

ORIGINAL ARTICLE

Effects of Absorbable Gelatine Sponge on Middle Ear Mucosa Alone and with Corticosteroids and Antibiotics: An Animal Study

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Objective: The aim of this study is to investigate histopathologic changes that take place in middle ear mucosa after the use of absorbable gelatine sponge (AGS), and to find out if AGS combined with corticosteroids and/or antibiotics have an influence on these changes.

Study design: Animal study

Materials and Methods: Thirty-two adult guinea pigs were divided into four groups, with eight ears in each group. In control group, nothing was inserted to middle ear after traumatization of mucosa (group A). In study groups, sponges soaked with physiologic saline (group B), dexamethasone (group C) or dexamethasone and levofloxacin (group D) was inserted to middle ear after mucosal abrasion. Animals were sacrificed at the end of fourth week and fibrosis, inflammation, microscopic residue of AGS and the signs of foreign body reaction in middle ear mucosa were analyzed under light microscopy.

Results: No fibrosis was observed in the study group and inflammation was visible in two ears. Sponge residue was seen in two ears in group B, in two ears in group C and in one ear in group D. Signs of inflammation, fibrosis and foreign body reaction was observed in all study groups more or less, and even if the difference between study groups is not statistically significant, all histopathologic changes were slighter in group D.

Conclusion: Since we cannot avoid the use of AGS in otologic surgery, we believe soaking sponges with antibiotics and steroids is the best way to reduce its undesirable effects like fibrosis and foreign body reaction.

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Introduction

Absorbable gelatine sponge (AGS) was introduced by Correl and Wise as an absorbable hemostatic agent in 1945. Initially, it was used for hemostasis. Very shortly thereafter, this material found its place as a scaffolding substance used to support the tympanic membrane grafts and ossicular chains in tympanoplasty surgery [1].

Although AGS is commonly used unexpected fibrosis and adhesions were found in patients who underwent "second look" tympanomastoidectomy after canal-wall-up surgery or revision tympanoplasty [1]. Similar findings, even new bone formation in the middle ear have also been reported in experimental studies [1-3].

However, we need supporting substances in otosurgery, and since the mechanical properties of gelatin sponge are excellent for this purpose, it would be valuable if its inflammatory reactions could be reduced. In order to minimize the adverse effects of AGS in the middle ear, agents such as various antibiotic or corticosteroid solutions and hyaluronic acid have been used in animal studies by different researchers, and histopathologic changes have been studied [4, 5, 6]. There are limited number of studies in the literature focused on foreign body reaction and fibrosis developed in middle ear after the use of AGS. Severe foreign body reaction and fibrosis could decrease

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success rates of tympanoplasties, and the use of AGS could be one of the most important cause for failure of the operation.

The purpose of this study is to find out the severity of the foreign body reaction and the degree of fibrosis after use of AGS in traumatized middle ear cavity, as well as to investigate whether AGS combined with corticosteroid and/or antibiotic solutions would have an influence on these effects.

Materials and Methods

Thirty-four healthy, adult guinea pigs each weighing between 300-400 grams were used in the study. The pigs were obtained from Neuroscience Investigation Unit of Van Yuzuncu Yıl University Faculty of Medicine. The institutional ethics committee approved the study protocol (approval number 2000/04-1, date 24.10.2000).

The animals were anesthetized with intramuscular injection of 100 mg/kg ketamine hydrochloride. The ear was prepared with povidine solution and draped in a sterile manner. Any debris existing in external ear canal was removed by using operation microscope. All procedures were performed under aseptic conditions. A retroauricular sulcus incision was made to right ears of guinea pigs to access posterior wall of the external ear canal, and an incision was made to ear canal to obtain a sufficient viewpoint. The left ear served as a control. The animals were divided into 4 groups, each group was consisted of 8 guinea pigs. Myringotomy was performed by the help of a pick, and middle ear mucosa of all guinea pigs were traumatized. Then AGS material, cut into small pieces (1 to 2 mm) and soaked with different solutions, was inserted into the middle ear cavity via myringotomy hiatus until the middle ear is completely filled and the sponges reached the tympanic membrane in groups B, C and D. Nothing was inserted to middle ear cavity in group A.

Group A. Nothing was inserted to middle ear cavity after traumatization of middle ear mucosa.

Group B. Sponges soaked with physiologic saline were placed into middle ear cavity

Group C. Sponges soaked with dexamethasone were inserted to middle ear cavity.

