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Mucosal trauma induced apoptosis in guinea pig middle ear: Comparision of hemostatic agents

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ABSTRACT

Objective: The aim of this study is to compare the effects of the absorbable gelatin sponge (AGS), microporous polysaccharide hemospheres (MPH), and Ankaferd on wound healing after middle ear trauma and to evaluate their ototoxicity in an experimental guinea pig model.
Methods: Middle ear mucosal trauma was created in 21 healthy adult guinea pigs. MPH, Ankaferd, and AGS were applied into the right tympanic bulla of the guinea pigs (7 ears for each treatment modality). The left ears of the seven animals were used as the sham group. At the fourth postoperative week (28–30 days), the guinea pigs were decapitated. Apoptosis was investigated, and the expression of Bcl-xl, Apaf, p53, cytochrome 3, and caspase 3 were evaluated.
Results: The Ankaferd and AGS groups demonstrated significantly lower epithelial thickness, inflammation, and capillary dilatation than did the control group ($p < 0.001$, <0.001, /0.001, <0.001/, 0.005, and 0.005, respectively). A statistically significant decrease in Bcl-xl staining was observed in the middle ears of animals treated with MPH ($p = 0.003$). There was significantly higher caspase 3 expression in the Ankaferd and AGS groups than in the control group ($p < 0.001$ and $p = 0.002$, respectively).
Conclusion: Light microscopy indicates that Ankaferd and AGS create less inflammation and increased caspase expression, which seems to induce inflammatory cell apoptosis. Ankaferd seems to be a promising hemostatic agent in otology.

1. Introduction

Packing material (PM) in middle ear surgery is used widely. The main aim of using PM is to provide support to the tympanic membrane and ossicular grafts, aerate the middle ear cavity, and provide hemostasis [1]. Some PM has been shown to decrease adhesion and limit scarring following middle ear mucosal trauma [2].

An ideal middle ear PM as described by Shen et al. should be biocompatible; cause minimal inflammation, adhesion, and foreign body responses; be nonosteogenic; be nonallergenic; promote hemostasis; and prevent adhesion and fibrosis [1]. The authors stated that no such material has been found.

The absorbable gelatin sponge (AGS) is an antihemostatic material commonly used as scaffolding material to support grafts used in tympanoplasty. However, fibrosis and adhesion have been reported after gelfoam packing [3]. Most studies investigated the effect of materials in the middle ear and their capacity to cause fibrosis and adhesion.

Ankaferd Blood Stopper® (ABS) is a unique folkloric medicinal plant extract that has historically been used in Turkish traditional medicine as a hemostatic agent [4]. It has been reported that ABS effectively reduces intraoperative bleeding [5]. Compared with saline, ABS-treated cutaneous wounds were superior in terms of inflammatory scoring, the type I/type III collagen ratio, and the wound contraction rate [6].

Microporous polysaccharide hemospheres (MPH) (commercially available as Arista and Hemaderm; Medafor, Inc., Minneapolis, MN) is a unique hemostatic agent that does not interfere with healing or intact sinonasal mucosa in the rabbit model [7]. According to a human study, the use of MPH after sinus surgery

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does not increase synechiae formation and does not appear to deleteriously affect the healing of postoperative sinus cavities [8].

Apoptosis is a form of cell death essential for tissue homeostasis and development. It is also used as a cellular response to stress or pathogens. Moreover, apoptosis is responsible for the removal of inflammatory cells and the evolution of granulation tissue into scar tissue in the wound-healing process [9]. Apoptosis is important in the process of healing. Inadvertent middle ear mucosa trauma during ear surgery can lead to troublesome hemorrhage and eventually unwanted scar tissue that can result in postoperative hearing loss. The purpose of this study was to compare the effects of three hemostatic materials (AGS, MPH, and ABS) on wound healing after middle ear trauma and to evaluate their ototoxicity in an experimental guinea pig model.

2. Material and methods

All animals were treated in accordance with protocols approved by the National Guidelines for the use and care of Laboratory Animals. Twenty-one healthy adult guinea pigs weighing 880 to 1200 g with no external or middle ear pathology were used.

The animals were anesthetized by intramuscular administration of xylazine (Basilazin, 5 mg/kg; Animedica GmbH, Senden-Bosensell, Germany) and ketamine (Ketalar, 50 mg/kg; Eczacıbaşı Warner Lambert, Istanbul, Turkey).

2.1. Surgical procedure

The posterior wall of the external ear canal (EEC) was identified microscopically, and the skin of the posterior wall of EEC was elevated using an elevator. After elevation of the inferior wall of the EEC, the inferiorly located bony structure (i.e., tympanic bulla) was approached. The posteroinferior aspect of the tympanic bulla was then perforated with a sharp pick, and the hole was adequately dilated. The middle ear cavity was entered with a pick, and mucosal trauma was created. MPH (Arista AH Medafor Inc., Minneapolis, MN), ABS (Ankaferd Healthcare Products, Istanbul, Turkey) and AGS (GelSpon-P; Eucare Pharmaceuticals, India) were applied into the right tympanic bulla of the guinea pigs (seven ears per material). The left ears of the seven animals were used as the sham group.

