Specific characteristics of peritoneal leucocyte populations during sterile peritonitis associated with icodextrin CAPD fluids

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Abstract

Background. Icodextrin dialysate used for peritoneal dialysis contains an iso-molar glucose polymer solution, which provides sustained ultrafiltration over long dwell times and is considered a valuable approach to reduce intraperitoneal glucose exposure. However, several side effects have been described, including abdominal pain and allergic and hypersensitivity reactions. Also, reactions compatible with chemical peritonitis have been reported. Over the period of a few months (January 2002–May 2002), a remarkable increase in the number of continuous ambulatory peritoneal dialysis (CAPD) patients using icodextrin dialysate diagnosed with sterile peritonitis was observed in our unit.

Methods. Five of the CAPD patients using icodextrin dialysate in our unit and diagnosed with sterile peritonitis were screened for leucocyte count and leucocyte differentiation during a follow-up period of 77 ± 23 days. In addition, expression of CD14, a receptor for lipopolysaccharide (LPS), on the peripheral and peritoneal monocyte population was analysed. These results were compared to CAPD patients suffering from bacterial peritonitis.

Results. The peritoneal leucocyte count of CAPD patients using icodextrin dialysate and diagnosed with sterile peritonitis did not decrease significantly before treatment with icodextrin dialysate was interrupted, whereas it currently disappeared within 2–4 days in proven bacterial peritonitis. The sterile, cloudy icodextrin effluent contained an excess of macrophages on the day of diagnosis, whereas in bacterial peritonitis essentially an increase in the granulocyte population was observed. No elevation in the eosinophil population was observed. In contrast to bacterial peritonitis, we observed no increase in CD14 expression on the peripheral and peritoneal macrophages on the day of presentation and during the follow-up period.

Conclusions. Specific batches of the icodextrin CAPD fluids contain a macrophage chemotactic agent, which causes a sustained inflammatory state in the peritoneal cavity. Because no increase in the expression of the LPS receptor CD14 could be observed, the increased peritoneal leucocyte count is probably not caused by LPS or LPS-like (possibly peptidoglycan-like) contamination.

Keywords: icodextrin dialysate; peritoneal dialysis; sterile peritonitis

Introduction

Icodextrin is a glucose polymer and a high molecular weight osmotic agent. Icodextrin is applied as an osmotic agent for peritoneal dialysis, essentially because it provides a sustained ultrafiltration over long dwell times [1]. This sustained osmotic effect relies on the large size of these molecules, which results in prolonged retention of osmotic agent in the peritoneal cavity and ultrafiltration in a colloid osmotic manner. Icodextrin dialysate probably produces this effect via intercellular pores, which in addition leads to increased transport and clearances of smaller proteins such as β2-microglobulin [2]. Icodextrin is also considered as an attractive approach to reduce the exposure of the patient and the peritoneal membrane to glucose [3].

However, several side effects of icodextrin solutions have been described, including abdominal pain and, rarely allergic and hypersensitive reactions. Also, reactions suggesting chemical peritonitis have been reported. Several case studies described sterile peritonitis associated with the use of icodextrin solutions in CAPD patients [4–6].

Between January 2002 and May 2002, a remarkable increase in the number of CAPD patients using
icodextrin dialysate were diagnosed with sterile peritonitis in our unit. Five of those patients were included in a follow-up study evaluating the total leucocyte count, the leucocyte differentiation and the expression of CD14 on the peritoneal macrophage and peripheral monocyte populations. CD14 is a differentiation antigen and a receptor for lipopolysaccharide (LPS) and peptidoglycan (PGN) [7,8]. LPS and PGN are known to upregulate their own receptor [9,10]. CD14 plays a role in the transduction of activation signals for the production of cytokines. In this context, CD14 plays an important role in the innate immune response towards infective agents and could be an interesting marker to distinguish between bacterial or bacterial-like and sterile peritonitis.

This report describes the characteristics of sterile peritonitis caused by icodextrin solutions used in peritoneal dialysis.

Subjects and methods

Subjects

The Renal Division, University Hospital of Ghent, Belgium has currently 50 continuous ambulatory peritoneal dialysis (CAPD) patients under treatment; 26 patients of this population are treated with icodextrin dialysate (EXTRANEAL™, Baxter SA, Lessines, Belgium) during an overnight dwell. From January 2002 to May 2002 an increased prevalence (total number, n = 8) of episodes of sterile peritonitis among the patients treated with icodextrin dialysate was observed. Five of the latter patients are included in this report (two males and three females, mean age 68 ± 11 years). The patients had been exposed to icodextrin dialysate for 27 ± 16 months (range: 1–41 months). Sterile peritonitis was diagnosed if peritoneal leucocyte count (> 100/mm³) counts were elevated and bacteriological cultures remained negative.

