Peritoneal Changes after Exposure to Sterile Solutions by Catheter

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ABSTRACT
Most current animal models that are used to study effects of long-term peritoneal exposure to dialysis solutions use an indwelling catheter for daily injections. It was hypothesized that the presence of a foreign body in the peritoneal cavity (PC) might alter the inflammatory response to the solutions and that the response would depend on exposure duration. For addressing these, long-term injections were carried out for 2 to 8 wk in 90 Sprague-Dawley rats: 40 via a subcutaneous port connected to a silicone catheter tunneled to the PC, 40 via direct needle injection, and 10 noninjected, age-control rats. Daily volumes were 30 to 40 ml of filter-sterilized, bicarbonate-buffered solutions that contained 4% dextrose. After 2, 4, 6, and 8 wk, anesthetized rats underwent transport experiments with a chamber affixed to the abdominal wall to determine mass transfer coefficients of mannitol (MTCmannitol) and albumin (MTCBSA), osmotic filtration flux (Josm), and hydrostatic pressure–driven flux. After the rats were killed, tissues were collected for measurement of peritoneal thickness, vascular density, and immunohistochemical staining. ANOVA demonstrated significant (P < 0.01) differences in thickness, vessel density, MTCmannitol and MTCBSA among the groups at the various time intervals and in overall means. Differences among the groups were less pronounced for hydrostatic pressure–driven flux and Josm. Vessel density, MTCmannitol, MTCBSA, and Josm were dependent on injection duration (P < 0.01). There were marked differences between the needle injection and catheter injection groups at various intervals in the expression of three cytokines. It is concluded that the histologic and functional response depends on the duration of injection with animals that are exposed for as little as 2 wk demonstrating alterations. These findings confirm the hypothesis that the presence of a PC catheter increases inflammatory response to sterile solutions as evidenced by the structural and functional changes in the peritoneal barrier.


Exposure of the peritoneum to sterile solutions that contain glucose results in changes in cellular characteristics, histology, and barrier function. Long-term peritoneal dialysis (PD) without evidence of peritonitis alters the phenotypic appearance of the human peritoneum in 8 to 12 mo. Biopsy studies of long-term (>3 yr) PD patients have demonstrated marked thickening over time of the subcompact zone, a region between the mesothelial cells and their basement membrane, with corresponding changes in the underlying microvasculature. Exposure of patients to glucose-based dialysis solutions resulted in significant functional changes in transperitoneal transport in a 2-yr study of patients who were treated with automated PD. Studies in animal models parallel these changes and have demonstrated benefit of solutions that are thought to be more biocompatible.

Studies in our laboratory using an animal model with a subcutaneous injection port connected to a silicone catheter tunneled to the cavity demonstrated angiogenesis and thickening of the perito-
neum after 8 wk of daily exposure to sterile solutions. A portion of the control group was made up of animals that had a catheter but were exposed only to a small volume of a dilute heparin solution (10 μ/ml isotonic Krebs bicarbonate) once a week. Despite having no exposure to clinical or glucose solutions, the animals with catheters did show minor structural alterations in the peritoneum. A recent study by Musi et al., who performed daily intraperitoneal injections without a catheter, demonstrated far less striking differences in the peritoneum after exposure to various dialysis solutions. This suggested that experimental findings in animal models might depend on the presence of a catheter.

Catheters are foreign bodies inserted through the abdominal wall into the cavity. Bacteria as well as human cells are known to grow in a biofilm and coat these catheters. Although biofilm is often associated with an infected catheter, careful observations in PD patients have demonstrated severe biofilm formation without detectable infection. We therefore hypothesized that the catheter may have a significant effect on the inflammatory process in the peritoneum, as evidenced by the thickness of the peritoneum and the vascular density. We further hypothesized that inflammatory change might produce different effects that depend on the duration of chronic exposure to PD solutions. Variable structural changes would lead to changes in transperitoneal transport of solutes and water. We therefore carried out careful studies of daily injection of the same sterile glucose solution for 2, 4, 6, and 8 wk in rats with and without catheters to study the early manifestations of the inflammatory process. The results support our hypotheses.

