ORIGINAL ARTICLE

Changes in the Middle Ear Mucosa of Guinea Pigs After the Insertion of Preserved Teeth: A Pathologic Study

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OBJECTIVE: In this study, we analyzed the pathologic changes in the middle ear mucosa after the insertion of preserved allograft teeth in guinea pigs by examining serial sections under the light microscope.

MATERIALS AND METHODS: Sixteen pigmented guinea pigs were included in this 2-stage study. In the first stage, 2 guinea pigs were killed, and a preservation technique that included autoclave sterilization was applied to their teeth. The proximal 3 quadrants of the dentition, including the root and pulpa, were separated from the distal quadrant, (approximately 1 mm3). In the second stage, a fragment was placed in the middle ear mucosa of the 12 remaining guinea pigs. The guinea pigs were coupled randomly, and was sacrificed sequentially on study days 3, 5, 10, 20, 40, and 60. The temporal bones were excised, and sections were obtained. The preparations were stained with hematoxylin-eosin and were analyzed under the light microscope.

RESULTS: Although the pathologic changes surrounding the teeth were concordant with acute inflammation on the third and fifth postsurgical days, evidence of chronic inflammation was first noted on the 10th day after surgery. That inflammation subsided on the 20th day, and a fibroepithelial capsule encircled the teeth. Assessments on the 40th and 60th postsurgical days revealed that the inflammation was progressively resolving.

CONCLUSION: The middle ear mucosa did not reject the preserved allograft teeth; instead, the implants were accepted as autologous tissue. No formation of new bones or resorption occurred during the brief follow-up period.

A successful tympanoplasty requires a ossicular chain to conduct sound from the intact tympanic membrane to the inner ear. In patients with otitis media, which causes the destruction of the bone chain, and in patients in whom the ossicles have been destroyed, reconstruction of the bone chain is one of the most critical stages of treatment.

In the last 2 decades, the development of biological and synthetic prostheses for the replacement of the bone chain has made it possible to perform more successful bone chain reconstruction operations (ossiculoplasties). Research on this subject is still being conducted. Preserved teeth have been used as an implant material in ossicular reconstruction. The aim of this study was to analyze the pathologic changes caused by allograft preserved teeth in the middle ear mucosa of guinea pigs.

MATERIALS AND METHODS

In this study, which analyzed the response of the middle ear mucosa to the preserved allograft teeth, 16 adult male pigmented guinea pigs were used. The animals were obtained from the Pendik Veterinarian Faculty in Istanbul, Turkey. Each guinea pig weighed between 350 and 400 g.

The study was performed in 2 stages. In the preliminary stage, 2 pigmented guinea pigs were killed after having received a general anesthetic (ether). The teeth of those 2 animals were extracted. A preoperative preservation technique that has been used in human allograft ossicles was applied to these teeth. In that preservation technique, the teeth were placed in 4% formaldehyde (pH, 5.6) for 72 hours. They were then sterilized for 20 minutes at 135°C and were preserved in 0.5% formaldehyde at a pH of 7 at 40°C[4]. In this first of 2 stages, the teeth were thus preserved.

In the second stage of the study, the remaining 14 guinea pigs received intramuscular ketamine 65 mg/kg and xylazine hydrochloride 12.5 mg/kg after a subcutaneous injection of 0.2 mg/kg atropine sulfate. The guinea pigs were moved to a dark room, where they remained for 5 minutes to adapt to anesthesia. The environmental temperature was kept above 20°C. The animals were fixed on the operating table with a rubber band between their teeth. Their eyes were closed, and their legs were attached to the table. Oxygen support was provided. The anesthesia allowed 30 minutes for the procedure. A 2-mm round speculum was placed in the outer ear canal, and the tympanic membranes were examined with a surgical microscope (Opmi 99, Carl Zeiss, Jena,West Germany).The outer ear canal of each guinea pig was washed with warm saline and cleansed with povidone-iodine (Betadine). Paracentesis of the tympanic membranes was performed with a pediatric scalpel. One guinea pig in which a massive hemorrhage in the tympanic membrane was detected and another with an unidentified foreign object in the external ear canal were excluded from the study. Twelve subjects thus remained.

