Mice that Lack Endothelial Nitric Oxide Synthase Are Protected against Functional and Structural Modifications Induced by Acute Peritonitis

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Abstract. Pharmacologic studies suggest that the release of nitric oxide (NO) by endothelial NO synthase (eNOS) contributes to functional alterations of the peritoneal membrane (PM) induced by acute peritonitis. In this study, peritoneal permeability parameters in a mouse model of peritoneal dialysis were characterized, and the effects of eNOS deletion on the PM structure and permeability at baseline and after catheter-induced bacterial peritonitis were examined. Exposure of C57BL/6 mice to standard dialysate yielded a transport of urea and glucose, a sodium sieving, and a net ultrafiltration that were remarkably similar to the values obtained in rats. In comparison with controls, mice with catheter-induced peritonitis were characterized by structural changes in the PM (mononuclear cells infiltrate, vascular proliferation), upregulation of endothelial and inducible NOS, increased permeability for urea and glucose, decreased ultrafiltration, and increased protein loss in the dialysate. Comparison of eNOS wild-type and knockout mice revealed that the permeability modifications and structural changes induced by acute peritonitis were significantly reversed in eNOS knockout mice, resulting in a net increase in ultrafiltration. In contrast, the deletion of eNOS in mouse peritoneum was not reflected by permeability modifications or structural changes at baseline. These results are the first to take advantage of a knockout mouse model to demonstrate directly the crucial importance of eNOS in the permeability and structural modifications caused by acute peritonitis. The characterization of this mouse model suggests that genetically modified mice represent useful tools to investigate the molecular bases of the peritoneal changes during peritoneal dialysis.

Despite technological advances and accumulating clinical experience, acute peritonitis remains the most frequent and serious complication of peritoneal dialysis (PD) (1). Understanding the molecular mechanisms that operate in acute peritonitis thus is an essential goal to reduce the functional and structural changes associated with the condition. Studies in rat and rabbit models of PD have demonstrated that transport across the peritoneal membrane (PM) depends on (1) the intrinsic permeability to each solute and (2) the effective peritoneal surface area (EPSA) reflecting the number of perfused capillaries within the peritoneum (2). The capillary endothelium, which expresses both the endothelial nitric oxide (NO) synthase (eNOS) and the water channel aquaporin-1 (AQP1), constitutes the major barrier for solutes and water transport during PD (3,4). Acute peritonitis is characterized by an increased EPSA, with increased permeability for small solutes and glucose, a faster-than-normal dissipation of the osmotic gradient, a decrease of free-water permeability, and a loss of ultrafiltration (UF) (5,6). These modifications are associated with mononuclear cell infiltrate and vascular proliferation within the PM (6).

During the past decade, NO has emerged as a crucial mediator involved in countless biologic processes, including regulation of vascular tone and permeability (7), interference with growth factors to modulate angiogenesis (8), and posttranslational control of protein activity by S-nitrosylation (9). All of these properties are relevant for the PM, because a significant NOS activity, mostly as a result of the Ca\(^{2+}\)-dependent eNOS, is detected in the peritoneum (6,10). The exact nature of the role of NO and eNOS in the PM remains debated (11). In control conditions, NOS inhibitors showed no effect on peritoneal permeability parameters (12,13). In acute peritonitis, a condition characterized by increased NOS activity as a result of upregulation of eNOS and inducible NOS (iNOS) (6,14), addition of the NOS inhibitor \(\text{N}^{\delta}\text{-nitro-L-arginine methyl ester (L-NAME)}\) to the dialysate resulted in a significant reduction of peritoneal permeability with an improvement of UF (14).

One limitation of the current rat and rabbit models of PD is that investigations of the molecular mechanisms of peritoneal permeability in vivo are mostly based on intervention studies using pharmacologic agents, blocking antibodies, or various expression systems (15). These models are often limited by the lack of specificity or the side effects of most interventions, as
well as the transient efficacy of expression systems, including adenoviral vectors (15,16). The use of genetically modified mice to investigate the molecular counterparts of PD could provide an attractive alternative to the above models. Although smaller in size, mice are economical and easy to breed and maintain, and an extensive number of knockout or transgenic mice have now been used for investigations that may be relevant for the PM.

