMENTAL STUDY

Does topical N-acetylcysteine application after myringotomy cause severe otorrhea?

Miringotomi sonrası uygulanan topikal N-asetilsistein ciddi otore sebebi midir?

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Objectives: The effect of topical N-acetylcysteine (NAC) application was investigated on the healing of acute experimental tympanic membrane perforations.

Materials and Methods: Twenty guinea pigs were used in this study. Under intraperitoneal ketamine anesthesia, incisional myringotomies were performed in the posterosuperior quadrant of the tympanic membranes with a straight otologic hook. The diameter of the perforations was approximately 2 mm. Perforations in both ears were treated with freshly prepared sponges soaked in either 0.1 ml 0.9% NaCl solution (10 control animals) or 0.6 mg/0.1 ml NAC (10 animals) for three consecutive days. All the tympanic membranes were examined by otomicroscopy on the third, fifth, seventh, and ninth days.

Results: In the control group, all the perforations were completely closed at the end of nine days. During the same period, only 40% of the perforations were completely closed in the NAC group. The remaining ears exhibited otorrhea by the third day.

Conclusion: N-acetylcysteine may cause severe otorrhea in the healing process of tympanic membrane perforations. Further studies including histopathological examinations are required to elucidate this condition.

Key Words: Acetylcysteine; ear, middle/surgery; middle ear ventilation; tympanoplasty.

Amaç: Akut, deneysel, travmatik timpan membran perforasyonlarının iyileşmesinde N-asetilsistein kullanımının etkileri incelendi.

Gereç ve Yöntem: Çalışmada 20 hayvan (guinea pig) kullanıldı. İntraperitoneal ketamin anestezisi altında, düz otolojik hook yardımıyla timpan membranların posterosuperior kadranlarına insizyonel miringotomi yapıldı. Perforasyonların çapı yaklaşık 2 mm idi. Kobaylar iki gruba ayrıldı. Kontrol grubuna (10 hayvan, 20 kulak) 0.1 ml %0.09 NaCl emdirilmiş spongostanlar, çalışma grubuna ise 0.6 mg/0.1 ml N-asetilsistein emdirilmiş sponglar her gün değiştirilerek üç gün boyunca dış kulak yolundan perforasyon üzerine yerleştirildi. Her iki grubun timpan membranları 3, 5, 7 ve 9. günlerde kulak mikroskobu ile değerlendirildi.

Bulgular: Kontrol grubundaki perforasyonların tamamının dokuzuncu günde kapandığı izlendi. N-asetilsistein uygulanan grupta perforasyonların ancak %40'ı kapandı, geri kalan kulakların tümünde üçüncü günden itibaren otore izlendi.

Sonuç: Timpan membran perforasyonlarının iyileşmesinde N-asetilsistein kullanımı yoğun otore sebebi olabilir. Bu konuyla ilgili histopatolojik incelemeleri de içeren daha kapsamlı çalışmalar gerekmektedir.

Anahtar Sözcükler: Asetilsistein; orta kulak/cerrahi; orta kulak ventilasyonu; timpanoplasti.

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Perforation of the tympanic membrane (TM) most commonly arises as a result of either otitis media or trauma. Clinically the most common manifestations of TM perforation are conductive hearing loss and chronic infection.

Most acute TM perforations (90%), especially those involving less than a quarter of the TM area, close spontaneously.[1-4] Perforations in which a greater area of the TM is involved and/or accompanied by pathologic changes in the middle ear require surgical closure. Alternative to surgery, topical hyaluranic acid, growth factors such as epidermal and fibroblast were recently reported as successful in the treatment of traumatic TM perforations.[5-9]

N-acetylcysteine (NAC) is a chemical agent which has anti-inflammatory, mucolytic and free radical scavenging properties. N-acetylcysteine exhibits these effects via its biochemical structure which includes a free thiol group.

The goal of this study was to investigate the effects of NAC on the healing of acute experimental traumatic TM perforation by observing the duration of perforation closure clinically.

MATERIAL AND METHODS

We performed the study in 20 healthy guinea pigs. The animals weighed between 750-1000 g. All animals were anaesthetized with ketamine hydrochloride (50 mg/ml) intraperitoneously and isoflurane. After anesthesia, incisional myringotomies were performed on the posterosuperior quadrant of the tympanic membranes with straight otologic hook (Fig. 1). The mean diameter of the perforations was approximately 2 mm. All of the myringotomies were performed with the operating microscope. The guinea pigs were divided into two groups. The control group (10 animals, 20 ears) received 0.1 ml topical 0.9% NaCl solution soaked in spongostan and the study group (10 animals, 20 ears) received 0.6 mg/0.1 ml topical NAC soaked in spongostan into the external ear canal daily for 3 days. The tympanic membranes were examined in the study and control groups with otomicroscopy at 3rd, 5th, 7th, and 9th days under brief isoflurane anesthesia. Myringotomy patency was recorded during each observation. The presence of otorrhea and crusting was also noted. The closure times of myringotomy sites of the study and control groups were evaluated with "Fisher's exact chi-square" test.