The preserved teeth were washed with saline. With the use of a drill, the proximal 3 quadrants of the dentition, including the root and pulpa, were separated from the distal quadrant. The distal quadrant was further sectioned into irregular fragments (approximately 1 mm3). In the next stage of the study, a needle was used to place a fragment into the middle ear mucosa of each of the 12 guinea pigs, all of which had received a general anesthetic. A single dose of cefazolin sodium 30 mg/kg was administered after manipulation.

According to the study plan, the guinea pigs were coupled randomly, and the couples were killed sequentially after having received a general anesthetic (ether) on study days 3, 5, 10, 20, 40, and 60. The temporal bones of the sacrificed guinea pigs were excised.

The samples were fixed in 10% formaldehyde. After the decalcification procedure, which was performed in the Dr. Lutfi Kirdar Training and Research Hospital Clinic of Pathology, macroscopic images of the temporal bones were obtained (Figure 1). The samples were processed routinely. After the paraffin blocking procedure had been performed, twenty 4-mm slices were obtained via a spinning microtome for pathologic examination. The slices were stained with hematoxylin-eosin. Pathologic examination was performed under a light microscope by the same pathologist.

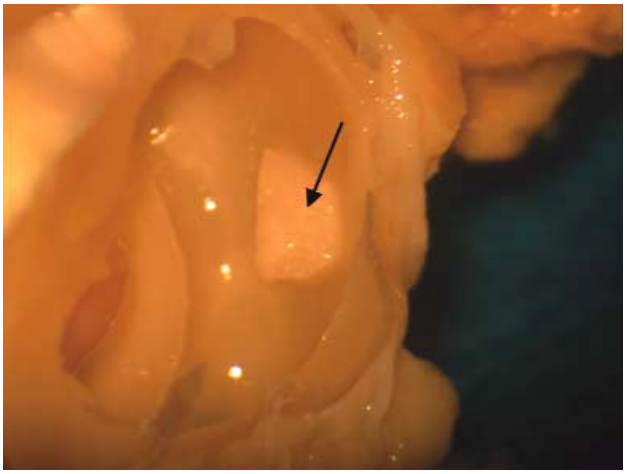

Figure 1: Macroscopic images of a tooth in the tympanic bulla obtained from guinea pigs killed on the third postoperative day. A tooth fragment (arrow) is visible.

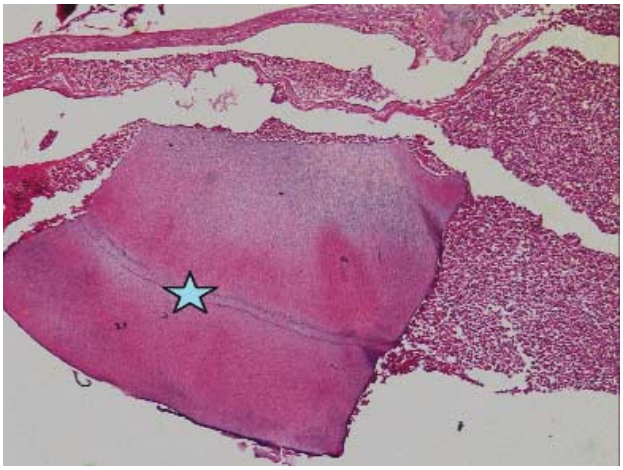

Figure 2: A histologic image of acute inflammation around a tooth embedded in the tympanic bulla of a guinea pig killed on the third postoperative day (hematoxylin-and-eosin stain, original magnification x100). Note the tooth fragment (star).

RESULTS

All animals were anesthetized before manipulation. There were variations in the initiation and the duration of the anesthetic effect. Some animals required the administration of additional anesthetic. No adverse reactions to the anesthetic drugs were observed.