RESULTS

Bacterial Cultures and Cell Counts after Injection Period

Table 1 shows the number of positive cultures and the number of rats analyzed in each group (n = 10 rats per injection group per duration of injection). Surprising, the age-control (AC) group, which was not administered an injection except just before the transport experiment, had three positive cultures.

Table 1. Number of infected rats from each experimental group (positive cultures) and the number of rats analyzed

<table>
<thead>
<tr>
<th>Experimental Group, Duration</th>
<th>Positive Cultures</th>
<th>No. Analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>NI, 2 wk</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>NI, 4 wk</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>NI, 6 wk</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>NI, 8 wk</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>CI, 2 wk</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>CI, 4 wk</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>CI, 6 wk</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>CI, 8 wk</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>AC (no injection)</td>
<td>3</td>
<td>7</td>
</tr>
</tbody>
</table>

*AC, age control; CI, catheter injection; NI, needle injection.

Although spontaneous bacterial peritonitis cannot be ruled out, the cell counts in each of these rats was low (mean 71 cells/mm³) and the positive cultures were likely the result of accidental bowel perforation at the intraperitoneal injection, just before the transport experiments. A relatively low cell count was also observed for nearly half of the rats that received a needle injection (NI) and whose culture was positive. All culture-positive rats were deleted from the analysis. No adhesions were noted in any of the rats, including those with positive cultures.

Figure 1 displays the total white blood cell (WBC) count for each group and the fraction of neutrophils in each WBC. Except for the 8-wk groups, the catheter injection (CI) group had higher WBC count than the NI group, but the differences were statistically nonsignificant. Fractions of neutrophils were greater in the CI group and were statistically different from the NI group in the two-way ANOVA (P < 0.0001). The overall means for the CI and NI groups were significantly higher that that of the AC group (P < 0.0001).

Structural Changes in the Peritoneum

Figure 2 displays the means ± SEM of the thickness of the submesothelial compact zone for NI rats, CI rats, and AC rats. The mean thickness for the NI rats was not significantly different from that for the AC rats. However, the thickness for the CI rats was significantly different (P < 0.0001) from that for the NI rats and approximately two times that for the AC rats (P < 0.0001).

Figure 3 shows the vascular densities of the subcompact zone for each group in terms of number of vessels per linear millimeter of peritoneum. When the peritoneal vessel number (CD31 staining) per square millimeter of peritoneum was plotted versus duration of injection, the plot was the same as in Figure 3, with significant differences between the CI and NI groups.
groups (data not shown, \( P < 0.0001 \), two-way ANOVA). Overall means for CI rats are significantly greater than those for either NI or AC rats \( (P < 0.0001) \). At each time interval, the CI rats have a greater vessel number than the NI rats. The differences between the injection groups are statistically significant \( (P < 0.0001) \).

Immunohistochemical Studies of the Peritoneum
Immunohistochemical stains of the various groups demonstrate different patterns, which depended on the presence of a catheter. Table 2 is the semiquantitative measurement of the stains in each group, and Figure 4 shows typical images of the three cytokines. Both the CI and NI groups demonstrated significantly greater staining than untreated AC rats \( (P < 0.005) \) for each of the cytokines. In a two-way ANOVA, the CI rats had increased staining over the NI rats \( (P < 0.02) \), but within each injection group, there was no significant trend in staining for basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and TGF-\( \beta \)1 with duration of injection.

