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**HISTOLOGIC STUDY**

# The effect of systemic prednisolone on propylene glycol-induced otitis media in guinea pig

## Guinea pigde propilen glikol ile oluşturulan otitis mediada sistemik prednisolonun etkisi

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**Objectives:** In this histopathologic study, we investigated the effect of systemic prednisolone on propylene glycol-induced otitis media in guinea pig ear.

**Materials and Methods:** Ten adult guinea pigs received systemic prednisolone (10 mg, IM, single dose) together with a single application of 60% propylene glycol (0.2 ml, per ear) to both middle ears. Ten control guinea pigs were given a single application of 60% propylene glycol (0.2 ml) in the left ear, and a single application of saline (0.2 ml) in the right ear. Four weeks later, the animals were sacrificed and temporal bones were removed and prepared for histological evaluation. Paraffin-embedded specimens were horizontally sectioned at 7 microns. One of each five sections was stained with hematoxylin and eosin, and studied under light microscopy.

**Results:** In the control group, otitis media developed in six of nine propylene glycol-administered ears (66%), whereas all saline-treated ears remained intact. In the prednisolone group, only one (6%) of 16 ears developed otitis media and cholesteatoma. The difference was statistically significant.

**Conclusion:** Our results suggest that systemic prednisolone has a potent effect to prevent propylene glycol-induced otitis media in guinea pig ears.

*Key Words:* Otitis media/chemically induced; prednisolone/therapeutic use; propylene glycols/toxicity.

**Amaç:** Bu çalışmada guinea pig orta kulağında propilen glikol ile oluşturulan otitis mediada sistemik prednisolonun etkisi histopatolojik olarak araştırıldı.

**Gereç ve Yöntem:** Çalışma grubunda sistemik prednisolon (10 mg, İM, tek doz) uygulanan 10 adet erişkin guinea pigin her iki ortakulağına tek doz, 0.2 ml %60 propilen glikol verildi. Kontrol grubundaki 10 guinea pigin sol kulaklarına tek doz 0.2 ml %60 propilen glikol, sağ kulaklarına tek doz 0.2 ml saline uygulandı. Propilen glikol uygulamasından dört hafta sonra hayvanların yaşamı sonlandırıldı. Çıkarılan temporal kemikler histopatolojik inceleme için hazırlandı. Parafine gömülen örneklerden horizontal planda yedi mikronluk kesitler alındı. Her beş kesitten biri hemotoksilen-eosin ile boyanıp, ışık mikroskobu ile incelendi.

**Bulgular:** Kontrol grubunda saline uygulanan kulaklarda patolojik değişiklikler görülmezken, propilen glikol uygulanan dokuz kulağın altısında (%66) otitis media gelişti. Prednisolon grubunda ise 16 kulağın sadece birinde (%6) otitis media ve kolesteatoma gelişimi gözlendi. Bu fark anlamlı bulundu.

**Sonuç:** Sonuçlarımız, guinea pig kulağında propilen glikol ile oluşturulan otitis medianın sistemik prednisolon tedavisiyle önlenebileceğini göstermiştir.

*Anahtar Sözcükler:* Otitis media/kimyasal yolla oluşturulan; prednizolon/terapötik kullanım; propilen glikol/toksisite.

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Several studies on otitis media and cholesteatoma formation following intratympanic application of chemical irritants in laboratory animals have been published. In ethiopathogenetic studies, talc-fibrin mixtures, quinine, streptococcus pneumonia, cortisporin otic suspension were used as substances or Eustachian tube obstruction and external ear canal ligation as procedures.[1,2] Cortisporin utilizes propylene glycol as a solvent and penetrance enhancer. Several laboratory studies have shown that propylene glycol alone produces marked inflammatory changes in the middle ear cavity that lead to cholesteatoma formation in laboratory animals.[3-5]

In many studies systemic and local anti-inflammatory agents were used to investigate their effects in inhibiting inflammation and the development of otitis media and cholesteatoma in middle ears treated propylene glycol. The effect of ventilation tube implantation, systemic isoretinoinin, systemic cyclophsphamide, topical hyoluronic acid and topical steroids on development of propylene glycol-induced otitis media and cholesteatoma were investigated in animal experiments using chinchilla and/or rat.[1,6-9] It was concluded that 5- fluorouracil does tend to reduce the proliferation of tympanic membrane epidermis and connective tissue, thereby reducing the likelihood of cholesteatoma formation.[10] Only Sennaroglu et al.,[9] have shown that topical prednisolone application was effective on propylene glycol-induced otitis media and hence experimental cholesteatoma production in rat ear.