At the time the patients presented with signs of peritonitis [day 0 (d0)] the peritoneal effluent and a blood sample were collected. A routine peritoneal leucocyte count was obtained using a Coulter counter (Analis, Ghent, Belgium) and the peritoneal leucocyte population was differentiated based on forward and side scatter and fluorescence properties using a FACSscan flow cytometer (Becton Dickinson, San Jose, CA, USA). In addition, CD14 expression on peritoneal macrophages and peripheral monocytes was analysed. The above-mentioned parameters were then further followed for 71 ± 21 days (range: 36–87 days) at variable time points, on the occasion of an ambulatory consultation. The follow-up time includes periods of interruption of icodextrin dialysate for 27 ± 16 months (range: 1–41 months). The responsible micro-organisms were identified as: two Gram-negative (three Staphylococcus aureus and one Streptococcus viridans) strains.

Figure 1A illustrates that in the case of sterile peritonitis, the peritoneal leucocyte count in the group subsequently diagnosed with sterile peritonitis exceeded 100 leucocytes/mm³, with a mean value of 1358 ± 1006 leucocytes/mm³ (range: 130–2900 leucocytes/mm³). The patients with bacterial peritonitis had a mean peritoneal leucocyte count of 1512 ± 1004 leucocytes/mm³ (range: 400–2900 leucocytes/mm³) (P = NS). The responsible micro-organisms were identified as: two Gram-negative (Bacteroides fragilis and Enterobacter cloacae) and four Gram-positive (three Staphylococcus aureus and one Streptococcus viridans) strains.

Figure 1B illustrates the leucocyte count on days 2 (d2), days 4–8 (d4-8) and later (d +) [18.8 ± 6.6 days (range: 12–28 days)] with the results obtained in six CAPD patients with bacterial peritonitis. The latter diagnosis was based on a peritoneal leucocyte count exceeding 100/mm³ combined with a positive culture result. For the levels of CD14 expression on peritoneal macrophages and peripheral monocytes an additional comparison was made with six stable CAPD patients who had no signs of peritonitis.

Leucocyte differentiation and expression of CD14 on peritoneal macrophages

Expression of CD14 was assessed by direct immunofluorescence. Peritoneal leucocytes were diluted or concentrated to approximately 1000/mm³. One hundred microlitres of sample (blood or peritoneal effluent) was incubated for 20 min with Simultest™ Leucogate™ (Becton Dickinson) at room temperature in the dark. Simultest™ Leucogate™ contains fluorescein isothiocyanate (FITC)-conjugated CD45 monoclonal antibodies (Anti-HLe-1) and phycoerythrin (PE) conjugated CD14 monoclonal antibodies (Leu™-M3). Subsequently, 2 ml of lysing solution was added. After washing procedures, the cells were submitted to flow cytometric analysis (FACScan®; Becton Dickinson). Fluorescence was standardized by microbeads (Calibrite™ particles; Becton Dickinson) with amplification and the voltage kept constant throughout the procedures. Analysis was performed on 10 000 events (detector threshold FSC-H:200, parameter FSC-H:1.00). The cell populations were differentiated according to forward and right-angled light scatter and fluorescence properties. Background binding was estimated by isotype-matched negative control antibodies (Simultest™ Control; Becton Dickinson).

Statistical analysis

Data are presented as means ± SD. A paired Wilcoxon or unpaired Mann–Whitney test was used and statistical significance was accepted with P-values < 0.05.

Results

Leucocyte count

On the day the patient presented with signs of peritonitis, the peritoneal leucocyte count in the group subsequently diagnosed with sterile peritonitis exceeded 100 leucocytes/mm³, with a mean value of 1358 ± 1006 leucocytes/mm³ (range: 130–2900 leucocytes/mm³). The patients with bacterial peritonitis had a mean peritoneal leucocyte count of 1512 ± 1004 leucocytes/mm³ (range: 400–2900 leucocytes/mm³) (P = NS). The responsible micro-organisms were identified as: two Gram-negative (Bacteroides fragilis and Enterobacter cloacae) and four Gram-positive (three Staphylococcus aureus and one Streptococcus viridans) strains.
In sterile peritonitis, after interruption of the treatment with icodextrin dialysate, and in accordance with the start of antibiotic treatment in the case of bacterial peritonitis, the leucocyte count decreased significantly from 574 ± 518 to 114 ± 123 leucocytes/mm³ within 4–8 days ($P < 0.05$). However, the mean count remained still in excess of 100 leucocytes/mm³ ($P = 0.0625$ vs antibiotic treatment in bacterial peritonitis after the same interval).

