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MAIN ARTICLE

Histological analysis of the effects of anti-adhesive haemostatic agents on the middle ear of the guinea pig

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Abstract

Introduction: Adhesion formation is a frequent and serious post-operative problem in ENT surgery. This study assessed the effect of two anti-adhesive haemostatic agents on an experimental guinea pig model.

Materials and methods: The middle-ear mucosa of 14 guinea pigs was exposed to surgical trauma. After surgery, Arista™ AH was injected into the right middle ear of seven animals, while Ankaferd Blood Stopper was injected into the right middle ear of the other seven animals. The left ears were left untreated and regarded as the control group. The three groups were compared by histological examination at post-operative week 4.

Results: In each of the three groups, consolidation of the lamina propria and epithelium mucosae, increments in the number of active fibroblasts, collagen fibrils and inflammatory cells, and increased vascular dilation were observed on haematoxylin and eosin-stained sections, and were more prominent in the control and Ankaferd Blood Stopper groups. Epithelial thickness and capillary vasodilation were significantly lower in the Arista™ AH group compared with the control and Ankaferd Blood Stopper groups ($p < 0.008$).

Conclusion: Arista™ AH may prevent the formation of adhesions in middle-ear surgery. Further experimental studies are required to determine its ototoxic potential.

Key words: Guinea Pigs; Ear, Middle; Surgery-Induced Tissue Adhesions

Introduction

Post-operative adhesion formation is a common and serious problem in ENT and other fields of surgery. For instance, intestinal adhesions can cause severe manifestations, such as ileus; in obstetrics and gynaecology, ovarian or intra-tubal adhesions lead to infertility and ectopic pregnancies, while in cardiovascular surgery, pericardial damage can lead to potentially fatal tamponade secondary to post-operative adhesions.[1] Inflammation occurring due to mucosal trauma in the middle ear can result in the formation of post-operative adhesions and related complications, such as ossicular fixation, retraction of graft and residual tympanic membrane.

Therefore, numerous haemostatic and anti-adhesive agents have been developed to prevent adhesions. An ideal anti-adhesive material has to be a non-adhesive product of adequate dimensions and without any potential risk of infection or adverse effects on the healing process.[2] Many products, such as blood clots, lyophilised plasma, Sepragel™ Sinus, Seprafilm® and Gelfoam® have been used to coat the middle ear to prevent adhesions.[3] In animal experiments, Sepragel and Seprafilm have been reported to reduce adhesions with good tolerability and without ototoxic effects.[4] The application of Gelfoam, which had been used widely in middle-ear surgery since the 1950s, has been abandoned because of its inflammatory and adhesion-increasing effects.[5]

The objective of our study was to evaluate the anti-inflammatory and anti-adhesive effects of two anti-adhesive products, Arista™ AH (a plant-based powder containing microporous polysaccharide hemospheres; Medafor Inc, Minneapolis, Minnesota, USA) and Ankaferd Blood Stopper (Ankaferd İlaç Kozmetik A.Ş., Istanbul, Turkey); both have well-known haemostatic and anti-adhesive properties on mucosal layers. To our knowledge, this is the first study reporting the use and effects of these products in otological practice.

Materials and methods

Approval for the study was obtained from the Animal Ethics Committee of Dokuz Eylül University, Turkey.

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The study protocol was also approved by the Animal Ethics Committee of Dokuz Eylül University.

The study was completed in two stages: a surgical and a histopathological stage. The surgical stage was performed at the Dokuz Eylül University Animal Laboratory. Fourteen 6-week-old guinea pigs weighing between 350 and 900 g were used in the study. Before surgery, the external auditory canals and eardrums of all guinea pigs were intact and not showing any middle-ear pathology. Anaesthesia was administered with intraperitoneal xylazine (Basilazin, 5 mg/kg; aniMedica GmbH, Senden, Germany) and ketamine (Ketalar, 50 mg/kg; Pfizer Inc, New York, New York, USA) injections. The incision site was numbed with local lidocaine hydrochloride 20 mg/ml and adrenaline 0.0125 mg/ml (this is a mixed drug containing both lidocaine and adrenaline and its market name is "jetobain") (Adeka, Samsun, Turkey) injections.

