Original Article

Inflammatory responses to Hydroxyapatite implants in middle ear in rats

YE Qing1,2, JIANG Yi1,2, WANG Xiao-yan1,2, ZHENG Ke-fei1,2
1 Department of Otolaryngology, Fujian Provincial Hospital, Fuzhou 350001, China
2 Department of Otolaryngology, Fujian Medical University, Fuzhou 350001, China

Abstract Objective To study local inflammatory response after implantation of hydroxyapatite synthetic ossicular prosthesis. Methods Hydroxyapatite granules were implanted in the bulla in 32 rats. Sham surgical procedures were performed in 10 rats as the control. Animals were sacrificed at 1 to 300 days after surgery. Bulla sections, stained with HE and Mallory’s azan, were examined for numbers and percentages of various inflammatory cell types. Results Slightly more inflammatory reaction was seen in animals with the implant than in the controls, mostly during the early stage following the implantation procedure. Few inflammatory cells were observed at later times. There were satisfactory fibrosis in both implanted and control ears. Conclusion The results indicate that hydroxyapatite synthetic prosthesis is a biocompatible implantation material in the middle ear. Nonetheless, the presence of inflammatory reaction immediately following implantation implies that control of infection is important in the early times after the implantation procedure.

Key words Middle ear; Hydroxyapatite; Synthetic auditory prosthesis; inflammatory response

Introduction

Various biomaterials are being widely used in clinical practice1. Knowledge of how a material interacts within the environment of the body, particularly at the implant/body interface and in the surrounding tissues, is necessary to determine its applicability as an implant material. One factor among several that determine the success of an implant is the inflammatory reaction at the recipient site2,3.

Hydroxyapatite, a calcium phosphate compound [Ca10(PO4)6(OH)2], has broad otological application in reconstruction of the auditory ossicular chain and in obliteration of the mastoid cavity due to its biocompatibility, mechanical and chemical properties. Few studies, however, have focused on quantifying inflammatory reactions in the middle ear mucosa following hydroxyapatite implantation. This study is to study histological evidence of inflammatory reactions in the middle ear mucosa in rats implanted with hydroxyapatite granules, a synthetic material identical in composition and density to that currently used in reconstructive ear surgery.

Materials and methods

Implant material

Hydroxyapatite granules, measuring approximately 1.0 × 1.5 mm, weighing between 3.5 and 4.0 mg, were prepared from commercially available synthetic ossicles (Asahi Optical, Tokyo, Japan). X-ray diffraction analysis was used to verify that granules contained 99.56% Ca10(PO4)6(OH)2 and 0.44% CaO. Prior to implantation, granules were sterilized in an autoclave for 30 min at 121℃.

Animal and surgical procedure
All experimental animal procedures were approved by the Animal Care and Use Committee of Fujian Medical University Provincial Clinical College.

Forty-two 8-week-old female specific pathogen-free Wistar rats, weighing between 140 and 150 g., were used in this study. Rats were anesthetized by intraperitoneal injection of 35 mg/kg pentobarbital sodium. Under sterile conditions, an incision of approximately 7 mm in length was made 3–4 mm behind the root of the left auricle. The surface of the bulla was exposed by blunt dissection with mosquito forceps. A bone curette was used to carefully open a hole of approximately 2~3 mm. The bulla cavity was filled with hydroxyapatite granules in 32 rats. A control group of ten rats underwent the same procedure without implantation of hydroxyapatite granules.

Four rats from the implant group, and two rats from the control group were sacrificed by decapitation under general anesthesia on each of the following times: Days 1, 3, 7, 14 and 30 following surgery. The remaining animals from the implant group were sacrificed in groups of four at 90, 180 and 300 days following surgery.

