View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Kidney International, Vol. 65 (2004), pp. 654–660

DIALYSIS – TRANSPLANTATION

Icodextrin-induced peritonitis: Study of five cases and comparison with bacterial peritonitis

FATOUMA TOURÉ, SYLVIE LAVAUD, MEHDI MOHAJER, FRANÇOIS LAVAUD, ERIC CANIVET, PHILIPPE NGUYEN, JACQUES CHANARD, and PHILIPPE RIEU

Departments of Nephrology, Allergology, and Hematology, University Hospital of Reims; and Centre National de la Recherche Scientifique (CNRS FRE 2534), Reims, France

Icodextrin-induced peritonitis: Study of five cases and comparison with bacterial peritonitis.

Background. An epidemic of aseptic peritonitis related to the presence of peptidoglycan contaminant in some batches of icodextrin solution (Extraneal®, Baxter Healthcare Corporation) occurred in Europe in the first six months of 2002.

Methods. By case-control study we examined the clinical and biologic features of 5 patients with icodextrin-induced peritonitis (group AP) and compared them with 7 patients with bacterial peritonitis (group BP) recruited in our clinical center between January and June 2002.

Results. Diagnosis of icodextrin-induced peritonitis was confirmed in all cases by a positive reintroduction test with contaminated batches of icodextrin. No recurrence was observed on re-exposure to icodextrin free of peptidoglycan. Skin tests were positive with contaminated icodextrin in 2 of 5 patients, while they were negative with icodextrin solution free of peptidoglycan (<0.6 ng/mL). During peritonitis, serum level of C-reactive protein (CRP) was lower in group AP (42.4 ± 34 mg/L) than in group BP (135 ± 59 mg/L) ($P = 0.01$). Leukocyte number in peritoneal dialysis effluent was lower in group AP (284 ± 101/mm^3), with a lower neutrophil/monocyte ratio (N/M = 0.67) than in group BP (1410 ± 973/mm^3; N/M = 4) ($P < 0.05$). A low number of peritoneal fluid eosinophilia (11 ± 8%) was detected in group AP.

Conclusion. Icodextrin-induced peritonitis was associated with a burst of intraperitoneal cytokines. The phenotype of peritoneal neutrophils was different between aseptic and bacterial peritonitis, indicating that inflammatory stimuli that activate neutrophils in both types of peritonitis are clearly distinct. Finally, peritoneal injury measured by weight gain, peritoneal permeability, and CA125 concentration seemed to be less severe during icodextrin-induced peritonitis than during bacterial peritonitis.

Key words: aseptic peritonitis, peritoneal dialysis, peptidoglycan, neutrophils, cytokines, icodextrin.

Received for publication January 22, 2003
and in revised form July 11, 2003, and August 18, 2003
Accepted for publication August 25, 2003

© 2004 by the International Society of Nephrology

Peritoneal dialysis (PD) is now an established alternative in the treatment of end-stage renal disease (ESRD). Because peritoneal dialysis is simple to perform and is less expensive than hemodialysis, it has gained worldwide popularity. Ninety percent of patients who require dialysis in Mexico, 50% of those in the United Kingdom, 40% of those in Canada, and between 10% to 20% of patients in Europe and in the United States participate in a PD program. Approximately 130,000 patients worldwide are maintained on chronic peritoneal dialysis [1].

Current peritoneal dialysis solutions use glucose as the osmotic agent. However, glucose-containing solutions have some disadvantages. First, the rapid absorption of glucose leads to progressive dissipation of the osmotic gradient, so that effective ultrafiltration lasts for only 2 to 3 hours. Second, daily absorption of glucose from dialysate may lead to long-term metabolic complications such as hyperinsulinemia, hyperlipidemia, and obesity. And third, advanced glycation end-products that develop under the influence of glucose have been incriminated in the long-term deterioration of the peritoneal membrane. Because of the need of alternative osmotic agents to overcome the disadvantages associated with glucose-containing solution, starch-derived glucose polymer has been developed [2].

Icodextrin is a soluble glucose polymer derived from maltodextrin that in turn was derived by partial hydrolysis of starch. It is formulated as a 7.5% aqueous solution with electrolytes (Extraneal®; Baxter Healthcare, Maurepas, France). It was first introduced into clinical practice in the United Kingdom in 1994 and is now currently used by more than 9000 patients worldwide. Icodextrin has been recently approved by the FDA. Icodextrin solution has been shown to be more effective than 1.5% and 2.5% dextrose solution in net ultrafiltration during the long-dwell dialysis period [3, 4]. Icodextrin is expected to be more biocompatible than glucose-based solution because it is an iso-osmotic solution with reduced glucose degradation product content [5].

