Effects of the Composition of Peritoneal Dialysis Fluid on Chemiluminescence, Phagocytosis, and Bactericidal Activity In Vitro

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Commercial fluid used for peritoneal lavage in peritonitis and in peritoneal dialysis suppressed the activity of peripheral blood leukocytes as measured by chemiluminescence, phagocytosis, and bacterial killing. Suppression was found to be due to the low pH and high osmolality of the fluid. The pH was adjusted to noninhibitory levels in vivo within 30 min, whereas osmolality changes were less rapid and remained at inhibitory levels for fluids of high dextrose concentration (4.25%). Chemiluminescence was the most sensitive assay for inhibitory effects of pH and osmolality, as well as for urea and heparin. The metabolic waste product urea at levels normally found in dialysate and heparin at concentrations routinely added to fluid inhibited only chemiluminescence, whereas creatinine and added insulin were not inhibitory. High fluid volume also resulted in a decrease in efficiency of bacterial killing. These results suggest some changes to be made in the treatment of peritonitis and in peritoneal dialysis.

Patients in end stage renal disease suffer from an increased susceptibility to infections (8). Although the mechanism of this increased susceptibility is not clear, inhibition of various cellular immune functions, including phagocytosis, has been implicated (1). Management of end stage renal disease requires the removal of waste products (primarily urea and creatinine) from the blood stream, and this can be achieved by dialysing the blood of the patients in hemodialysis machines. An alternative is dialysis through the body's own membrane, the membrane of the peritoneal cavity (9). This method basically requires the introduction through a peritoneal cannula of 2 liters of dialysis solution which remains in the peritoneum for several hours and is then removed, the process to be repeated four to five times a day. The manipulations have to be carried out under meticulously sterile conditions since the risk of peritonitis caused by bacteria is high. In fact, various groups have reported peritonitis as a major complication of peritoneal dialysis (9).

Peritoneal defense depends on two basic mechanisms. The intraperitoneal circulation of fluid carries particles toward stomata on the surface of the diaphragm from where they are transported to the lymphatics and rapidly appear in the thoracic duct and ultimately the blood stream (5). In situ bacterial elimination is carried out by the resident phagocytic cells which are primarily mononuclear but which change during peritonitis to a predominantly polymorphonuclear cell population (7). This population can reach very high numbers; we have observed peritoneal fluids from cases of peritonitis containing $10^9$ to $10^{10}$ cells per 2-liter fluid exchange.

Due to the basic principle of peritoneal dialysis, patients have large quantities of fluid in the peritoneal cavity. The changing of this fluid at regular intervals depletes the peritoneal cavity of large numbers of inflammatory cells during peritonitis and alters the physiological environment. Commercial dialysis fluids have an initial pH of 5.2, with osmotic concentrations varying from 275 to 479 mosmol/kg. The large fluid volume (2 liters) also increases the volume/surface area ratio and probably disrupts the normal intraperitoneal fluid circulation as well. To develop a rational therapy of peritonitis, we investigated the effects of the components of dialysis fluid on the activity of polymorphonuclear leukocytes by measuring chemiluminescence (CL), phagocytosis, and bacterial killing by these cells.

The results in this paper also show that although CL was the most sensitive of the three assays used, it was also the one most subject to irrelevant or modifying influences and should, therefore, always be supported by functional assays.

MATERIALS AND METHODS

PBL. Blood from normal donors was collected into heparinized vacutainer tubes and allowed to settle for
1 h at 37°C. The plasma supernatant, rich in peripheral blood leukocytes (PBL) was removed and washed before use. For CL experiments, PBL underwent a hypotonic lysis step to remove erythrocytes.