Group D. Sponges soaked with levofloxacin + dexamethasone mixture were placed into middle ear cavity.

Ampicillin/sulbactam was administered for a week postoperatively for prophylaxis. The animals were reanesthetized and sacrificed by using 10 to 15 mg succinylcholine chloride and the temporal bones were harvested at the end of fourth week. After fixing in formaline solution for 24 hours, the specimens were placed in 10% formic acid decalcifying solution for two days. Each specimen was then cut according to the standard histologic technique; semi-thin sections were cut on a microtome then stained with hematoxylin and eosin.

Under light microscopy, fibrosis, inflammation, microscopic residue of AGS and the signs of foreign body reaction were analyzed.

Degree of fibrosis was divided in three categories; no fibrosis was scored as "0", mild fibrosis was scored as "1" and moderate fibrosis was scored as "2".

Foreign body reaction was classified as focal or diffuse. No foreign body reaction was scored as "0", focal foreign body reaction was scored as "1" and diffuse foreign body reaction was scored as "2".

If there was an inflammation, it was defined as acute or chronic.

For AGS residue, while no residue was scored as "0", slight residue was scored as "1" and moderate residue as "2" subjectively.

Chi-square and ANOVA tests were used for comparison of the degree of fibrosis and foreign body reaction between groups. Post-hoc (Dunnett) verification test was applied to groups A and B when a significant difference was found between groups in the aspect of foreign body reaction. Chi-square test was used for comparison of the degree of AGS residue and inflammation between groups.

Results

The animals of all groups survived the surgery in excellent condition. Otomicroscopically, there was no evidence of infection, such as hyperemia or otorrhea. After the animals killed, histologic changes regarding fibrosis, foreign body reaction, inflammation and AGS residue were evaluated. These findings are summarized in Table 1.

Table 1. Histopathologic changes observed in guinea pigs

	Guinea pig	AGS residue	Fibrosis	Inflammation	Foreign body reaction
Group A	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
	4	-	-	-	-
	5	-	-	Acute	-
	6	-	-	Acute	-
	7	-	-	-	-
	8	-	-	-	-
Group B	1	Moderate	Mild	-	Diffuse
	2	-	Mild	-	Diffuse
	3	Mild	-	-	Focal
	4	-	-	Acute	-
	5	-	Moderate	Acute	Diffuse
	6	-	-	-	-
	7	-	-	-	-
	8	-	-	-	-
Group C	1	Moderate	-	Acute	-
	2	Mild	-	-	-
	3	-	Mild	-	-
	4	-	-	-	-
	5	-	-	-	Focal
	6	-	-	-	Focal
	7	-	-	-	Focal
	8	-	-	-	Focal
Group D	1	-	-	-	-
	2	-	-	-	Focal
	3	-	Mild	-	-
	4	Mild	-	-	-
	5	-	-	-	-
	6	-	-	-	-
	7	-	-	-	-
	8	-	-	-	-

In groups B and C, we found slight sponge residue in one ear and moderate residue in another, while in group D, sponge residue was observed in only one ear slightly (Figure 1). There was no sign of sponge residue in the rest 19 ears. The difference was not statistically significant between groups ($p>0.05$).

In group B, there was mild fibrosis in two ears, moderate fibrosis in one ear. In groups C and D, there was mild fibrosis in one ear each (Figure 2). No

fibrosis was observed in control group (group A). There was no significant difference between groups (chi-square: 5.074, Pearson chi-square: $p=0.457$, likelihood ratio P value=0.392).

Acute inflammation findings were detected in two ears in groups A and B and in one ear in group C (Figure 3). There was no sign of inflammation in group D. The difference between groups was not statistically significant ($p>0.05$).

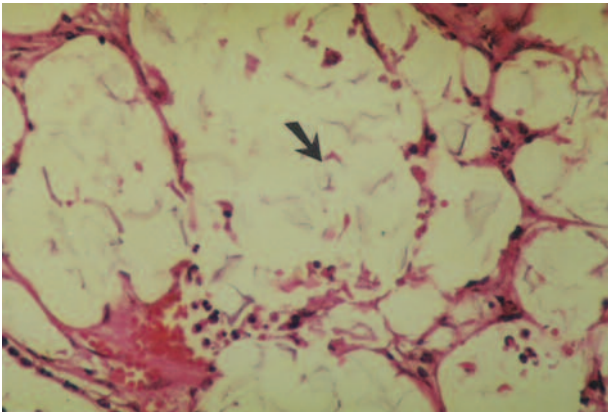


Figure 1. Light microscopic pictures showing sponge residue in middle ear cavity in an animal from group D (arrow). HE100

