Peritoneal defense in continuous ambulatory versus continuous cyclic peritoneal dialysis

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Peritoneal defense in continuous ambulatory peritoneal dialysis versus continuous cyclic peritoneal dialysis. Several centers have reported a lower rate of peritonitis among adult patients on continuous cyclic peritoneal dialysis (CCPD) as compared to those undergoing continuous ambulatory peritoneal dialysis (CAPD). Preliminary results of our ongoing prospective randomized study comparing CAPD-Y with CCPD also suggest a lower peritonitis incidence among CCPD-treated patients. To investigate whether the two dialysis regimens could result in differences in local host defense, we studied peritoneal macrophage (PMO) function and effluent opsonic activity in eight patients established on CAPD-Y matched with eight chronic CCPD patients. Since short and long dwell times are inherent to both dialysis modalities, and we previously found that dwell time has an impact on PMO function and effluent opsonic activity, patients were studied after both a short (4 hr) and a long (15 hr) dwell time. In both patient groups PMO phagocytic capacity increased significantly with dwell time ($39 \pm 3.3\%$ at 4 hr vs. $58 \pm 4.2\%$ at 15 hr in CAPD patients, and 40 ± 3.9 vs. $72 \pm 3.3\%$ in CCPD patients; $P < 0.01$), as did PMO peak chemiluminescence response (31 ± 4.9 vs. 77 ± 7.2 counts $\cdot$ min^{-1}/10^4 cells in CAPD, and 22 ± 3.9 vs. 109 ± 21.2 counts $\cdot$ min^{-1}/10^4 cells in CCPD; $P < 0.01$) and effluent opsonic activity (41 ± 7.6 vs. $73 \pm 5.8\%$ in CAPD and 39 ± 6.2 vs. $70 \pm 5.9\%$ in CCPD; $P < 0.01$). However, no significant difference was found in either variable between CAPD and CCPD patients when dwell times were equal. In conclusion, no differences were observed in PMO function or effluent opsonic activity between matched CAPD-Y and CCPD patients when dwell times were equal. In both patient groups prolongation of dwell time enhanced PMO function as well as effluent opsonic activity, thereby providing a better host defense. The improvement in peritoneal defenses may, in part, be responsible for the lower peritonitis incidence observed among CCPD-treated patients.

Peritoneal dialysis has become an increasingly applied treatment modality for patients with end-stage renal disease. However, bacterial peritonitis still is the major complication of continuous ambulatory peritoneal dialysis (CAPD), resulting in relatively high morbidity and drop-out rates [1]. Several centers applying continuous cyclic peritoneal dialysis (CCPD) on a large scale have reported significantly lower rates of peritonitis among adult CCPD patients as compared to those undergoing CAPD [2, 3]. Preliminary results of our ongoing prospective

randomized study comparing CAPD and Y-connector (CAPD-Y) with CCPD also suggest a lower peritonitis incidence among CCPD-treated patients [4]. The lower peritonitis incidence observed in CCPD as compared to CAPD has primarily been associated with the fewer (dis)connections needed in CCPD. However, local defense mechanisms may also play an important role in the prevention of and recovery from bacterial peritonitis; in this respect ingestion and killing of microorganisms by peritoneal macrophages are thought to be essential [5].

Because the dialysis regimens inherent to CAPD and CCPD, respectively, differ primarily in their dwell time periods preceding exchange procedures, we studied PMO function and effluent opsonic activity after both a short (4 hr) and a long (15 hr) dwell time in eight CAPD-Y patients matched with eight CCPD patients.