**Reagents and solutions.** Heparin (Hepalean; Harris Laboratories, Toronto, Canada), urea (BDH Chemicals, Toronto, Canada), sodium lactate, sodium acetate, creatinine (Fisher Scientific Co., Fairlawn, N.J.), and insulin (Connaught Laboratories, Willowdale, Ontario) were diluted in the appropriate medium. Luminol (5-amino-2,3-dihydro-1,4-phenalazine dine; Eastman Kodak Co., Rochester, N.Y.) was dissolved in fetal calf serum by incubating 1 ml of fetal calf serum plus 0.1 g of Luminol at room temperature for 1 h on a rotator and then filtering the mixture through a 0.45-µm membrane to remove insoluble particles. Control medium was phosphate-buffered balanced salt solution with Earle salts. Dianeal fluids containing 0.5 to 4.25% dextrose were obtained from Baxter Travenol Laboratories, Malton, Ontario. Complete Dianeal solutions containing 0.5 to 4.79 mosmol/kg contained the following (in milligrams per 100 milliliters): NaCl, 567.0; CaCl₂, 22.1; MgCl₂, 15.2; sodium lactate, 392.0; and dextrose, 500.0 to 4,250.0. For experiments measuring the effects of lactate, acetate, dextrose concentration, and osmolality, a basal Dianeal solution (osmolality, 275 mosmol/kg) was used and contained the following (in milligrams per 100 milliliters): NaCl, 767.0; CaCl₂, 22.1; MgCl₂, 15.2; and dextrose, 500.0.

CL. CL was measured essentially by the method of Allen et al. (2). The reaction was carried out in 5-ml plastic scintillation minivials (Fisher Scientific Co.) containing 10⁶ PBL, 300 × 10⁶ heat-killed Escherichia coli opsonized with a 1:5 dilution of fresh normal human serum, and 0.01 ml of fetal calf serum-Luminol in a total volume of 3 ml. The vials were counted in a Beckman LS 8000 scintillation counter in the single-photon count program. A stable base line was obtained before addition of opsonized bacteria. The amount of CL was calculated as the total number of counts over the base-line value accumulated during the first 10 min after addition of opsonized bacteria. This figure was found to be approximately proportional to the number of counts obtained over a 1-h period. Actual counts averaged around 15 × 10⁶ counts per 10 min. All experiments were repeated a minimum of three times.

**Phagocytosis.** The method used to measure phagocytosis was a modification of that described by Walker and Demus (11). Briefly, 5 × 10⁶ PBL and 5 × 10⁵ ¹⁹Cr-labeled sheep erythrocytes plus a 1.500 dilution of rabbit anti-sheep erythrocyte serum (Flow Laboratories, McLean, Va.) in a total volume of 0.2 ml were incubated in triplicate in plastic tubes (12 by 75 mm, no. 2052; Falcon Plastics Co., Oxnard, Calif.) for 1 h in a 37°C shaking water bath. Saline (1.8 ml) was then added to each tube which was then centrifuged at 700 × g for 10 min, and the supernatant was aspirated. Unengested sheep erythrocytes were lysed by addition of distilled water to the pellets, and osmolality was restored after 30 s with the addition of 10× saline. The pellets obtained after recentrifugation were counted in a Beckman Gamma 4000 counter. The percentage of phagocytosis was calculated by using the formula (cpm in pellet + total cpm) x 100.

**Bactericidal assay.** Bactericidal assay was performed essentially as described by Hooke et al. (6). Briefly, 10⁶ colony-forming units of E. coli E/2/64, a temperature-sensitive mutant which grows at 25°C but not at 37°C, plus 10% fresh normal human serum were added to 10⁶ PBL to give a total volume of 1 ml (the mutant is not killed by incubation at 37°C). This concentration of PBL (10⁶/ml) was chosen because bacterial killing values obtained range from 60 to 95% as compared with greater than 99% under optimal conditions of cell concentration. This produced a more sensitive assay for inhibitory conditions. In experiments of osmolality, E. coli were opsonized with a 1:5 dilution of fresh normal human serum and washed before their addition; the cultures contained no serum. The mixture was incubated in triplicate in plastic tubes (12 by 75 mm, no. 2054; Falcon Plastics) on a rotator for 1 h at 37°C. Samples were taken at 0 and 1 h, diluted in distilled water and incubated at room temperature for 30 min to lyse the granulocytes. Samples were then plated on nutrient agar and incubated for 48 h at room temperature.