Table 2. Semiquantitative scoring of immunohistochemistry for injection groups

<table>
<thead>
<tr>
<th>Duration of Injection</th>
<th>VEGF&lt;sup&gt;b&lt;/sup&gt;</th>
<th>bFGF&lt;sup&gt;b&lt;/sup&gt;</th>
<th>TGF-( \beta )&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NI</td>
<td>Cl</td>
<td>NI</td>
</tr>
<tr>
<td>2 wk</td>
<td>0.6 ± 0.2</td>
<td>1.8 ± 0.6</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>4 wk</td>
<td>0.5 ± 0.1</td>
<td>2.4 ± 0.8</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td>6 wk</td>
<td>1.6 ± 0.2</td>
<td>1.5 ± 0.6</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>8 wk</td>
<td>1.7 ± 0.2</td>
<td>1.3 ± 0.4</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>AC VEGF</td>
<td>0.3 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC bFGF</td>
<td>0.2 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC TGF-( \beta )</td>
<td>0.2 ± 0.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Scoring of immunohistochemical stains as follows: No stain, 0; minimal stain, +1; moderate stain, +2; large stain, +3; and intense stain, +4. bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor.

<sup>b</sup> \( P < 0.005 \), one-way ANOVA of cytokine staining versus AC, NI, and CI groups; \( P < 0.02 \), two-way ANOVA of cytokine staining versus treatment groups (NI and CI) and weeks of treatment.
Within the NI and CI groups, there was a decreasing and significant trend in osmotic filtration flux $\left( I_{\text{osm}} \right)$ versus duration of injection ($P < 0.003$). Within time injection intervals, there were no significant differences; neither were there significant differences among the overall means for AC, CI, and NI rats. Solution osmolalities varied between 477 and 493 mOsm/kg among all of the groups.

Fluid flow across the peritoneum as a result of hydrostatic pressure is displayed in Figure 8. Although there was a decrease in mean flux from weeks 2 to 4 in each injection group, the trends in each group were not statistically significant. There were no differences between injection groups at each injection interval. The overall means of the CI and NI groups were significantly different from AC rats ($P < 0.0001$).

**DISCUSSION**

**Effect of Catheter on Peritoneal Structure and Its Transport Properties**

Significant differences in peritoneal structure, histology, and function were noted between the two injection techniques. The peritoneal thickness, vascular density, and transport of both small solutes and albumin revealed marked differences between NI and CI rats. There were higher mean values for total WBC in the CI rats with a significantly higher fraction of neutrophils than in the NI group. Cytokine staining was more intense in CI rats during the first 4 wk of solution exposure. Functionally, the $MTC_{\text{mannitol}}$ and the $MTC_{\text{BSA}}$ were higher in the CI group than in the NI group. The solution was designed

**Figure 4.** Comparison of vascular endothelial growth factor (VEGF; A), basic fibroblast growth factor (bFGF; B), and TGF-β (C) staining (brown) in CI and NI groups at 2, 4, 6, and 8 wk. Bars = 100 μm. AC rats did not receive injections and were held for 8 wk with subsequent transport experiments and tissue sampling. All tissue samples are from rats with negative fluid and catheter cultures and were sampled at a location remote from the location of the catheter. Magnification, ×400.
to have a high concentration of glucose (4%), high osmolality and low glucose degradation products (presumed but not measured), and neutral pH. The solution resulted in inflammation in both animal models, but no statements can be made concerning direct effects of the makeup of the solution because there was no comparison of different solutions. Because the same solution was used in each group, the different results point to the catheter as the cause of the variation in measurements.

The catheter seems to set up inflammation before the infusions of the solution and may interact with the solution to enhance peritoneal inflammation. Isolated leucocytosis with an elevated fraction of neutrophils has been found after catheter implantation in humans.11 We observed this in earlier experiments7 in animals with the same catheter but no infusions other than a weekly infusion of dilute heparin. Although heparin infusions are considered to be anti-inflammatory,12,13 the use of a heparinized catheter decreased bacterial colonization but not the degree of inflammation or peritonitis rate over 4 wk of twice-daily exchanges.14 Injection of isotonic, buffered solutions has been observed to promote inflammation in the abdominal wall peritoneum15 but to preserve capillary morphology in the omentum16; the latter study was performed with heparin in the solution, which could have influenced the inflammatory response.

