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ORIGINAL ARTICLE

EFFECTS OF BONAIN'S SOLUTION (LOCAL ANAESTHETIC) OF THE TYMPANIC MEMBRANE AND EXTERNAL AUDITORY CANAL OF RABBIT

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Abstract

The tympanic membrane (TM) and external auditory canal (EAC) of the rabbits were studied microscopically after application of Bonain's solution (local anaesthetic) on 21 rabbits including six controls. Bonain's solution was administered into both sides of the rabbits and was massaged for at least 3 minutes. Experimental animals were divided into three groups of 5 rabbits each. The rabbits of first, second and third group were sacrified at 48 hour, 10 days and 20 days respectively. The specimens taken at 48 hours after exposure of Bonain's solution showed intraepithelial hemorrhage in the squamous epithelium of the EAC, and the TM showed hemorrhage and curvatures. The specimens taken at 10 days showed split of the basal membrane in the epidermal layer and intraepithelial mononuclear cell infiltration in the EAC. Thickening in the epidermal layer and mononuclear cell infiltration were also seen in the TM. The specimens taken at 20th day showed degeneration and pus on the epithelial layer in the EAC, thickening and hyperplasia in the epidermal layer of TM were observed as well. Controls were normal. This study confirms that Bonain's solution damages the TM and EAC when used as a local anaesthetics in rabbits and similar damage may be caused in humans thus its use should be limited and with caution.

Key Words = *Bonain's solution – Tympanic membrane – External auditory canal – Rabbit.*

Introduction

Ear drops containing antibiotics, steroids or anaesthetic agents are used for different purposes e.g. relief of pain, myringotomy and the treatment of otitis media in the external and middle ear cavities.[1] In contrast to the beneficial effects of the ear drops, they have some adverse effects on the skin and TM with which they come into contact.[2] They damage or even destroy the entire epidermal layer of the TM after local application. Moreover, changes could even be recorded in the epithelial lining of the middle ear as well after such treatment.[2]

The TM is covered on one side by a keratinizing stratified squamous epithelium and the other side by a single layer of squamous epithelium. Between these two layers of the TM, there is a collagenous fibre layer. EAC is also covered by a keratinizing stratified squamous epithelium. Rabbit TM resembles with the human tympanic membrane. The squamous epithelium of the TM is a barrier to effective topical anaesthesia, but all cell membranes give destructive-type permeability to lipoid-soluble (nonpolar) substances.[3]

Bonain's solution is one of these compounds and is a mixture of phenol, menthol and cocaine. It is used for local anaesthesia during tympanostomy tube insertion or myingotomy in adults. After application of solution on the TM, anaesthesia starts in a few minutes. However, this solution is believed to damage the TM structurally and is further apt to provoke pronounced inflammatory reactions in the skin of the EAC.[4,5]

In this study, we investigated the TM and EAC of the rabbit after application of Bonain's solution containing phenol and reported the microscopical changes in the TM and EAC at various time intervals.

Materials and Methods

A total of 15 adult New Zealand rabbits, each weighing approximately 3.5 Kg were used. All animals were anaesthetized with 30 mg of ketamine hydrochloride administered subcutaneously. A tube was placed into the external ear canal and Bonain's solution was administered on both sides of the rabbits.



Fig. No. 1: Intraepithelial hemorrhage in the EAC (48th hour; 33X, H.E.).

After delivery of the solution, the tragus was massaged for at least three minutes to aid penetration of the solution to the TM. In the control group, no treatment of any sort was administered to the rabbits. In the study, the animals were divided into 3 groups. After application of Bonain's solution into the EAC, the animals in the first, second and third group were sacrificed at 48th hour, 10th day and 20th day respectively. Control were sacrificed at 48th hour, 10th day and 20th day and each group consisted of 2 rabbits.

Temporal bones including EAC, TM and middle ear were removed from the skull and a hole was made in the ventral portion of the bullae to allow introduction of fixative into the middle ear cavity. Then, the temporal bones were immediately fixed in 10% buffered formalin solution. After cleaning of surrounding connective tissue, the temporal bones were decalcified in accordance with Nitric Acid Method I, and washed in tap water, dehydrated in alcohols series and then paraffin-embedded sections were cut in 5 micron thick and stained with hematoxylin, eosin and Van Gieson, and were examined under the light microscope.[6]

Results

Both the TM and the meatal skin were preserved and the sections of the TM were made following the dermis of the EAC. The external surface of the TM was distinguished from the inner surface of it. The dermis and bony canal of the EAC were identified.