Methods

Patients and study design

Eight stable CAPD-Y patients [4 men and 4 women, median age 55 years (35 to 76) and on CAPD for a mean $\pm$ SD period of 26.6 ± 14 months] matched with eight CCPD patients [5 men, 3 women, median age 53.5 years (37 to 73) and on CCPD for a mean $\pm$ SD of 25.8 ± 12.9 months] were studied. The etiologies of end-stage renal failure were glomerulonephritis (2 patients), interstitial nephritis (2 patients), nephrosclerosis (2 patients), diabetic nephropathy (2 patients) for the CAPD-Y group, and glomerulonephritis (2 patients), interstitial nephritis (3 patients), nephrosclerosis (2 patients) and diabetic nephropathy (1 patient) for those on CCPD. At time of study entry, there was no evidence of peritonitis (that is, no symptoms and clear effluents with less than 100 WBC/mm^3 for at least 4 weeks prior to participation). In a randomized cross-over setting each patient performed the first exchange (2 liters of Dianeal®, containing 2.27% glucose, Baxter Ltd., Thetford, UK) of study day one after a dwell time of 4 or 15 hours. The next day, the patients who had performed the first exchange of study day one after a dwell time of 4 hours, exchanged their first bag (2 liters of Dianeal®, containing 2.27% glucose) after a dwell time of 15 hours and vice versa. The peritoneal effluents were collected and studied for total WBC and differential count, IgG and C3 concentration, as well as opsonic activity for *Staphylococcus aureus*. Functional studies of PMO isolated from the effluents

included both their phagocytic capacity and their ability to mount a respiratory burst upon stimulation. The study had been approved by the Ethical Review Committee of the Free University Hospital and informed consent was obtained from all patients.

Phagocytic cells

Peritoneal macrophages (PMO) were isolated from the effluents as previously described [6]. Final resuspensions were made in Hank's balanced salt solution containing 0.1% gelatin (GHBSS) to a concentration of 5 × 10⁶ PMO/ml. Total and differential cell counts were performed on all samples. Viability was assessed by the trypan blue exclusion test. Test-control phagocytes were peripheral blood polymorphonuclear leucocytes (PMN) isolated from 50 ml sterile, heparinized venous blood of healthy donors using dextran sedimentation and a Ficoll gradient [7], and were also used at a concentration of 5×10^6 cells/ml.

Bacteria

A clinical isolate of *S. aureus*, obtained from a CAPD patient with peritonitis was used. Bacteria were radiolabeled by growing them in 10 ml of Mueller-Hinton broth (Difco Laboratories, USA) containing 20 μCi ³H-adenine (ICN, USA) in a shaking incubator for 18 hours at 37°C [10]. Subsequently, bacteria were washed three times with phosphate-buffered saline (PBS) and resuspended in GHBSS to a concentration of 5×10^8 cfu/ml [8].

Opsonins and opsonization procedure

A pool of human serum (HPS) obtained from ten healthy (HBs-antigen and HIV antibodies negative) donors, stored at $-70°C$, was used as source of opsonins. Immediately prior to use, serum was thawed and diluted to a final concentration of 5% in GHBSS. Suspensions of bacteria (0.1 ml containing approximately 5×10^7 microorganisms) were mixed with 0.9 ml of 5% HPS and incubated in a shaking water bath for 30 minutes at 37°C. Opsonization was stopped by adding 2.5 ml ice-cold PBS. Subsequently, the suspensions were centrifuged at 3000 rpm for 15 minutes, the supernatants discarded, and the bacterial pellets resuspended in 1 ml GHBSS and kept at 4°C until use (preopsonized bacteria).

Cell-free effluents were also stored at $-70°C$ until they were likewise used as source of opsonins to test their opsonic activity. The effluents, however, were used undiluted. In addition part of the serum and effluents were heated (56°C for 30 min) to deplete them of heat-labile opsonins prior to their use.

Phagocytosis assay

The uptake of preopsonized *S. aureus* by PMO was determined using an assay that has been described in detail [7]. In brief, 100 μl of bacteria preopsonized with 5% HPS was mixed with 100 μl of PMO in a final bacteria to phagocyte ratio of 10:1. In parallel, donor PMN incubated with the same preopsonized cocci were run as control. Phagocytosis was allowed to proceed for 60 minutes in a shaking incubator at 37°C. Phagocytosis was stopped by adding 2.5 ml of ice cold PBS. Non-phagocyte-associated bacteria were removed by centrifugation (1200 rpm for 5 min), washing three times with ice cold PBS followed by three cycles of centrifugation (1200 rpm for 5 min). The phagocyte-associated radioactivity was determined by liquid scintil-lation counting [9]. Phagocytosis was expressed as percentage uptake of total radioactivity added, which was determined in a separate vial [7].