There is an abundant literature on the effects of bacterial biofilm on catheters and its link to peritonitis.9,17 The catheter was cultured with the fluid in each rat at the end of the injection period, and only culture-negative rats were used in the reported studies. Although the 48-h culture should have produced bacterial colonies if planktonic bacterium was present, most biofilms require electron microscopy or specialized confocal microscopy for their study.18 Clinical studies have reported that biofilm formation does not require the presence of infection,10 but our study did not include examination of the catheters with electron microscopy.19 Without more detailed study, we can only assume that the catheters resulted in a greater degree of structural changes in the peritoneum and parallel alterations in transport rates of solutes than the corresponding NI rats. It is possible that different catheter materials...
may vary the inflammatory response, but test of bacterial adherence to three biomaterial surfaces demonstrated differences between organisms rather than materials. These findings suggest that careful examination of the animal models that are used to test biocompatibility of solutions is necessary for correct interpretation of the effects that can be exclusively attributed to a particular solution on the peritoneum and its barrier function.

The catheter that we used was made of silicone and designed for intravenous insertion. We used sterile technique to implant the catheter and waited until the skin over the titanium port was completely healed before initiating injection. Use of the catheter before this often resulted in wound dehiscence. That the catheter was in the peritoneal cavity for 3 to 4 wk with a weekly flush of heparin and saline likely set up a reaction in the peritoneum to the commencement of the daily injections. We did not determine the condition of the peritoneum or its transport function before the injections. Therefore, we cannot with absolute certainty quantify its interactive effect with the solution on our results. However, it is clear from the 2- and 4-wk results that the processes of peritoneal thickening and angiogenesis in the subcompact zone and inflammation were enhanced in the CI rats when compared with the NI rats. We note that many of our patients undergo catheter placement with a weekly heparin flush for 3 to 4 wk before their training to do PD.

We speculate that the catheter is a platform for the activation of mesothelial cells that are chronically shed from their basement membrane and their transition from an epithelial phenotype to a fibroblastic type with amplification of cytokine production within the cavity. Inflammatory substances that are secreted by these cells would be absorbed through the peritoneum to "wash out" these mediators may exacerbate the inflammatory response in this animal model.

Time Course of Inflammatory Reaction from Sterile Solutions

A major goal was the study of the early time course of the inflammatory reaction of the peritoneum to the sterile solution. The solution was carefully prepared and filtered to minimize glucose degradation products, which arise in the heat sterilization of glucose solutions. The solution was refrigerated and cultured before and after use; none of these cultures grew bacteria. Before injection, the solution was handled in a laminar airflow hood and injected daily into anesthetized rats to investigate their effect on the structure and function of the peritoneal barrier. As illustrated in Figure 1, there was no clear pattern of WBC versus time, and the total WBC of the CI and NI groups were not significantly different from that of the AC group.

The transport data and immunohistochemistry did display significant changes with time. Presumably because of the presence of the catheter for 3 to 4 wk before long-term injections, the CI group had marked differences from the NI group in the progression of bFGF, VEGF, and TGF-β1, with the NI rats lagging ≥4 wk behind the CI group. In the 2-wk groups, the CI rats displayed the greatest thickness, which subsequently decreased with time and on the average was significantly greater than that of AC rats. The mean thickness in the NI rats did not vary significantly with duration of injection; neither did these rats have a significant difference from the AC rats (see Figure 2). This again demonstrates the effect of the catheter. In the NI group, the vascular density increased between 4 and 6 wk, whereas the CI group showed a decrease after the initial 2-wk period, demonstrating possible differences in cytokine expression versus time. MTCmannitol, MTCBSA, and Josm also tended to decrease in magnitude after the first 2 to 4 wk and had significant trends over the 8 wk. This pattern was also seen in the fluid fluxes and MTCBSA of the NI rats. Both injection groups demonstrated significant differences in vascular density, solute transport, and cytokine staining from noninjected AC rats.