Section preparation

Following decapitation, the middle ear was filled with formalin via a needle through the tympanic membrane, while the entire head was immersed in 10% phosphate-buffered formalin for 3 days. After decalcification for 3 days, the bulla was removed under stereoscopic microscope, dehydrated in a series of ethanol, embedded in paraffin, and then sectioned at a thickness of 6 μm. Sections were stained with haematoxylin and eosin (HE), and Mallory's azan.

Histological examination

Sections were photographed under light microscopy using color slide film. Inflammatory cells were identified and counted on enlarged images projected via a slide projector. Percentages of various cell types were calculated.

Results

Mild inflammatory reactions were observed in the mucosa in both the implant and control groups at all time points studied. Erosion and degeneration of epithelia were observed at the recipient sites. Inflammatory cells included neutrophils, macrophages and lymphocytes (Fig. 1).

Fibroblasts, fibrocytes, and, in some cases, capillaries were visible. No foreign body giant cells were seen in either group at any time point studied (Tables 1, 2).

Inflammatory reactions from 1–30 days after implantation were consistent with acute inflammatory response, especially at the 1st day. Cell counts revealed significant numbers of neutrophils (3.3%), lymphocytes (14.2%) and macrophages (11.5%). Fibroblasts totaled 15.6% and fibrocytes 14.5%. At 3 days, there were a sharp decrease in the percentages of lymphocytes and macrophages and absence of neutrophils.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Averaged percentages of cell types in the middle ear mucosa after hydroxyapatite implantation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>3.3</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>14.2</td>
</tr>
<tr>
<td>Macrophages</td>
<td>11.5</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>15.6</td>
</tr>
<tr>
<td>Fibrocytes</td>
<td>14.5</td>
</tr>
<tr>
<td>Other cells*</td>
<td>40.9</td>
</tr>
</tbody>
</table>

* No foreign body giant cells were observed.
** Other cells: These include mucosal epithelial cells, plasma cells and unidentified cells.
Table 2  Averaged percentages of cell types in the middle ear mucosa after sham procedure in control animals (%)*

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>1 day</th>
<th>3 days</th>
<th>7 days</th>
<th>14 days</th>
<th>30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>1.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>8.1</td>
<td>1.9</td>
<td>1.4</td>
<td>1.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Macrophages</td>
<td>5.0</td>
<td>1.1</td>
<td>0.5</td>
<td>0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>14.4</td>
<td>23.1</td>
<td>17.5</td>
<td>15.9</td>
<td>16.1</td>
</tr>
<tr>
<td>Fibrocytes</td>
<td>13.3</td>
<td>26.7</td>
<td>30.7</td>
<td>32.6</td>
<td>33.3</td>
</tr>
<tr>
<td>Other cells**</td>
<td>57.9</td>
<td>47.2</td>
<td>49.9</td>
<td>49.7</td>
<td>49.5</td>
</tr>
</tbody>
</table>

* No foreign body giant cells were observed.
** Other cells: These include mucosal epithelial cells, plasma cells and unidentified cells.

Fibroblast and fibrocyte numbers were increased. Between 7 and 30 days after implantation, specimens showed further decrease in the numbers of macrophages and lymphocytes, and no neutrophils. There were fewer fibroblasts and slightly increased number of fibrocytes.

Inflammatory reactions observed in the control group were similar to that of the implant group, but to a lesser degree. Within 3 days following surgery, lymphocyte and macrophage numbers had also decreased rapidly in the control group, and neutrophils were absent. Between 3 and 7 days, the control group showed a greater reduction in lymphocyte and macrophage numbers than did the implant group.

In the implanted animals, inflammatory reactions 90–300 days after surgery showed that low numbers of lymphocytes and macrophages, closely resembling that of the control group at 30 days. The number of fibroblasts continued to decrease between during this period, while that of fibrocytes increased, reaching a maximum at 300 days (Fig. 2).

Hydroxyapatite is the main inorganic constituents of mature bone, providing optimal biocompatibility as a bone replacement in otolaryngological and other surgeries, such as implantation of a synthetic auditory ossicle. Successful implantation of a middle ear prosthesis is reliant on the biological response evoked in the mucosa of the middle ear, and influenced by factors such as the anatomical and immunohistological architecture of the middle ear, as well as mechanical and chemical properties of the implant biomaterial.

