Effect of indomethacin on peritoneal protein loss in a rabbit model of peritonitis

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Background. Although various inflammatory mediators have been previously shown to be released into the peritoneal cavity during peritonitis in peritoneal dialysis patients, those that are involved in governing changes in peritoneal permeability to small solutes and protein remain incompletely defined.

Methods. We determined the importance of prostanoid production in the enhanced protein loss observed during acute peritonitis by inhibition experiments using indomethacin, an inhibitor of cyclooxygenase activity. The association between changes in peritoneal permeability and the generation of inflammatory mediators after adding Escherichia coli to peritoneal dialysate was first examined in series 1 experiments. Series 2 experiments then determined the effect of intraperitoneal administration of indomethacin (75 μg/mL) on changes in peritoneal permeability after adding E. coli to peritoneal dialysate. All experiments were performed in male New Zealand White rabbits (2.6 to 3.4 kg body weight) using an eight-hour dwell of dialysate containing 2.5% glucose. Peritoneal permeability to creatinine and protein was assessed by time-dependent changes in the dialysate to plasma concentration ratios of these solutes.

Results. Series 1 experiments showed enhanced leukocyte migration into the peritoneal cavity and increased peritoneal permeability to protein during bacterial challenge that was accompanied by an increase in the dialysate concentrations of prostaglandin E2 (PGE2), 6-keto-PGF1α, and interleukin-8, but not nitrate + nitrite (a measure of local nitric oxide production). Inhibition of prostanoid production by intraperitoneal administration of indomethacin in series 2 experiments resulted in lower dialysate concentrations of PGE2 and 6-keto-PGF1α and in lower peritoneal permeability to protein, both to control levels. No effect of indomethacin on transperitoneal migration of leukocytes or the generation of interleukin-8 was observed.

Conclusions. Enhanced production of prostaglandins likely plays an important role in governing the increase in peritoneal permeability to protein during acute, bacterial peritonitis in the rabbit.

Peritonitis causes significant morbidity in chronic peritoneal dialysis patients [1, 2]. During the acute phase of peritonitis, there is rapid migration of leukocytes into the peritoneal cavity and an increase in the permeability of the peritoneum or peritoneal membrane to both protein [3] and small solutes. The latter change in peritoneal permeability also results in a loss of ultrafiltration capacity and decreased clearances of urea and creatinine [4, 5]. The mechanisms governing changes in peritoneal permeability during peritoneal inflammation remain incompletely defined.

Local production of inflammatory mediators within the peritoneum [6], some of which can direct leukocyte migration into the peritoneal cavity [7], has been associated with changes in peritoneal permeability during peritonitis. For example, Steinhauer and Schollmeyer showed that peritoneal dialysate concentrations of prostaglandin E2 (PGE2), 6-keto-PGF1α, PGF2α and thromboxane B2 increased during episodes of peritonitis in continuous ambulatory peritoneal dialysis (CAPD) patients and that dialysate concentrations of PGE2 and 6-keto-PGF1α correlated with the increased loss of proteins into the dialysate [8]. The importance of inflammatory mediators in regulating changes in peritoneal permeability during peritonitis in CAPD patients was further supported by Zemel et al, who showed that increased transperitoneal transport of protein and small solutes correlated with enhanced appearance rates of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and prostaglandins in peritoneal dialysate [9]. In that study, increases in transperitoneal transport of small solutes correlated best with dialysate IL-6 concentrations, and changes in peritoneal permeability to protein correlated best with dialysate PGE2 concentrations. An association between the appearance of certain prostaglandins and complement proteins in

Key words: prostanoid, bacterial peritonitis, inflammatory mediators, cyclooxygenase, interleukins, nitric oxide.

Received for publication October 20, 1999
and in revised form July 10, 2000
Accepted for publication July 13, 2000

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the peritoneal cavity and enhanced protein loss rates has also been demonstrated in the rabbit during zymosan-induced peritonitis [10]. Other recent studies have proposed that local production of nitric oxide can mediate changes in transperitoneal transport of both protein and small solutes during peritonitis in the rat [11–13]. Despite these previously reported statistical correlations, the relative importance of prostaglandins, cytokines, and nitric oxide in altering peritoneal permeability during peritonitis remains unclear.