Characterization of Animal Models

Comparison of our results with studies from other laboratories is made difficult because of the different techniques of injection, including volume and frequency, and because of the method of measuring transport via a chamber instead of the whole cavity. As far as we know, no one group has performed the side-by-side comparison of CI versus NI contained in this article. It is interesting to note the varying results from two other laboratories. One group performed twice-daily NI of commercial solutions with and without glucose degradation products (GDP); they observed no significant differences in solute transport, ultrafiltration, or tissue thickness but showed higher vascularity with the more biocompatible solution. A second group implanted polyurethane catheters connected to a subcutaneous port and subsequently administered injection to the animals twice daily for 12 to 20 wk; they observed improved ultrafiltration and less angiogenesis with the more biocompatible solutions. From these two studies, the reader arrives at completely different conclusions concerning the effects of low-GDP, more “biocompatible” solutions. Our study with a presumed low-GDP solution points to the presence of a catheter, or “foreign body,” in the cavity as an additional factor in the immune response of the peritoneum and suggests that a broader examination of interactions between the catheter and immune elements of the peritoneal cavity are required for full characterization of the usefulness of relatively expensive, “biocompatible” solutions.

CONCISE METHODS

Animals and Experimental Groups

Sprague-Dawley female rats (200 to 300 g; Charles River Laboratories, Wilmington, MA) were divided into three groups. One group of rats (NI) received direct intraperitoneal injections daily for durations of 2, 4, 6, or 8 wk. The other group of rats (CI) received daily peritoneal injections through a subcutaneous port connected to a peritoneal
catheter for durations of 2, 4, 6, or 8 wk. A third group was used as age controls (AC) and followed for 8 wk. The relative age of the rats in each group was similar. No animal lost weight during the period of injection. Average weight gains in both groups were 21 g at 2 wk, 30 g at 4 wk, 43 g at 6 wk, and 45 g at 8 wk.

The experimental protocols for this study were approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center and were carried out in accordance with both the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health and the guidelines of the Animal Welfare Act.

Catheter Implantation

Aseptic techniques were used to implant a catheter for long-term PD in each rat that was to receive the daily injections through the catheter. The peritoneal catheter was placed in the lower right quadrant of the abdomen and connected to a subcutaneous chamber (Rat-o-Port CP6-0S; Access Technologies, Skokie, IL), as described in our previous publication. The chamber is made from implant grade 4 titanium, and the silicone tubing is the same as is used in human applications (Dow Corning Medical-Grade Silicone Rubber, Corning, NY). The material is of similar makeup as the Silastic silicone rubber, which is used in silicone dialysis catheters and intravenous catheters for human use. The daily injections of dialysis solution were not started until the skin wound over the port was completely healed (usually 3 to 4 wk after implantation, which is not unlike the placement of a catheter in humans). Heparin (10 U in 1 ml of PBS) was injected at weekly intervals during this healing period to maintain catheter patency.

Daily Injection of Dialysis Solution

To minimize animal activity that might increase the potential for contamination during this process, each rat was anesthetized with isoflurane (1 to 3%, 3 to 5 min daily exposure). Each rat was given 30 to 40 ml of sterile dialysis solution daily for up to 8 wk. The volume of PD solution used in these studies was selected by scaling the typical volume used in humans (approximately 2 L) by the factor (body weight). The rationale and justification for this scaling criteria are detailed in by Dedrick and colleagues. From previous experiments, this volume will touch the entire peritoneum during the 24 h period of injection, but the contact area at any one time will be a maximum of approximately 40% of the anatomic peritoneum. The peritoneal pressures observed with this volume are well within the range seen in humans.