**RESULTS**

**In vivo changes in Dianeal solution.** To study the changes in composition of Dianeal during in vivo dwell time, peritoneal dialysate was removed at intervals through a T tube under sterile conditions. The pH was measured directly at bedside, and fluid for osmolality determinations was taken to the laboratory on ice. A representative experiment with 4.25% Dianeal is shown in Fig. 1. The pH rose rapidly from 5.2 to approximately 6.8 within 30 min and equilibrated at around 7.2 after 1 h. The osmolality changes were not so rapid and did not reach physiological limits even within 5 h. The pH changes were essentially identical, and the osmolality changes were proportionately different with Dianeals of lower dextrose concentration.

In preliminary experiments, PBL incubated in Dianeal solutions were suppressed 98.8 ± 4.1% in their CL response (0.07 × 10⁶ counts per 10 min compared to 14.7 × 10⁶ counts per 10 min in medium). We therefore decided to study the effects of single variables on CL, phagocytosis, and bactericidal activity.

**Effects of pH and osmolality.** Dianeal solution has a starting pH of 5.2 and an osmolality ranging from 275 to 479 mosmol/kg for Dianeal solutions containing 0.5 to 4.25% dextrose. A basal solution containing only the salts in Dianeal plus 0.5% dextrose was used in which the pH was adjusted with dibasic phosphate and the osmolality was adjusted with NaCl or dextrose (see description of basal solution above). When the pH was varied over the range 5.2 to 7.4, CL was completely suppressed at pH 5.2 to 6.0 but increased rapidly as the pH was increased above 6.4 (Fig. 2A). Phagocytosis was significantly suppressed (P < 0.05) at pH 6.0 (Fig. 2B) but was not as sensitive to pH changes as was CL, partly.
due to the higher background obtained with this assay. Bacterial killing was less sensitive to pH, with no difference observed at pH 6.2 but a decrease to 17.2% of control ($P < 0.001$) observed at pH 5.2 (Fig. 2).

Raising the osmolality of the medium decreased PBL function in all three assays (Fig. 3). Increasing amounts of dextrose were added to the basal Dianeal solution. CL was most sensitive to changes in osmolality. Statistically significant ($P < 0.05$) suppression was observed at dextrose concentrations of 1.5% or higher for CL and 2.5% or higher for phagocytosis and killing. Essentially identical results were obtained when osmolality was increased by using NaCl (data not shown), and suppression of bactericidal activity was even slightly increased, demonstrating that inhibition was due to the increasing osmolality and not to the high dextrose concentration. Because of the reported inhibitory effects of high glucose concentration on phagocytosis by PBL (3), osmolality was maintained at serum levels (275 to 300 mosmol/kg) with NaCl and glucose concentration was increased to ascertain whether a high sugar concentration by itself was inhibitory. Figure 4 shows that increasing the glucose concentration from 0.1 to 4.25% did not suppress phagocytosis.

Effect of lactate. Adjustment of 0.5% Dianeal to pH 7.2 (osmolality, 275 mosmol/kg) resulted in a low CL response ($P < 0.05$), whereas phagocytosis and bactericidal activity were not inhibited when compared with the control (Table 1). This was found to be due to interference with the Luminol-dependent chemiluminescence reaction. Lactate was added to a cell-free system containing only saline, 0.01% fetal calf serum–Luminol, and 0.02% H$_2$O$_2$. CL was suppressed immediately upon addition of 100 and 400 mg of lactate per 100 ml (89.1 and 97.4% suppression, respectively; concentration in Dianeal is 392 mg/100 ml).
Effects of osmolality on activity of PBL. The osmolality of a basal Dianeal solution was increased by the addition of dextrose, and the cells were assayed for CL (A), phagocytosis (B), and bactericidal activity (C).

**Fig. 3.** Effects of osmolality on activity of PBL. The osmolality of a basal Dianeal solution containing various concentrations of dextrose. Osmolality was kept constant at 275 to 300 mosmol/kg with NaCl.