Corticosteroids are known as the most potent anti-inflammatory drugs. They inhibit all types of allergic, inflammatory or immunologic responses.

The present study was designed to evaluate the effect of systemic prednisolone, as a systemic anti-inflammatory agent, on the development of otitis media following propylene glycol application in guinea pig ear.

## MATERIALS AND METHODS

Twenty adult guinea pigs (10 control, 10 study group), weighing from 500 to 870 g. were included. Animals were anesthetized with 30 mg of subcutaneous ketamine hydrochloride. The 0.2 ml of 60% propylene glycol solution or saline was instilled into the middle ear by injection through a small hole made in the superior aspect of the bulla. Propylene glycol was given to study group animals bilaterally, and to left ears of control group animals with a single application. Saline was given only to right ears of control group animals with a single application. Control materials for this study were obtained from a previous investigation.[11] Tympanic membranes were intact. Periosteum, subcutaneous tissue and skin were sutured with absorbable thread. After these procedures, each animal from the study group was administered one dose of 10 mg/methyl prednisolone acetate, by intramuscular injection. Chloramphenicol sodium succinate, 10 mg, was subcutaneously given to all animals, every day during 10 days, to prevent post surgical infections. Eight animals in the study group, and 9 animals in the control group were alive 4 weeks following the application of solutions.

Four weeks after the application of propylene glycol, the animals were deeply anesthetized with pentobarbital (60 mg/kg, subcutaneously) and decapitated. After otoscopic examination, the temporal bones were removed. The temporal bones were then processed for examinations under light microscopy. Each temporal bone was fixed in 10% formalin for a week, then decalcified in 10% formic acid for three weeks. After each was embedded in paraffin, specimens were serially sectioned horizontally to obtain sections 7 micron thick. One of every five sections was stained with hematoxyline and eosin, mounted on a glass slide, and studied under light microscopy. Histological findings and anatomical details were noted. Before starting the investigation, the experimental protocol for this research was reviewed and approved by the Institutional Review Board for Experimental Medical Research at the University of Istanbul in Istanbul, Turkey.

## RESULTS

In the control group, nine of the middle ears (100%) treated with saline and 3 of the middle ears (33%) treated with propylene glycol exhibited normal anatomic details (Table I).

In the experimental group that received prednisolone, 14 middle ears (87%) treated with propylene glycol were normal.

### Inflammatory findings in the middle ear

Six of the nine ears (66%) in the control group treated with propylene glycol developed otitis media. Five ears showed granulation tissue and one ear

TABLE I

PATHOLOGIC FINDINGS IN GUINEA PIG MIDDLE EAR AND COCHLEA

| Treatment | Control group Ear no: 9 Normal saline | Control group Ear no: 9 Propylene glycol | Prednisolone group Ear no: 16 Propylene glycol |
|---|---|---|---|
| Findings | | | |
| In the middle ear | | | |
| Normal | 9 | 3 | 14 |
| Inflammation: | – | 6 | 1 |
| Granulation tissue | – | 5 | 1 |
| Effusion | – | 1 | 1 |
| Perforated tympanic membrane | – | 1 | 1 |
| Thickening bone | – | 5 | – |
| Osteogenesis | – | 5 | – |
| Cyst formation in bone of bulla | – | 5 | – |
| Adhesion | – | 1 | 1 |
| Fibrosis | – | – | 1 |
| Keratinizing epithelium and cholesteatoma | – | – | 1 |
| Edema | – | – | 1 |
| Hemorrhage | – | – | 1 |
| In cochlea: | | | |
| Fibrosis and reactive osteogenesis | – | – | 1 |

showed purulent effusion as a histological evidence of inflammation in this group (Fig. 1).

In the study group that received prednisolone only one ear (6%) treated with propylene glycol had purulent effusion.

**Morphological changes in the middle ear**

In the control group treated with propylene glycol, one ear had perforated tympanic membrane, one ear had middle ear adhesion, five ears had thickening of the bone, new bone formation (osteogenesis) and cyst formation in bone of bulla.

In the study group that received prednisolone one ear had perforated tympanic membrane, adhesion and fibrosis, and another one ear had edema and hemorrhage without inflammation (Fig. 2).

**Presence of keratinizing epithelium in the middle ear**

As an unexpected result, none of the ears treated with propylene glycol in the control group had keratinizing squamous epithelium or cholesteatoma. In the prednisolone group, one ear showed keratinizing squamous epithelium within the middle ear cavity on a layer of granulation tissue (Fig. 3, 4).

