Failure of anti-inflammatory agents to modulate infection-induced pelvic adhesive disease in rabbits

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Summary. A suspension of *Neisseria gonorrhoeae* was injected into rabbit uterine horns to induce pelvic adhesive disease. There was no statistical difference in the formation of adhesions between the rabbits that were given anti-inflammatory agents and those that received no therapy. This study suggests that anti-inflammatory agents will offer no benefit as adjunctive therapy for the prevention of infection-induced pelvic adhesive disease.

Introduction

Over 10% of couples in the U.S.A. are involuntarily infertile. Pelvic inflammatory disease (PID), with its resultant pelvic adhesive disease, is the reason for female infertility in as many as 50% of these cases. Current therapy for PID involves bedrest and antibiotic treatment. Despite the use of different antibiotic regimens, 20% of patients with PID subsequently become infertile (Westrom *et al.*, 1979).

Several investigators have successfully used postoperative ibuprofen in preventing surgically induced pelvic adhesions (Kapur *et al.*, 1969; Larsson *et al.*, 1977; Seigler *et al.*, 1980; Nishimura *et al.*, 1983, 1984). Other research has shown no benefit from this agent or from the use of corticosteroids (diZerega & Hodgen, 1980; Holtz, 1982; Harris & Daniell, 1983). No one has studied the effect of modulating the inflammatory response produced by pathogenic bacteria and subsequent formation of adhesions.

Decreasing the inflammatory response to a bacterial inoculum may result in fewer pelvic adhesions and increase the fertility rate. Nonsteroidal anti-inflammatory agents have not been used to prevent adhesion formation in PID therapy. A possible consequence of such therapy could be exacerbation of the pelvic infection by interfering with the immune response. This study was designed to determine the effect of two anti-inflammatory agents on the formation of infection-induced adhesions in rabbits.

Materials and Methods

Virginal, 12-week-old New Zealand White rabbits, weighing 2.5–3.0 kg, were obtained from a local rabbit farm. The rabbits were housed in individual cages and were given food and water *ad libitum*. After induction of anaesthesia with 35 mg ketamine/kg and 5 mg xylazine/kg i.m., the rabbits' abdomens were shaved and a povidone-iodine preparation was applied before sterile draping. General anaesthesia was maintained with halothane and oxygen while the rabbit uterus was exposed through an incision in the lower abdomen. A single suture of 2-0 plain catgut was placed around the left uterine horn 2 cm distal to the cervix. Inoculum (5 ml) was injected into the left uterine horn of 33 rabbits. Ten rabbits received normal saline (9 g NaCl/l) only and served as controls. The abdominal wall was then closed using a routine two-layer closure (Hammill *et al.*, 1984).

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Five groups of animals were used. Group 1 (N = 9) was the preliminary group used to establish the technique. This group was re-explored at intervals varying from 6 h to 6 weeks to study the natural history of this experimental manipulation (Table 1). The other groups were part of the protocol to determine the effect of anti-inflammatory agents on infection-induced pelvic adhesive disease. The animals in Groups 2–5 were treated in a randomized, blind manner so that the observer did not know which animals received a bacterial inoculum and normal saline and which animals received anti-inflammatory agents and no therapy. Group 2 (N = 10) received normal saline as the inoculum and no adjunct therapy. Group 3 (N = 7) received the bacterial inoculum and no adjunct therapy. Group 4 (N = 9) received the bacterial inoculum, plus adjunct therapy consisting of ibuprofen (Motrin: Upjohn, Kalamazoo, Michigan), 70 mg/kg i.m. for 2 doses, 6 h apart, beginning 6 h before the inoculum was injected. A maintenance dose of 20 mg/kg i.m. was given every 6 h for 4 days. This dose was estimated to deliver higher serum levels than could be achieved in humans and was similar to a dose used in a previous investigation with good results (Nishimura et al., 1983). Group 5 (N = 8) received the bacterial inoculum and adjunct therapy consisting of methyl prednisolone (Solu-Medrol, Upjohn), 38 mg/kg i.m. administered immediately before the operation and followed by 25 mg/kg given every 6 h for 4 days. All animals in Groups 2–5 were reexplored after 6 weeks and examined macroscopically for adhesions. Those rabbits with no adhesions were scored as grade 0, filmy, avascular adhesions as grade 1, and dense, vascular adhesions as grade 2 (Hulka et al., 1978).