In this study, we have characterized the parameters of peritoneal permeability, the structure of the PM, and the expression of NOS isoforms and endothelial markers (AQP1 and CD31) in C57BL/6J mice, both in basal state and after catheter-induced bacterial peritonitis. We then show that deletion of eNOS in this genetic background had significant protective effects on the permeability and structure of the PM exposed to acute peritonitis. These data substantiate the role of eNOS in the regulation of peritoneal permeability and demonstrate the usefulness of genetically modified mouse models in studies of peritoneal transport and physiology.

Materials and Methods

Animals, Experimental Groups, and Acute Peritonitis Model

Experiments were conducted using male C57 BL/6J mice (Iffa Credo, Brussels, Belgium). The C57 BL/6J eNOS"+/" (wild-type [WT]) and eNOS"−/−" (knockout [KO]) mice were generated as described previously (17) and obtained from the Jackson Laboratory (Bar Harbor, ME). Male Wistar rats, aged 8 to 10 wk, were used for comparative studies (6,10,14). All animals had access to appropriate standard diet and tap water ad libitum. The experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the local Ethics Committee.

A group of 12 control mice was used to characterize the permeability parameters at the basal state, in comparison with those of control rats (n = 12) and mice with acute peritonitis (n = 6). Another group of mice with acute peritonitis (n = 6) was used to assess the differential leukocyte counts in the dialysate at day 1, day 3, and day 7. In a second series of experiments, 11 pairs of eNOS WT and KO mice matched for age were investigated in control conditions (n = 5) or after acute peritonitis (n = 6). Acute peritonitis was generated by insertion of a peritoneal catheter as described previously (6,14). At day 0, mice were anesthetized with ketamine (100 mg/kg subcutaneously; Merial, Brussels, Belgium) and xylazine (10 mg/kg subcutaneously; Bayer, Brussels, Belgium). A silicone catheter (Terumo, Leuven, Belgium) was implanted into the peritoneal cavity without aseptic precautions and subcutaneously tunneled to the neck. A daily infusion of 2 ml of dialysate (3.86% Dianecal; Baxter, Nivelles, Belgium) was performed for 6 d. In addition, animals were intraperitoneally administered Staphylococcus epidermidis (107/ml colon-forming units, diluted in 3.86% dialysate) at day 1 and day 3. On day 7, mice were submitted to a 2-h PD exchange to measure permeability parameters followed by tissue sampling.

Peritoneal Permeability Measurement and Tissue Sampling

A peritoneal equilibration test similar to that used in rat (6,14) was used to investigate peritoneal permeability parameters in mice. After anesthesia with ketamine and xylazine, mice were placed on a thermopad at 37°C. The right common carotid artery was cannulated for the measurement of mean arterial BP using an isotonic BP transducer (Harvard Apparatus, Holliston, MA). The right jugular vein was catheterized for saline infusion (0.9% NaCl, 0.3 ml/h). After 30 min of stabilization, a silicon catheter (Ventflon 22 GA, Baxter) was inserted into the peritoneal cavity and 2.0 ml of a standard dialysate (Dianecal; Baxter) containing either 3.86% or 7% glucose was instilled. Blood and dialysate samples (100 μl) were taken from the carotid artery and the PD catheter at time 0 and at 30 min, 60 min, and 120 min of dwell time. Before each sampling, 50 to 100 μl of the dialysate was flushed back and forth, and the abdomen was agitated gently to facilitate fluid mixing. Hematocrit was measured before PD exchange. At the end of the dwell, the dialysate was recovered from the peritoneal cavity through the catheter, whereas the remaining portion was subsequently removed from the open cavity with gauze tissues that were weighted. As described previously (6), net UF corresponds to the difference between the total volume of dialysate collected (sampling through catheter and dialysate weight) and the volume instilled in the cavity. The intra-assay and interassay variability parameters of the latter method in mice are 6% and 5%, respectively.