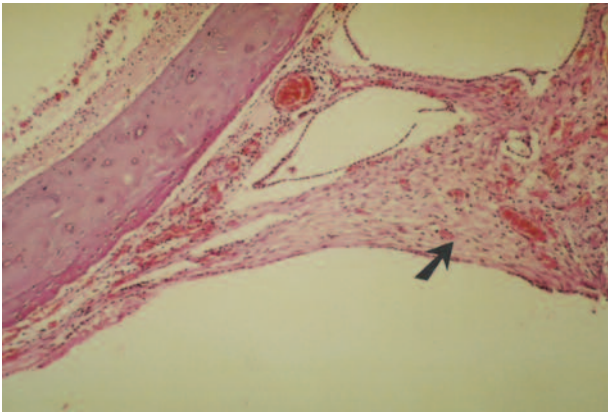


Figure 2. The middle ear cavity with mild fibrosis in group B (arrow). HE25

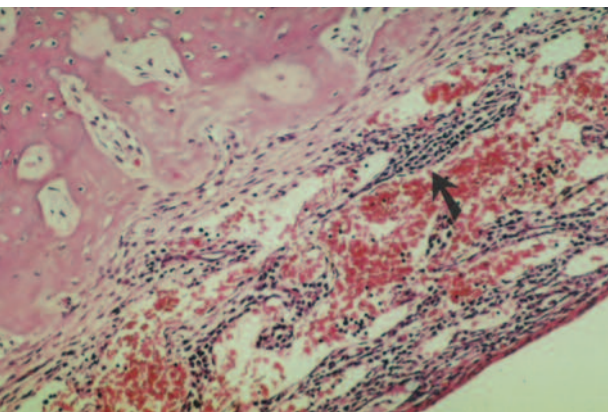


Figure 3. Light microscopic picture of inflammatory cells in group B (arrow). HE25

There were findings of foreign body reaction in four ears in group B (3 diffuse, 1 focal), in four ears in group C (all focal) and in one ear in group D (focal)

(Figure 4). There was a statistically significant difference between groups ($p=0.09$). Single way variance analysis (ANOVA) and post-hoc Dunnett verification test was applied to groups. While there was a significant difference between groups A and B, we could not find any significant difference between groups B and C, groups B and D and groups C and D.

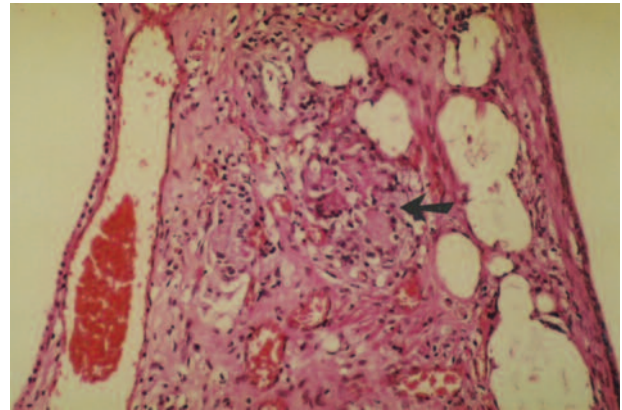


Figure 4. Foreign body reaction in the middle ear (arrow). HE50

Discussion

Absorbable gelatin sponge has been used widely in otologic surgery especially as a supporting substance to support the tympanic membrane grafts and ossicular chain and as a closing material of the oval window in stapes surgery. However, there still exist some controversies on its use. Since the introduction of AGS, numerous studies have been performed to clarify effects of AGS on surgical outcome and some of them showed significant histopathological changes in middle ear which are attributable to use of AGS [1,3,4,7-9].

Severe inflammation and fibrosis in postoperative period could be one of the reasons of unsuccessful results in tympanoplasty [4,10]. Several animal studies have been performed to investigate formation of fibrous tissue in AGS filled middle ears, but the results are controversial. Liening et al. [3] showed that AGS promotes fibrosis at a significantly higher rate than absorbable gelatin film or collagen-absorbable hemostat. Harris [11] demonstrated the invasion of gelfoam with fibroblasts in 72 hours after stapedectomy resulting connective tissue formation on oval window in his study performed on cats. On the other hand, Withers et al. [12] placed AGS in a cats middle ear with intact mucosa and found no secondary

fibrosis or residue of AGS after 6 weeks. Conversely, Doyle-Kelly^[8], Hellstrom et al.^[1] and Bahadır et al.^[9] found fibrous tissue formation and fibroblast infiltration in normal middle ear mucosa after AGS use. In his study on 31 dog ears, Joseph^[4] noted that significant fibrosis was observed in all ears that AGS was used, if the mucosa was abraded, and mixing AGS with dexamethasone could not prevent fibrous tissue formation. He also suggested that infection has no effect on fibrosis, because he also observed fibrous tissue formation in ears without infection that he used antibiotic ointment.