We have developed a rabbit model of acute peritonitis that elicits both enhanced leukocyte migration into the peritoneal cavity and increased peritoneal permeability to protein during the first eight hours of infection (abstract; Liu et al, J Am Soc Nephrol 5:462, 1994). In the present study, we describe the local release of certain inflammatory mediators into the peritoneal cavity in this model and determine the importance of prostanoid production in controlling protein permeability during peritonitis by inhibition experiments using indomethacin, an inhibitor of cyclooxygenase (COX) activity.

**METHODS**

**Experimental design**

Two separate series of peritoneal dialysis experiments were performed in male New Zealand White rabbits weighing between 2.6 and 3.4 kg. Series 1 experiments compared transperitoneal solute transport rates and dialysate concentrations of inflammatory mediators after the addition of $1 \times 10^6$ colony forming units (cfu) of *Escherichia coli* (*E. coli*; catalog number 25922, *N* = 7; American Type Culture Collection, Rockville, MD, USA) to the peritoneal cavity with those from control rabbits without bacteria added to the dialysate (*N* = 7). Series 2 experiments compared transperitoneal solute transport rates and dialysate concentrations of inflammatory mediators with (*N* = 4) and without (*N* = 4) intraperitoneal administration of indomethacin (Merck, West Point, PA, USA) at a concentration of 75 µg/mL to dialysate containing $5 \times 10^6$ cfu of *E. coli*. In all experiments, rabbits underwent an eight-hour exchange using 120 mL of dialysate containing 2.5% glucose (Dianeal PD-2; Baxter Healthcare, Deerfield, IL, USA).

**Surgical procedure and experimental protocol**

The surgical procedure and protocol for evaluating peritoneal permeability in these experiments were similar to those previously published [14, 15]. Rats were fasted overnight and were anesthetized by an intramuscular injection of acepromazine maleate (16 mg/kg) and xylazine hydrochloride (3 mg/kg), followed 10 minutes later by an intramuscular injection of ketamine hydrochloride (50 mg/kg). A carotid artery and jugular vein were cannulated for blood sampling and the infusion of solutions, respectively. The peritoneal cavity was then penetrated, and a peritoneal catheter (Sil-Med Corporation, Tauton, MA, USA) was inserted and secured with a purse-string suture. No additional anesthesia was given after surgery was completed.

After surgical preparation, a baseline arterial blood sample was obtained. A washout exchange of the peritoneal cavity was then performed using 100 mL of Normosol R® (Abbott Laboratories, North Chicago, IL, USA) for 15 minutes. Normosol R® solutions had a pH of 6.8 and contained 140 mEq/L sodium, 5 mEq/L potassium, 98 mEq/L chloride, 27 mEq/L acetate, 3 mEq/L magnesium, and 23 mEq/L gluconate. A priming dose of 100 mg of creatinine (Sigma Chemical, St. Louis, MO, USA) dissolved in 20 mL of Normosol R® was infused intravenously during a 10-minute interval. This was followed by a constant infusion of a Normosol R® solution containing 1.65 mg/mL creatinine at a rate of 1.15 mL/min to maintain a plasma creatinine concentration of approximately 10 mg/dL. After drainage of the washout exchange by gravity, 120 mL of the dialysis solution (Dianeal PD-2) were instilled into the peritoneal cavity containing *E. coli* and indomethacin as required by the experimental design. All solutions were warmed to 37°C before infusion into the rabbit.

