MORPHOLOGICAL REACTIONS OF WISTAR RAT TISSUES EXPOSED TO ACETAL RESIN IMPLANTS

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Abstract

Morphological changes developed in rat bucal mucosa, parotid glands, and lymphoid nodes following 6 weeks exposure to dental acetal resin implants were compared to metal/titanium, acrylic implants, and sham-operated rats. Morphological evaluation of bucal mucosa of rats exposed to acetal resin showed an increased accumulation of lymphoid and macrophage-like cells compared to sham-operated rats. Eosinophils were found in bucal mucosa and in parotid glands of rats exposed to acetal resin, although this change did not differ significantly from sham-operated rats. Morphological findings with respect to granular reaction and proliferation were similar in acetal resin exposed and sham-operated rats. Acetal resin implants may induce inflammatory and potentially allergic reactions at the site of implantation.

Key words: rats, dental implants, acetal resin, side effects.

Continuous advances in science of materials, combined with ever increasing demands and expectations from both doctors and patients, have contributed to the market launches by dentistry materials manufacturers of ever more refined and modern products. Synthetic, as well, as metal alloys are widely used in dental prosthetics. The most common denture materials are derived from methyl polymetacryl, which is classified as an acrylic substance (5, 6). Tolerance of dental materials is an issue, which draws attention of many research groups (1, 3, 8, 9).

Acetal resin is a thermoplastic polymer with a monomer-free crystalline structure and is a product of formaldehyde polymerisation. The resin is characterised by a high abrasion resistance, an excellent tensile and shock strength, a high elasticity, and a low thermal conductivity, and it ensures an appropriate rigidity for the denture base. According to the manufacturer, acetal resin is neither toxic, nor allergenic. The resin is manufactured in 14 shades and can be used for the majority of dental prosthetic work, including novel and non-standard structures, which can be produced thanks to the application of thermal injection technology. Acetal resin is relatively new to dentistry, but the range of application is already broad: metal-based dentures, aesthetic clasps for standard metal-based dentures, partial butt joints, bridges and bridges on telescopic crowns, prosthetic components on implants, occlusal splints and stabilisation splints, and retaining elements on struts, which also represent the strengthened basis of the denture structure, among others. Denture components made of the material are marked by a high chemical and a mechanical stability and a high resistance parameters for a pH range of 4 to 9.

Acetal resin is used for elastic dentures as it has 30% elasticity compared to the 6% elasticity of chromium-cobalt alloy. The introduction of removable dentures made of chromium-cobalt-molybdenum alloy, acetal resin, or acrylic substances into the oral cavity means a short- or a long-term contact with foreign material (4, 10). Following the incorporation of the denture, advantageous conditions exist for a conflict to appear between the host and the prosthetic material used. Pathological changes may take the form of atrophy, hypertrophy, or inflammatory processes (4, 9, 10).

The aim of the study was to evaluate morphological changes in bucal mucosa, parotid glands, and lymphoid nodes of rats exposed to acetal resin implants in comparison to metal/titanium and acrylic implants, and sham-operated rats.

Material and Methods

Implanted material. Acetal resin plates were prepared from T.S.M. Acetal Dental (Pressing Dental S.r.I, Dogana, San Marino) The prepgs were stacked
and molded under the temperature of 220°C and pressed in 5.5 atm. The value of Young’s module of acetal resin determined during bending tests was 2.6 GPa. Such a material was further used for the preparation of implants. Acetal resin plates, 2x2x1 mm of size, were cut, washed in deionised water, and autoclaved before implantation. Plates of identical size were prepared from metal (titanium) and acryl. Prior to implantation, all plates were immersed in 2% Virkon solution for 20 min, and then flushed with 3% hydrogen peroxide solution.

**Animals.** Wistar rats (males, weighing 426 ±27 g) were kept under the standard environmental conditions and had free access to standard laboratory chow and drinking water. The total of 46 rats were used for the experiments. The rats were observed over the course of 6 weeks for general condition and alertness. The rats were weighed before the procedure and every 2 weeks. The implanted material did not impair their ability to feed, as their weight growth corresponded to their age. Ethical permission was obtained from the Animal Research Ethics Committee of the Pomeranian Medical University. The guidelines of the local Ethical Committee for the animal experiments were followed.