Allergic skin reactions to icodextrin have been reported since the beginning of its commercialization. Prevalence of the skin reactions—exfoliative or blistering—varies from 2.5% to 15% [6, 7]. In about one third of cases, rashes are considered serious enough to stop icodextrin therapy. More recently, a syndrome of sterile peritonitis has also been described in association with icodextrin use [8–16]. In most of the cases, patients present with cloudy dialysates and without clinical symptoms. Few patients have abdominal discomfort and fever. One case experienced severe abdominal pain, diarrhea, vomiting, and marked ultrafiltration [10]. Prevalence of icodextrin-related sterile peritonitis is not clearly established. The first cases were reported in 1999, and an epidemic of aseptic peritonitis occurred in Europe in the first six months of 2002 [10, 14, 16, 17]. According to Baxter's pharmaco-vigilance, sterile peritonitis to icodextrin was reported in less than 1% of patients before January 2001, while prevalence reached more than 10% during the first six months of 2002. Investigation from Baxter revealed that increased episodes of sterile peritonitis in 2002 were associated with a high amount of peptidoglycan contaminant in certain batches of Extraneal® [14]. Recalling contaminated batches by Baxter Healthcare allowed the prevalence of sterile peritonitis back to decrease to 1%.

It is critical to recognize icodextrin-related sterile peritonitis in order to avoid unnecessary antibiotic prescription, or even catheter removal, as previously reported [8]. It is also important to gain insight into the mechanisms involved in peritoneal inflammation to identify factors contributing to bioincompatibility. We therefore examined the clinical and biologic features of 5 patients with icodextrin-induced peritonitis and compared them with 7 patients with bacterial peritonitis recruited in our clinical center during the same period.

METHODS

Patients

The annual peritonitis rate at the center was 37.2 in 2000 (months × patients/peritonitis) and 35.12 in 2001 (data from the Registre de Dialyse Péritonéale de la Langue Française). Between January 2000 and June 2001, 49 patients were undergoing peritoneal dialysis; 5 were admitted to the nephrology unit for aseptic peritonitis. Peritonitis was diagnosed when cloudy effluent was present due to an increase leukocyte count above 100/mm^3. Concentrated dialysates were then cultured on aerobe-anaerobe blood agar and chocolate agar for 5 days. Repeated cultures of the effluent and blood were negative in these 5 patients. Icodextrin was incriminated because recovery of peritonitis was obtained 48 hours after discontinuation of icodextrin and recurrence occurred on re-exposure. We therefore considered that these 5 patients had aseptic peritonitis related to icodextrin. Seven patients hospitalized in the same period for bacterial peritonitis were studied (4 *Staphylococcus epidermis*, 2 *Echerichia coli*, and 1 *Streptococcus B*).

Skin tests

Two lots of icodextrin solution were used: sample 1 numbered 01J19G32 (icodextrin contamined with 10 ng/mL peptidoglycan), and sample 2 numbered 02D22G33 (icodextrin with less than 0.6 ng/mL of peptidoglycan).

Skin tests were performed according to the usual procedures for drug allergy [18]. Hypersensitivity reactions were first tested by skin prick-test (SPT) using icodextrin solution dilution (1/10 in NaCl 4% containing phenol). SPT were compared to a positive control (histamine 10 mg/mL) and to a negative control (NaCl 4% containing phenol). They were analyzed after 20 minutes. Intradermal (IDR) administration was performed with 0.05 mL of successive dilutions of icodextrin native solution (1/1000, 1/100, and 1/10 in NaCl 4% containing phenol). Tests were read by the same observer (FL). The positivity was assessed by the presence of erythema and wheal at 20 minutes and then 6 hours, 12 hours, 24 hours, and reviewed at 48 hours. Results were given in median size of papula/erythema in millimeters. All patients were tested with sample 1. In patients with positive skin test, a new testing with sample 1 (right arm) and sample 2 (left arm) were performed. Five control subjects (non-atopic, without renal disease) from the medical staff gave their informative consent for testing sample 1 and 2 by SPT and IDR to eliminate false-positive reaction. All 5 were negative.

Peritoneal injury

Peritoneal permeability was studied during peritonitis (day 2) and 2 months after recovery using a peritoneal equilibration test (PET) as previously described [19], with slight modification. Briefly, a standardized 4-hour dwell was performed with a glucose-free but osmotic solution—icodextrin. Dialysis fluid (30 mL) was sampled at 0, 10, 20, 30, 60, 120, 180, 240 minutes, and blood samples were drawn twice at 0 and 240 minutes. Transport parameters for low-molecular-weight solutes were calculated as mass transfer area coefficient (MTAC) using the model of Gared [20]. For β_2 microglobulin, albumin, and immunoglobulin G, solute transport was calculated as the peritoneal clearance, that is, the amount of protein drained, divided by the product of the mean of the concentration of each protein in serum and the dwell time. The ratio:transport solute during peritonitis/transport solute after recovery reflects the intensity of peritoneal lesions [19, 21].

At the end of the PET (at day 2 and at 2 months after recovery), 5 mL of peritoneal effluent were collected for chemiluminescent determination of CA125

concentration (Cryptor; Brahms, S'Ouen l'Aumône, France). The ratio:CA125 during peritonitis (at day 2)/CA125 after recovery (at 2 month) reflects the intensity of peritoneal lesions [22].