Three milliliters of blood and 4 mL of dialysate were obtained at 0, 2, 4, 6, and 8 hours after complete infusion of the dialysate into the peritoneal cavity. One hundred microliters were taken from each of these dialysate samples to determine total and differential leukocyte counts, and the remainder was centrifuged to ensure complete removal of particles and cells. The supernatant was collected immediately into tubes containing sodium ethylenediaminetetraacetic acid, pH 7.2, and indomethacin (10 mmol/L and 5.6 µmol/L final concentrations, respectively) and was stored at −70°C until assays were performed. Additional 1 mL dialysate samples were obtained at 0.5, 1, and 3 hours after complete infusion of the dialysate for determination of small solute and protein concentrations only. After taking the last samples, the peritoneal cavity was drained by gravity for five minutes, and the drained volume was measured to the nearest milliliter using a graduated cylinder. The experiment was then terminated by a barbiturate overdose.

**Analytical techniques**

Leukocytes in dialysate were counted using a hemocytometer. The percentage of neutrophils in the dialysate was determined by using Wright’s stain (Sigma Chemical). Plasma and dialysate concentrations of creatinine, glucose, and protein were measured using an automated analyzer (Beckman CX3, Fullerton, CA, USA). Creatinine concentrations were corrected for high glucose concentrations as described previously [15].

Prostaglandin E$_2$ and 6-keto-PGF$_{1α}$ concentrations in
the dialysate were determined by using commercially available immunoassays according to the instructions of the manufacturer (R&D Systems, Minneapolis, MN, USA). The minimum detection limit for both prostanoids was 36.2 pg/mL. IL-8 was measured in peritoneal dialysate by using an enzyme-linked immunosorbent assay (Endogen, Cambridge, MA, USA) with a minimum detection limit of 2 pg/mL. To assess local production of nitric oxide, the sum total of both nitrate and nitrite (hereafter referred to as nitrate + nitrite) concentrations was determined using a colorimetric assay (Cayman Chemical, Ann Arbor, MI, USA) with a minimum detection limit of 2.5 μmol/L. The latter assay required two steps. The first is the conversion of all nitrate to nitrite using nitrate reductase, and the second is the evaluation of nitrite concentration using the Griess reagents.

Calculations

Peritoneal permeability to creatinine and total protein was evaluated by calculating the dialysate to plasma (D/P) concentration ratio at each sampling time. When the concentration in plasma was not measured at a given dialysate sampling time, the value was interpolated from the bracketing concentrations. Creatinine and protein clearances averaged over the eight-hour dwell were calculated by multiplying D/P for creatinine and total protein, respectively, by the drained volume and dividing by the dwell time. Peritoneal permeability to glucose was evaluated by calculating the ratio of the dialysate concentration at each sampling time to the dialysate concentration immediately after complete infusion of the dialysis solution into the peritoneal cavity (D/D₀).

Statistics

All results were expressed as mean ± SEM. The significance of differences in the measured parameters as a function of dwell time between groups was first assessed by analysis of variance (ANOVA) with repeated measures. If this test indicated significant differences between groups, the significance of differences at each sampling time was assessed using a Student t test for unpaired data. P values less than 0.05 were considered significant.

RESULTS

The effect of adding E. coli to peritoneal dialysate on the time dependence of dialysate leukocyte counts and the D/P protein concentration ratios is shown in Figures 1 and 2, respectively. The addition of E. coli to the peritoneal cavity resulted in a substantial increase in dialysate leukocyte counts after four hours and a significant increase in the D/P protein concentration ratio at one, two, and eight hours of the dwell. The increase in dialysate leukocyte counts was primarily due to neutrophils; the percentage of leukocytes in the dialysate that were neutrophils at eight hours after the addition of E. coli to the peritoneal cavity was 92 ± 3% (P < 0.01, different from the 51 ± 10% in control rabbits). Drained volume and creatinine clearance were marginally higher (P = 0.078 and P = 0.088, respectively) after intraperitoneal addition of E. coli than in control rabbits, and protein clearance was significantly higher after intraperitoneal addition of E. coli.
neal addition of *E. coli* than in control rabbits (Table 1). These data show that the addition of *E. coli* to the peritoneal cavity resulted in enhanced leukocyte migration into the peritoneal cavity and increased peritoneal permeability to protein. These results are similar to those previously reported by ourselves (abstract; Liu et al, *J Am Soc Nephrol* 5:462, 1994) and others [10] after intraperitoneal addition of an inflammatory stimulus in the rabbit.

