Histology of the rat vas deferens after injection of a non-occlusive chemical contraceptive*

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Summary. An alternate co-polymer of styrene and maleic anhydride was dissolved in dimethylsulphoxide and injected into the vas deferens of rats. The polymer was retained in the vas deferens and the morphological changes detected were confined to the mucosa. When the polymer was removed by flushing dimethylsulphoxide, the mucosal structure became normal within 2 weeks.

Introduction

There is a need to develop non-occlusive and reversible methods of male sterilization. Mistro et al. (1979) have described a technique using a polymer. The polymer, an alternate co-polymer of styrene and maleic anhydride, was dissolved in dimethylsulphoxide (DMSO) and injected into the rat vas deferens; the pH was lowered sufficiently to kill the spermatozoa passing through. It was effective as a contraceptive in rats for a period of 180 days. The polymer does not degrade and can be flushed out with DMSO and fertility is restored about 3 weeks later (unpublished observations).

Although such clinical observations are encouraging, we have now examined the morphological changes in the rat vas deferens after injection of the polymer and its subsequent removal by flushing with DMSO.

Materials and Methods

Experiment 1

The method of Singh, Ray, Vasudevan, Verma & Guha (1979) was used. Adult albino rats (Wistar strain) were obtained from the Institute’s experimental animal facility and divided into two groups of 15 each. In one group, pellets of polymer (made from the monomers: BDH, U.K.) sterilized with methyl alcohol were implanted subcutaneously on either side of the lower back. Rats in the control group were similarly implanted with Silastic (Dow Corning, Midland, Michigan, U.S.A.) pellets. The implanted material and the surrounding tissues were removed from 3 animals of each group on Days 3, 7, 14, 21 and 90 after operation and were fixed in 10% buffered formalin. Sections (5 μm) were cut and stained with haematoxylin and eosin, and the tissue reactions to the polymer and Silastic were studied.

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**Experiment 2**

Albino rats weighing 150–200 g and aged 16–18 weeks were treated as follows: Group I rats were injected with 0.01 g polymer in 0.03 ml DMSO; Group II rats received 0.03 ml DMSO; and those in Group III were injected with 0.03 ml saline (9 g NaCl/l).

The rats were anaesthetized by ether and a single cut was made ventrally, just above the urethral opening, to expose the vas deferens on both sides. The ampullary portions of the vas deferens (i.e. distal region) were located beneath the fat bodies, and the duct is of greater diameter here than in other regions (Hamilton & Cooper, 1978; Kennedy & Heidger, 1979). Care was taken that the injections were made at the same level of the vas deferens in all the animals. Although the polymer flowed along the vas deferens after the injection, the flow was more towards the urethral side because of the pressure gradient.

From each group, 3 rats were killed on Days 3, 7, 14 and 21 after injection and the other 5 rats of Group I were killed on Days 40 (one), 60 (two), 90 (one) and 150 (one). The ampullary regions of the vas deferens were exposed, removed and fixed in 10% buffered formalin. Sections (5 µm) were stained with haematoxylin and eosin.

**Experiment 3**

Polymer (0.001 g in 0.03 ml DMSO) was injected into the vas deferens of 15 rats as above. After 21 days, the ampullary region was re-exposed and the polymer was flushed out with 0.03 ml DMSO. The rats were killed at 0 h, 24 h, 3 days, 7 days or 14 days after flushing. The 21-day period was chosen in relation to the results of Exp. 2. The tissue was fixed, sectioned (5 µm) and stained as above.

**Results**

**Experiment 1: compatibility of tissue with the polymer**

The tissue reaction to subcutaneous implants of the polymer was less and more localized than that to the Silastic implant at all times studied. At Days 3 and 7, the cellular reaction was predominantly a deposition of polymorphonuclear leucocytes and fibrin. A prominent macrophagic response was observed from Day 14 onwards. Foreign body giant cells were few on Day 14 but had increased by Day 90 after the implantation.

**Experiment 2: changes after polymer injection**

No morphological alterations of the vas deferens were observed for the animals in Groups II and III. In Group I rats the morphological alterations were confined chiefly to the mucosa (see Table 1). The polymer remained as amorphous material lying in the lumen throughout the period of study. It did not infiltrate the wall of the vas deferens at any stage. Flattening of the normal

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**PLATE 1**

**Fig. 1.** Vas deferens 14 days after injection of polymer. The polymer is present in the lumen and the mucosa is denuded and flattened. H & E, ×200.

**Fig. 2.** At 21 days after injection of the polymer the mucosal cells are showing degenerative changes. H & E, ×200.

**Fig. 3.** Immediately after removal of the polymer the vas deferens lumen is patent. The mucosal lining is flat with focal ulceration. H & E, ×200.