Surgical procedure

The posterior wall of the right external auditory canal was identified microscopically and the skin of the posterior wall of the external auditory canal was elevated. Following elevation of the inferior wall of the external auditory canal, the bony structure located inferiorly (i.e. the tympanic bulla) was reached. Then, the postero-inferior aspect of the tympanic bulla was perforated with a sharp pick and the hole was dilated. The middle-ear cavity was reached with a pick to create mucosal trauma. Thus, the middle-ear mucosa and the outer mucosal aspect of the cochlea facing the middle ear were traumatised. The middle ear was penetrated through the bone window in the tympanic bulla of the right ear. Then, Arista™ AH was injected into the middle ear of seven guinea pigs using the manufacturer's applicator. Ankaferd Blood Stopper was injected into the middle ear of the other seven guinea pigs. The left ears of the 14 guinea pigs were left untreated and they formed the control group. The incision site was sutured and covered with a dressing.

At post-operative week 4 (28–30 days post-surgery), the guinea pigs were euthanised using higher doses of the anaesthetising agents previously described, and then decapitated. The temporal bones were carefully excised without harming the cochlear bones and then fixed in 10 per cent formalin solution (Figure 1).

Tissue specimens that had been fixed in formalin solution for 24–48 hours were dehydrated in calibrated ethanol solution, washed with xylene and embedded in paraffin. Paraffin-embedded tissue blocks from the middle ear, promontory of the tympanic cavity and cochlea were cut into 5-mm-thick sections. The sections were stained with haematoxylin and eosin and histological examination was performed under a light microscope (BX40; Olympus, Tokyo, Japan). Images were saved as digital media in a computerised system (Windows XP Pro/Image Pro Plus, version 3.0; MediaCybernetics Inc., Rockville, Maryland, USA). Two independent observers classified the intensity of the inflammation

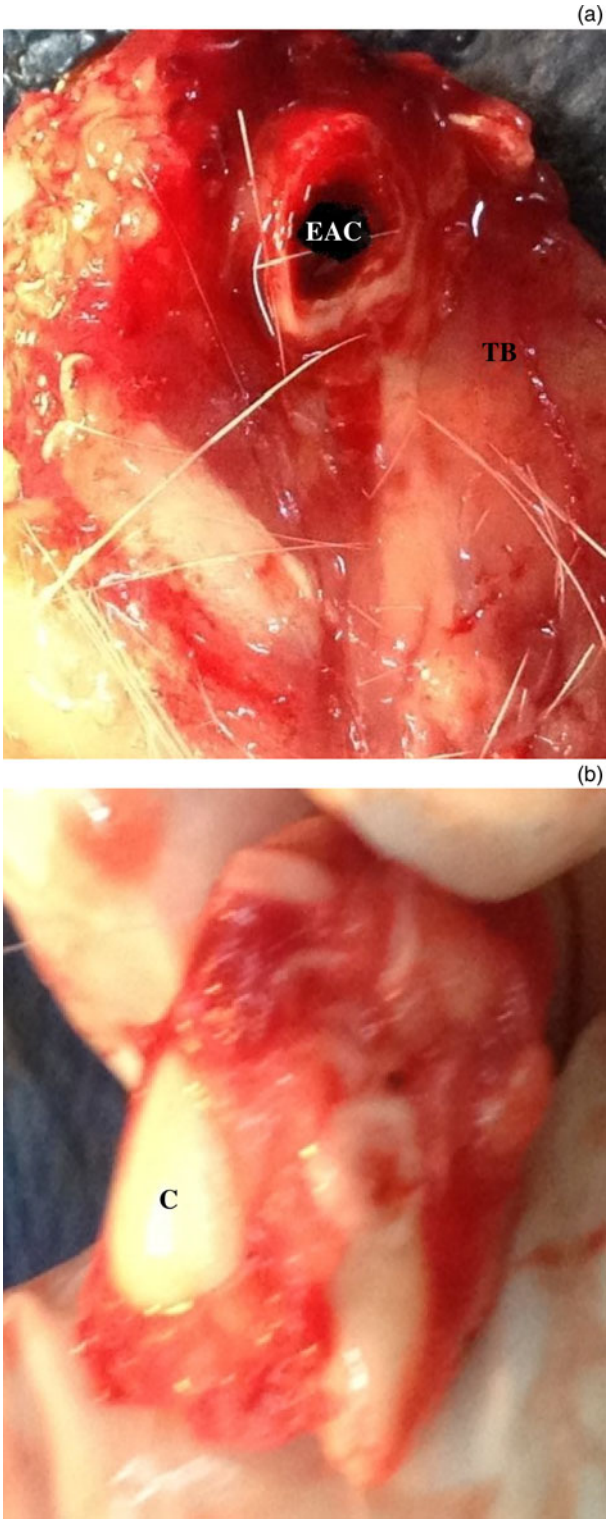

FIG. 1

(a) Lateral and (b) medial views of the temporal bone following resection. Care was taken to leave the skin of the cochlea and external auditory canal intact. EAC = external auditory canal; TB = tympanic bulla; C = cochlea

observed in the middle- and inner-ear sections as mild (+), moderate (++) or severe (+++).