The specimens taken at 48th hour after Bonain's solution application, the squamous epithelium of the EAC showed scarcely damaged in one sample of five and intraepithelial hemorrhage in the other four samples. TM showed hemorrhage and binding in its shape. The fibrous layer was oedematous and swollen (Fig. Nos. 1 and 2) and after 10 days the specimens of the EAC showed separation of the basal membrane in the epidermal layer and intraepithelial mononuclear cell infiltration in all samples. TM showed thickening in the epidermal layer, and intraepithelial infiltration together with necrosis of epithelial cells in most samples (Fig. No. 3). Fibrous layer was thickened and epithelial cells of the inner epithelium were also swollen (Fig. No. 4). Twenty days after Bonain's solution appli-



Fig. No. 2: Severe oedema in the TM (48th hour; 132X, H.E.).



Fig. No. 3: Intraepithelial infiltration and necrosis of the epithelial cells in the TM (10th day; 132X, H.E.).

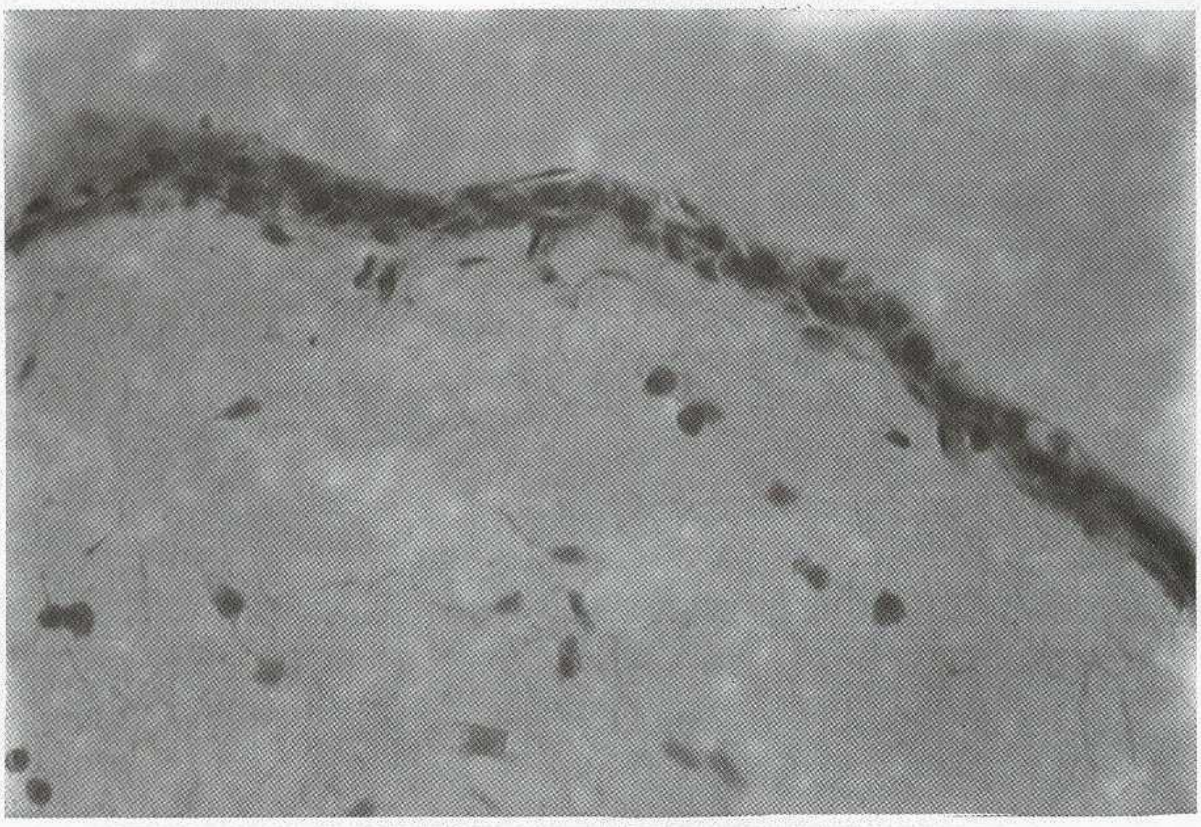


Fig. No. 4: Swelling in the cells of inner epithelial layer and oedema in the fibrous layer of the TM (10th day; 132X, H.E.).