Assessment of effluent opsonic activity for S. aureus

The uptake of radiolabeled *S. aureus*, preopsonized in undiluted effluent, by PMN, was taken as a measure of effluent opsonic activity (see phagocytosis assay). Bacteria, preopsonized with 5% HPS, were used as controls in each experiment. The concentrations of IgG and C3 in effluent were measured nephelometrically using a Beckmann protein assay (Beckmann, Mijdrecht, The Netherlands).

Chemiluminescence assay

The microchemiluminescence assay described by Mills, Rholl and Quie [10] was modified and used to measure the oxidative metabolic responses of PMO obtained after different dwell times. Amplification of the chemiluminescence signal was obtained by adding luminol (0.03 mmol/ml in GHBSS; Sigma Chemical, St. Louis, Missouri, USA). Chemiluminescence mixtures, in transparent plastic scintillation vials, contained 800 μl GHBSS, 100 μl PMO or PMN (1×10^5 cells/ml), and 70 μl luminol. Phagocyte chemiluminescence was activated by adding 100 μl phorbol myristate acetate (PMA; 50 ng/ml in GHBSS; Sigma Chemicals). Backgrounds were measured in separate vials devoid of stimuli (100 μl GHBSS instead of PMA). After stimulation, counts were obtained every two minutes for 30 minutes at 37°C. Results were recorded as the peak number of counts per minute per 10^4 cells after subtracting background counts.

Data analysis

Paired two-tailed Wilcoxon signed rank tests were used for the comparison of the matched CAPD-Y versus CCPD patients at a given dwell time and for the comparison of short versus long dwell time within either dialysis modality, where the patients served as their own controls. A probability of 5% was chosen as level of significance.

Results

The effluent total white cell count from both CAPD and CCPD patients increased significantly with dwell time; at 4 hours and 15 hours the WBC was 2.6 ± 0.46 versus 6.7 ± 0.64 × 10⁶ cells/liter, respectively, in CAPD patients, and 2.2 ± 0.38 versus 6.2 ± 0.66 × 10⁶ cells/liter, respectively, in CCPD patients ($P < 0.01$). However, there was no change in cellular composition of the effluents; the overall white cell differentiation was 89% PMO (range 82 to 93%), 7% lymphocytes (4 to 9%), 3% PMN (2 to 5%) and 1% mesothelial cells (0 to 2%). Viability was always over 90%. PMO obtained after a 15-hour dwell time period revealed a significantly better uptake of *S. aureus* as compared to PMO obtained after a dwell of 4 hours, whereas no significant difference was found between PMO phagocytic capacity of CAPD and CCPD patients when dwell times were equal (Table 1).

The same held true for PMO chemiluminescence response, reflecting the ability of PMO to mount a respiratory burst, that is, to produce toxic oxygen radicals upon stimulation with PMA: PMO derived from both CAPD and CCPD patients after a dwell time of 15 hours revealed a significantly higher peak

Table 1. The effect of dwell time on PMO function in CAPD and CCPD

PMO function	Dwell time (hr)	CAPD	CCPD
% Uptake *S. aureus*	4	39 ± 3.3	40 ± 3.9
	15	58 ± 4.2[a]	72 ± 3.3[a]
Peak CL response	4	31 ± 4.9	22 ± 3.9
	15	77 ± 7.2[a]	109 ± 21.2[a]

Data are mean ± SEM. Abbreviation is: CL, chemiluminescence in counts per min per 10^4 cells.