Dialysate white blood cells (WBCs) were counted using a hemocytometer (Marienfeld, Lauda, Germany), and differential leukocyte counts were obtained from peritoneal cells plated to a glass slide by cytopsin (Thermoshandon, Pittsburgh, PA) and stained with Papanicolaou. Urea, glucose, sodium, and total protein were assayed using a Kodak Ektachem DT60 II and DTE II analyser (Eastman Kodak Company, Rochester, NY). PD parameters were also obtained in rats,

Table 1. Clinical and biological parameters in the different groups of C57BL/6J mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Body Weight (g)</th>
<th>Hematocrit (%)</th>
<th>Plasma Urea (mg/dl)</th>
<th>Dialysate WBC (10^3/ml)</th>
<th>MAP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>29.3 ± 0.6</td>
<td>45.8 ± 0.7</td>
<td>29.7 ± 1.2</td>
<td>99.6 ± 10.4</td>
<td>ND</td>
</tr>
<tr>
<td>Peritonitis</td>
<td>6</td>
<td>29.0 ± 0.9</td>
<td>43.0 ± 1.0</td>
<td>32.8 ± 1.7</td>
<td>890.7 ± 76.5</td>
<td>ND</td>
</tr>
<tr>
<td>eNOS WT</td>
<td>5</td>
<td>25.3 ± 0.4</td>
<td>45.0 ± 1.8</td>
<td>38.5 ± 1.7</td>
<td>50.0 ± 8.0</td>
<td>70.0 ± 5.6</td>
</tr>
<tr>
<td>eNOS KO</td>
<td>5</td>
<td>24.8 ± 2.0</td>
<td>43.8 ± 1.2</td>
<td>44.0 ± 5.7</td>
<td>74.0 ± 12.0</td>
<td>85.0 ± 6.0</td>
</tr>
<tr>
<td>eNOS WT-p</td>
<td>6</td>
<td>26.2 ± 0.9</td>
<td>44.6 ± 1.0</td>
<td>31.0 ± 2.4</td>
<td>760.0 ± 73.1</td>
<td>71.2 ± 3.1</td>
</tr>
<tr>
<td>eNOS KO-p</td>
<td>6</td>
<td>23.7 ± 0.8</td>
<td>44.0 ± 0.9</td>
<td>40.2 ± 6.0</td>
<td>755.0 ± 51.5</td>
<td>78.2 ± 6.3</td>
</tr>
</tbody>
</table>

a WBC: white blood cells; MAP: mean arterial blood pressure; eNOS WT-p, eNOS wild-type peritonitis; eNOS KO-p, eNOS knockout peritonitis; ND, not determined. All parameters were recorded before starting the dwell (day 7).

b P < 0.05 versus control; c P < 0.05 versus WT; d P < 0.05 versus KO.
using 15 ml of standard dialysate containing either 3.86% glucose (n = 6) or 7% glucose (n = 6) as described previously (6,14).

At the end of the dwell, animals were killed and peritoneum samples were processed for fixation and protein extraction as described previously (6,10,14). Samples from the visceral and parietal peritoneum were fixed for 3 h at 4°C in 4% paraformaldehyde in 0.1 M phosphate buffer, rinsed, and embedded in paraffin. Samples from the visceral peritoneum were dissected, snap-frozen in liquid nitrogen, and stored at −80°C.

**Antibodies**

The NOS isoforms were detected with monoclonal antibodies against eNOS, neuronal NOS (nNOS), and iNOS (Transduction Laboratories, Lexington, KY) (10). Other antibodies included a rabbit antibody against AQP1 (Chemicom International, Temecula, CA), a goat antibody against CD31 (Santa Cruz Biotechnology, Santa Cruz, CA), a rabbit antibody against vascular endothelial growth factor (VEGF; Santa Cruz), a goat antibody against vascular cell adhesion molecule-1 (VCAM-1; Santa Cruz), and a monoclonal antibody against β-actin (Sigma, St. Louis, MO).

**Tissue Staining and Immunohistochemistry**

Trichrome blue staining and immunostaining were performed as described previously (6,14). After blocking in 0.3% H2O2 and incubation with 10% normal serum, sections were incubated successively for 45 min each with the primary antibody, biotinylated IgG, and avidin-biotin peroxidase (Vector Laboratories, Burlingame, CA). The MOM immunodetection kit (Vector) was used for monoclonal antibodies. Antigen retrieval was performed by incubating sections for 30 min at pH 5.8, in a water bath heated at 97°C, before rinsing in water. Immunolabeling was visualized using aminooethylcarbazole (Vector). Sections were viewed under a Leica DMR microscope (Leica, Heerbrugg, Switzerland). The specificity of immunolabeling was confirmed by incubation without primary antibody and with nonimmune IgG (Dako).