The solution that was administered for PD was prepared weekly from Krebs-Ringer solution with 4% glucose (average osmolality 480 to 500 mOsm). The composition of the Krebs-Ringer solution was as follows (in mM): 120 NaCl, 10 KCl, 2 CaCl2, 25 NaHCO3, 0.28 KH2PO4, and 1.2 MgSO4. The dialysis solution was sterile filtered (0.2-µm filter) in a laminar-flow hood and stored at 4°C until use. The solution was presumed to be low in GDP and to be more biocompatible than commercial solutions, which are typically heat-sterilized with a low pH. Bottles of dialysis solution were handled throughout with aseptic technique, and syringes were filled within a laminar-flow hood just before injection. Each solution was cultured for bacteria at the first use of the bottle and at the end of each bottle. All solution cultures were negative.

Needle Injection.

The lower abdominal wall was prepared with alcohol and the solution was administered to rats in the NI group by direct intraperitoneal injection with a syringe and 23-G needle. Skin puncture was limited to the lower quadrants of the abdomen, with the left and right lower quadrants used alternately for the daily injections. Care was taken to avoid puncture of the intestine or subcutaneous injection of the dialysis solution. Injection sites were inspected daily. One rat developed a subcutaneous hematoma, which resolved during a 7-d period and did not affect the peritoneal cavity.

Catheter Injection.

Dialysis solution was administered through the subcutaneous port to rats in the CI group. The skin over the port was sterile-prepared with alcohol, and Huber needles were used to inject the solution. Tissue around the injection port was examined carefully for any sign of swelling or scabbing as a result of infection or bleeding.

Each rat was carefully monitored at daily injections and weighed weekly. Weight loss (>10%), abnormal activity (lack of movement in the cage), or development of a hematoma or apparent infection at the injection site or port was noted in a written log.

Bacterial Cultures before Transport Studies

After the requisite duration (2, 4, 6, or 8 wk) of daily injections, each rat was anesthetized by intramuscular injection of sodium pentobarbital (60 mg/kg). After sterile preparation of the abdomen, 30 ml of isotonic sterile Krebs solution was injected intraperitoneally. After 10 min, using sterile technique, an incision was made in the midline of the abdominal wall. The residual peritoneal fluid and catheter (if appropriate) were collected for cell count and bacterial culture. Cells were stained with Wright’s stain and counted with a hemacytometer.

Fluid was cultured on TSA II 5% SB plates (Becton Dickinson Microbiology Systems, Cockeysville, MD) for 48 h at 37°C. Positive bacterial cultures were identified by inspection of the plates.

Transport Studies

Polyethylene catheters (PE-50) were placed in the femoral vein to maintain anesthesia with additional pentobarbital and in the femoral artery for collection of blood samples during the transport studies. Temperature was maintained at 37°C, and BP was monitored to ensure that the mean pressure remained >80 mmHg (average mean arterial pressures varied between 90 and 110 mmHg).

For carrying out transport studies of solutes and water across the peritoneum, a plastic chamber was affixed to the serosa of the abdominal wall to examine the transport characteristics of the peritoneum using the procedures described previously. The chamber was affixed to the upper left quadrant of the abdominal wall remote from the location of the catheter tip or needle punctures. The chamber removes the peritoneal contact area as a variable and therefore provides a measure of the intrinsic mass transfer characteristics of selected portions of the peritoneum; recent data in rodents have shown...
similitude in the measurements with the chamber method in mice and rats. Because the peritoneal contact area may be altered by inflammation, there may not be a direct correlation between chamber measurements and transport studies performed with the traditional whole-cavity technique.

Small Solute Transport and Osmotic Filtration.
The osmotic water flux from the blood flowing through the tissue into the chamber and the transport of a small solute, [14C]mannitol, from the chamber into the tissue were measured for a period of 120 min. The MTC was calculated from the experimental data by our previously published method.27,28

Protein Transport.
Transport of protein from the blood into the chamber was determined during the same 120-min period by intravenous injection of FITC-labeled BSA (FITC-BSA) as a bolus (40 mg/ml). Changes in the plasma concentration and chamber concentration were measured during the 120-min period. The albumin flux and MTC were calculated from these measurements as described previously.7 All calculations of transport rates were performed as previously published.27,28