At the fourth postoperative week (28–30 days), the guinea pigs were decapitated. Their temporal bones were excised without harming the cochlear bones and fixed in a 10% formaldehyde solution.

2.2. Histology

Tissue specimens were fixed in formaldehyde solution for 24 to 48 h, dehydrated in a series of calibrated ethanol solutions, cleansed with xylene, and embedded in paraffin. From paraffin blocks prepared from the mucosa of the middle ear promontorium and cochlea were cut into 5-μm-thick sections for histological examination. The cut sections were stained with hematoxylin and eosin (H&E) for histologic examination under a light microscope (BX 40; Olympus, Tokyo, Japan), and the images were entered into a computerized system. Two independent observers classified the intensity of inflammation, epithelial thickness, capillary vasodilatation observed in the middle ear, and inflammation observed in the inner ear as mild (+), moderate (++), moderate to severe (+++), or severe (++++). In the inner ear stria vascularis and organ of Corti, cell degeneration was also classified as described above by two independent observers.

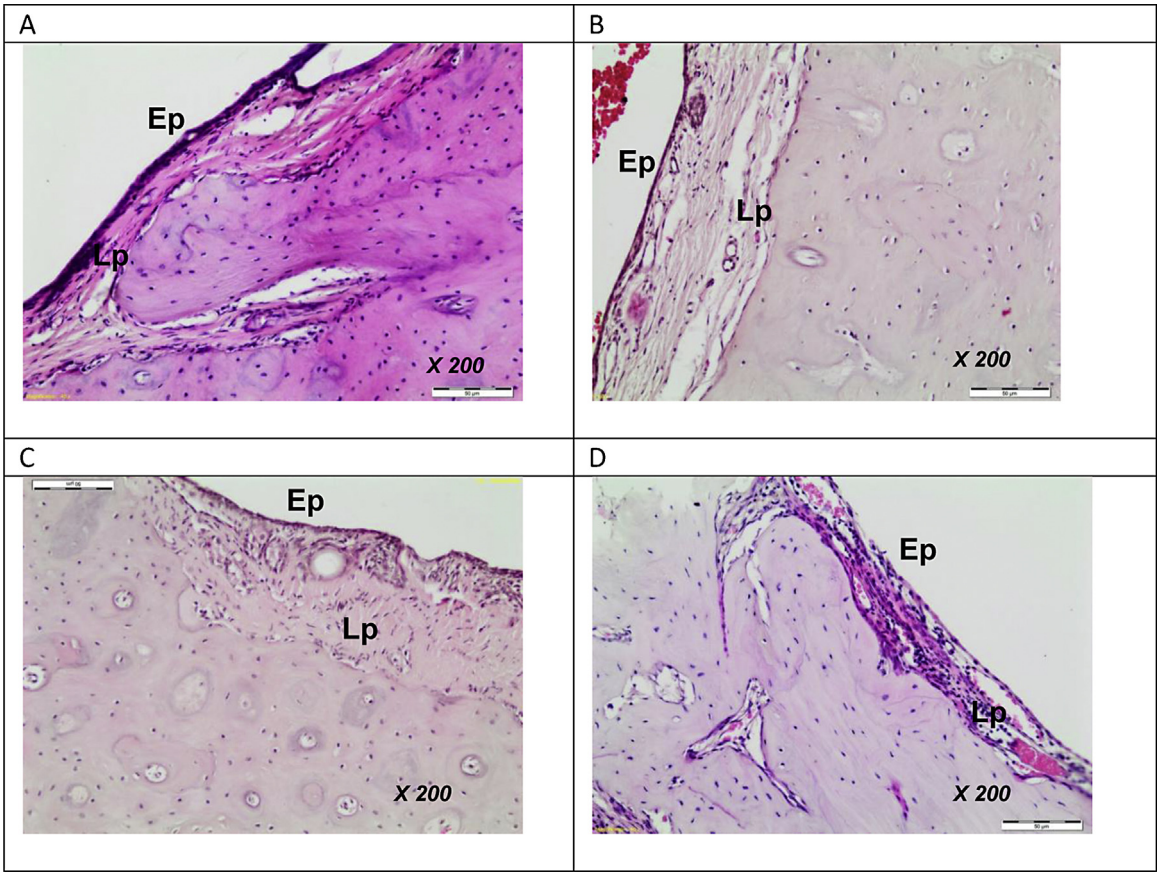

Fig. 1. In the middle ear H&E sections, increased epithelial thickness, increased edema, stasis in the capillaries of the lamina propria, and increased inflammatory cells can be seen in the control and MPH groups ((A) control group; (B) ABS; (C) AGS; (D) MPH; Ep, epithelium; LP, lamina propria).