**Implantation procedure.** Rats were divided into test group with acetal resin plates (n=13) and two control groups, which obtained metal/titanium plates (n=10) and acryl plates (n=13). Sterile plates were implanted under general anaesthesia induced by intravenous ketamine hydrochloride (130 mg per 1 kg of body weight) injection. An incision was made in the mucous membrane of the cheek, the plate was implanted under the membrane, and the incision stitched with absorbable suture. The sham-operated rats (n=10) received no plates implanted, an incision was made in the mucous membrane of their cheeks and stitched with dissolving suture to eliminate the possibility of the tissues reacting to the stitching. Following 6 weeks, the experiment was terminated and the implanted plates with surrounding tissues were excised for a histological evaluation.

**Tissue preparation for morphological evaluation.** Specimens of tissues surrounding the implanted plate including the mucous membrane of the cheek, and the parotid gland in the vicinity of the implanted plate. Additionally, neck lymph nodes were taken for pathological examination. The tissues were placed in 4% buffered formalin, dehydrated, and embedded in paraffin. Paraffin sections of 4 μm were prepared and stained with haematoxylin and eosin.

**Morphological evaluation.** The sections were examined at different magnifications under the light microscopy. The specimens of bucal mucosa were evaluated for the presence of inflammatory cell infiltration, fibroblast proliferation, signs of keratosis, and presence of multinuclear (giant) cells by two independent investigators. The intensity of morphological changes was arbitrary judged as mild, moderate, and severe. Inflammatory cells within the infiltrates were distinguished as lymphocytes, macrophages, and eosinophils on the basis of morphological appearance. The parotid glands were evaluated for the influence of toxic substances contained in the prosthetic material.

**Statistical analysis.** For statistical evaluation of the results, values of continuous variables (haemoglobin etc.) and rank variables (e.g. degree of intensity of inflammation features in histopathological examination) were compared along four groups of rats using Kruskal-Wallis test, subsequently values of particular groups were compared using Mann-Whitney test. For all calculations the results were considered as significant at P<0.05.

**Results**

**Morphological findings in the bucal mucosa.** Morphological evaluation was performed in the sections of bucal tissues surrounding the implanted plates. The following changes in the surrounding tissue were scored: keratosis of striated squamous epithelium and inflammatory infiltration composed of histiocytes, fibroblasts, and leukocytes (Fig. 1 a). Arbitrary scoring of these changes in relation to the implanted material is presented in Table 1.

In the microscopic images of the sham-operated group specimens, dermal and subdermal connective tissues demonstrated a smoothly transition into the mucous membrane pattern (Fig. 1 b). The mucous membrane had a well-developed corneal layer, similar to derma. The transition between skin and mucous membrane was composed of small histiocytes and few neutrophils. The sections obtained from rats implanted with acetal resin had significant lymphoid and macrophagal infiltration of the mucous membrane as compared to the sham-operated rats, while fibroblast infiltration was similar. Lymphocyte infiltration of bucal mucosa exposed to acetal resin implants was significantly more pronounced, when compared to sham-operated rats and those exposed to acrylic implants. Additionally, acetal resin implanted rats had significantly less cysts and/or pseudocysts compared to acrylic and sham-operated rats. In one sample from acetal resin exposed rats, a cyst lined with stratified squamous epithelium was noticed.

**Morphological changes in the lymph nodes.** Morphological evaluation of the neck lymph nodes indicated a lacunar reaction in the lymph nodes of four (31%) acetal resin implanted rats.

Essentially, the histological changes in the lymph nodes of rats implanted with acetal resin indicated only a modest increase in connective tissue proliferation as compared with sham-operated rats and those with implanted plates prepared with other dental materials (see Table 1). Granular reaction in the lymph nodes was similar independently of the type of dental material implanted.
Table 1