**Morphological changes in the cochlea**

In the study group that received prednisolone, one ear showed fibrosis and reactive l finding in cochlea.

## DISCUSSION

The effects of topical propylene glycol on pathologic conditions of the middle and inner ear have been investigated by several clinical investigators. When discussing the results of former studies, it should be noted that it is not healthy to compare our study with other studies because these studies have worked with different experimental animals and used different combinations and concentrations of chemical irritant propylene glycol and different surgical techniques. Although the initial interest focused on the inner ear effects, pathologic changes were found in the middle ear as well. Morizono et al.[12] reported a reduction in cochlear micro phonics after the application of propylene glycol in the middle ear of animals. Also, this inflammatory response involved all connective tissues in the ear. While, the bone of bulla was thickened, reactive new bone formation were observed. They demonstrated that propylene glycol produces chronic inflammation to

the middle ear and tympanic membrane, leading to hyperplasia of tympanic membrane epidermis and eventual epidermal invasion through the intact lamina propria into the middle ear cavity and keratinizing to produce cholesteatoma.[3-5,13,14] In accordance with these previous studies, in this study, ears showed inflammatory changes of the middle ear mucosa and bone, such as granulation tissue, inflammation, thickening of bone, osteogenesis, cyst formation in bone of bulla, fibrous adhesions, perforation of tympanic membrane, and in the inner ear, such as fibrosis and reactive osteogenesis. But, we observed as unexpected results, that inflammatory changes did not lead to cholesteatoma formation and inner ear changes were not found in guinea pigs after the application of propylene glycol and in control group and in prednisolone group one ear showed fibrosis and reactive osteogenesis in cochlea. Previous works have shown that middle ear application of propylene glycol in guinea pigs or chinchillas produces inflammatory changes depend on concentration or number of repeated application 2, 10.5, 50, 60 and 90%.[1,4,5,10] Vassali et al.[4] found development of otitis media in 6 of 7 ears and cholesteatoma in 4 of 7 ears treated with 10.5% concentration of propylene glycol and they found development of otitis media in 2 of 7 ears and no cholesteatoma treated with 2% concentration of propylene glycol. Masaki et al.[5] used a ingle application of 60% propylene glycol to produce chronic inflammation of the middle ear and tympanic membrane. Other studies focused on the development of

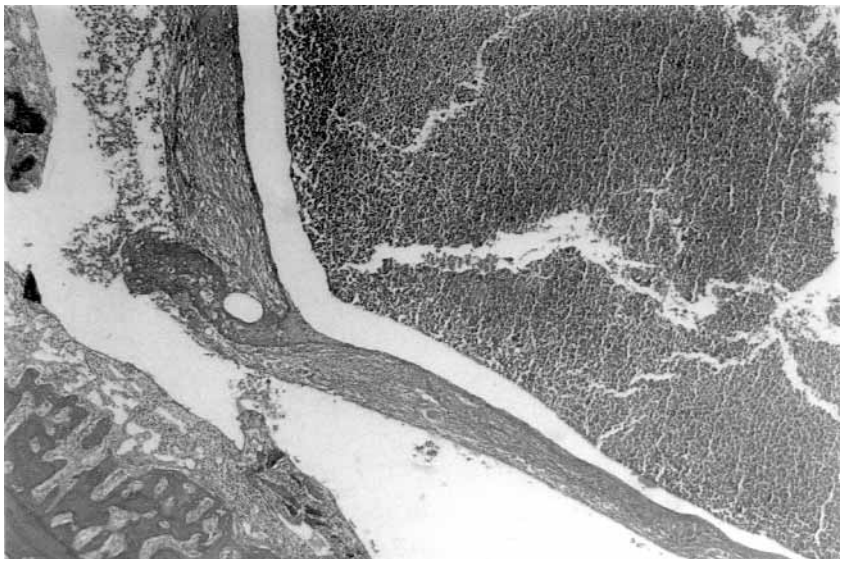

***Fig. 1 -*** *Otitis media, perforation of tympanic membrane and epithelial proliferation were seen in propylene glycol-applied guinea pig ear from the control group (H-E x 40).*