On the other hand, a significant increase in the peritoneal leucocyte count was again observed (224 ± 99 vs 54 ± 30 leucocytes/mm³; $P < 0.05$) on the day of the first check-up [within 7 days (range: 3–11 days)] after the restart of the treatment with icodextrin dialysate.

**Peritoneal cell differentiation**

**Peritoneal macrophage population.** In the case of sterile peritonitis in the patients on icodextrin dialysate, the differentiation of the peritoneal leucocyte population on the day of presentation (d0), revealed an excess of peritoneal macrophages of 18.3 ± 12.9% (range: 6.2–38.7%) compared to 4.0 ± 2.5% (range: 1.1–8.0%; $P < 0.05$) in the case of bacterial peritonitis.

Figure 2 illustrates that during the follow-up period, no further significant difference in the percentage of peritoneal macrophages between sterile and bacterial peritonitis was observed.

In the case of sterile peritonitis, the percentage of peritoneal macrophages only significantly decreased and normalized after interruption of the treatment with icodextrin dialysate, from 15.9 ± 4.3 to 8.8 ± 4.5% ($P = 0.05$) (Figure 2A). In contrast, in the case of bacterial peritonitis, a significant increase in the percentage of peritoneal macrophages after the start of antibiotic treatment from 4.0 ± 2.5 to 10.2 ± 7.3% ($P < 0.05$) was observed (Figure 2B).

After the restart of the icodextrin dialysate treatment, on the day of the first consultation, an excess of peritoneal macrophages (18.9 ± 9.8%) was again observed.
**Peritoneal granulocyte population.** On the day of presentation, the percentage of peritoneal granulocytes was elevated but significantly lower in the case of sterile peritonitis [72.7 ± 10.1% (range: 59.4–86.9%)] compared to bacterial peritonitis [88.6 ± 7.4% (range: 77.3–95.8%; \( P = 0.01 \)].

During the follow-up period, a longer and more sustained percentage elevation of peritoneal granulocytes was observed before and even after interruption of the icodextrin dialysate, which is in contrast with a steady decrease of the percentage of peritoneal granulocytes in the case of bacterial peritonitis [from 88.6 ± 7.4 to 44.4 ± 24.9% on day 2 (\( P = 0.062 \)), and to 16.2 ± 17.9% (\( P < 0.01 \)) on days 4–8] (Figure 3A and B).

**Peritoneal eosinophil population.** No significant increase in the eosinophil population was observed (data not shown).

**CD14 expression on peritoneal and peripheral macrophages.** CD14 expression on peritoneal macrophages is elevated compared to peripheral monocytes independently of the presence of peritonitis.

On the day the patients presented with signs of peritonitis (d0: sterile and bacterial), no significant increase in the expression of CD14 on peritoneal macrophages was observed compared to peritonitis-free CAPD patients.

In contrast, although in the case of bacterial peritonitis a significant increase in CD14 expression on peripheral blood monocytes was observed on d0, we could not find a significant effect of sterile peritonitis on the expression of this parameter compared to the CD14 expression on peripheral blood monocytes of peritonitis-free CAPD patients (Figure 4).

During the follow-up period, no change in expression of CD14 could be observed on peritoneal macrophages in the case of the sterile peritonitis associated with icodextrin dialysate, even after a rechallenge with icodextrin dialysate and this in contrast to an increased expression of CD14 on peritoneal macrophages during bacterial peritonitis with a maximum 2 days after the start of treatment from 1992 ± 1239 to 3670 ± 2331 MFI (\( P < 0.05 \)) (Figure 5A and B).

**Discussion**

Over a short time period, a remarkable increase in the number of CAPD patients using icodextrin dialysate was diagnosed with sterile peritonitis in our unit. Icodextrin is applied in CAPD patients with ultrafiltration problems because of its associated advantage of a sustained ultrafiltration over a long dwell time.

Five of the eight CAPD patients diagnosed with sterile peritonitis were included in a follow-up study analysing peritoneal leucocyte count and differentiation in addition to the expression of CD14 on peritoneal macrophages and peripheral monocytes. The elevated peritoneal leucocyte count only significantly decreased after withdrawal of the treatment with icodextrin dialysate, which was presumed after...
receiving the negative culture results (Figure 1A). In addition, when recovery was presumed and the treatment with icodextrin dialysate was restarted, this resulted again in an increase of the peritoneal leucocyte count.