Statistical analysis

Data were analysed using the SPSS software program, version 16.0 for Windows (SPSS Inc., Chicago,

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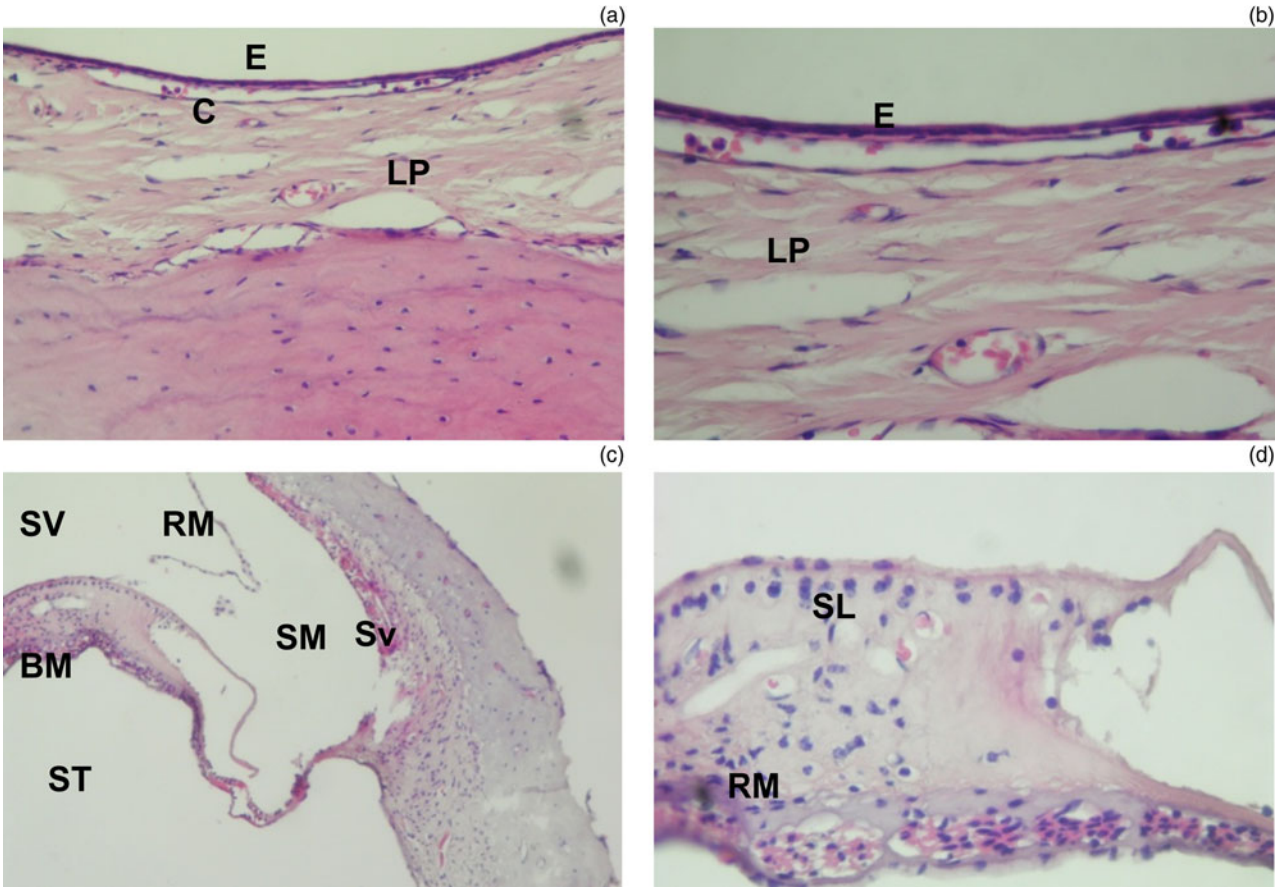

