The Retroperitoneum Protects Prosthetic Graft Material from Intraperitoneal Contamination: An Experimental Study

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Objectives. To evaluate the ability of the retroperitoneum to serve as a barrier, against bacterial contamination, between the peritoneal cavity to the retroperitoneal space.

Methods. Seventy rats had a small piece of knitted Dacron graft placed in the retroperitoneal space and $10^6–10^9$ colony forming unit (cfu) Enterococcus faecalis was injected into the peritoneal cavity. In half the retroperitoneal (RP) group, the retroperitoneum was preserved and in the remainder, the open peritoneal (OP) group, needle holes were created. Grafts were harvested after 1, 4, or 7 days and cultured for E. faecalis. A blood sample was collected from three rats in each group for culture before the graft was harvested.

Results. Graft infection did not develop in any rat injected with $10^6$ or $10^7$ cfu in the RP group, while seven out of the 10 graft cultures of the OP group grew E. faecalis ($P=0.003$). In rats injected with $10^8$ or $10^9$ cfu, five out of the 10 graft cultures in the RP group and eight out of 10 in the OP group grew E. faecalis. All blood cultures were negative when the injected bacterial count was $10^7$ cfu or less. One out of the three blood cultures was positive at $10^8$ cfu, and all were positive at $10^9$ cfu.

Conclusions. These results suggest that an intact retroperitoneum acts as a protective barrier against intraperitoneal bacterial contamination, particularly when blood cultures are negative.

Keywords: Retroperitoneum; Graft infection; Enterococcus faecalis.

Introduction

Preoperative evaluation of elderly patients for aortic surgery occasionally uncovers gastrointestinal pathology that requires surgery, and vascular surgeons are forced to decide how to treat two diseases. Some surgeons stress the safety of simultaneous transperitoneal operation, while others are reluctant to perform two procedures simultaneously, because of the risk of graft infection by gastrointestinal surgery. Advocates of simultaneous surgery report that great care is taken not to soil the abdominal cavity and the retroperitoneum is closed tightly. However, meticulous closure inevitably creates numerous needle holes that obviously are large enough for bacteria to pass through. Komori et al. performed simultaneous operations through two incisions: a transperitoneal incision to operate on the gastrointestinal tract and a retroperitoneal incision to resect the AAA. They emphasized the safety of this method with respect to prevention of graft infection. No scientific study on the risk of graft infection in simultaneous operations has been published.

The purpose of this study was to compare the protective capacity, against bacterial diffusion from the abdominal cavity, of the intact retroperitoneum and the needle hole perforated retroperitoneum. The former mimics the situation in retroperitoneal approach to the aorta and the latter the transperitoneal approach with tight suture closure.

Methods

Seventy male Sprague–Dawley rats, weighing 250–300 g, were used in this experiment. The animals were housed in cages at 23 °C with a 12-hour light-dark cycle and were given tap water and standard feed. All experimental protocols and animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals (Washington, National Academy Press, 1996) and were approved by the Institutional
Animal Care and Use Committee of the Nagoya University Graduate School of Medicine.

The rats were divided into two groups. A retroperitoneal (RP) group: an intact peritoneum group (model of the retroperitoneal approach to the aorta) and an open peritoneal (OP) group (a perforated peritoneum group which is the model of the transperitoneal approach).

Operations were performed under ether anesthesia followed by intraperitoneal injection of 15 mg of pentobarbiturate with patent blue dye (Wako Pure Chemical Industries, Ltd) 1% dissolved in distilled water. The retroperitoneum was exposed without injury through a small incision in the back just lateral to the spine. The retroperitoneum was defined as intact when dye was observed through the retroperitoneum without leakage. In the RP group, the integrity of the retroperitoneum was preserved. In the OP group, three needle holes were made in the retroperitoneum using a 21 gauge needle.