Neutrophil activation

CD11b, CD16, CD35, CD43, CD62, and CD63 expression were determined at day 2 on whole blood and on neutrophils from peritoneal cavity. Briefly, 100 µL of EDTA-anticoagulated blood from patients (5×10^5 neutrophils) was used for each determination. Blood was labeled with the primary antibody (obtained from Immunotech; Beckman Coulter, Fullerton, CA, USA) under saturating conditions for 30 minutes at 4°C. Nonspecific binding of antibody was determined on cells incubated with an irrelevant class-matched control antibody. Cells were washed twice with phosphate-buffered saline (PBS) supplemented with bovine serum albumin (BSA) (0.1%) and sodium azide (0.1%), and then labeled during 30 minutes at 4°C with the secondary antibody (FITC-$F(ab')_2$ fragments of goat anti-mouse IgG (Valbiotech, Paris, France). Erythrocytes were lysed in 1% NaCl for 15 minutes at 4°, and cells were fixed with 1% formaldehyde. Neutrophils from the peritoneal cavity were obtained by centrifugation of 50 mL of the night effluent. Neutrophils (5×10^5) were labeled as for the blood samples except erythrocytes lysis. Samples were analyzed by flow cytometry. Analysis was focused on granulocytes, identified by their forward and right angle scatter features. The cut-off for positive fluorescence was the 99th percentile of the distribution of the cells labeled with the irrelevant antibody. The mean fluorescence intensity was quantified on the positive population and collected by using logarithmic amplification. Results for each receptor were expressed as the ratio:peritoneal cavity mean fluorescence/peripheral blood mean fluorescence.

Intracellular production of H_2O_2 by neutrophils was determinated using the fluorescent dye 2′,7′-dichlorofluorescin diacetate (DCFHDA), purchased from Acros (Geel, Belgium). Neutrophils from the peripheral blood and from the peritoneal cavity effluent were isolated by a one-step density gradient centrifugation on Polymorphprep (Nycomed, Oslo, Norway), according to the manufacturer's instructions. Cells were washed and resuspended in the culture medium. Neutrophils (10^6) in 1 mL PBS, 0.1% BSA, Ca^{2+} 1 mmol/L, were incubated with DCFHDA (5 µmol/L final) for 15 minutes at 37°C. Neutrophils were immediately analyzed by flow cytometry, and fluorescent emission at 510 nm was measured. Results were expressed as the ratio:peritoneal cavity mean fluorescence/peripheral blood mean fluorescence.

Measurement of cytokine concentrations in the peritoneal dialysis effluent (PDE). Sandwich enzyme-linked immunosorbent assays were used to measure interleukin (IL)-1, interleukin 1 receptor antagonist (IL1ra), IL-4, IL-5, IL-6, IL-8, tumor necrosis factor-α (TNF-α), and monocyte chemoattractant protein-1 (MCP-1) in the PDE (R&D Systems, Minneapolis, MN, USA). Measurements were made on the night effluent for 3 consecutive days, and for each patient the value of the cytokine was the mean of these 3 determinations. Cell-free PDE was collected and stored at −70°C until analysis. All samples were assayed at the same time to avoid interbatch variation. The lowest limits of sensitivity for the assays were 1 pg/mL (IL-1), 22 pg/mL (IL1 ra), 10 pg/mL (IL-4), 3 pg/mL (IL-5), 0.7 pg/mL (IL-6), 10 pg/mL (IL-8), 4.4 pg/mL (TNF-α), and 5 pg/mL (MCP-1). Intra-assay coefficient of variation was less than 8% for all assays.

Statistics

The results are given as mean values ± 1 standard deviation unless mentioned otherwise. The data were analyzed with multivariate analysis of variance for repeated measures. An independent samples *t* test and Mann-Whitney test were also used. The statistical analysis was made on a personal computer using Analyse it Software (Analyse it Software, Ltd., Leeds, UK). A P value below 0.05 (two-tailed) was considered to indicate a significant difference.

RESULTS

Aseptic peritonitis

Five episodes of aseptic peritonitis occurred in our hospital between January and June 2002 among 49 patients maintained on chronic PD. Icodextrin solution was used in all 5 case-patients and in 30 patients on PD. In 5 case-patients, cloudy effluent was the predominant finding. Two patients had fever and abdominal pain. No skin rash was observed. Repeated cultures of the effluent and blood were negative. Icodextrin was incriminated in all five patients because recovery of peritonitis was obtained 48 hours after discontinuation of icodextrin and recurrence occurred on re-exposure. To determine whether an immunoallergic mechanism was involved in this reaction, we performed skin test with icodextrin solution (sample 1, lot number 01J19G32). SPT remained negative in all cases, while intradermal tests were positive in two of five patients. In these two patients, wheal/flare ratio was doubtful for dilution 1/1000 (papulas 5 and 7 mm) and significant for dilution 1/100 with reactions of 12/18 mm and 15/20 mm, respectively. Papulas only appeared at 6 hours, with a maximal intensity at 24 hours, and remained unchanged until 48 hours.