**Fig. 4.** By 14 days after removal of the polymer, the mucosa is folded and lined by pseudostratified ciliated columnar epithelium. H & E, ×200.
mucosal folds was observed as early as Day 3. The normal pseudostratified ciliated epithelium changed to 2–3 layers of low cuboidal cells with loss of cilia. Focal degenerative changes were observed in mucosal cells at Day 21 after injection (Pl. 1, Fig. 2). Focal ulceration and denudation of mucosa was observed from Day 7 (Pl. 1, Fig. 1). The lamina propria was oedematous in earlier stages. Later, mononuclear cell and macrophage infiltration occurred mostly in association with denudation of mucosa. The muscle and serosal layers remained normal. The above changes reached a maximum intensity by Day 21 and then remained static. Spermatozoa were observed in many specimens caught up in the polymer in the lumen but none was seen in the wall of the vas deferens or outside.

**Table 1.** Morphological changes in the rat vas deferens after injection of polymer

<table>
<thead>
<tr>
<th>Days after injection</th>
<th>No. of rats</th>
<th>Lumen</th>
<th>Folds</th>
<th>Epithelial cells</th>
<th>Continuity</th>
<th>Lamina propria</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3</td>
<td>Polymer, spermatozoa, inflammatory cells</td>
<td>Flat</td>
<td>2–3 layers</td>
<td>Intact</td>
<td>Oedema</td>
<td>Normal</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>Polymer, spermatozoa, inflammatory cells</td>
<td>Flat</td>
<td>2–3 layers, low cuboidal</td>
<td>Focal ulceration (1)</td>
<td>Mononuclear cell infiltration</td>
<td>Normal</td>
</tr>
<tr>
<td>14</td>
<td>3</td>
<td>Polymer, spermatozoa, inflammatory cells</td>
<td>Flat</td>
<td>2–3 layers, low cuboidal</td>
<td>Denudation (1)</td>
<td>Macrophages at site of denudation (1)</td>
<td>Normal</td>
</tr>
<tr>
<td>21</td>
<td>3</td>
<td>Polymer, spermatozoa, inflammatory cells</td>
<td>Flat</td>
<td>Single layer, low cuboidal; focal degenerative changes</td>
<td>Partial denudation (3)</td>
<td>Macrophage reaction minimal</td>
<td>Normal</td>
</tr>
<tr>
<td>40–60</td>
<td>3</td>
<td>Polymer</td>
<td>Flat</td>
<td>Single layer, low cuboidal</td>
<td>Denudation (1)</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>90–150</td>
<td>2</td>
<td>Polymer</td>
<td>Flat</td>
<td>Single layer, low cuboidal</td>
<td>Focal denudation (2)</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Figures in parentheses give the number of animals showing the change.

**Table 2.** Morphological changes in the rat vas deferens after removal of the polymer

<table>
<thead>
<tr>
<th>Time after washing</th>
<th>No. of rats</th>
<th>Lumen</th>
<th>Folds</th>
<th>Epithelial cells</th>
<th>Continuity</th>
<th>Lamina propria</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>3</td>
<td>Patent</td>
<td>Flat</td>
<td>1–2 layers, low cuboidal</td>
<td>Denudation of mucosa (2)</td>
<td>Normal</td>
</tr>
<tr>
<td>24 h, 3 days</td>
<td>3</td>
<td>Patent</td>
<td>Flat</td>
<td>1–2 layers, low cuboidal</td>
<td>Normal (2)</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Patent</td>
<td>Flat</td>
<td>3–4 layers, cuboidal</td>
<td>Normal (2)</td>
<td>Normal</td>
</tr>
<tr>
<td>7 days</td>
<td>3</td>
<td>Patent</td>
<td>Flat</td>
<td>Columnar (3)</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Patent</td>
<td>Flat</td>
<td>Normal (3)</td>
<td>Ciliated (2)</td>
<td>Normal</td>
</tr>
<tr>
<td>14 days</td>
<td>3</td>
<td>Patent</td>
<td>Normal</td>
<td>Pseudostratified columnar</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>and ciliated (3)</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Figures in parentheses give the number of animals showing the change.
Experiment 3: changes after polymer removal

The morphological alterations produced in the mucosa by the polymer were reversible (Table 2). The mucosa appeared normal by Day 14 after washing out the polymer in all the animals (Pl. 1, Fig. 4). Polymer was not seen in the vas deferens lumen of any animal and the lumen was patent (Pl. 1, Fig. 3). Regeneration of epithelial cells of the mucosa covering was complete without any ulceration. The mucosal lining cells became columnar and mucosal folds began to appear in some animals by Day 7.

Discussion

The results of Exp. 1 show that the polymer does not cause excessive incompatibility reactions in rats. The morphological changes detected after the injection of the polymer into the vas deferens were specific to the polymer; no changes were seen in rats given intravasal infusions of saline or DMSO alone. The morphological alterations occurred as early as 3 days after infusion and reached a peak by 21 days but were still manifest at 150 days. In spite of the extent of the changes in the mucosa, when the polymer was removed after being in situ in the vas for 21 days, the mucosal morphology became normal by 14 days after washing. This correlates well with our clinical observations (unpublished) that fertility returned at 3 weeks after removal of the polymer.

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References


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