A 25-mm² square piece of knitted Dacron graft (Gelsoft, Sulzer Vascutek, UK) was placed next to the retroperitoneum. Immediately after graft placement, a small midline laparotomy was made, and saline (2 ml) containing one of four doses of Enterococcus faecalis (10⁶, 10⁷, 10⁸, or 10⁹ cfu) was injected into peritoneal cavity, five rats in each group. Inoculations below 10⁶ cfu did not produce consistent results in the preliminary study. The piece of Dacron graft was harvested, without injuring the retroperitoneum, on the first postoperative day, and was cultured. A blood sample was collected from the femoral vein of the rat for culture before the graft was harvested in three rats for each group of the doses of bacteria in the RP group. In the serial study, grafts from rats receiving 1×10⁸ E. faecalis were harvested serially on post-operative days 1, 4, and 7, five rats in each experimental group.

Liver, kidney, and retroperitoneum were resected for pathologic examination. All rats were sacrificed after harvesting the specimens by an overdose of ether anesthesia. All procedures were done aseptically without administration of antibiotics.

Microbiologic and pathologic studies

The bacterial strain used in this study, E. faecalis 1116, was isolated from the blood of a septic patient. To obtain a constant bacterial concentration, the bacteria was cultured at 37°C for 3 h in heart infusion broth. This culture consistently yielded a bacterial concentration of 5×10⁸ cfu per milliliter. Subsequently this culture was diluted 10⁴, 10⁵, or 10⁶-fold with saline.

The harvested grafts were soaked in 5 ml of saline, the saline solution was stirred for 10 min before 100-fold dilution with saline. This suspension was cultured on agar plates at 37°C for 48 h, and the number of colonies was counted. Blood (0.1 ml) also was cultured on agar plates. The colony was cultured on an EF agar plate (Nissui Pharmaceutical, Japan) and its identity as E. faecalis was confirmed from its color. If a colony of E. faecalis was identified, the graft was considered to be contaminated.

Resected specimens were stained with hematoxylin-eosin for routine histopathology.

Statistical analysis was done with \(\chi^2\) test. \(P < 0.05\) was considered significant.

Results

On post-operative day 1, no animal in the RP group (0/10) had a positive graft culture for E. faecalis if the inoculum was \(10^7\) cfu or less, while seven out of 10 in the OP group had positive graft cultures at this inoculum, \(P = 0.003\). When the number of injected bacteria was \(10^8\) cfu or more, positive graft cultures were seen in both groups. No significant difference was seen in either group (Table I). All three blood cultures were negative when inoculum was \(10^7\) cfu, whereas one of three was positive at \(10^8\) cfu and all three were positive at \(10^9\) cfu. Three out of four graft cultures in the rats of positive blood culture were positive and none of the five graft cultures in rats of negative blood culture was positive.

No positive graft culture was observed in the RP group at inoculums of \(10^7\) cfu during week 1. In contrast, graft cultures in the OP group were positive in 3/5 animals on day 1, in 4/5 on day 4, and in 1/5 on day 7, \(P < 0.001\) for summation analysis over the 7 day period.

Infiltration of neutrophils around the graft was observed when the graft culture was positive but was not seen when the culture was negative (Fig. 1). Microabscesses were found in the liver and the kidney when the bacterial inoculum was \(10^9\) cfu (Fig. 2).

<table>
<thead>
<tr>
<th>Bacterial inoculum (cfu)</th>
<th>RP group (n = 20)</th>
<th>OP group (n = 20)</th>
</tr>
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<tbody>
<tr>
<td>(10^0)</td>
<td>0% (0/5⁰)</td>
<td>60% (3/5⁰)</td>
</tr>
<tr>
<td>(10^1)</td>
<td>0% (0/5⁰)</td>
<td>80% (4/5⁰)</td>
</tr>
<tr>
<td>(10^2)</td>
<td>20% (1/5)</td>
<td>60% (3/5)</td>
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<tr>
<td>(10^3)</td>
<td>80% (4/5)</td>
<td>100% (5/5)</td>
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RP, retroperitoneum; OP, open peritoneum.
* \(P = 0.003\) analyzing the first and second row together.
Simultaneous surgery for gastrointestinal pathology in patients undergoing resection of an AAA is controversial. In 1960, Ochner et al. analyzed their experience with 640 aneurysmectomies that had been performed in combination with another abdominal or pelvic operation, including 480 appendectomies, 184 lumbar sympathectomies, 51 cholecystectomies, 12 gastric resections, and eight colorectal resections. They concluded that aneurysmectomy combined with another operation does not increase morbidity and mortality. Since then other authors have reported successful simultaneous operations. On the other hand, Szilagyi et al. formulated guidelines for the treatment of patients with malignant diseases and aneurysms in which they recommended staged procedures. Lobatto et al. surveyed the opinions of 46 professors of general and vascular surgery in the United States. One third of the respondents favoured excision of the carcinoma first, one third stated they would excise the aneurysm first, and the remaining stated that they would withhold their decision until the time of laparotomy. Only two would have attempted to perform aneurysmectomy and colectomy simultaneously. No published report has documented a higher incidence of aortic graft infection in patients undergoing simultaneous gastrointestinal surgery and aneurysmectomy.