[a] $P < 0.01$ vs. 4 hr; Control phagocytes (PMN; $N = 8$) phagocytized 86 ± 1.97% of *S. aureus*, and their chemiluminescence response was 954 ± 64.3 counts · $min^{-1}/10^4$ cells

Table 2. Effluent opsonic activity and IgG- and C3 levels after different dwell times

	Dwell time (hr)	CAPD	CCPD
Total opsonic activity (OA) %	4	41 ± 7.6	39 ± 6.2
	15	73 ± 5.8[a]	70 ± 5.9[a]
Heat-stable OA %	4	33 ± 5.9	33 ± 4.4
	15	48 ± 5.1[b]	41 ± 5.6[b]
Effluent IgG *g/liter*	4	0.05 ± 0.010	0.06 ± 0.012
	15	0.17 ± 0.070[a]	0.18 ± 0.041[a]
Effluent C3 *g/liter*	4	<0.01	<0.01
	15	0.013 ± 0.0031[a]	0.014 ± 0.0041[a]

Data are mean ± SEM.

[a] $P < 0.01$ vs. 4 hr; [b] $P > 0.1$ vs. 4 hr; Control OA (5% HPS) was 83% and 31%, respectively, for unheated and heated serum.

chemiluminescence as compared to PMO harvested from the same patients after a dwell time of 4 hours, whereas no significant difference was observed in chemiluminescence response of PMO obtained from CAPD versus CCPD patients after the same dwell time (Table 1). Since IgG is a major heat-stable opsonin and C3 is an important component of the heat-labile opsonic system, IgG and C3 concentrations were determined in all peritoneal dialysis effluents. As shown in Table 2, the mean effluent IgG level increased significantly with dwell time, and did not differ between CAPD and CCPD patients at a given dwell time. C3 was below the lower limit of detection (0.01 g/liter) in all 4 hour effluents, and could be detected in all 15 hour effluents (Table 2).

In both patient groups effluent opsonic activity was significantly higher in the 15 hour effluents than in the 4 hour effluents (Table 2). Heat-stable effluent opsonic activity also increased with dwell time, however, not significantly so, suggesting that the major increase in effluent opsonic activity with dwell time was due to heat-labile component(s). That the opsonic requirement of the *S. aureus* strain used in this study is largely dependent on heat-labile factor(s) was also reflected in the results obtained when heated versus unheated 5% HPS was used as opsonic source (31% vs. 83% uptake, Table 2).

Discussion

This comparative study in patients on long-term continuous peritoneal dialysis revealed no significant differences in effluent white cell count, differentiation, viability, peritoneal macrophage function and effluent opsonic activity between CAPD patients and those undergoing CCPD when dwell times were equal. However, in both patient groups prolongation of the dwell time was associated with improvements in peritoneal macrophage functions as well as in the opsonic activity of the effluent fluids. Thus longer dwell times are accompanied by an improvement in the phagocytic defense of the peritoneal cavity. Bacterial peritonitis still is the major complication in patients with end-stage renal failure maintained on chronic peritoneal dialysis [1]. Since the peritoneal macrophage, as the predominant cell in dialysate, constitutes the first line of cellular defense against bacterial invasion into the peritoneal cavity [5], investigations targeting at the effect of peritoneal dialysis on their functions are essential. It appears that PMO from most CAPD patients, when tested in vitro in dialysate-free media, are able to function as efficiently as normal PMN or monocytes [5, 11–13]. In the in vivo setting of peritoneal dialysis, however, every dialysate exchange compromises peritoneal host defenses by washing out and diluting cells and opsonins. In addition, with every exchange a phagocytotoxic milieu is created by the high osmolality and low pH of fresh glucose-based dialysis solutions [14]. After instillation of dialysis fluid into the peritoneal cavity, dialysate pH adjusts to levels which are noninhibitory for peripheral blood leucocytes after 30 minutes; the changes in osmolality, however, are less rapid and may remain at inhibitory levels for fluids of high glucose concentrations [14]. Thus, especially during the initial period of a dwell, the peritoneal cells operate under extreme unphysiologic conditions. If this causes impairment of essential cellular functions an at least transitory increased risk of infection may result. Indeed, several in vitro studies have demonstrated that exposure to fresh peritoneal dialysis fluid (1.) reduces the viability of lymphocytes [15], PMN [16] and PMO [17]; (2.) inhibits the phagocytic and bacterial killing capacity of PMN [14] and of PMO [16]; (3.) impairs their respiratory and oxidative metabolism [18, 19]; (4.) inhibits leucocytes to release inflammatory mediators in response to adequate stimuli [20]. Peritoneal dialysis effluents obtained after dwell times of 30 to 180 minutes have been shown to suppress both PMO and PMN phagocytic capacity as well, whereas effluents obtained after an overnight dwell did not affect phagocytosis [16], suggesting phagocytic function to be depressed for clinically relevant periods of the CAPD cycle. In a previous study in CAPD patients [21], we demonstrated a significant increase in the percentage of macrophages phagocytizing opsonized sheep red blood cells and unopsonized latex beads when dwell time increased from 1.5 to 15 hours. The present study, using a bacterial challenge, reveals that the impairment of peritoneal host defense mechanisms decreases significantly later in the dwell time, once the dialysate has reached physiologic pH and its osmolality has decreased.