<table>
<thead>
<tr>
<th>Morphological parameter</th>
<th>Sham-operated (n=10)</th>
<th>Metal/titanium (n=10)</th>
<th>Acetal resin (n=13)</th>
<th>Acryl (n=13)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histology of the mucous membrane</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Giant cells</td>
<td>0%</td>
<td>0%</td>
<td>15%</td>
<td>36%</td>
<td>0.04</td>
</tr>
<tr>
<td>Cysts/pseudocyst</td>
<td>30%</td>
<td>18%</td>
<td>8%</td>
<td>100%</td>
<td>0.005</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>0.9 ± 0.9</td>
<td>1.1 ± 0.5</td>
<td>1.0 ± 0.8</td>
<td>1.0+0.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Lymphoid cells</td>
<td>0.3 ± 0.5</td>
<td>0.6 ± 0.7</td>
<td>1.2 ± 0.7**</td>
<td>0 ± 0</td>
<td>0.0003</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.2 ± 0.4</td>
<td>0.4 ± 0.9</td>
<td>0.4 ± 0.9</td>
<td>0+0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Macrophages</td>
<td>0.1 ± 0.3</td>
<td>0.55 ± 0.69</td>
<td>0.6 ± 1.0*</td>
<td>0 ± 0</td>
<td>0.037</td>
</tr>
<tr>
<td><strong>Histology of parotid glands</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoid cells</td>
<td>1.4 ± 0.8</td>
<td>1.5 ± 0.7</td>
<td>1.4 ± 1.0</td>
<td>0 ± 0**</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0%</td>
<td>0%</td>
<td>8%</td>
<td>0%</td>
<td>n.s.</td>
</tr>
<tr>
<td>Connective tissue proliferation</td>
<td>0 ± 0</td>
<td>0.5 ± 0.7 *</td>
<td>0.2 ± 0.6</td>
<td>0 ± 0</td>
<td>0.026</td>
</tr>
<tr>
<td><strong>Histology of the lymph nodes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sinus reaction</td>
<td>0.4 ± 0.5</td>
<td>0.8 ± 1.0</td>
<td>0.9 ± 0.8</td>
<td>0 ± 0</td>
<td>0.025</td>
</tr>
<tr>
<td>Granular reaction</td>
<td>0.1 ± 0.4</td>
<td>0.3 ± 0.8</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Morphological changes in the bucal mucosa, lymph nodes, and parotid glands were scored arbitrary as (-)=0, (+)=1, (++)=2, (+++)=3. The individual values within each group were summarised and presented as a mean ±standard deviation. Comparison between the groups was done by Kruskal-Wallis statistics and is presented in the last column. Comparison of each group to sham-operated rats was calculated by Mann-Whitney test.

* P<0.05; ** P<0.01; n.s.- not significant.

**Fig 1. a, b** – Histological evaluation of bucal mucosa exposed to the acetal resin implant (a) compared to the one excised from the sham-operated rat in the control group (b). An infiltrate consisting of fibroblasts, histiocytes, and relatively numerous eosinophils was found in the stroma, but few cells around the foreign body are visible in acetal resin exposed tissue. (H.E., 50 x).

**Fig. 2. a.** Histological findings in the parotid gland exposed to acetal resin implant. Inflammatory infiltration composed of lymphoid cells is visible in the stroma, as well as fibrous tissue proliferation and few eosinophils. b. The tissue of the parotid gland of sham-operated rats showed no such changes. (H.E., 50 x).
Morphological findings in the parotid glands. Morphological evaluation of the parotid glands showed the inflammatory infiltrate with dominance of lymphoid cells in the stroma (Fig. 2 a). In two (15%) rats having acetal resin implants, the proliferation of the fibrous tissue and stromal swelling was observed. Isolated eosinophils were found to be present in one rat. These changes were not sufficient to reach the significant difference from the groups implanted with other materials and sham-operated group (Fig. 2 b).

Discussion

The inflammation caused by the implanted dental material, both acetal resin and to less extend titanium/metal, was marked by moderate inflammatory infiltration consisting of lymphoid cells, fibroblasts, and macrophages. No formation of granuloma tissues and only insignificant number of multinuclear cells were noted. The histological specimens from the neck lymph nodes of acetal resin implanted rats did not differ from the sham-operated rats. Parotid tissues have been previously suggested to be highly sensitive to exposure to chemical substances, as well as, to dental implanted materials. Parotid tissue reactions were recognised for instance by changes in salivation (4, 10). Therefore, morphology of the salivary glands of rats, which were subjected to a variety of dental materials for 6 weeks, was examined. A moderate lymphoid cell infiltration was observed in parotid tissues. Proliferation of connective tissue was only minor. The changes in the parotid tissue of rats exposed to acetal resin were similar to those seen in sham-operated rats. Our findings differ from those presented by Ebadian et al. (2), who observed an increased rate of inflammation in groups implanted with acrylic materials. The study contained no group implanted with acetal resin material. Another study compared changes in epoxy resin and titanium implanted tissues, and showed significant modification of the exposed tissues in rats and pigs (7). However, the experimental model used in the latter study differed from the one used in the present investigation with respect to implanted materials and to the evaluated tissues.

In summary, the presented study has demonstrated that acetal resin may induce lymphoid cell infiltration in bucal mucosa and in parotid tissue, which in some cases is associated with the presence of eosinophils and proliferation of connective tissues.

References