**Quantification of Vascular Immunoreactivity**

The use of CD31 to quantify microvascular density has been previously reported in detail (18,19). Peritoneal sections stained for CD31 were viewed through a Zeiss microscope coupled to a DAGE-MTI CCD 72 camera (Michigan City, IL) and analyzed through a KS400 system (Kontron, Munich, Germany). The stained areas of the visceral peritoneum were digitized, to outline the vessel walls. The intensity of the mononuclear cells infiltrate was graded as 0, absent; 1, moderate; 2, mild; and 3, severe in WT versus KO eNOS mice (n = 5 in each group).

**Statistical Analyses**

Data are presented as mean ± SEM. Comparisons between results from different groups were performed using two-tailed t test or Mann-Whitney U test, as appropriate.
Results

Clinical and Biological Parameters

Mice were similar in terms of body weight, hematocrit, plasma urea, and glycemia at baseline (Table 1), and none of them died prematurely during the protocol. In comparison with controls, mice with acute peritonitis were characterized by positive dialysate cultures and cloudy dialysates with increased WBC counts (Table 1). None of the cultures obtained from controls, eNOS WT, and eNOS KO mice was positive. The differential leukocyte counts in the dialysate of mice with peritonitis showed that the majority (60 to 80%) of inflammatory cells were polymorphonuclear leukocytes at day 1. That proportion decreased to 34 to 45% at day 3 and further to 8 to 20% at day 7, in parallel with a progressive increase in mononuclear leukocytes (macrophages and lymphocytes). A comparison between eNOS WT and KO mice did not show any significant difference in the differential leukocyte count at day 7 (data not shown). As expected (17), eNOS KO mice were characterized by a mean arterial BP higher than eNOS WT mice in control conditions and, to a lower extent, during acute peritonitis (Table 1).

Characterization of Peritoneal Permeability in Mouse: Effects of Acute Peritonitis and eNOS Deletion

Control mice were submitted to a 2-h exchange with 3.86% versus 7% glucose dialysate (Figure 1, Table 2). Exposure to both dialysates induced a progressive increase in the dialysate-to-plasma ratio for urea (Figure 1A), a progressive reabsorption of glucose from the dialysate (Figure 1B), and a fall in the dialysate-to-plasma ratio of sodium during the first 30 min of the dwell (sodium sieving; Figure 1C). At drainage, the dialysate was clear in all animals. Exposure to 7% dialysate induced a significant increase in the sodium sieving (Figure 1C, Table 2), whereas it had no effect on other parameters (Figure 1, A and B, Table 2). It must be noted that the PD parameters obtained in mice were remarkably similar to those obtained in rats, as shown by similar values for cumulative urea and glucose transport, sodium sieving, and net UF (normalized to body weight; Table 2).

The effects of acute peritonitis were investigated in control and eNOS mice (Figure 2, Table 3). Acute peritonitis induced a major increase in peritoneal permeability for urea, a faster glucose absorption from the dialysate, and a loss of the sodium sieving in control and eNOS WT mice (Figure 2). In both groups, the changes were reflected by a similar increase in the cumulative transport of urea and glucose, a fall in UF, and an increased protein loss in the dialysate (Table 3). Thus, the magnitude of the changes induced by acute peritonitis was similar in control and eNOS WT mice. However, in comparison with the latter, eNOS KO mice with acute peritonitis were characterized by a significant reduction in the hyperpermeability to urea and glucose, a significant increase in UF and sodium sieving, and a decreased protein loss in the dialysate (Figure 2, Table 3). At baseline, the deletion of eNOS was not reflected by changes in peritoneal permeability parameters (Table 3).

Structural Changes in the Mouse Peritoneum: Morphology and Immunostaining

Morphologic examination of the visceral and parietal peritoneum showed that acute peritonitis in mice was reflected by a massive, submesothelial infiltrate of mononuclear cells, together with a discrete edema (Figure 3). These structural modifications were similar in eNOS WT and KO mice, although the mononuclear cell infiltrate was less marked in the latter (median intensity of the mononuclear cells infiltrate, 3.0 versus 2.0; n = 5; P = 0.033, Mann-Whitney U test).