The effect of intraperitoneal addition of *E. coli* on dialysate concentrations of inflammatory mediators is shown in Figure 3 and Table 1. Figure 3 shows the time dependence of 6-keto-PGF$_{1\alpha}$ (Fig. 3A) and PGE$_2$ (Fig. 3B) concentrations in the dialysate during series 1 experiments. Intraperitoneal addition of *E. coli* resulted in an increase in the dialysate concentration of 6-keto-PGF$_{1\alpha}$ after six hours and of PGE$_2$ after eight hours. There was no increase in nitrate + nitrite concentrations in the dialysate with dwell time in series 1 experiments, and intraperitoneal addition of *E. coli* did not result in higher dialysate nitrate + nitrite concentrations than those determined in control rabbits (Table 1). Dialysate concentrations of IL-8 were not consistently detectable in peritoneal dialysate until eight hours of the dwell. Intraperitoneal addition of *E. coli* resulted in a substantial increase in dialysate IL-8 concentrations after eight hours (Table 1).

Figures 4 and 5 show the time dependence of dialysate leukocyte counts and D/P protein concentration ratios in series 2 experiments in which *E. coli* was added to the dialysate for both groups of rabbits. There was no effect of intraperitoneal administration of indomethacin on the increase in dialysate leukocyte counts with dwell time (Fig. 4), and the percentage of leukocytes in the dialysate that were neutrophils was 95 ± 1% with or without intraperitoneal administration of indomethacin. In contrast, intraperitoneal administration of indomethacin resulted in lower D/P protein concentration ratios (Fig. 5), lower creatinine and protein clearances (Table 2), and lower drained volumes of dialysate (Table 2). These findings show that intraperitoneal administration of indomethacin resulted in peritoneal permeability to protein similar to that observed in control rabbits not exposed to bacteria in the dialysate (compare with the results from series 1 experiments).

As expected, intraperitoneal administration of indomethacin blocked prostanoid production during acute peritonitis, as reflected by 6-keto-PGF$_{1\alpha}$ and PGE$_2$ concentrations in the dialysate (Fig. 6 and Table 2) that were similar to those in control rabbits not exposed to bacteria in the dialysate. In contrast, dialysate concentrations of nitrate + nitrite and IL-8 were not influenced by intraperitoneal administration of indomethacin.

Figure 7 shows the time dependence of D/P concentration ratios for creatinine during both series 1 and 2 experiments. There was no effect of any study intervention on either D/P creatinine or D/D$_0$ glucose (data not shown). These findings demonstrate that the addition of bacteria

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**Table 1.** Calculated and measured parameters after an 8-hour dwell in series 1 experiments

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control rabbits</th>
<th>Intraperitoneal administration of <em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Drained dialysate volume <em>mL</em></td>
<td>90 ± 9</td>
<td>117 ± 11</td>
</tr>
<tr>
<td>Creatinine clearance <em>mL/min</em></td>
<td>0.19 ± 0.02</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>Protein clearance <em>µL/min</em></td>
<td>9.6 ± 1.1</td>
<td>16.1 ± 1.4</td>
</tr>
<tr>
<td>Dialysate 6-keto-PGF$_{1\alpha}$ <em>ng/mL</em></td>
<td>10.9 ± 1.4</td>
<td>14.5 ± 2.2</td>
</tr>
<tr>
<td>Dialysate PGE$_2$ <em>pg/mL</em></td>
<td>202 ± 20</td>
<td>426 ± 67</td>
</tr>
<tr>
<td>Dialysate nitrate/nitrite concentration <em>µmol/L</em></td>
<td>3.6 ± 1.0</td>
<td>5.7 ± 1.4</td>
</tr>
<tr>
<td>Dialysate IL-8 concentration <em>pg/mL</em></td>
<td>0.3 ± 0.3</td>
<td>115.7 ± 43.5</td>
</tr>
</tbody>
</table>

Mean ± SEM values are shown.