Hydrostatic Pressure–Driven Convection.
The chamber was washed with Krebs-Ringer solution and then filled to a depth of 6 cm with Krebs-Ringer solution that contained 5% BSA, 0.05% Evans blue dye, and 125I-IgG (1 μCi/ml) to determine hydrostatic pressure–driven water transport. The volume of the chamber solution and the concentration of 125I-IgG were measured every hour for a period of 3 h. At the end of this period, the chamber was removed and the tissue under the chamber was identified from the staining with Evans blue dye. Tissue from this region was recovered, and its radioactive content was measured to estimate transport of 125I-IgG into the tissue. The hydrostatic pressure–driven flux of water was calculated from these measurements as described previously.7 All calculations of transport rates were performed as previously published.27,28

Materials
The tracer molecule that was used for small solute experiments was 14C-mannitol purchased from Moravek Biochemicals (Brea, CA), and the tracer was stated to be at least 97% pure by the manufacturer and was used as received. 14C-mannitol was detected by liquid scintillation (Packard Tricarb 2500TR, Ramsey, MN).

FITC-BSA was purchased from Sigma Chemicals (St. Louis, MO) and was used as delivered. Fluorescence was detected with a Turner TD700 spectrophotometer. Checks of label purity7 have demonstrated that there is no detectable separation of the fluorescence label from the protein.

125I-IgG (anti-rabbit, immunoabsorbed for human and rat antigens) was purchased from Amersham (Piscataway, NJ) and purified daily according to our previously published method.30 Detection was carried out by γ counting (Packard Cobra II Auto-Gamma Counter).

Histology and Image Analysis
As in our previous publication,7 trichrome staining was used for determinations of the submesothelial compact zone thickness, and CD31 immunohistochemistry was used to stain endothelial cells for determination of apparent vascular density.

All quantitative measurements were averaged from observations of three independent observers. Samples of abdominal wall tissue were collected in a standardized manner for the evaluations to minimize variation as a result of the sampling process. Samples were taken from the right upper quadrant of the abdominal wall opposite the site for the transport studies and remote from the location of the CI or NI so as to exclude effects from mechanical irritation. Three to five horizontal sections from the midportion of the abdominal wall were prepared. Sections of peritoneal tissue were excised and immediately fixed in neutral pH-buffered 4% formalin solution and stained as described previously.31

Expression of growth factors was evaluated using standard immunohistochemistry protocols. Antibodies to VEGF, bFGF, and TGF-β1 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). After deparaffinization and rehydration, tissue sections were processed for quenching (treatment with 3% hydrogen peroxide for 30 min), antigen retrieval (steam heating sections for 30 min in Ag Retrieval Citra Plus buffer; BioGenex, San Ramon, CA), and biotin blocking (DakoCytmation, Carpinteria, CA). Incubation with blocking solution (VectaStain system [Vector Laboratories, Burlingame, CA] or Protein Block Serum-Free, Avidin-Biotin Block system [Dako, Carpinteria, CA]) was performed to block nonspecific binding. Sections were incubated with primary antibody at 4°C overnight and processed for treatment with the secondary antibody after washing with buffer solution. An avidin-biotinylated hors eradish peroxidase reagent and 3,3′-diaminobenzidine tetrahydrochloride were used for detection. The sections were counterstained with hematoxylin. The degree of tissue staining with antibodies to these growth factors was evaluated as follows: No stain, 0; minimal stain, +1; moderate stain, +2; large stain, +3; and intense stain, +4. At least five sections of each group were graded and averaged for the single score.

Statistical Analyses
Data are presented as the means ± SEM. Data from rats with positive bacterial cultures were excluded from the analysis. Statistical analyses of effects of different groups and the duration of injection were performed using two-way ANOVA. Overall means for each injection group and the AC group were compared with a one-way ANOVA. The probability of a type I error of P < 0.05 was considered significant. All tests were performed using NCSS-97 (Kaysville, UT).

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DISCLOSURES
None.
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