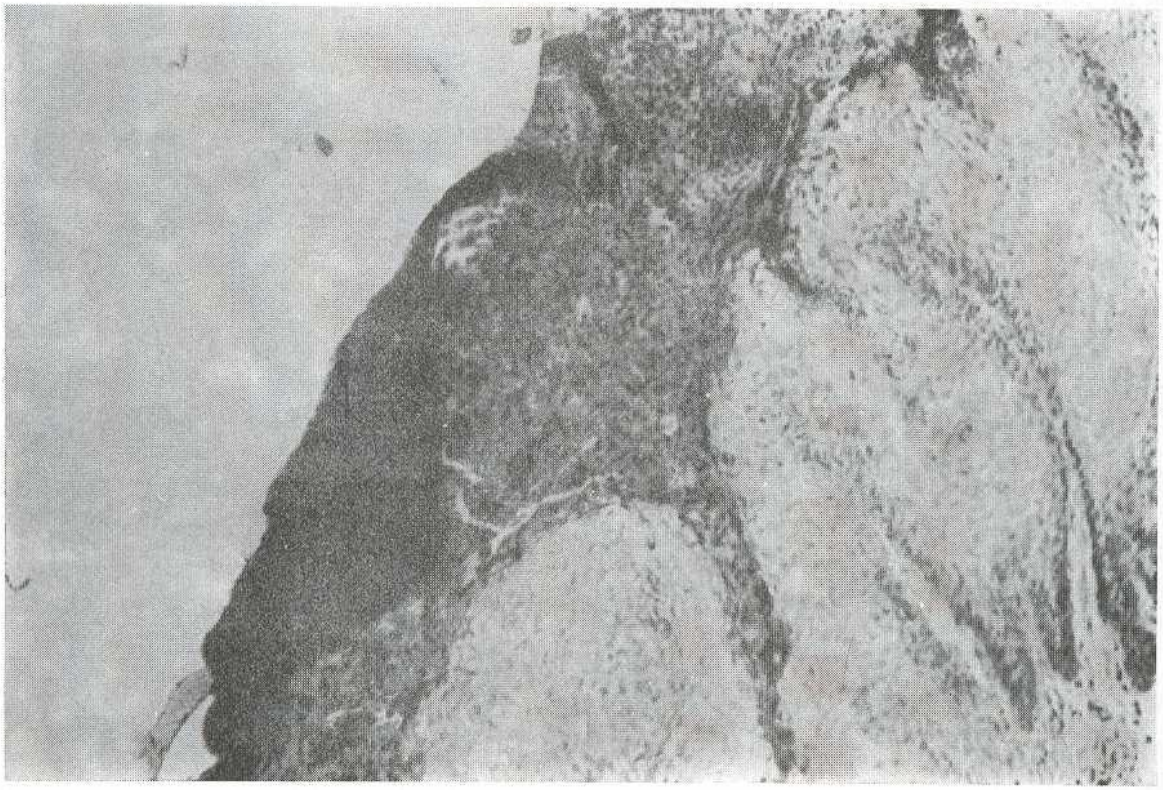


Fig. No. 5: Degeneration and mass with pus on the epithelial layer of the EAC (20th day; 33X, H.E.).