In June 2002, we learned from Baxter Healthcare Corporation that icodextrin solution used in our center contained abnormal amounts of peptidoglycan. This peptidoglycan was issued from *Bacillus acidocaldarius*, a bacteria found to contaminate the starch used in the production of icodextrin. The concentration of peptidoglycan in icodextrin solution varied from 3 to 10 ng

Table 1. Clinical characteristics of the patients with bacterial (group BP) and aseptic (group AP) peritonitis

Parameter	Group BP	Group AP	
Patients *number*	7	5	
Age *years*	76 ± 15.3	67 ± 17.6	NS
Gender *F/M*	5/2	2/3	
Treatment time *months ± SD*	20 ± 11	9 ± 4.2	NS
Modality	CAPD: 6/APD: 1	CAPD: 5	
Icodextrin exposure *months*	11 ± 5	8.4 ± 4.3	NS
Residual diuresis *mL ± SD*	1230 ± 586	590 ± 174	$P = 0.03$
Weight before peritonitis *kg*	71.82 ± 16.7	76.56 ± 11.0	NS

Abbreviations are: APD, automated peritoneal dialysis; CAPD, continuous ambulatory peritoneal dialysis; NS, no significance.

Table 2. Biological parameters at admission of the patients during bacterial (group BP) and aseptic (group AP) peritonitis

Parameter	Group BP	Group AP
Number of cells in PDE/mm^3	1228.3 ± 978	284 ± 101[a]
Neutrophils/monocytes (PDE ratio)	4.58 ± 2.95	0.67 ± 0.55[b]
Eosinophils in PDE %	Undetectable	11 ± 8
Systemic leukocytosis/mm^3	10320 ± 3027	9700 ± 4130
CRP *mg/L*	135.1 ± 53.1	42.4 ± 34[c]

Abbreviations are: PDE, peritoneal dialysis effluent; CRP, C-reactive protein.
[a] $P = 0.03$
[b] $P = 0.02$
[c] $P = 0.003$

per milliliter (silkworm larvae plasma test [23]). Baxter Healthcare recalled contaminated batches of Extraneal®, and we next obtained lots of icodextrin solution free of peptidoglycan (<0.6 ng/mL). To test whether the new icodextrin solution free of peptidoglycan might be reintroduced in our "allergic" patients, we first performed skin tests with different batches of Extraneal®. In the two previously positive patients, intradermal tests were negative with icodextrin solution free of peptidoglycan (<0.6 ng/mL, sample 2), while they were positive with contaminated icodextrin (10 ng per milliliter of peptidoglycan, sample 1). We therefore reintroduced icodextrin solution free of peptidoglycan for peritoneal dialysis in all five patients. Intraperitoneal administrations of these new solutions were not complicated by aseptic peritonitis.

Clinical and biologic features

To examine the clinical and biologic features of the aseptic peritonitis, the 5 patients with icodextrin-induced peritonitis (group AP) were matched with 7 patients with bacterial peritonitis (group BP) recruited in our clinical center during the same period.

Age, time on peritoneal dialysis, and duration of icodextrin exposition were the same in both groups (Table 1). Systemic and peritoneal inflammation was less severe in aseptic peritonitis than in bacterial peritonitis (Table 2). Serum level of CRP and leukocyte number in peritoneal dialysis effluent was significantly lower in group AP than in group BP ($P < 0.05$). Leukocyte formula was also different, with a lower neutrophil/monocyte ratio in group AP (N/M = 0.67) than in group BP (N/M = 4) ($P < 0.05$). Furthermore, all patients of group AP had a low number of peritoneal fluid eosinophilia (11 ± 8%), while no patient of group BP had eosinophils detected in peritoneal dialysis effluent (Table 2). No peripheral eosinophilia was detected in any patient.

Peritoneal injury

Peritonitis results in an acute injury to the peritoneal membrane that is characterized by an increase of solute clearances and a release of CA125 from damaged mesothelial cells [19, 22]. Peritoneal transport kinetic and CA125 peritoneal dialysate concentration were studied during peritonitis and after recovery to evaluate peritoneal injury (Table 3). These tests have been performed in 5 patients with bacterial peritonitis and in only 2 patients with aseptic peritonitis. On bacterial peritonitis, MTACs were higher during peritonitis than after recovery, as indicated by the peritonitis:recovery (P:R) ratio. As published [19], the peritonitis-induced augmentation of the MTAC values was most marked for the high-molecular-weight proteins (albumin and immunoglobulin G) (Table 3). In both patients with aseptic peritonitis, albumin and IgG P:R ratios were lower than those of patients with bacterial peritonitis. In addition, we also found that the increment in CA125 concentration in both patients with aseptic peritonitis was lower than mean elevation of CA125 measured in patients with bacterial peritonitis. Because these measurements were performed in only two cases with aseptic peritonitis, statistical analysis could not be made. However, weight gain during peritonitis, which reflects alterations in the peritoneal transport with decreased removal of water from the body, was significantly lower in group AP than in group BP. These results suggest that peritoneal injury was less severe in aseptic peritonitis than in bacterial peritonitis.