The bacterial inoculum used was a pure culture of a peritoneal isolate of Neisseria gonorrhoeae (obtained by culdocentesis from a patient with gonococcal PID). Identification of the organism was verified by growth on selective media (chocolate agar and Thayer–Martin). Gram's stain, colonial morphology and biochemical (sugar fermentation) evaluation. An inoculum of 10⁷ colony-forming units/ml, confirmed by quantitative culturing, was suspended in normal saline (9 g NaCl/l). After re-exploration, all animals in Group 1 had an aspirate as well as a tissue homogenate of the infected uterine horn cultured for aerobic and anaerobic bacteria. Aerobic cultures were plated onto sheep blood agar and chocolate agar which were incubated at 37°C in air and 5% CO₂, respectively. Anaerobic cultures were plated onto prereduced blood agar and were incubated in an anaerobic chamber at 37°C for 7 days.

The results were analysed using the Fisher exact probability test. The control group and the infected animals that were not treated with anti-inflammatory agents were compared for the presence or absence of adhesions using the hypothesis that no effect would be demonstrated. Subsequent comparisons were made of the infected animals that were not treated with anti-inflammatory agents and those two groups that were treated with ibuprofen or methyl prednisolone, respectively.

**Results**

All animals in Group 1, except the rabbit explored 6 weeks after inoculation, showed evidence of an inflammatory response to the bacterial inoculum. These animals had a distended, erythematous left uterine horn distal to the ligature. A fibrinous exudate covered the pelvic organs as early as 6 h after inoculation. Side-to-side agglutination of the uterine horns and involvement of the oviducts occurred in this inflammatory reaction. The pelvic organ agglutination could be easily separated manually at this time. A thicker, coalescing exudate was noted 3 days post-operatively. A Gram stain of the uterine horn aspirate showed numerous white blood cells. In 2 of the 8 aspirates, the white blood cell count was 100 000/ml and 230 000/ml, respectively. Permanent fibrinous adhesions were first noted in the animal examined 7 days after inoculation (see Table 1).

<table>
<thead>
<tr>
<th>Time of exploration</th>
<th>No. of rabbits</th>
<th>Culture</th>
<th>Inflammation</th>
<th>Adhesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 h</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>24 h</td>
<td>2</td>
<td>—</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>3 days</td>
<td>3</td>
<td>—</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>7 days</td>
<td>1</td>
<td>—</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>42 days</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>+</td>
</tr>
</tbody>
</table>

+= present; — = absent.
Culture of the aspirate, as well as a tissue homogenate of the uterine horn, showed no evidence of a persistent gonococcal infection. The gonococcus was present in 2 rabbits explored 6 h after the inoculum was injected but was not found in any of the 7 remaining animals in Group 1 (see Table 1). Cultures for other aerobic and anaerobic bacteria were negative. Antibiotic therapy in these experimental rabbits was believed to be unnecessary.

The effect of anti-inflammatory therapy on the formation of adhesions is shown in Table 2. There was a significant difference in the formation of adhesions in Groups 2 and 3. There was no statistical difference in adhesions in animals in Groups 3, 4, and 5.

Discussion

Studies of Neisseria gonorrhoeae in rabbit oviduct cell culture show no pathological effect (Taylor-Robinson et al., 1974; Johnson et al., 1977). The sequelae noted in the present study can therefore be presumed to be entirely secondary to the inflammatory response of the experimental rabbits. The inflammation was associated with a fibrinous exudate which coated the pelvic viscera and became permanent fibrous adhesions in most (87.5%) of the infected animals. Not all bacteria will be associated with the same inflammatory response. Chlamydial infections are likely to be associated with a greater lymphocytic response than was appreciated in this animal model. Different infections may therefore be associated with a different response to therapy with anti-inflammatory agents.

Adhesions were found in 2 of the animals in the control group. Since all the animals were subjected to a surgical procedure with suture placement, this is not a totally unexpected finding. Foreign body reaction to the suture or the normal reparative response to a surgical wound are possible explanations for this finding. Although samples from these animals were not cultured, uniformly negative aerobic and anaerobic cultures in the pilot group (Group 1) after 6 h suggest that bacterial contamination as a cause for these adhesions is unlikely.

Adhesions after acute peritonitis have been described in the medical literature since the early 1800s (Baillie, 1833). Surgeons and pathologists have considerable experience with early fibrinous exudate which causes side-to-side agglutination of intra-abdominal and/or pelvic structures. This fibrinous exudate either reabsorbs or becomes organized with the ingrowth of capillaries and fibroblasts into permanent fibrous adhesions (Ellis, 1971).