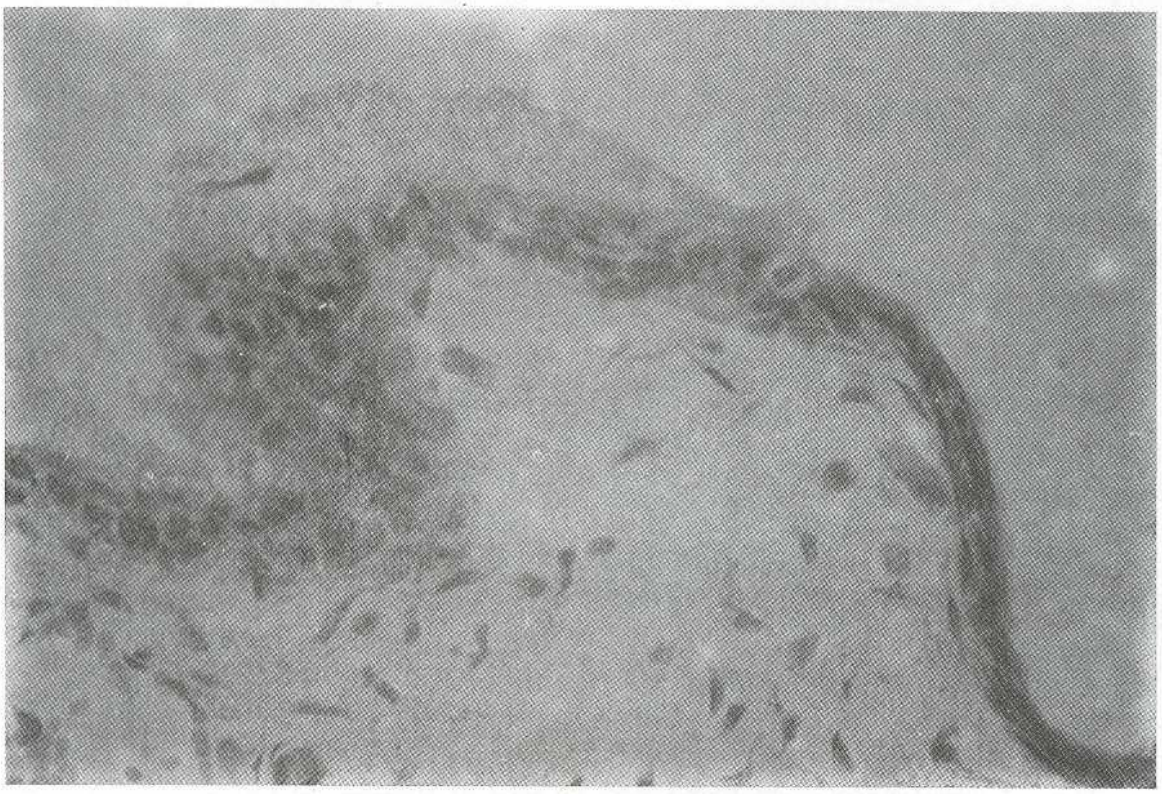


Fig. No. 6: Thickening and hyperplasia of the TM (20th day; 132X, H.E.).

cation the specimens of the EAC showed degeneration and mass with pus on the epithelial layer of the EAC showed thickening of all three layers and marked hyperplasia in the epidermal layer (Fig. No. 6). The bone of the EAC exhibited no pathological alterations and were normal.

No noticeable alteration of the EAC, TM, and middle ear were observed in the control specimens.

Discussion

Topical application of solutions containing phenol to the skin and TM may cause toxicity if used liberally. Phenol is the most active part of Bonain's solution and the effectiveness of Bonain's solution lies in the action of the phenol that destroys the overlying epithelium on the TM, exposing the nerve endings to the cocaine.[7] This destructive action of the phenol can extend to nearby structures in the TM, raising questions about the ability of the TM to subsequently heal. Phenol's propensity for a chemically induced inflammatory response and its irreversibility has limited its use.[8]

Since the external surface of the TM is a continuation of the external ear canal skin, the basic desquamating and keratinizing prosesses are the same as in the TM.[9]

The solution also deepithelializes portion of the EAC, destroying its self-protective mechanism. This irritating effect of phenol can cause a significant chemical irritation, as well as infectious, otitis externa.[8]

Phenol causes changes and creates great hazards when it is applied on the skin.[10] Keratinization is a synthesis of protein and lipid in desquamating epithelium. Phenol causes protein denaturation and coagulation of surface keratin. This coagulation is relatively rapid and irreversible and results in local devacularization and also destruction of nerve endings, thereby providing local anaesthesia.[11]

The most noticeable alterations occur in the dermis. In addition, the complete destruction of the epidermis and its re-formation from the epithelial appendages account for the accessory benefits of the peel above and beyond the smoothings effect. It is also quite clear that elastin fibers abundantly regenerate, forming a network of branched fine fibers often paralleling the distribution of the collagen bundies.[12]

In 1970, Spira et al[13] applied phenol to the thighs of six paraplegics and noted dermal thickening with deposition of new collagen.

In our study, the external and middle ear cavities of all animals were free of effusion and also no stratified squamous epithelium or evidence of keratin formation was found in the specimens. No pathological changes were present in the TM or external and middle ear cavities of the control specimens. A consistent pattern of structural changes in the TM and EAC was found to occur between 48 hours and 20 days. There was histological differences between those animals killed at 48 hours, 10th day and 20th day. It has been shown in the specimens of the tenth and twentieth days that the histological findings were dramatically increased in the TM and EAC. The inflammatory reaction was more severe than the specimens taken at forty-eight hours. Changes were observed in each of the three layers of the TM.

Masaki et al[14] and Schmidt[3,4] observed similar changes after application of local anaesthetics to the lateral side of the tympanic membrane in rats. According to Schmidt,[3,4] the TM was nearly normal at three weeks after exposure to local anaesthetics with light microscope. However, it has been shown that considerable hyperplasia of the fibrous layer with numerous fibroblasts was present in the same specimens of the three weeks' samples investigated by electron microscope. In our study, we observed that the changes of the TM and EAC persisted in the specimens taken on 20th day as well.

The results of this histological investigation demonstrate that Bonain's solution produce marked alterations of the TM and EAC. These alterations have been shown in both layers of the TM and epidermal layer of the EAC.

The use of Bonain's solution for local anaesthesia in patients may contribute to TM and EAC complications.

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