* Higher than in control animals (P < 0.05)

**Fig. 3.** Dialysate 6-keto-PGF$_{1\alpha}$ (A) and PGE$_2$ (B) concentrations as a function of dwell time during series 1 experiments. Results are shown after addition of *E. coli* to the peritoneal cavity (○) and in control rabbits (□). Error bars denote ± SEM, and *P < 0.05 and **P < 0.01 in dialysate concentrations after intraperitoneal addition of *E. coli* compared with control values, respectively.
to the peritoneal cavity of the rabbit does not substantially alter peritoneal permeability to small solutes in the initial stage (the first 8 hours) of peritonitis and that any differences in creatinine clearance in this model are largely due to differences in drained volume.

**DISCUSSION**

During peritonitis in CAPD patients and in animal models, a number of inflammatory mediators are generated locally and released into the peritoneal cavity [6–13]. The current experiments show that dialysate concentrations of 6-keto-PGF\(_1\alpha\), PGE\(_2\), and IL-8, but not nitrate + nitrite, are elevated during acute peritonitis induced by the addition of *E. coli* to the peritoneal cavity of the rabbit. It should be noted that these concentrations are relatively low for inflammatory sites, largely because of the large volume of dialysate present within the peritoneal cavity. Although *E. coli* is not the most common pathogen causing peritonitis in CAPD patients [1], the magnitude of the increase in dialysate concentrations of 6-keto-PGF\(_1\alpha\), PGE\(_2\), and IL-8 is comparable to that observed during infection of the peritoneal cavity with other organisms in CAPD patients [8, 9, 16, 17]. While increased dialysate concentrations of nitrate + nitrite have been reported in one study of peritonitis in CAPD patients [18], an enhanced local production of nitric oxide was not observed by others [19]. The observations from the current study suggest that *E. coli*-induced peritonitis in the rabbit elicits some of the same inflammatory mediators as during peritonitis in CAPD patients. Furthermore, these data show that production of nitric oxide is not a feature of *E. coli*-induced peritonitis in the rabbit, unlike that observed during peritonitis induced by lipopolysaccharide or zymosan in the rat [11, 12].

Prostaglandins (mainly PGI\(_2\) and PGE\(_2\)) are generated by the oxidation of arachidonic acid by COX and are multifunctional mediators of inflammation. The vasoactive properties of prostaglandins have been recognized for many years [20, 21], and they exert their vasoactive activities by binding to specific cell surface receptors. This leads to activation of adenylate cyclase and subsequently to elevated intracellular cyclic adenosine monophosphate levels, which may in turn activate specific protein kinases. Prostaglandins, by themselves, do not alter
vascular permeability to macromolecules; rather, they can act synergistically with other inflammatory mediators to enhance vascular permeability [22, 23]. Indomethacin blocks the conversion of arachidonic acid to biologically active prostanoids by inhibiting the activity of both isoforms of COX. It should be noted that the immunomodulatory properties of indomethacin are not entirely the result of its inhibition of the COX system and prostanoid production [24]; thus, the findings of the current study may be due to other actions of indomethacin, such as alteration of second-messenger pathways [25] and cytokine production [26].

Previous work in CAPD patients and in animal models has shown that peritoneal inflammation is accompanied by an enhanced appearance of prostanoids in the peritoneal cavity and increased peritoneal permeability to protein [8–10]. Despite these statistical correlations, attempts to prove a causal relationship between prostanoid production and increased peritoneal permeability have not been convincing. Steinhauer and Schollmeyer observed lower rates of protein loss and lower dialysate concentrations of PGE₂ and 6-keto-PGF₁α after intraperitoneal administration of indomethacin in four CAPD patients who were initially resistant to antibiotic treatment [8]; however, the results from these four patients were not compared with those in the control group during the same phase of peritonitis. More recently, Zemel et al also observed lower rates of protein loss (but not statistically different from control values) and lower dialysate concentrations of 6-keto-PGF₁α, PGE₂, and thromboxane B₂ after intraperitoneal administration of indomethacin in 11 episodes of peritonitis in nine CAPD patients than in an untreated (by indomethacin) historical control group [27]. Comparison between the experimental and control groups might not have been valid in this study because the infections in the experimental group were more severe than in the control group, as reflected by substantially higher dialysate leukocyte counts (6267 × 10⁶/L vs. 860 × 10⁶/L, respectively).