Immunostaining showed that acute peritonitis was associated with an increased signal for eNOS in capillary endothelium (Figure 4, A and B), whereas no specific staining was detected in eNOS KO mice (Figure 4C). Contrasting with the absence of signal in control mice, a strong staining for iNOS was detected in mononuclear cells infiltrating the peritoneum of mice with acute peritonitis (Figure 4, D and E). Of note, the intensity of iNOS staining was lower in eNOS KO versus eNOS WT mice (compare Figure 4, F and E). Acute peritonitis was reflected by a marked increase in the immunoreactivity for CD31, located in both endothelial cells and macrophages (Figure 4, G and H). The signal for CD31 was lower (intensity and density) in eNOS KO versus eNOS WT mice (compare Figure 4, I and H). The morphologic and immunocytochemical modifications induced by acute peritonitis were similar in the visceral and parietal peritoneum.

Quantification of vascular density and relative endothelial area in the visceral peritoneum (Figure 5) confirmed that the

| Table 2. Peritoneal permeability parameters in control mice and ratsa |
|-------------------|---|-------------------|-----------------|---|-------------------|
| Dialysate (%) Glucose | N | AUC D/P Urea | AUC D/D0 Glucose | Na⁺ Sieving (%) | BW (g) | Net UF/BW (ml/kg) |
|-------------------|---|-------------------|-----------------|---|-------------------|
| Mice              |   |                   |                  |   |                   |
| 3.86%             | 6 | 62.6 ± 1.3        | 60.9 ± 1.5       | 6.7 ± 0.1 | 29.3 ± 0.6        | 24.9 ± 1.5 |
| 7%                | 6 | 63.5 ± 2.1        | 59.8 ± 1.5       | 12.4 ± 0.4b | 29.6 ± 0.7        | 39.5 ± 1.3b |
| Rats              |   |                   |                  |   |                   |
| 3.86%             | 6 | 56.1 ± 1.5        | 66.2 ± 1.5       | 8.7 ± 0.2 | 305 ± 13          | 25.3 ± 1.3 |
| 7%                | 6 | 59.7 ± 1.8        | 63.3 ± 0.8       | 13.6 ± 0.6b | 307 ± 16          | 41.4 ± 2.4b |

a AUC, area under the curve (0–120 min); BW, body weight; UF, ultrafiltration (volume out–volume in).

b P < 0.05 versus 3.86% dialysate.
deletion of eNOS in mice had no effect on both parameters at baseline. Peritonitis induced a significant increase in vascular density and relative endothelial area in both eNOS WT and KO mice, but the effect was significantly attenuated in eNOS KO mice. Peritonitis induced a significant increase in the mean radius of stained vessels in both eNOS WT-p (63 ± 8 versus 198 ± 19 μm; P = 0.03) and eNOS KO-p (64 ± 21 versus 115 ± 16 μm; P = 0.03) mice. The increase, which was predom-
inantly observed in intermediate and large-sized vessels, was significantly attenuated in eNOS KO-p versus eNOS WT-p mice (115 ± 16 μm versus 198 ± 19 μm, respectively; P = 0.03).

Expression of NOS Isoforms and AQP1 in the Mouse Peritoneum: Immunoblotting

Acute peritonitis induced a significant upregulation of the endothelial (140 kD) and inducible (130 kD) NOS isoforms,
and a discrete upregulation of AQP1 (relative optical density versus control, 141 ± 17%; \( P = 0.20 \)) in the mouse peritoneum (Figure 6A). As described previously (10), the anti-iNOS antibody cross-reacted with eNOS (see the upper band on the gel), but the specific band corresponding to iNOS (lower band of the gel) was identified by its molecular mass (130 kD) and
co-migration with macrophage extract (positive control). The induction of iNOS was variable (6,10), ranging from weak to very strong. In control conditions, eNOS and iNOS isoforms were not detected in the peritoneum of eNOS KO mice, whereas the expression of AQP1 was similar in eNOS WT and KO mice (data not shown). Acute peritonitis in eNOS WT and KO mice was reflected by a variable induction of iNOS in the peritoneum (Figure 6B). Of note, the expression of iNOS was lower in eNOS KO (relative optical density versus eNOS WT, 64 ± 22%; P = 0.55), whereas that of AQP1 was higher (relative optical density versus eNOS WT, 175 ± 54%; P = 0.42). A weak expression of nNOS was also detected within the peritoneum of control and eNOS mice and showed no significant variation in case of acute peritonitis (data not shown). Preliminary experiments (limited to three pairs of eNOS mice with peritonitis) also indicated that there was a 20% decrease in the expression of the adhesion molecule VCAM-1 in the peritoneum of eNOS KO mice versus eNOS WT mice with peritonitis (data not shown).