The slices obtained from the temporal bones were examined pathologically with regard to inflammatory response, the epithelial sheet around the dental implant, the fibrous capsule, foreign-body reaction, the resorption of teeth, the presence of tympanic bullae, and the development of new bone structure.

Acute inflammation characterized by the predominance of polymorphonuclear leucocytes (Figure 2) was prominent in the guinea pigs killed on study days 3 and 5. Chronic inflammation with a predominance of macrophages and plasma cells was notable after study day 10. Lymphocytes were also present around the teeth.

The inflammation subsided after the 20th day of the study, and a fibroepithelial capsule had begun to develop around the prostheses (Figure 3). Progressive resolution of the inflammation was noted on the 40th day, and the inflammation had resolved completely by study day 60. The cells of the epithelial tissue overlying the implant were simple, single-lined cuboidal cells that

Figure 3: A histopathologic image of the fibroepithelial capsule forming around a tooth in the tympanic bulla of a guinea pig killed on the 20th postoperative day (hematoxylin-and-eosin stain, original magnification _100). A tooth fragment (star) and the fibroepithelial capsule forming around the tooth (arrow) are shown.

resembled the cells of the middle ear mucosa. These epithelial cells had no cilia. No goblet cells were present among the epithelial cell lines. The fibrous capsule underlying this epithelial layer consisted of a few lines of fibroblasts and collagen and was parallel to the teeth (Figure 4). Small blood vessels were present in the fibrous layer. Teeth were attached to the middle ear mucosa, and a fibroepithelial capsule surrounded them.

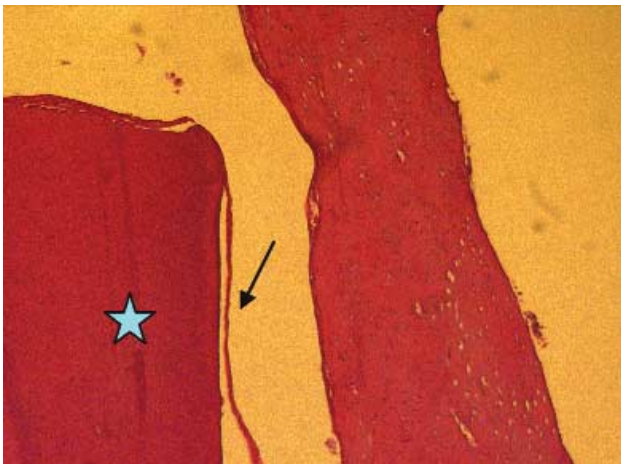

Figure 4: A histopathologic image of a fibroepithelial capsule that has formed around a tooth in the tympanic bulla of a guinea pig killed on the 40th postoperative day (hematoxylin-and-eosin stain, original magnification x400) and on the 60th postoperative day (hematoxylin-and-eosin stain, original magnification x100). Note the tooth fragment (star) and the fibroepithelial capsule forming around the tooth (arrow).

In the serial sections, no new bone development was noted at the contact point of the teeth and the tympanic bullae. Giant cells, which indicated the resorption of the implants and a foreign-body reaction, were not present during the follow-up period. No bacterial clusters suggestive of infection were detected in the tissue sections.

DISCUSSION

Middle ear implants should be inexpensive, easily obtained, adequately rigid, easily formed, and biologically accepted by the recipient organism. They should also preserve or increase hearing. Although many researchers believe that implants made from autologous ossicular bone or temporal bone cortex are the best materials for middle ear implants, we suggest that the perfect material for use in that type of otologic surgery has not yet been discovered.

Zini first introduced and used teeth as an alternative material for ossicular reconstruction More than a decade later in an animal study, Kirtane and colleagues[2,3] confirmed the inertness of human teeth and compared the tissue reaction evoked by teeth to that elicited by a homologous human incus on the thigh of a white mouse. Those authors subsequently used preserved allograft human teeth in 20 patients. No evidence of rejection was observed in their subjects, in whom the longest follow-up was more than 2 years. The hearing improvement was satisfactory in 15 patients, in whom the air-bone gap was reduced less than 15 dB.[2,3]

Huttenbrink and Lindorfer performed ossiculoplasty on a sample of 400 human patients in whom teeth were used with successful results. In a retrospective analysis, those authors examined the short-term results (ie, at least 1 year after implantation) of that procedure and were satisfied with teeth as an implant material[4].