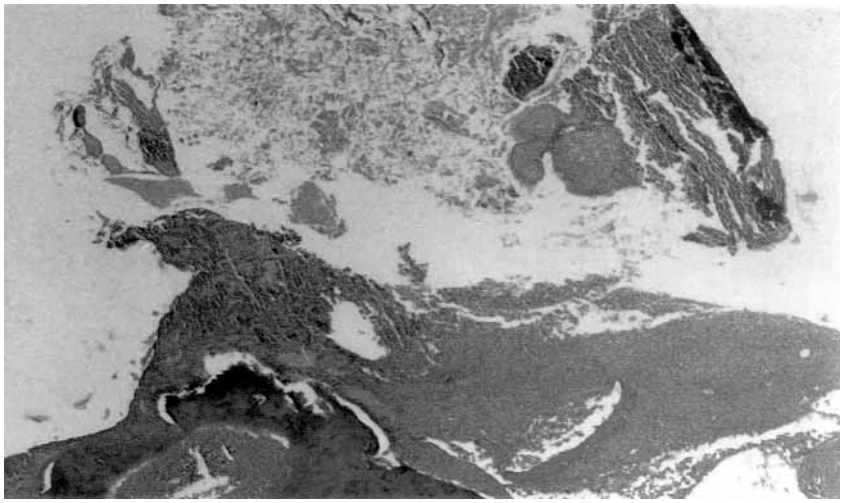

***Fig. 2 -*** *Otitis media and osseous degeneration in the cochlea of a prednisolone-treated ear (H-E x 40).*

cholesteatoma and they have also found similar results. Wright et al.,[13] reported that cholesteatomas could be produced in 60% to 70% of all animals by the application of 50-60% propylene glycol solution to the middle ear. Jove et al.,[1] used 90% propylene glycol solution for five treatments and after six weeks they reported that none of the ears treated with propylene glycol was normal in appearance. Sennaroglu et al.,[9] found that propylene glycol produced inflammation in 95% and cholesteatoma in 33% of the middle ears treated with 50% propylene glycol solution, three times at 5 day intervals. All these studies indicated that, the concentration of propylene glycol is important in the set-up of experimental cholesteatoma production. Repeating administrations of propylene glycol may be as important as the concentration, in the set-up of cholesteatoma. The majority of these studies had repeated propylene glycol application more than three times. In this investigation, propylene glycol engendered otitis media in 66% of the middle ears and unexpectedly, inflammatory changes did not lead to cholesteatoma formation in guinea pigs after of application propylene glycol in control group. This may be due to single application and low concentration of propylene glycol to the middle ears of guinea pigs.

In most of these studies, propylene glycol solution contained neomycin sulfate, polymyxin B or gentamicin as a topical antibiotic agent. The antibi-

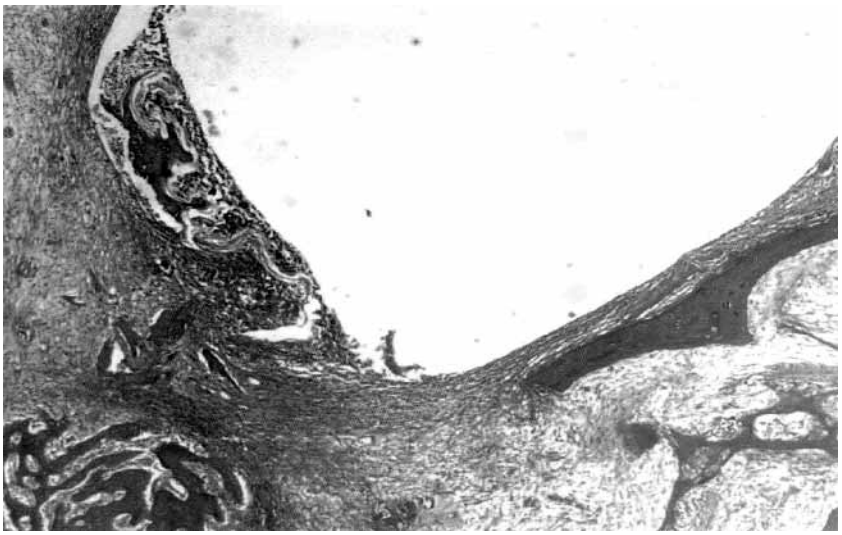

***Fig. 3 -*** *Otitis media, granulation tissue, keratinizing epithelium, and cholesteatoma were observed in one ear treated with prednisolone (H-E x 40).*