Several approaches towards the control of subsequent formation of adhesions have been summarized by Ellis (1971). They include (1) prevention of the deposition of fibrin in the peritoneal exudate, (2) removal of fibrin which has already formed by enzymic or mechanical means, (3) inhibition of fibroblastic proliferation, and (4) separation of potential agglutinating surfaces with the use of high molecular weight substances. Steroidal and nonsteroidal anti-inflammatory agents should limit the deposition of fibrin in the peritoneal exudate by inhibiting inflammation and fibroblastic migration, proliferation and organization (Ellis, 1971; Harris & Daniell, 1983; Stangel et al., 1984).

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**Table 2. Adhesion formation in rabbits in Groups 2–5**

<table>
<thead>
<tr>
<th>Grade of adhesions</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

Fisher exact test: 2 vs 3, \( P = 0.01 \); 3 vs 4, \( P = 0.85 \); 3 vs 5, \( P = 0.47 \).
This study fails to demonstrate the effectiveness of the nonsteroidal anti-inflammatory agent, ibuprofen, or a steroid, methyl prednisolone, in decreasing pelvic adhesive disease after a pelvic infection. These results confirm the findings of Falk (1965) who used steroids to modulate the inflammatory response of women with acute PID. Despite an improved clinical response to the acute disease process, the overall subsequent infertility rate remained unchanged. Falk (1965) considered that the damage that occurred before the patient was treated was responsible for this failure. Our study suggests that these modulating agents are not effective, even when they are present in the body before the infection occurs.

There are other explanations for the ineffectiveness of the anti-inflammatory agents in preventing pelvic adhesions in the animals used in the study. Among them are (1) a large bacterial inoculum, (2) a therapeutic course that was too short, and/or (3) local concentrations of the agents that never reached a therapeutic level.

Previous studies associated with the therapeutic effectiveness of ibuprofen were concerned with the prevention of surgically induced adhesions (Kapur et al., 1969, 1972; Larsson et al., 1977; Siegler et al., 1980; Holtz, 1982; Nishimura et al., 1983, 1984). The surgery was confined to a limited area. In our study, a large bacterial inoculum was placed in the uterine horn. This allowed ascending spill into the pelvic peritoneal cavity, bathing the pelvic organs with organisms and inflammatory exudate. The subsequent formation of adhesions suggests that no clinically significant inhibition of inflammation occurred. A large inoculum would further decrease possible ameliorating effects of the anti-inflammatory agents. Further study with a smaller inoculum is indicated, since the large number of organisms used in this study are not found in the endometrial cavity or Fallopian tubes of women with PID.

In the present study, the formation of permanent fibrous adhesions was not noted in Group 1 until 7 days after inoculation. Maximal collagen biosynthesis appears to be 4–5 days after surgery and large amounts of nonsulphated glycosaminoglycans are present at 4 days (Nishimura et al., 1984). Deposition of these substances is associated with the formation of permanent adhesions. Our study design attempted to modulate the acute inflammatory response to our bacterial inoculum. Therapy was discontinued at the end of Day 4. The two anti-inflammatory agents did not seem to be effective in blunting inflammation or inhibiting collagen or glycosaminoglycan synthesis as was evidenced by the formation of significant pelvic adhesions. It is possible that the modulating agents were successful in partly inhibiting the production of these substances, but that the early discontinuation of the anti-inflammatory agents may have allowed a renewed synthesis of these compounds and therefore the subsequent formation of adhesions. More prolonged therapy may be able to alter permanent adhesion formation by continued interference with collagen and glycosaminoglycan synthesis (Nishimura et al., 1984).

It is possible that concentrations of ibuprofen and methyl prednisolone never reached a therapeutic level, despite the large doses given to the rabbits in the study. In fact, ibuprofen may have been present in concentrations which actually potentiate leucocyte recruitment (Nishimura et al., 1984). Serum concentrations were not measured in our study animals, but parenteral administration of the agents should have ensured good systemic absorption. Decreased blood flow to the infected area, secondary to thrombosis of inflamed vessels, could have prevented local concentrations of the agents from reaching a therapeutic level.

The views expressed herein are those of the authors and do not necessarily reflect the views of the United States Navy or the Department of Defense.

References


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Received 2 July 1986