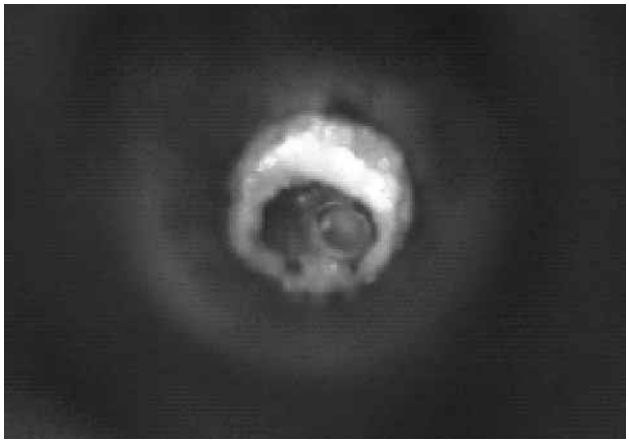

Fig. 1 - *The myringotomies were performed on posterosuperior quadrant of the tympanic membranes.*

RESULTS

In the control group, 100% of the perforations were completely closed by 9 days. In the NAC group, 40% of the perforations were completely closed by 9 days; the remaining of the study group had still otorrhea beginning from the third day. No otorrhea was observed in the control group. Significant differences were observed between two groups in the number of otorrhea ($p<0.001$). The healing scheme for each group is drawn in Fig. 2.

DISCUSSION

Topical application of NAC was recommended in otolaryngology practice for resolving middle ear effusions or for preventing myringosclerosis.[10] During myringotomy, as a result of hyperoxic condition, there will be an increased production of O_2 derived free radicals.[11,12] Oxygen radicals cause tis-

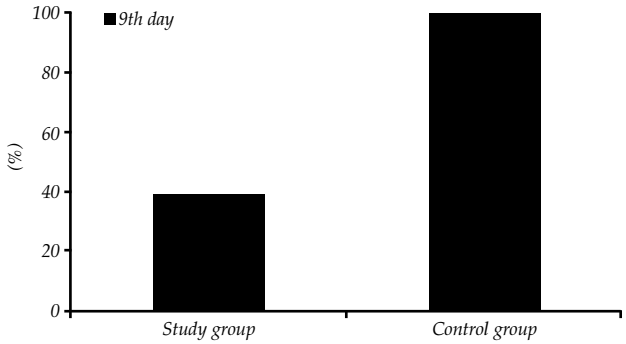

Fig. 2 - *N-acetylcysteine does not improve the healing of the tympanic membrane.*

sue damage by lipid peroxidation of cellular and organelle membranes, distruption of intracellular matrix and alteration of important protein enzymatic processes. These agents not only damage the lipids but also produce lipid hydroperoxides, secondary intermediates that can lead to a chain reaction of lipid peroxidation.[13-15]

Nitric Oxide (NO) is a small, short-lived oxygen free radical involved in cellular signaling and known to play a role in inflammation. Kampf and Roomans[16] demonstrated that the antioxidant NAC inhibited NO production. Pepperl et al.[17] indicated that hyperoxia can up regulate the NO pathway in stimulated alveolar macrophages through the increased production of intracellular reactive oxygen species. N-acetylcysteine inhibited the NO pathway both in normotoxic and hyperoxic conditions. Ozcan et al.[10] found an increase in NO levels after myringotomy and they demonstrated that NAC prevents the increase of NO levels.

In this study, we hypothesized that during myringotomy the production of free radicals increases and these radicals cause tissue damage and prevent the healing of the TM. N-acetylcysteine has a free radical scavenging property and by application of NAC topically, we could decrease the effect of free radicals on the healing process. However; NAC did not significantly affect the speed of TM healingand the normal sequence of healing in TM was altered because of severe otorrhea.

These results may be explained by some other effects of NAC. Ovesen et al.[18] described cytotoxic effects of NAC in a dose dependent way on phagocytes and fibroblasts. Kharazmi et al.[19] found that NAC at concentrations higher than $3x10^{-2}$ M causes cytotoxicity on neutrophils and monocytes. Some opthalmologists described the toxic effects of NAC in different concentrations in corneal wound healing.[20] Another explanation of severe otorrhea may be reducing the NO levels. Although NO is a free radical and causes tissue damage, it has ability to kill pathogens and it may be important in the host defense against ear infections.[21] Ovesen et al.[22] described increased otorrhea in their patients receiving topical NAC against placebo for otitis media with effusion. Interestingly, the dosage and the application time of NAC used in these investigations, was higher than our dosage and application time.

Finally; topical application of NAC does not improve the TM healing. On the contrary, it may cause severe otorrhea. For this reason it may delay the healing process of the TM.

CONCLUSION

Topical application of NAC was recommended in otolaryngology practice for resolving middle ear effusions or for preventing myringosclerosis. In this study, we presented a clinical observation in the healing process of TM perforations and we determined that NAC may cause otorrhea. Further studies with histopathological examination are warranted.

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