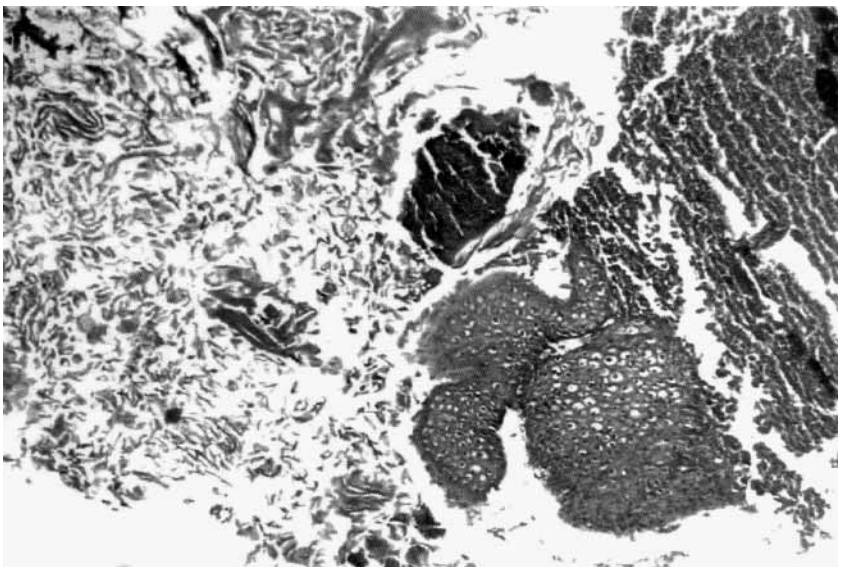

***Fig. 4 -*** *Cholesteatoma material consisting of infiltrating leukocytes, epithelial edema, and keratinizing epithelium (H-E x 100).*

otics were included in order to reduce the occurrence of middle ear infections in the experimental animals. Masaki et al.[5] concluded that, it seems doubtful that these antibiotics played a significant role in producing the epidermal invasion they observed, since cholesteatoma development has not been found in previous laboratory studies in which neomycin and polymixin B alone were applied to the middle ear cavity. According to them, relatively little is known regarding the early stages of cholesteatoma development after deposition of irritants into the middle ear cavity. We prefer to exclude the effect of topical antibiotics on guinea pig middle ear. The present study was therefore designed to investigate the changes in guinea pigs' middle ears after application of 60% propylene glycol to middle ear alone followed by chloramphenicol sodium succinate to prevent post surgical infections subcutaneously. Thereby, we only investigated propylene glycol effect on the middle ear of guinea pig.

Recently, studies have been directed toward the investigation of agents that may modify the inflammatory response and thereby reduce the incidence of cholesteatoma formation in animal models. Previous studies have investigated the effect of ventilation tube implantation, systemic isoretinoinin, systemic cyclophosphamide, topical hyaluronic acid on the development of propylene glycol-induced otitis media and cholesteatoma in mostly chinchilla ear and reported that, they had no effects on cholesteatoma development.[1,6,7,8] Wright et al.[10] concluded that, 5-fluorouracil does tend to reduce the proliferation of tympanic membrane epidermis and connective tissue, thereby reducing the likelihood of cholesteatoma formation in the experimental model. In a recent study on propylene glycol-induced inflammation and cholesteatoma in rat ears, conducted by Sennaroglu et al.,[9] it was found that topical prednisolone application suppressed inflammation and hence the formation of cholesteatoma in long-term chronic inflammations. In other two studies, cortisporin otic suspension, which contains neomycin, polymyxin B, hydrocortisone and 10.5% propylene glycol, was used as a steroid- containing topical otic preparation, in order to produce middle ear inflammation and cholesteatoma.[4,5] These findings showed that, cholesteatoma could be produced in the presence of hydrocortisone topically. Based on this, it was reported that, it is not possible to draw any conclusions from these results about the anti-inflammatory role of steroids in regard to experimental cholesteatoma.[9]

In the present study, otitis media developed in 6 out of 9 ears (66%) in the control group, while only 1 out of 16 ears (6%) receiving systemic prednisolone had otitis media. There were no clinical or experimental studies on the effects of systemic steroid on otitis media in the literature. This is the first study demonstrating the effects of systemic steroids on experimental otitis media. Systemic corticosteroids strongly inhibited inflammatory effects of propylene glycol. Following these results, we conclude that systemic steroids were effective on propylene glycol-induced otitis media however we could not reach a conclusion about the preventive effects of systemic steroids on the development of cholesteatoma.

Using systemic steroids as anti-inflammatory agents in humans, may improve chronic otitis media without cholesteatoma. We need more studies to reach conclusions regarding the development cholesteatoma. If pathogenesis becomes clear with new discoveries, the management of chronic otitis media and cholesteatoma will be more medical than surgical. Further studies are needed in order to clarify the effects and the optimal usage of systemic steroids on otitis media.

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