FIG. 2

Photomicrographs of the control group samples, showing staining of the middle ear at (a) × 100 and (b) × 400 (H&E) and cochlea at (c) × 100 and (d) × 400 (H&E). These demonstrate thickening of the epithelium and lamina propria, and capillary dilation. Irregularities, inflammation, vascularisation in the organ of Corti situated on the cochlear basal membrane and increased vacuolisation of the stria vascularis are also seen. C = capillary; E = epithelium; LP = lamina propria; SV = scala vestibuli; BM = basilar membrane; ST = scala tympani; RM = Reissner's membrane; SM = scala media (cochlear duct); Sv = stria vascularis (of cochlear duct); SL = spiral limbus

Illinois, USA). All differences associated with a p value of 0.05 or less were considered statistically significant. Continuous variables were presented as mean ± standard deviation. Parametric tests were applied to data with normal distributions and non-parametric tests were applied to data with questionable normal distributions. The Shapiro–Wilk test was used to assess the distribution of the data. The three groups were compared using the non-parametric Kruskal–Wallis test. Significant data ($p < 0.08$) were subjected to a post-hoc Bonferroni correction with a 95 per cent confidence interval.

Results

For each group, epithelial thickness (scoring: 1 = normal thickness; 2 = mild thickening; 3 = marked thickening) and capillary dilation (scoring: 1 = absent; 2 = mild increase; 3 = marked increase) in the middle and inner ears were quantitatively evaluated and tabulated (see Table I). In each of the three groups, consolidation of the lamina propria and epithelium mucosae, increments in the number of active fibroblasts, collagen fibrils and inflammatory cells, and increased vascular dilation were seen on haematoxylin and eosin-stained sections, and were more prominent in the control and Ankaferd Blood Stopper groups (Figures 2–4). Epithelial thickness and capillary vasodilation were significantly lower in the Arista™ AH group, when compared to the control and Ankaferd Blood Stopper groups ($p < 0.008$). In the Ankaferd Blood Stopper group, these changes were lower when

TABLE I

EPITHELIAL THICKNESS AND VASCULAR DILATION SCORES FOR THE MIDDLE AND INNER EARS OF THE THREE GROUPS

Group	Epithelial thickness score			Vascular dilation score		
	1	2	3	1	2	3
Control ($n = 14$)	0	3	11	0	4	10
Ankaferd Blood Group ($n = 7$)	1	5	1	0	6	1
Arista™ AH ($n = 7$)	5	2	0	5	2	0

Epithelial thickness scores: 1 = normal thickness; 2 = mild thickening; 3 = marked thickening.
Vascular dilation scores: 1 = absent; 2 = mild increase; 3 = marked increase.
$p = 0.04$ for epithelial thickness, $p = 0.038$ for vascular dilation, $p < 0.008$ for Arista™ AH.