Neutrophil activation

To gain insight into the mechanism of activation of cells found in the peritoneal cavity during peritonitis, we examined surface expression of several receptors on neutrophils. Neutrophil migration toward inflammatory tissue sites is associated with phenotypic change. Some membrane molecules disappear from the cell surface (CD16, CD43, and CD62), while others receptors increase (CD11b, CD35, CD63) [24]. These surface transformations vary with inflammatory mediators that activate neutrophils. On day 1 of peritonitis, surface expression of CD11b, CD16, CD35, CD43, CD62, CD63 of neutrophils in peripheral blood and peritoneal dialysis effluent were quantified by immunostaining and fluorescent-activated cell sorting (FACS) analysis.

Table 3. Peritoneal injury during bacterial (group BP) and aseptic (group AP) peritonitis

	BP Group (*N* = 5, mean values)			AP Group (*N* = 2, values for the 2 patients)		
	P	R	P/R	P	R	P/R
MTAC urea	28.7 ± 12.7	20.6 ± 6.5	1.4 ± 0.2	61.4 20.31	25.06 25.65	2.5 0.8
MTAC creat	15.6 ± 6.6	13.5 ± 5.4	1.3 ± 0.5	22.26 13.01	13.43 16.33	1.7 0.8
Cl $_{\beta 2m}$	2131 ± 423.6	1489.6 ± 725.3	1.6 ± 0.6	2533 2297	1713 1845.1	1.5 1.2
Cl $_{Alb}$	171 ± 53.4	85.5 ± 19.7	2.0 ± 0.4	282.2 152.8	229.4 194.8	1.2 0.8
Cl $_{IgG}$	91.5 ± 28.7	51.2 ± 14.0	1.9 ± 0.5	191.41 96.91	142.8 143.6	1.3 0.7
CA 125 *U/mL*	80.2 ± 61	19 ± 14.5	4.3 ± 2.1	67 37	27 23	2.5 1.6
Weight gain at admission *kg*		3.38 ± 0.35 (*N* = 7)			1.8 ± 1.25[a] (*N* = 5)	

Abbreviations are: P, peritonitis; R, recovery; PR, peritonitis/recovery ratio; MTAC, mass transfer area coefficient (mL/min); $Cl_{\beta 2m}$, clearance of beta-2 microglobulin (μL/min); Cl_{Alb}, clearance of albumin (μL/min); Cl_{IgG}, clearance of immunoglobulin G (μL/min).
[a] $P = 0.02$.

Respiratory burst of neutrophils was determined by fluorometry. We found that activated, peritoneal-infiltrating neutrophils were distinct from resting, circulating neutrophils because they had an up-regulation of CD11b, CD35, and CD63, a down-regulation of CD16, CD43, and CD62, and an increased intracellular generation of hydrogen peroxide (Fig. 1). No difference was observed for the markers of neutrophil degranulation (CD11b, CD35, CD63) between group AP and group BP (Fig. 1A). In contrast, we found that neutrophil down-regulation of CD16, CD43, and CD62 were significantly more pronounced in group AP than in group BP (Fig. 1B). Generation of respiratory burst products by peritoneal-infiltrating neutrophils was lower in aseptic peritonitis than in bacterial peritonitis but the difference did not reach statistical significance (Fig. 1C). Our results showed that inflammatory stimuli that activate neutrophils in both type of peritonitis are clearly distinct.

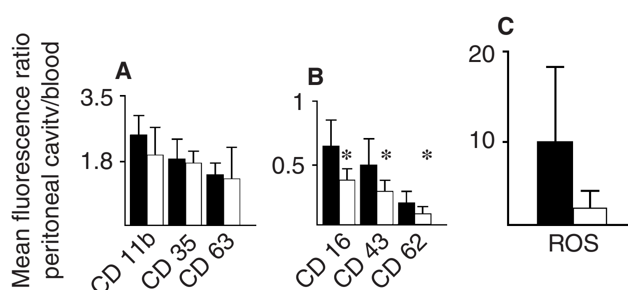

Fig. 1. Neutrophil phenotypic change associated with neutrophil transmigration to peritoneal cavity during bacterial peritonitis (closed bar) and aseptic peritonitis (open bar). Surface expression of CD11b, CD16, CD35, CD43, CD62, and CD63 on neutrophils from peripheral blood and peritoneal dialysis effluent were quantified by immunostaining and FACS analysis. Reactive oxygen species (ROS) production by blood and peritoneal neutrophils were detected by flow cytometry analysis using 2′,7′-dichlorofluorescin diacetate (DCFHDA). Results are expressed as the ratio (peritoneal cavity/blood) of mean fluorescence intensity. Data are given as mean (bar) + SD (error bar) of at least three experiments. (*A*) Up-regulation of CD11b, CD35, and CD63 on peritoneal neutrophils. (*B*) Down-regulation of CD16, CD43, and CD62 on peritoneal neutrophils, ∗, $P < 0.05$ for comparing aseptic peritonitis versus bacterial peritonitis. (*C*) Up-regulation of ROS production by peritoneal neutrophils. ROS production was lower in aseptic peritonitis than in bacterial peritonitis, but not statistically significant ($P = 0.06$).