**Determination of VEGF Concentrations in the Plasma and Dialysate**

A specific ELISA was used to determine the concentrations of VEGF in the plasma and dialysate of the eNOS mice in control conditions and after catheter-induced acute peritonitis (Table 4). The deletion of eNOS in mice was not reflected by significant changes in VEGF concentrations. Peritonitis induced a major increase in dialysate VEGF in both eNOS WT and eNOS KO mice; the increase in that parameter was slightly blunted in eNOS-KO mice, but the difference with eNOS WT mice was NS (36 ± 11 versus 61 ± 24 pg/ml, respectively; P = 0.42). Immunoblotting studies also failed to demonstrate a significant difference in the expression of VEGF in peritoneal samples from eNOS WT and KO mice (Figure 7).

**Discussion**

In this study, we describe the structure, permeability parameters, and expression of NOS isoforms and endothelial markers in the PM of a mouse model of PD at baseline and after catheter-induced bacterial peritonitis. The latter condition is reflected by major structural and functional changes in the PM, similar to those observed in PD patients. These alterations are significantly reversed in eNOS KO mice. Importantly, the deletion of eNOS in mouse has no effect on the PM at baseline, demonstrating the importance of a tight regulation of this enzyme.

The permeability of the PM was investigated in C57 BL/6J mice, which represent one of the most common genetic backgrounds for gene-targeted mutations. The permeability for small solutes, the reabsorption of glucose, the free-water permeability (as assessed by the sodium sieving), and the net UF normalized for body weight were actually similar in mice and rats exposed to the same dwell. Moreover, exposure to higher osmolality was reflected by a similar increase in sodium sieving and net UF in both species (Table 2, Figure 1). We next used a model of catheter-induced bacterial peritonitis (6,14) to investigate structural and permeability modifications in the mouse. To modelize further the situation encountered in PD-
related peritonitis, the catheter contamination from skin flora was completed by an inoculum of coagulase-negative Staphylococci—the most common organism responsible for peritonitis in PD patients (14,21). As compared with controls, mice with peritonitis showed cloudy dialysate with positive cultures and high WBC counts in the dialysate with a majority of polymorphonuclear leukocytes at day 1 followed by a progressive increase in macrophages and lymphocytes; mononuclear cells infiltrates and vascular proliferation in the peritoneum at day 7 (Figures 3 through 5); upregulation of eNOS and iNOS (Figures 4 and 6); increased permeability for urea and glucose, with a loss of sodium sieving (Figure 2); and a combination of decreased UF and increased protein loss in the dialysate (Table 3). These changes, which confirm our previous observations in rat models (6,14), are characteristic of acute peritonitis in PD patients (5). These functional and structural studies thus demonstrate the relevance of the mouse as a model for studying peritoneal transport at baseline and during acute peritonitis.

The role of NO in the regulation of peritoneal permeability is suggested by its influence on vascular tone and permeability, particularly during infection or inflammation (7,22), as well as its interaction with angiogenic growth factors such as VEGF (8). Addition of the NO donor nitroprusside to the dialysate increases EPSA in rat models (23) and stable PD patients (24). An increased release of NO is also involved in the increased EPSA and the loss of UF that are observed during acute peritonitis (25). An upregulation of eNOS and iNOS has been
documented in a rat model of acute peritonitis (6,14), and addition of L-NAME to the dialysate has been shown to restore UF and decrease protein loss in this model (14). However, the acute nature and the lack of specificity of these pharmacologic interventions against NOS limit the relevance of these earlier studies. Considering that the endothelium lining peritoneal vessels is the major functional barrier during PD and because eNOS accounts for most of the NOS activity in the peritoneum (6,10), it was tempting to investigate the permeability and morphology of the peritoneal blood vessels and the basal PD conditions (Table 2, Figure 3). These data confirm previous studies. For instance, eNOS-deficient mice show impaired angiogenesis (28,29). Deficiency in eNOS also impairs myocardial angiogenesis (30) or angiogenesis in the ischemic hindlimb (31). The exact mechanism of the impaired angiogenesis in these conditions remains to be determined. There are no significant modifications of VEGF levels in mice that lack eNOS (32) (Table 4, Figure 7), but information about the state of VEGF receptors in these mice is lacking. Nevertheless, the role of eNOS in mediating angiogenesis induced by VEGF and other growth factors (33,34) is particularly important for the PM, because neovascularization is a major cause for functional changes and loss of UF in PD patients (35).