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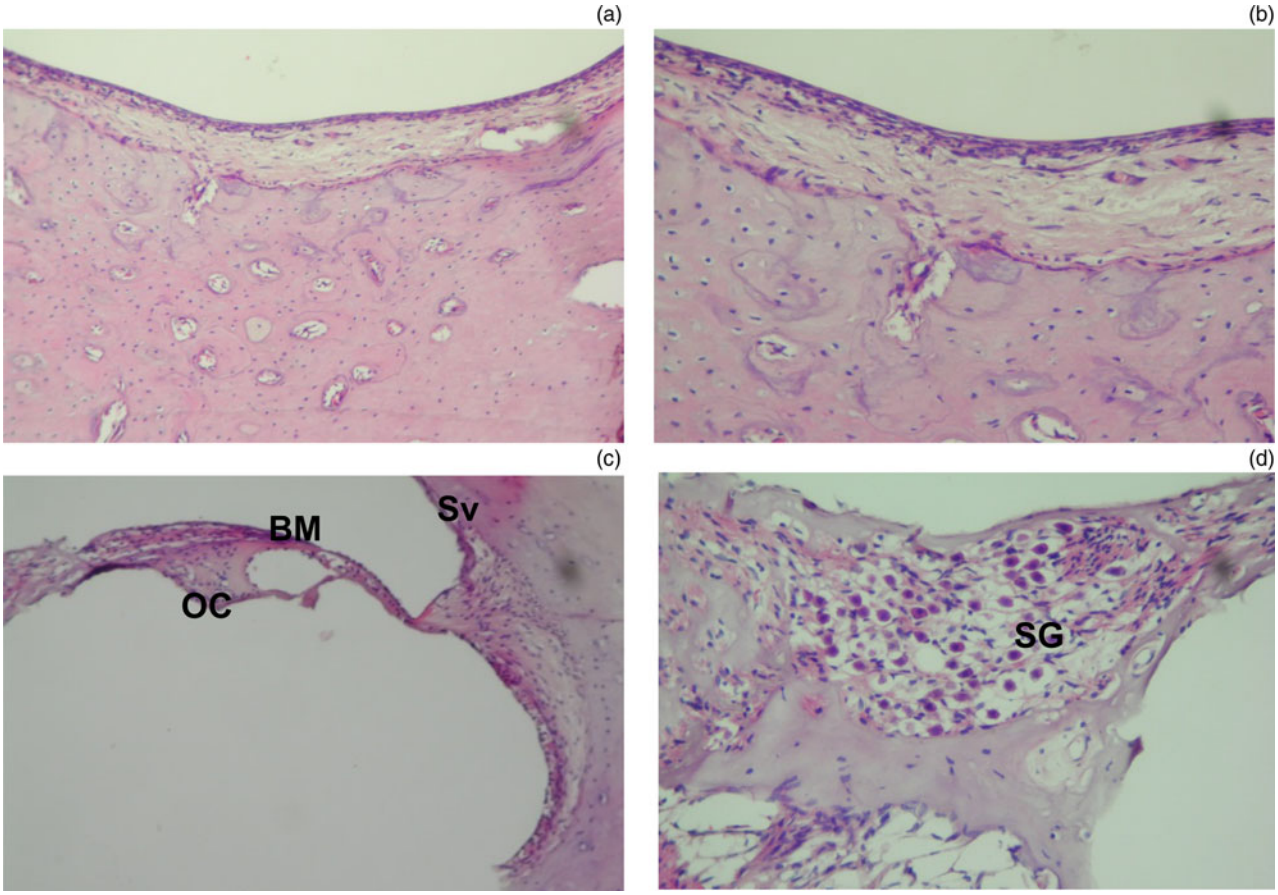

FIG. 3

Photomicrographs of the Arista™ AH group samples, showing staining of the middle ear at (a) × 40 and (b) × 100 (H&E) and cochlea at (c) × 40 and (d) × 100 (H&E). These photomicrographs demonstrate minimal vascular dilation, reduced epithelial thickening and a lower number of inflammatory cells in this group. OC = organ of Corti; BM = basilar membrane; Sv = stria vascularis (of cochlear duct); SG = spiral ganglion

compared to the control group, but the difference was not statistically significant ($p > 0.008$) (Table II).

Discussion

Various methods and agents have been used to prevent the formation of post-operative adhesions. Peri-operative preventive measures, such as minimisation of surgical trauma, precise tissue dissection and avoidance of any remaining foreign substances in the middle-ear cavity, desiccation of mucosal surfaces, excessive hot irrigation (warm irrigations usually use to clean intrabdominal spaces and also could be use in middle ear so excessive hot irrigation without paying attention could be a reason for postoperative fybrosis and syneschias), redundant use of retractors, cautery and laser application are notable factors which may help decrease the formation of adhesions.[6] Up to the present time, pharmacological agents, such as corticosteroids, non-steroidal anti-inflammatory drugs and fibrinolytic agents have been administered locally without any distinct beneficial effects. As a surgical barrier, blood clots, lyophilised plasma, Sepragel, Seprafilm, Gelfoam and Silastic® sheet packing have been used to coat the middle ear; however, owing to extensive inflammation and adhesion, the use of Silastic sheet packing and Gelfoam in clinical practice remains controversial.[5]

Gelfoam had been widely used in middle-ear surgery since the 1950s, but its use has now been abandoned.[5] Reports about Sepragel and Seprafilm have stated that, in animal experiments, they were well tolerated and had favourable effects on adhesions without any ototoxic effect in the middle ear.[5,6] GYNECARE INTERCEED®, which is commonly used in daily practice in obstetrics and gynaecology, is a promising agent for use in middle-ear procedures.[6] In recent years, cellular linkage proteins, such as intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, which have been held responsible for the development of adhesions, and substance P and neurokinin 1 (tachykinin receptor 1) receptor antagonists, which reduce intra-abdominal adhesion formation and play a role in the formation of free oxygen radicals, have steadily become the focus of research interest.[7]

In this study, we aimed to assess and compare the anti-adhesive properties of Arista™ AH and Ankaferd Blood Stopper, which are well known for their haemostatic capabilities, though there are few studies on their anti-adhesive role in ENT practice.