Cytokine profiles

To further investigate the mechanism involved in aseptic peritonitis, we measured cytokine profiles in peritoneal dialysate effluent. We detected high amount of IL-1, IL-1ra, IL-6, IL-8, MCP-1, and TNF-α in dialysate effluent of patient with aseptic peritonitis (Table 4). Concentrations of these cytokines were higher than those of bacterial peritonitis. The difference was statistically significant for IL-1ra, MCP-1, and TNF-α (Table 4). IL4 and IL5 were not detected in dialysate effluent of patient with aseptic and bacterial peritonitis.

DISCUSSION

Peritonitis is a major complication of peritoneal dialysis. Peritonitis usually is caused by bacterial infection, but sterile peritonitis does occur. Culture-negative peritonitis may result from: (*1*) concomitant use of antibiotics or infection caused by non-culturable agent; (*2*) presence of chemical irritants such as methylene blue, acetaldehyde, or chlorhexidine [25–27]; (*3*) hypersensitivity reaction to intraperitoneally administered heparin, antibiotics, plasticizers, or other constituents of the peritoneal dialysis system [28, 29]; or (*4*) contamination of dialysate solution by bacterial product(s) such as endotoxin [30, 31]. Other bacterial products could lead to peritoneal inflammation. In this paper, we reported an outbreak of sterile peritonitis caused by peptidoglycan. Evidence such as presence of high amount of peptidoglycan in batches of icodextrin solution associated with peritonitis, immediate disappearance of the symptoms after discontinuation of

Table 4. Cytokine profile in peritoneal cavity during bacterial (group BP) and aseptic (group AP) peritonitis

	Group BP Mean ± SD, ($N = 5$)	Group AP Mean ± SD, ($N = 4$)	Statistical analysis (Mann-Whitney test)
IL-1 *pg./mL*	6.9 ± 9.1	42 ± 4.63	NS
IL-1ra *pg./mL*	465.4 ± 142.8	3925 ± 3486.2	$P = 0.01$
IL-4 *pg./mL*	<10	<10	–
IL-5 *pg./mL*	2.1 ± 0.8	4.4 ± 3.2	NS
IL-6 *pg./mL*	2036 ± 1606.1	14803 ± 16646	NS
IL-8 *pg./mL*	462 ± 592	2637.5 ± 2360.3	NS
MCP-1 *pg./mL*	748.4 ± 293.8	7843.7 ± 7308	$P = 0.03$
TNF *pg./mL*	3.1 ± 1.7	42.7 ± 36	$P = 0.01$

Abbreviations are: IL, interleukin; IL-1ra, interleukin 1 receptor agonist; MCP-1, monocyte chemoattractant Protein-1; TNF, tumor necrosis factor; NS, not significant.

contaminated icodextrin, and absence of recurrence on re-exposure to icodextrin-free of peptidoglycan, strongly suggest the role of peptidoglycan as the etiologic factor in this aseptic peritonitis. Interestingly, a recent paper reported recurrence of sterile peritonitis on rechallenging with new batches of icodextrin [32]. One explanation may be that the batches of icodextrin used for rechallenge still contained very low amount of peptidoglycan. Although since June 2002, Extraneal® batches are guaranteed to contain less than 7.4 ng/mL of peptidoglycan, they are not guaranteed to be free of peptidoglycan. It is therefore possible that a very low concentration of peptidoglycan that is not sufficient to induce immunization may induce an immunologic response in sensitized patients.

Peptidoglycan is a component accounting for about 70% and 20% of the Gram-positive and Gram-negative bacterial cell wall, respectively. The main structural features of peptidoglycan are linear glycan chains interlinked by short peptides. These molecules, produced exclusively by bacteria, belong to the pathogen-associated molecular patterns (PAMPs) family [33]. Other examples of PAMPs are lipopolysaccharide (LPS), bacterial lipoprotein, and lipoteichoic acids. PAMPs are recognized by germline-encoded receptors on the surface of cells of the innate immune system. Toll-like receptor (TLR) proteins, homologs of the *Drosophila* protein Toll, have been found on the surface of mammalian cells and are important in the responses of phagocytes to PAMPs. TLR4 is critical for the response to lipopolysaccharide (LPS) of Gram-negative bacteria, while TLR2 is important for response to peptidoglycan of Gram-positive bacteria [33]. It is therefore possible that peptidoglycan induced peritoneal inflammation by binding to TLR2 express on cells of the innate immune system. Delayed type hypersensitivity to peptidoglycan is another mechanism that may also explain peptidoglycan-induced peritonitis. Immunization to peptidoglycan has been described [34, 35]. High amounts of peritoneal cytokines and predominance of peritoneal monocytes are compatible with both hypotheses. But positive skin tests obtained with very low amount of peptidoglycan (1/1000 dilution) and presence of peritoneal eosinophils during peritonitis argue for an allergic reaction. In addition, the fact that only 5 patients among 30 receiving the same batches of icodextrin experienced peritonitis is consistent with an immunoallergic process. Determination of the mechanism leading to peptidoglycan-induced peritonitis will require further investigations.