**Table 1. Effects of additives or waste products on CL, phagocytosis, and killing**

<table>
<thead>
<tr>
<th>Additive or waste product (concn)</th>
<th>% of control</th>
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<tr>
<td></td>
<td>CL</td>
</tr>
<tr>
<td>Heparin (500 U/liter)</td>
<td>63.0 ± 12.3</td>
</tr>
<tr>
<td>Insulin (8 U/liter)</td>
<td>104.8 ± 17.4</td>
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<tr>
<td>Urea (100 mg/100 ml)</td>
<td>56.2 ± 2.4</td>
</tr>
<tr>
<td>Creatinine (12 mg/100 ml)</td>
<td>97.5 ± 17.4</td>
</tr>
<tr>
<td>Lactate (392 mg/100 ml)</td>
<td>29.4 ± 3.5</td>
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</table>

* Results were statistically significant: P < 0.05.

Effects of insulin, heparin, and small molecules. Other than antibiotics, heparin and insulin are the most common additives to Dianeal when it is used in peritoneal dialysis. Table 1 shows the effects of these additives on PBL activity. Heparin suppressed CL but did not affect phagocytosis or bacterial killing at the concentration normally used, 500 U/liter. Heparin did not inhibit bactericidal activity at 1,000 U/liter (103.0 ± 5.5% control) but occasionally, though not significantly, inhibited killing at >5,000 U/liter (data not shown). Insulin is added directly to Dianeal in the case of diabetic patients on peritoneal dialysis and had no effect on CL, phagocytosis, or bacterial killing in the concentration normally used (8 U/liter) or at higher concentrations (data not shown).

Two of the major metabolic waste products which accumulate in Dianeal fluid during a 4-h dwell are urea and creatinine. Creatinine had no effect on CL, phagocytosis, or bacterial killing, whereas urea suppressed CL at concentrations often found in Dianeal (50 to 75 mg/100 ml) but had little or no effect on phagocytosis and bactericidal activity in this dose range (Table 1). Urea, as well as heparin, had no effect on CL in the cell-free system, even at high concentrations (100 mg/100 ml for urea and 10,000 U/liter for heparin).
Effects of fluid volume or cell concentration. Dianeeal does not inhibit bacterial killing once the pH and the osmolality are adjusted to physiological values (Fig. 5). In the case of peritoneal dialysis patients, one other potentially important parameter is the large fluid volume these patients carry in their peritoneal cavities. We, therefore, studied the effects of fluid volume or cell concentration on bacterial phagocytosis and killing. The total number of cells was held constant at $4 \times 10^6$ per tube, and the volume varied from 1 to 8 ml per tube. Figure 5 shows a rapid decrease in the ability to eliminate bacteria as fluid volume increases. Similarly, when the volume was held constant at 1 ml per tube and the cell concentration was varied from 0.5 to $4.0 \times 10^6$/ml, a parallel decrease in bacterial killing was obtained, indicating that effector cell density had to be high for PBL to efficiently kill the bacteria.

DISCUSSION

Dianeeal, which is used for peritoneal lavage in peritonitis and in peritoneal dialysis, has some undesirable effects on the anti-bacterial action of normal PBL. Preliminary studies with human peritoneal cells show similar results (data not shown). Dianeeal has a low pH, 5.2, which strongly inhibited all PBL functions tested. Although pH was adjusted in vivo within 30 min, this time interval may be crucial during peritoneal lavage, a time when host defenses must cope with a large bacterial inoculum.

The osmolality of Dianeeal varies from 275 to 479 mosmol/kg for solutions containing from 0.5 to 4.25% dextrose. It was found that Dianeeal containing 2.5% dextrose or higher inhibited all three functions tested: CL, phagocytosis, and bacterial killing. Although osmolality is adjusted to some extent in the peritoneum, it may never reach normal physiological levels during the dwell time.

The inhibition seen in Dianeeals of high dextrose concentration was due to the high osmolality. Bagdade et al. (3), using an assay system containing 80 to 90% normal or hyperglycemic serum, reported that serum glucose concentrations greater than 0.25% inhibit the phagocytic and bactericidal activity of PBL. Inhibition of killing and phagocytosis in this study was not caused by the high dextrose concentration because (i) similar results were obtained by increasing the osmolality with NaCl while keeping the sugar concentration constant and (ii) no inhibition was observed when the osmolality was kept constant and the dextrose concentration was varied from 0.1 to 4.25%. CL was also inhibited when the osmolality was increased by the addition of an amino acid solution (unpublished data).