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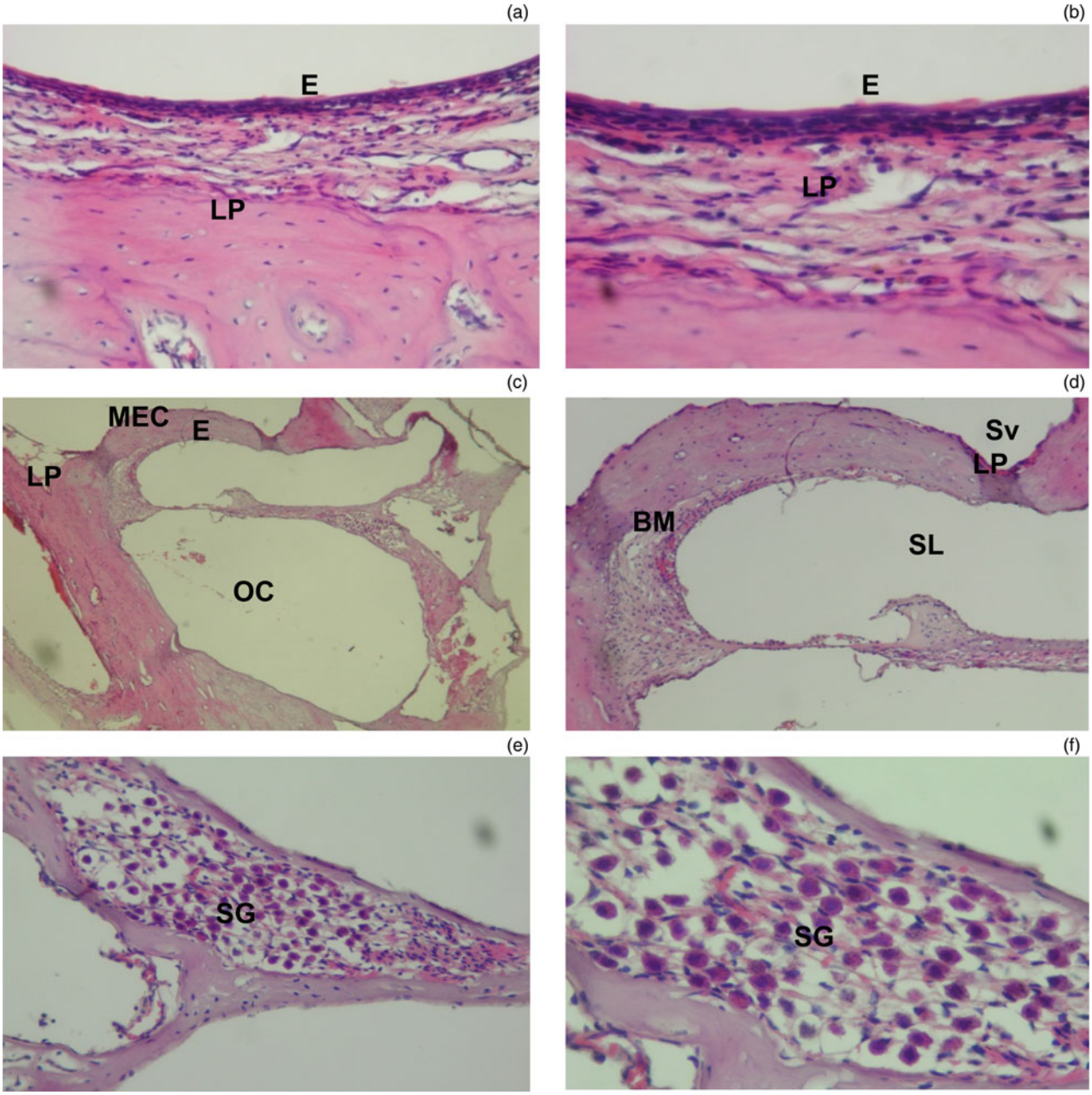

FIG. 4

Photomicrographs of the Ankaferd group samples, showing staining of the middle ear at (a) × 40 and (b) × 100 (H&E) and cochlea at (c) × 40 and (d) × 100 (H&E); panels (e) and (f) show staining of the spiral (cochlear) ganglion at × 100 and × 400 (H&E), respectively. These photomicrographs demonstrate reduced inflammation in the Ankaferd group relative to the control group, though this difference was not statistically significant. LP = lamina propria; E = epithelium; MEC = middle-ear cavity; OC = organ of Corti; BM = basilar membrane; SL = spiral limbus; Sv = stria vascularis (of cochlear duct); SG = spiral ganglion