Scientific studies have shown that translocation of enteric flora can cause systemic bacterial infections in patients with endotoxemia, malnutrition, immunosuppression, hemorrhagic shock, intestinal obstruction, thermal injury, trauma, and total parenteral nutrition. Woodcock et al. demonstrated that bacterial translocation occurs in patients undergoing AAA repair. They also reported incidences of graft infection possibly due to bacterial translocation. Mora et al. have demonstrated that sterile prosthetic materials implanted intraperitoneally become contaminated with enteric flora within 1 to 3 days, even though no bacterial overgrowth, perforation, or histological changes in the bowel were found. Therefore, bacteria migrate into the abdominal cavity

Fig. 1. Micrographs around the implant: haematoxylin and eosin stain of the graft. (A) Infiltration of neutrophils around the graft material was observed when the graft culture was positive. (B) No neutrophil infiltration was seen when the graft material culture was negative.

Fig. 2. Micrographs of kidney (left) and liver (right) to demonstrate neutrophil infiltration (micro abscess), when 10⁹ bacteria were inoculated: haematoxylin and eosin stain.
through intact bowel and can contaminate prosthetic materials in the abdominal cavity. Koratzanis et al. examined mesenteric lymph nodes in patients undergoing various intra-abdominal operations. They reported that the incidence of bacterial translocation was 5.9% with graft replacement post aortic aneurysms, 44.4% with colectomies, and 50% with gastrectomies. Zaporozhets et al. have demonstrated that the integrity of the bowel to bacterial migration is compromised by suturing, and that the peritoneal cavity is contaminated by bacteria within 6–8 h after gastrointestinal surgery. Such findings, common sense and clinical experience argue that the risk of graft infection is real in cases of simultaneous surgery.

Our study demonstrates that the intact retroperitoneum is capable of preventing intraperitoneal bacterial contamination and violation of retroperitoneal integrity by the creation of needle holes increases the incidence of graft infection, when the intraperitoneal bacterial challenge is insufficient to cause bacteraemia. High bacterial inoculations caused graft infection in this study even when the retroperitoneum was intact. Infection in this setting is most likely the result of bacteraemia. To confirm this hypothesis, a piece of graft was implanted in gluteus muscle in two rats. The graft was harvested and cultured on post-operative day 1. In both animals, graft and blood cultures were positive when the dose of injected bacterial count was 10^7 cfu. Thus, a high intraperitoneal bacterial count can cause bacteraemia and result in graft infection regardless of whether the retroperitoneum is intact. However, the bacterial inoculum used in this study may be much higher than occurs routinely in clinical situations uncomplicated by anastomotic leak or gross fecal contamination.

The most common pathogen causing early graft infection is Staphylococcus aureus, while Staphylococcus epidermidis is the most common cause of late graft infection. Gram negative bacteria, such as Escherichia coli and Pseudomonas spp., are virulent and cause a particularly dangerous type of graft infection. We used E. faecalis in this experiment because it is a common enteric flora and a likely contaminant in gastrointestinal surgery. Future work will examine how our findings are modified by antibiotic therapy.

A ‘tight closure’ of the retroperitoneum may be comforting to the surgeon, but the present study demonstrated usefulness of the intact retroperitoneum as a barrier against bacterial contamination from the peritoneal cavity to the retroperitoneal space under the condition of negative blood culture. Therefore, we recommend a retroperitoneal approach to repair of AAA with transperitoneal resection of intra-abdominal pathology when these lesions are treated concomitantly.

References


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