Even if it has been recently questioned [36], peritoneal inflammation observed during infection seems to be a major factor in reducing the life span of the membrane [37]. In the present study, peritoneal injury was evaluated by increment in permeability, CA125, and weight gain. In group AP, PET were only performed for 2 patients, so statistical analysis was not performed. Interestingly, augmentation of the MTAC of high-molecular-weight proteins and in CA125 levels was lower in these two patients compared with mean values obtained in BP group. This could indicate a less severe peritoneal injury in AP than in BP group, or at the opposite long-term impairment of peritoneal membrane in AP group. We found no clinical evidence in our patients that the peritonitis episode had caused irreversible damage to the peritoneum. In addition, weight gain was lower in AP group, despite the poor residual renal function. Taken together these results suggest a less severe acute peritoneal injury during aseptic peritonitis.

Icodextrin-induced peritonitis was associated with a burst of intraperitoneal cytokines. The high level of MCP-1 may account for the high number of monocytes. Despite high amounts of IL-8, a major neutrophil chemoattractant, the number of peritoneal neutrophils was lower in aseptic peritonitis than in bacterial peritonitis. These data suggest that other chemoattractants such as bacterial products (formylated peptides and LPS) may also play a role in neutrophil recruitment during bacterial peritonitis. Moreover, we found that the phenotype of transmigrated neutrophils was different between aseptic and bacterial peritonitis. This result indicates that activation signals leading to neutrophil activation and peritoneal inflammation are clearly distinct between both types of peritonitis. Tissue damage after acute bacterial infection partly result from excessive neutrophil infiltration and activation in the infected tissue [24]. Data from the literature suggest that peritoneal injuries during peritonitis are mediated by neutrophils [38]. The low number of neutrophils and the poor reactive oxygen species production by peritoneal infiltrating neutrophils during aseptic peritonitis may account for the weak peritoneal damage observed during icodextrin-induced peritonitis. It is interesting to note that despite a burst of intraperitoneal cytokines, aseptic peritonitis are associated with a small inflow of activated neutrophils and a weak peritoneal damage.

CONCLUSION

Our study shows that icodextrin-induced peritonitis was associated with: (*1*) a contaminating peptidoglycan in some batches of icodextrin; (*2*) a burst of intraperitoneal cytokines; (*3*) a small inflow of activated neutrophils; and (*4*) weak peritoneal damage. Further investigations are needed to determine whether peritoneal inflammation result from direct activation of cells of the innate immune system by peptidoglycan, or from delayed type hypersensitivity to peptidoglycan.

ACKNOWLEDGMENTS

F. Touré is supported by a doctoral fellowship from "Société de Néphrologie." This study was partly supported by a research grant from Baxter. We wish to thank A. Delou for excellent technical assistance. This work has been presented in part at a meeting of the American Society of Nephrology, Philadelphia, Pennsylvania, November 4, 2002.

Reprint requests to Philippe Rieu, M.D., Ph.D., Department of Nephrology, Hôpital Maison Blanche, 45 rue Cognacq-Jay -51092 Reims, France.
E-mail: prieu@chu-reims.fr