In many *in vivo* and *in vitro* studies performed so far, Ankaferd Blood Stopper, which is a standard mixture of *Thymus vulgaris* (thyme), *Glycyrrhiza glabra* (liquorice), *Vitis vinifera* (grape vine), *Alpinia officinarum* (lesser galangal) and *Urtica dioica* (nettle), has been shown to be an effective haemostatic agent.[8] In addition, reports have shown that, in ENT practice, Ankaferd Blood Stopper has decreased peri-operative bleeding and operative times in children undergoing tonsillectomies.[9] Many publications have shown its safe use for haemostatic control after thyroidectomy.[10] Only a few studies have questioned the anti-adhesive properties of Ankaferd Blood Stopper. In a relevant investigation, Cömert *et al*. reported that the intra-peritoneal application of Ankaferd Blood Stopper decreased intra-abdominal adhesions. They also added that the intra-peritoneal administration of Ankaferd Blood Stopper led to some minor changes in the lungs and serosal surfaces of the intestines, as well as minor architectural changes in the liver that were not considered toxic.[11] Odabaş *et al*. reported the concentration-dependent cytotoxic effects of Ankaferd Blood Stopper on cell cultures and showed that it was cytotoxic to human pulp fibroblasts.[12]

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TABLE II
NUMBER OF INFLAMMATORY CELLS IN THE THREE GROUPS

Controls (left ears, $n = 14$)														
Animal	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	K12	K13	K14
No. of cells (× 400)	28	20	32	16	24	21	32	15	37	29	35	31	34	27
Ankaferd Blood Stopper ($n = 7$)														
Animal	A1	A2	A3	A4	A5	A6	A7							
No. of cells (× 400)	22	15	20	16	24	32	21							
Arista™ AH ($n = 7$)														
Animal	AH1	AH2	AH3	AH4	AH5	AH6	AH7							
No. of cells (× 400)	12	5	29	7	5	8	14							

$p < 0.05$ for Arista™ AH and Ankaferd Blood Stopper groups. In the inter-group comparison: Arista™ AH, $p < 0.008$; Ankaferd Blood Stopper, $p > 0.008$.

Arista™ AH is a simple, safe and effective haemostatic agent that was recently approved by Food and Drug Administration (FDA) for use in cardiac, orthopaedic, spinal and general surgery, though not for use in cranial and ophthalmic interventions. It is a plant-based powder derived from purified plant starch that contains microporous polysaccharide hemospheres, a patented blood clotting technology; the powder rapidly exerts haemostatic effects and forms clots on contact with surfaces.[13] In animal studies, it has been shown that microporous polysaccharide hemospheres do not harm the intact epithelium, do not cause foreign substance reactions and do not increase the infection rate at the wound site.[14] In addition to its haemostatic and antibacterial properties, in controlled studies performed with several haemostatic anti-adhesive agents, various reports have shown that Arista™ AH alleviated peritoneal adhesions after intra-abdominal surgery.[15] In 2005, Arista™ AH was approved by the FDA for use in ENT surgery. Since then, it has been widely used in post-nasal packing after endoscopic sinus surgery, haemostatic control after adenoidectomy and tonsillectomy, and local haemostasis following neck dissections and tonsillectomy.[16] To the best of our knowledge, there have been no experimental animal studies or clinical experiences in the use of Arista™ AH in middle-ear procedures.

- **Post-operative adhesions can lead to ossicular fixation, retraction of graft and residual tympanic membrane in middle-ear surgery**
- **The anti-adhesive haemostatic agent Arista™ AH can be used to prevent the formation of adhesions in middle-ear surgery, but its ototoxic potential should be further investigated**

We designed this experimental animal study to examine the anti-adhesive properties of two agents with known haemostatic activities, to be used in the field of otology and especially in daily ENT practice.

As an outcome of our study, histopathologically, we noted a statistically significant decrease in epithelial thickness and capillary vasodilation in the Arista™ AH group when compared to the control and Ankaferd Blood Stopper groups ($p < 0.008$). These reductions were also present in the Ankaferd Blood Stopper group, but the difference, when compared to the control group, was not statistically significant ($p > 0.008$). The number of inflammatory cells increased in the control and Ankaferd Blood Stopper groups, while they were close to normal values in the Arista™ AH group.