Ogale and colleagues[5] performed ossicular reconstruction in 613 patients. In their study, various graft materials (autologous ossicles or tragal cartilage, homologous preserved ossicles, cortical bone, human dentin, cadaveric styloid process, prosthetic gold) were used for ossicular reconstruction. However, graft materials such as autologous and homologous ossicles have been found to have better uptake and long-term survival.

Ogale and colleagues used human dentin in 28 patients, and although the early results of that procedure were promising, a high rejection rate (7.14%) developed during long-term follow-up[5].

In our study, the middle ear mucosa accepted the allograft teeth as autologous tissue. No evidence of rejection was observed. However, the low antigenic property of the allograft teeth and the absence of a foreign-body reaction may be attributed to the small (1 mm3) size of the tooth fragments, to the preservation procedure performed before implantation, and/or to the brief follow-up period.

Although allograft materials have the advantage of being biologically acceptable, they carry the risk of transmitting the acquired immunodeficiency syndrome and slowly progressive viral infections such as Creutzfeldt-Jakob disease[6]. For that reason, the use of allografts is banned in many countries. Before any allograft is implanted, a detailed history from the donor must be obtained and a panel of serologic tests should be performed to exclude the possibility of transmitting

infection. In addition, a presurgical preservation technique should be used to treat the allograft. In our study, except for the preservation technique that included autoclave sterilization, the distal quarter of the dentition (which has a relatively less porous structure and does not contain nerve endings) was used as the allograft to minimize the chance of transmitting infection.

Kirtane and colleagues[2] examined previously implanted teeth in a patient who underwent 1 revision surgery and stated that the structure of the teeth was maintained histologically. However, Hartwein and colleagues detected resorption in each of 3 patients who underwent 1 revision[7]. Although resorption was complete in 1 of those 3 individuals, only histopathologic findings of resorption were identified in the remaining 2 patients. In animal studies, Stewart and colleagues showed that although resorption had occurred, it had progressed slowly over time, and the histologic demonstration of that resorption was revealed only by means of serial sectioning[8]. In our study, no histologic resorption was observed during the brief follow-up period.

Teeth that have been implanted without prior preservation have been shown to cause the extensive formation of new bone[8] and are prone to losing their osteogenic properties. The preservation technique that was applied to the teeth in our study included the use of chemicals as well as autoclaving[9]. No formation of new bone at the contact point of the teeth or the tympanic bullae was detected, perhaps as a result of the presurgical preservation technique used.

Ogale and colleagues reported that human teeth are difficult to shape[5]. However, Hartwein and Koch[10] and Huttenbrink and Lindorfer[4] indicated that a tooth is an easy substance to form. In addition, Huttenbrink and Lindorfer proposed that medical residents be educated about the formation of teeth during their study of diseases of the ear, nose, and throat so that tympanoplasty could be performed more quickly and more successfully[4].

Although the results of our study were not verified objectively, they revealed that teeth can be easily formed with currently available equipment of otologic intervention. During the drilling process, it was noted that the preserved teeth did not lose their mechanical properties. When evaluated with regard to cost, the most inexpensive implants were found to be autograft implants, which are obtained from the patients' own tissues. It takes approximately 15 minutes to prepare these implants in the operating room. Therefore, the expense can be calculated by including the cost of anesthesia and staff. Allograft teeth are similar in cost to autograft implants with exception of the additional charge for preservation.

Our study was performed in a very small group (12 guinea pigs), and the follow-up period (60 days) was brief. The use of preserved teeth as an alternative material in ossiculoplasty, especially when the use of autologous ossicles is not possible, should be evaluated. Specifically, the long-term results of the status of the teeth and the success in conducting sound after middle ear infection must be studied in a large number of samples.

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