REFERENCES

1. Pastan S, Bailez J: Dialysis therapy. *N Engl J Med* 338:1428–1437, 1998
2. Mistry C, Gokal R, Mallick N: Ultrafiltration with an isosmotic solution during long peritoneal dialysis exchanges. *Lancet* 178–182, 1987
3. Mistry C, Gokal R, Peers E: A randomized multicenter clinical trial comparing isosmolar icodextrin with hyperosmolar glucose solutions in CAPD. MIDAS Study Group. Milticenter Investigation of Icodextrin in Ambulatory Peritoneal Dialysis. *Kidney Int* 46:496–503, 1994
4. Posthuma N, Wee PT, Verbrugh H, *et al*: Icodextrin instead of glucose during the daytime dwell in CCPD increases ultrafiltration and 24-h dialysate creatinine clearance. *Nephrol Dial Transplant* 12:550–553, 1997
5. Cooker L, Holmes C, Hoff C: Biocompatibility of icodextrin. *Kidney Int* 62:34, 2002
6. Goldsmith D, Jayawardene S, Sabharwal N, Cooney K: Allergic reactions to the polymeric glucose-based peritoneal dialysis fluid icodextrin in patients with renal failure. *Lancet* 355:897, 2000
7. Divino Filho J: Allergic reactions to icodextrin in patients with renal failure. *Lancet* 355:1364–1365, 2000
8. Williams P, Foggensteiner L: Sterile/allergic peritonitis with icodextrin in CAPD. *Perit Dial Int* 22:89–90, 2002
9. Heering P, Brause M, Plum J, Grabensee B: Peritoneal reaction to icodextrin in a female patient on CAPD. *Perit Dial Int* 21:321–322, 2001
10. Pinerolo M, Porri M, D'Amico G: Recurrent sterile peritonitis at onset of treatment with icodextrin solution. *Perit Dial Int* 19:491–492, 1999
11. Tintillier M, Pochet J, Christophe J, *et al*: Transient sterile chemical peritonitis with icodextrin: clinical presentation, prevalence, and literature review. *Perit Dial Int* 22:534–537, 2002
12. Fiedler R, Schneider I, Buchmann J, *et al*: Does peritoneal dialysis predispose a diabetic patient to icodextrin-induced peritonitis? *Nephrol Dial Int* 17:1709, 2002
13. Reichel W, Schulze B, Dietze J, Mende W: A case of sterile peritonitis associated with icodextrin solution. *Perit Dial Int* 21:414–415, 2001
14. Gokal R: Icodextrin-associated sterile peritonitis. *Perit Dial Int* 22:445–448, 2002
15. Montagnac R, Slingeneyer A, Schillinger F: Aseptic peritonitis: Role of icodextrin. *Nephrol Dial Int* 16:435–436, 2001
16. MacGinley R, Cooney K, Alexander G, *et al*: Relapsing culture-negative peritonitis in peritoneal dialysis patients exposed to icodextrin solution. *Am J Kidney Dis* 40:1030–1035, 2002.
17. Williams P: Timely initiation of dialysis. *Am J Kidney Dis* 34:595–596, 1999
18. Brockow K, Romano A, Blanca M, *et al*: General considerations for skin test procedures in the diagnosis of drug hypersensitivity. *Allergy* 57:45–54, 2002
19. Krediet R, Zuyderhoudt F, Boeschoten E, Arisz L: Alterations in the peritoneal transport of water and solutes during peritonitis in continuous ambulatory peritoneal dialysis patients. *Eur J Clin Invest* 17:43–52, 1987
20. Pannekeet M, Imholz A, Struijk D, *et al*: The standard peritoneal permeability analysis: A tool for the assessment of peritoneal permeability characteristics in CAPD patients. *Kidney Int* 48:866–875, 1995
21. Davies S, Bryan J, Phillips L, Russel G: Longitudinal changes in peritoneal kinetics: the effects of peritoneal dialysis and peritonitis. *Nephrol Dial Transplant* 11:498–506, 1996
22. Pannekeet M, Zemel D, Koomen G, *et al*: Dialysate markers of peritoneal tissue during peritonitis and in stable CAPD. *Perit Dial Int* 15:217–225, 1995
23. Kobayashi T, Tani T, Yokota T, Kodama M: Detection of peptidoglycan in human plasma using the silkworm larvae plasma test. *FEMS Immunol Med Microbiol* 28:49–53, 2000
24. Witko-Sarsat V, Rieu P, Descamp-Latscha B, *et al*: Neutrophils: Molecules, functions and pathophysiological aspects. *Lab Invest* 80:617–653, 2000
25. Junor B, Briggs J, Forwell M: Sclerosing peritonitis—The contribution of chlorhexidrine in alcohol. *Perit Dial Bull* 5:101–104, 1985
26. Tuncer M, Sarikaya M, Sezer T, *et al*: Chemical peritonitis associated with high dialysate acetaldehyde concentrations. *Nephrol Dial Transplant* 15:2037–2040, 2000
27. Macia M, Gallego E, Garcia-Cobaleda I, *et al*: Methylene blue as a cause of chemical peritonitis in a patient on peritoneal dialysis. *Clin Nephrol* 43:136–137, 1995
28. Charney D, Gouge S: Chemical peritonitis secondary to intraperitoneal vancomycin. *Am J Kidney Dis* 17:76–79, 1991
29. Fracasso A, Coli U, Landini S: Role of plasticizers. *Trans Am Soc Artif Intern Organs* 33:676–682, 1987
30. Karanicolas S, Oreopoulos D, Izatt S, *et al*: Epidemic of aseptic peritonitis caused by endotoxin during chronic peritoneal dialysis. *N Engl J Med* 296:1336–1337, 1977
31. Mangram A, Archibald L, Hupert M, *et al*: Outbreak of sterile peritonitis among continuous cycling peritoneal dialysis patients. *Kidney Int* 54:1367–1371, 1998
32. Basile C, De Padova F, Montanaro A, Giordano R: The impact of relapsing sterile icodextrin-associated peritonitis on peritoneal dialysis outcome. *J Nephrol* 16:384–386, 2003
33. Aderem A, Ulevitch R: Toll-like receptors in the induction of the innate immune response. *Nature* 406:782–787, 2000
34. Katsuki M, Kakimoto K, Kawata S, *et al*: Induction of delayed-type hypersensitivity by the T cell line specific to bacterial peptidoglycans. *J Immunol* 139:3570–3572, 1987
35. von Mayenburg J, Heymer B, Dungemann H, *et al*: Studies on bacterial hypersensitivity in man. Interrelationship between skin reactions to bacterial peptidoglycan and serum peptidoglycan antibody titers. *Allergy* 37:249–258, 1982
36. Fubholler A, Zur Nieden S, Grabensee B, Plum J: Peritoneal fluid and solute transport: Influence of treatment time, peritoneal dialysis modality, and peritonitis incidence. *J Am Soc Nephrol* 13:1055–1060, 2002
37. Davies SJ, Bryan J, Phillips L, Russell GI: Longitudinal changes in peritoneal kinetics: The effects of peritoneal dialysis and peritonitis. *Nephrol Dial Transplant* 11:498–506, 1996
38. Andreoli SP, Mallet C, William K, *et al*: Mechanisms of polymorphonuclear leukocyte mediated peritoneal mesothelial cell injury. *Kidney Int* 46:1100–1109, 1994