Conclusion

Arista™ AH can reduce the effects of surgical trauma in middle-ear surgery. Our results have shown that it is a promising agent to prevent adhesions in middle-ear surgery. Further experimental studies are needed to determine its ototoxic potential.

References

1 Yener A, Bardakçı Y, Iriz E, Özoğul C, Yener N, Bayram H *et al*. Intraoperative changes in the pericardium: a study via electron microscope [in Turkish]. *Türk göğüs kalp damar cerrahisi dergisi* 2011;**19**:576–85
2 Hellebrekers BW, Trimbos-Kemper GC, van Blitterswijk CA, Bakkum EA, Trimbos JB. Effects of five different barrier materials on postsurgical adhesion formation in the rat. *Hum Reprod* 2000;**15**:1358–63
3 Hellström S, Salén B, Stenfors LE. Absorbable gelatin sponge (Gelfoam) in otosurgery: one cause of undesirable postoperative results? *Acta Otolaryngol* 1983;**96**:269–75
4 Jang CH, Park H, Cho YB, Choi CH. The effect of anti-adhesive packing agents in the middle ear of guinea pig. *Int J Pediatr Otorhinolaryngol* 2008;**72**:1603–8
5 Dogru S, Haholu A, Gungor A, Kucukodaci Z, Cincik H, Ozdemir T *et al*. Histologic analysis of the effects of three different support materials within rat middle ear. *Otolaryngol Head Neck Surg* 2009;**140**:177–82
6 diZerega GS, Tulandi T. Prevention of intra-abdominal adhesions in gynaecological surgery. *Reprod Biomed Online* 2008;**17**:303–6
7 Reed KL, Heydrick SJ, Aarons CB, Prushik S, Gower AC, Stucchi AF *et al*. A neurokinin-1 receptor antagonist that reduces intra-abdominal adhesion formation decreases oxidative stress in the peritoneum. *Am J Physiol Gastrointest Liver Physiol* 2007;**293**:544–51
8 Firat HC, Ozdemir O, Kosar A, Goker H, Haznedaroglu IC. *Annual Review of Ankaferd 08–09*. Istanbul: Naviga Publications, 2009;13–19
9 Teker AM, Korkut AY, Gedikli O, Kahya V. Prospective, controlled clinical trial of Ankaferd Blood Stopper in children

https://doi.org/10.1017/S0022215114001571 Published online by Cambridge University Press

undergoing tonsillectomy. *Int J Pediatr Otorhinolaryngol* 2009; **73**:1742–5
10 Guler M, Maralcan G, Kul S, Baskonus I, Yılmaz M. The efficacy of Ankaferd Blood Stopper for the management of bleeding following total thyroidectomy. *J Invest Surg* 2011;**24**: 205–10
11 Cömert M, Karakaya K, Barut F, Karadeniz Çakmak G, Uçan HB, Gültekin FA *et al*. Does intraabdominal use of Ankaferd Blood Stopper cause increased intraperitoneal adhesions? *Ulus Travma Acil Cerrahi Derg* 2010;**16**:383–9
12 Odabaş ME, Ertük M, Çinar Ç, Tüzüner T, Tulunoğlu Ö. Cytotoxicity of a new hemostatic agent on human pulp fibroblasts *in vitro*. *Med Oral Patol Oral Cir Bucal* 2011;**16**:e584–7
13 Emmez H, Tonge M, Tokgoz N, Durdag E, Gonul I, Ceviker N. Radiological and histopathological comparison of microporous polysaccharide hemospheres and oxidized regenerated cellulose in the rabbit brain: a study of efficacy and safety. *Turk Neurosurg* 2010:**20**;485–91
14 Antisdel JL, Janney CG, Long JP, Sindwani R. Hemostatic agent microporous polysaccharide hemospheres (MPH) does not affect healing or intact sinus mucosa. *Laryngoscope* 2008;**118**: 1265–9
15 Hoffmann NE, Siddiqui SA, Agarwal S, McKellar SH, Kurtz HJ, Gettman MT *et al*. Choice of hemostatic agent influences adhesion formation in a rat cecal adhesion model. *J Surg Res* 2009;**155**:77–81
16 Sindwani R. Use of novel hemostatic powder MPH for endoscopic sinus surgery: initial impressions. *Otolaryngol Head Neck Surg* 2009;**140**:262–3

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