Third, the protective effect of eNOS deletion on the intensity of the infiltrate and the induction of iNOS suggests that eNOS may regulate the vascular permeability of inflammatory mediators such as leukotrienes or histamine (7). As suggested by our preliminary observations, eNOS activity could interfere with the expression of adhesion molecules such as VCAM-1 (36). In turn, such an interference could modify leukocyte-

Table 4. Plasma and dialysate concentrations of VEGF in eNOS WT and KO mice in control conditions and after catheter-induced bacterial peritonitis

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 4)</th>
<th>Peritonitis (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>eNOS WT</td>
<td>eNOS KO</td>
</tr>
<tr>
<td>Plasma VEGF (pg/ml)</td>
<td>108 ± 13</td>
<td>138 ± 13</td>
</tr>
<tr>
<td>Dialysate VEGF (pg/ml)</td>
<td>1.0 ± 0.6</td>
<td>1.6 ± 0.9</td>
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</table>

*a* VEGF, vascular endothelial growth factor. The *P* value relates to comparisons between eNOS WT and eNOS KO mice within control and peritonitis conditions.

*b* *P* < 0.01, dialysate VEGF in eNOS WT-p versus eNOS WT.

*c* *P* < 0.01, dialysate VEGF in eNOS KO-p versus eNOS KO.

**Figure 7.** Expression of vascular endothelial growth factor (VEGF) in the peritoneum of eNOS WT and KO mice with peritonitis. Representative immunoblot for monomeric VEGF (21 kD; 1, 1000) in the visceral peritoneum of eNOS WT and KO mice with peritonitis (20 μg protein/lane, reducing conditions). Recombinant VEGF was used as positive control (lane C). The blots were probed with Ponceau red (Sigma) for transfer accuracy. The band corresponding to VEGF is detected in all samples from eNOS WT and KO mice with peritonitis. The expression of VEGF in the peritoneum is similar in both groups (relative optical density, eNOS KO-p versus eNOS WT-p mice, 115 ± 16%; *P* = 0.63).

First, they indicate that a tight regulation of eNOS is critical to regulate permeability parameters in PD. The positive effects observed in eNOS KO mice are very similar to those observed acutely with L-NAME addition to the dialysate (14), which supports the relative specificity of L-NAME toward constitutive NOS (27) and emphasizes the therapeutic potential of such NOS inhibitors for functional alterations encountered during acute peritonitis (14).

Second, our observation of a significant reduction in vascular proliferation in eNOS KO mice, mirrored by lower endothelial area and permeability parameters, completes several lines of evidence suggesting that eNOS plays a predominant role in growth factor–induced angiogenesis and vascular permeability. For instance, eNOS-deficient mice show impaired wound healing and angiogenesis in response to VEGF or angiopoietin-1 (28,29). Deficiency in eNOS also impairs myocardial angiogenesis (30) or angiogenesis in the ischemic hindlimb (31). The exact mechanism of the impaired angiogenesis in these conditions remains to be determined. There are no significant modifications of VEGF levels in mice that lack eNOS (32) (Table 4, Figure 7), but information about the state of VEGF receptors in these mice is lacking. Nevertheless, the role of eNOS in mediating angiogenesis induced by VEGF and other growth factors (33,34) is particularly important for the PM, because neovascularization is a major cause for functional changes and loss of UF in PD patients (35).
endothelial cell interactions (37) and inflammation at the microvascular level (38).

Finally, despite its spectacular effects, the deletion of eNOS does not totally reverse the permeability changes induced by acute peritonitis in mice. This incomplete reversibility is particularly evident for the free-water permeability and net UF, as already observed in acute studies with L-NAME (14). Considering that the water channel AQPI is upregulated in these conditions (Figure 6), the lack of total reversibility could be due to vasoactive substances and proinflammatory cytokines liberated during the course of infection/inflammation (39) or, alternatively, to posttranslational modifications of target proteins (9). Of interest, our studies also confirm the differential regulation of the two endothelial proteins eNOS and AQPI, as already observed in rat models (6,40) and PD patients (4).

In conclusion, we have characterized and validated a model of PD in mouse and provided a direct demonstration of the importance of eNOS for structural and functional changes associated with acute peritonitis. These results suggest that the use of genetically modified mice will be useful to characterize the molecular bases for the alterations of the PM during PD.

Acknowledgments

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