The current study demonstrates that intraperitoneal (IP) administration of indomethacin decreased the concentrations of 6-keto-PGF₁α and PGE₂ in the dialysate and decreased peritoneal permeability to protein. These observations strongly suggest that enhanced prostanoid production is directly involved in increasing peritoneal permeability to protein during acute peritonitis. Our results contrast with those previously reported by Forrest, Jose, and Williams, who evaluated the effect of prostanoid generation on peritoneal permeability to protein during zymosan-induced peritonitis in the rabbit [10]. These investigators observed a nonsignificant increase in dialysate...
sate concentrations of PGE$_2$ during peritonitis and reported that intravenous (IV) administration of indomethacin (1.8 mg/kg) only reduced albumin loss by 29%. The contrasting results between the current study and those of Forrest, Jose, and Williams may be due to differences in the nature of the inflammatory stimulus (E. coli vs. zymosan) or in the dose and route of administration (IP vs. IV) of indomethacin. The latter may be particularly relevant in this case since Rampart and Williams have shown that prostaglandins may have either anti-inflammatory or proinflammatory actions, depending on whether they are administered intravenously or subcutaneously, respectively [28].

The physiological mechanisms by which enhanced prostanooid generation increases peritoneal permeability to protein cannot be determined from this study. It is possible that the increase in peritoneal permeability to protein is due to the local vasodilation of blood vessels that leads to enhanced blood flow within capillaries actively involved in peritoneal transport. This increase in vasodilation may be expected to increase transperitoneal transport of small solutes by increasing the effective peritoneal surface area [29, 30]; however, recent work by Kim, Lothhouse, and Flessner has shown that enhanced blood flow does not necessarily increase peritoneal transport of small solutes in the rat [31, 32]. Our recent work has shown that inhibition of neutrophil migration into the peritoneal cavity, either by depleting circulating leukocytes or by blocking leukocyte adhesion to and migration through the peritoneal capillary wall, resulted in protein loss rates during acute peritonitis that were similar to those found in control rabbits without peritonitis [33]. Those studies showed that inhibition of neutrophil migration into the peritoneal cavity was sufficient to block the increase in protein loss during peritonitis. In contrast, the results of the current study show that inhibition of neutrophil migration into the peritoneal cavity is not necessary to block the increase in protein loss during peritonitis. The results of both of these studies are consistent with the known importance of prostanooids in potentiating the effect of neutrophils in increasing vascular permeability in other models of inflammation [34].

In summary, inhibition of COX activity and generation of 6-keto-PGF$_{1α}$ and PGE$_2$ by indomethacin decreases protein loss during acute peritonitis. Furthermore, COX inhibition with indomethacin had no effect on transperitoneal migration of leukocytes or the production of nitric oxide or IL-8. The present findings show that enhanced production of prostanooids likely plays an important role in governing the increase in peritoneal permeability to protein during acute bacterial peritonitis in the rabbit.

ACKNOWLEDGMENTS

This material is based on work supported by the Office of Research & Development (R&D), Medical Research Service, Department of Veterans Affairs (USA) and the Dialysis Research Foundation (Ogden, UT, USA). This work was presented in part at the 31st and 32nd Annual Meetings of the American Society of Nephrology that were held October 25–28, 1998, in Philadelphia, PA, USA, and November 4–7, 1999, in Miami Beach, FL, USA, and has been published in abstract form (abstracts: Peng et al, J Am Soc Nephrol 9:257A, 1998, and Peng et al, J Am Soc Nephrol 10:227A–228A, 1999). The technical assistance of Ms. Janice F. Gilson is gratefully acknowledged.

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