The opsonic activity of effluents obtained after a dwell time of 15 hours was significantly higher than that of effluents obtained after 4 hours, which can be ascribed to the rise in effluent IgG and C3 levels with prolongation of dwell time. In addition, PMO phagocytic capacity increased significantly with dwell time as did PMO chemiluminescence response, the latter reflecting the ability of PMO to mount a respiratory burst, that is, to produce toxic oxygen radicals, which plays a key role in bacterial killing [22].

The observed increase in PMO function with dwell time could be due to the inflow of new cells into the peritoneal cavity; these

newly arriving cells are exposed to a less detrimental milieu the later in the dwell time period they appear. During CAPD it has been estimated that 3 to 4 × 10⁷ peritoneal macrophages are lost each day in dialysate [5]. The PMO turnover rate in humans undergoing chronic peritoneal dialysis is, however, not known.

Recovery of resident macrophages from the detrimental effects of fresh dialysis fluids during the intraperitoneal dwell of such fluids may also contribute to the enhanced function of PMO obtained after longer dwell times, which would suggest that the impact of dialysis fluids on phagocyte functions is reversible. The finding that PMO obtained from CAPD patients incubated in tissue culture medium appear to express normal phagocytic function [5, 13] is in favor of the latter hypothesis. Anyway, longer dwell times that allow cells to recover and/or repopulation of the peritoneal cavity, potentially improve the host's resistance to peritonitis. The dialysis prescription inherent to CAPD (three exchanges after 4 to 6 hr during the day, and one exchange after 8 to 10 hr dwell overnight) differs from that of CCPD (one long diurnal dwell time followed by 3 to 4 exchanges after 1.5 hr dwell time during the night). When external occlusion is used in CCPD [23], open contact between the peritoneal cavity and the environment is limited to only once a day. This contamination-prone procedure is preceded by a long diurnal dwell time, which we have shown to significantly enhance the host defense status of the peritoneal cavity. CAPD not only requires more frequent (dis)connection procedures, but the connections are also preceded by shorter dwell times. Thus, the dialysis regimen prescribed may have an impact on peritonitis rates by its (dwell time related) effect on phagocytic defense in instances where small numbers of bacteria contaminate the peritoneal cavity. In conclusion, no differences in peritoneal host defense variables were found between chronic CCPD and CAPD patients when dwell times were equal. Prolonged dwell time enhanced effluent opsonic activity as well as PMO functions, thereby providing a better local phagocytic defense. As in CCPD with external occlusion the dwell time period between contamination-prone procedures is much longer (14 to 16 hr) than in CAPD, this might contribute to the lower peritonitis incidence among CCPD-treated patients.

Acknowledgments

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