In contrast to bacterial peritonitis, which is associated with a high polymorphonuclear leucocyte count and a low macrophage percentage, the sterile, cloudy icodextrin effluents were characterized by an excess of macrophages on the day of presentation (Figure 2). In addition, interruption of the icodextrin dialysate treatment was followed by a normalization of the percentage of peritoneal macrophages, but rechallenge again caused a rise in the percentage of peritoneal granulocytes is highly suggestive of a sterile peritonitis associated with icodextrin dialysate.

Macrophages are responsible for the release of substantial amounts of cytokines into the peritoneal fluid, leading to a profound intraperitoneal inflammatory status; this could have a negative impact on the peritoneal ultrafiltration and clearance capacity even in the absence of bacterial infection. These negative consequences especially occur if exposure to the cause of inflammation is prolonged, e.g. when sterile peritonitis is not expected. In addition, leucocyte counts remained slightly more elevated with an average in excess of 100 cells/mm³, even several days after withdrawal of icodextrin dialysate (Figure 1A).

The rapid appearance of macrophages into the peritoneum could point in the direction of the presence of a macrophage chemotactic agent in several batches of the fresh icodextrin solution. Our results indicate that, on the day of presentation of the CAPD patient with an elevated peritoneal leucocyte count (>100/mm³), a peritoneal macrophage population exceeding 10%, together with an elevated percentage of peritoneal granulocytes is highly suggestive of a sterile peritonitis associated with icodextrin dialysate.

No significant increase in the eosinophil population was observed, suggesting that an allergic reaction is highly unlikely.

Our results confirm the data described in a few isolated case studies in which a sterile peritonitis associated with the use of icodextrin dialysate was characterized by an increase in peritoneal leucocyte count and an excess of macrophages. Likewise, a normalization of the leucocyte count after withdrawal of the icodextrin dialysate and an often immediate increase after re-exposure [4–6] were observed. To our knowledge, our study is the first to analyse several patients at a time, and to compare this with patients suffering from bacterial peritonitis. This study also offers a more in-depth kinetic analysis of the evolution over time.

In addition, the differentiation antigen CD14, a receptor for LPS and PGN, was evaluated during the follow-up period (Figure 5). Several studies indicate that LPS influences the expression of CD14 [10–12], and there is at least one study where an increase in CD14 expression is reported for PGN [12]. Our finding that CD14 expression is significantly enhanced in the presence of a bacterial peritonitis caused by Gram-negative as well as by Gram-positive bacteria, also points in this direction. PGN is shed by Gram-positive bacteria; in contrast, LPS is released by Gram-negative bacteria.

In the case of bacterial peritonitis, we observed a significant increase in CD14 expression on peripheral blood monocytes on the day of presentation, compared to peripheral blood monocytes from peritonitis-free CAPD patients. In contrast, no change in CD14 expression on peripheral blood monocytes from patients diagnosed with sterile peritonitis was found (Figure 4).

CD14 expression on peritoneal macrophages from CAPD patients with bacterial and with sterile peritonitis, on the other hand, was not increased on the day of presentation compared to peritoneal...
macrophages from CAPD patients without peritonitis. During the follow-up period, however, bacterial peritonitis caused an increase in CD14 expression on peritoneal macrophages with a maximum on day 2 after the start of treatment, whereas no changes were observed in the case of sterile peritonitis associated with icodextrin dialysate (Figure 5). Hence, sterile peritonitis is not likely to be caused by LPS or a LPS-like contamination.

There has been some recent suggestion that the sterile peritonitis in CAPD patients using icodextrin solutions is caused by a PGN contamination (>150 ng/ml) (Baxter S.A./N.V., personal communication), as this group of compounds was detected in several specific batches related to sterile peritonitis. This suggestion does not fit with our findings of CD14 expression, since both LPS and PGN have been shown to upregulate CD14 expression; therefore, we recommend that causative factors other than PGNs be investigated. Alternatively, specific PGN fractions without CD14-inducing capacity might be at play.

However, an almost immediate increase in peritoneal leucocyte count and percentage of peritoneal macrophages after restarting the icodextrin dialysate treatment was observed using batches which were assured to contain a PGN concentration below 20 ng/ml. In some cases a permanent withdrawal from icodextrin dialysate treatment was necessary. This also adds to the suggestion of a responsible factor other than 'contaminated' icodextrin solutions.

In conclusion, some batches of icodextrin solution used for CAPD contain a macrophage chemotactic agent that, despite the absence of bacterial infection, causes a sustained inflammatory state in the peritoneal cavity. Because no increase in the expression of the LPS receptor CD14 could be observed, the increased peritoneal leucocyte count is probably caused by factors other than LPS, PGN or LPS/PGN-like contamination.

Conflict of interest statement. None declared.

References
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