This study seeks to examine the histological changes that occur in the middle ear mucosa following implantation of hydroxyapatite granules. The time points chosen to examine these changes is based upon our understanding of inflammatory
reaction, which, in general, consists of an acute stage lasting up to about 14 days after an injury, and a chronic (recovery) stage transpiring about 30 days subsequent to injury, after which few changes are evident.

Implantation of hydroxyapatite granules in the bullae of rats caused extremely low level inflammatory reactions. The microscopic changes evident in the control group were typical of that expected to follow a surgical intervention. At 1 day after surgery, higher percentages of inflammatory cells were evident in the implant group, reflecting an effect of the implant on the mucosa beyond that due to normal surgical intervention. At 3 days after implantation, percentages of inflammatory cells had decreased and were close to that of the control group, with no neutrophils in either groups. These changes indicate normal recovery from tissue injury, and suggest that, in the implant group, mucosal tissue adapted well to hydroxyapatite within a short period of time.

From 7 to 300 days after surgery, percentages of lymphocytes and macrophages in the implant group gradually decreased, becoming increasingly similar to those of the control group at 30 days. The relatively small number of macrophages and lymphocytes present in this control group is similar to that reported in immunological studies of normal middle ear mucosa. Thus, we infer that the results seen in the implant group at 300 days after surgery are nearly normal.

No foreign body giant cells were seen in either group at any time point studied. The mechanism for formation of foreign body giant cells through fusion of activated macrophages is not completely understood. Such formation is influenced by factors such as the pH of the environment, cytokines (including γ-interferon and interleukin-4) released by various cells and macrophage aggregation, and the chemical composition and physical properties of implanted materials. If an implant material has favorable biocompatibility, mechanical and medical properties, in addition to excellent host acceptance, foreign body giant cells may not appear. Their absence in this study suggests that hydroxyapatite is suitable for implantation in the middle ear.

Development of fibrosis is a criterion for biocompatibility of a biomaterial, and the appearance of mature fibrous connective tissue indicates satisfactory implantation. Macrophages play an important role in inflammatory response, stimulating proliferation of fibroblasts and fibrocytes during repair of a tissue lesion. Fibroblasts and fibrocytes have been observed in the submucosa of the middle ear soon after implantation, as a thin connective tissue layer consisting of fibroblasts, fibrocytes and associated collagen fibers. Fibroblasts and fibrocytes were observed in our study 1 day after implantation. Fibrocytes increased steadily between 3 and 300 days, to a level of 2.7 times of that observed on day 1. These results suggest that hydroxyapatite is a good material for implantation in the middle ear.

The acute inflammatory cell infiltrates evident in the present study were primarily lymphocytes. Migration of lymphocytes is probably due to the function and anatomy of the middle ear mucosa. Besides epithelial cells, fibroblasts and fibrocytes, there are many lymph capillaries, some wandering tissue phagocytes, and occasional plasma cells and lymphocytes in the mucosa of the middle ear. Middle ear mucosa has a stable and well-controlled immunological defense system involving secretion of immunoglobulin from plasma cells, and cytokines from macrophages, T and B cells, especially in inflamed middle ear mucosa.

Conclusions

The tissue reactions observed in the present study were similar to other experimental and clinical studies of hydroxyapatite implantation in the ear, which have reported low levels of inflammation and fibrosis. Combined with other reports of satisfactory integration of hydroxyapatite–bone formation, and the mild inflammatory reactions evident in the mucosal tissue after implantation of
hydroxyapatite granules in our study, we conclude that hydroxyapatite is suitable for use in ossiculoplasty or obliteration of the mastoid cavity. However, it should be emphasized that infection control is important after implantation, especially at early stage.

Acknowledgement

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References


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