We did not observe any fibrosis in the control group that we only traumatized the middle ear mucosa, but there was formation of fibrous tissue in all other groups. However, even if the difference was not statistically significant, fibrosis was slighter in groups where AGS was soaked with dexamethasone or

dexamethasone+antibiotic mixture. These results suggest that, even the use of corticosteroids do not prevent fibrosis completely in ears with abraded mucosa, they may reduce fibrous tissue formation and thus complications related to fibrosis in middle ear cavity postoperatively.

In the literature, there is no consensus on the resolving duration of AGS from middle ear cavity. Although in some studies complete resolution of AGS was seen in 15 days^[4,13,14], some others demonstrated AGS residue in the middle ear cavity up to 3 to 9 months^[1,15]. Kylander et al.^[14] showed polymorphonuclear leukocyte infiltration of gelfoam at the postoperative 31st hour and this process continued until all gelfoam is resolved at the end of second week and left its place to fibrous tissue formation. Bauer^[13] showed that AGS was absorbed in guinea pig middle ear after 15 days. On the other hand, in their study on rats, Hellstrom et al.^[12] noted that AGS was not completely resolved even after 2 or 3 months postoperatively. Schuknecht^[15] observed AGS residue in one of his patients postoperatively on the ninth month. He noted that the AGS was not resolved but infiltrated by surrounding soft tissues and its surface was covered with membrane. It was also shown that, AGS resorption time was not shortened after soaking AGS with antibiotic solutions^[8] or physiologic saline, corticosteroid and propylenglycol mixture^[4]. In our study, we found microscopic AGS residue only in five

middle ears at the end of fourth week, 19 ears were free of AGS. There was no significant difference between groups in the aspect of AGS residue degree, so we can suggest that using corticosteroid or antibiotic solutions has no effect on resolution duration of AGS.

Light and Prentice first reported that AGS caused a mild cellular reaction dominated by an invasion of polymorphonuclear cells. Hellstrom et al.^[1] also noted that AGS itself was invaded by polymorphonuclear leukocytes and was characterized by a collagen producing fibroblast proliferation of the vessels. Similar findings suggesting acute inflammation was also seen in our study, in groups A, B and C. We could not observe any finding of acute inflammation in the group that we used antibiotic solution, but the difference between groups was not statistically significant.

Dogan et al.^[7] compared foreign body reaction seen in middle ears filled with dry AGS and AGS soaked with hyaluronic acid, and they reported similar degree of foreign body reaction in both groups. However, they observed that number of foreign body cells were higher in quantity, they were greater in shape and their nuclei were larger in hyaluronic acid group. There was focal foreign body reaction in four of the ears in dexamethasone group, and there was focal reaction only in one ear in dexamethasone + levofloxacin group. The difference between the control group and study groups was statistically significant, but there was no statistically significant difference between study groups. We observed foreign body reaction in all groups that we filled middle ear with AGS, but the reaction was more diffuse in the group that AGS was soaked only with physiologic saline. Even if the reaction against foreign bodies is a biologically necessary process for defensive purposes, it might play a role in failure of the graft that is used in tympanoplasty if the reaction is intense. We cannot conclude that addition of steroid or antibiotic solution to AGS middle ear packing will reduce foreign body reaction, but non-significant trends in our data suggest that using them may reduce inflammation and fibrosis.

AGS causes several histopathological reactions in middle ear mucosa as our study, and similar previous studies have shown. It is evident that a reduction of postoperative changes in the connective tissue is

highly desirable and beneficial to the outcome of ear surgery. Since AGS is a widely accepted and still mostly used supporting substance in ear surgery, it is mandatory to reduce its undesirable effects. Even though our results are not statistically significant, when they and the literature are evaluated together, we think that less adverse effects might be achieved by using as small as possible AGS pieces, soaked with antibiotic and corticosteroid mixture. Further animal studies with more subjects have to be performed to find out the exact effects of the use of AGS on tympanoplasty outcomes.

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