Peritoneal dialysis patients frequently have additions made to the Dianeeal fluid, such as heparin, to prevent fibrin clots which may block their catheters, and insulin, in the case of diabetic patients. Neither heparin or insulin inhibited bacterial killing in the concentrations normally used. An additional factor to consider in dialysis patients is the accumulation of metabolic waste products such as creatinine and urea in concentrations up to 10x that of normal blood. Creatinine had no effect in any of the assays tested, whereas urea, like heparin, inhibited only CL at higher concentrations.

The results in this paper point out a great advantage of the CL assay and also dictate that great caution be used. In all cases in which inhibition was obtained, CL was the most sensitive assay. pH and osmolality effects were most apparent when measuring CL. This was most likely because the CL assay measures very early events in the cell-bacteria reaction. The phagocytosis assay used measures the number of particles within the cell at one point in time, whereas the bactericidal assay measures the destruction of bacteria that has taken place during a 1-h interval. These assays, therefore, allow the PBL to adjust to the medium or, more likely, to adjust their microenvironment. Heparin and urea inhibited only CL in the concentrations normally used. Both substances occasionally inhibited phagocytosis or killing at much higher concentrations, but this inhibition was not sig-

![Fig. 5. Effect of PBL concentration and fluid volume on bacterial killing. PBL ($4 \times 10^6$) were incubated in volumes ranging from 1.0 to 4.0 ml (○), or 0.5 to $4.0 \times 10^6$ PBL were incubated in 1.0 ml (●). Bacteria/PBL ratio was kept constant at 1:1 in all cultures.](http://iai.asm.org/)
significant or consistent. CL, therefore, can be a very sensitive indicator of an inhibitor and detects very early events in the reaction between cell and ligand. On the other hand, the suppression of CL by lactate was not caused by an inhibition of PBL-dependent generation of bactericidal products, such as O$_{2}^{-}$, -OH, and H$_2$O$_2$, which are responsible for CL and which increase during infection (4, 8). Lactate did not suppress phagocytosis or bactericidal activity but did block CL in a cell-free system utilizing H$_2$O$_2$ and Luminol, demonstrating the artifactual nature of this result. Neither heparin nor urea had any effect in the cell-free system.

Finally, the efficiency of bacterial killing was found to depend on a high cell/fluid ratio. Locomotion and phagocytosis by neutrophils is optimal when bacteria can be trapped against a surface (12), but patients on chronic peritoneal dialysis carry approximately 2 liters of fluid in their peritoneal cavities. The result is greater volume/surface area ratios with a possible consequent decrease in bacterial elimination. This was demonstrated in vitro by the rapid decrease in bacterial killing as the cell number/fluid volume ratio decreased, resulting from a decrease in phagocytosis due to an inability of PBL to trap bacteria between them. An additional adverse effect of the large fluid volume may be a low O$_2$ tension in the peritoneal fluid which is dependent on close proximity to blood capillaries. An important bactericidal mechanism in neutrophils is mediated by H$_2$O$_2$ produced via uptake of molecular O$_2$ (10). The large fluid volume and consequent low O$_2$ tension may result in a decrease in bactericidal activity of resident cells. In addition, the large fluid volume would be expected to interfere with normal bacterial elimination. Bacteria are cleared from the peritoneum via the diaphragmatic lymphatics and then transferred to the systemic circulation (5). This clearance is aided by the normal intraperitoneal fluid circulation and, therefore, a large fluid volume, such as 2 liters of dialysis fluid, would disrupt the intraperitoneal flow carrying bacteria upward to the diaphragmatic lymphatics and the subsequent clearance of the bacteria and appearance in the circulation. The absence of positive blood cultures in peritoneal dialysis patients with bacterial peritonitis tends to support this view.

In conclusion, these results suggest that dialysis patients should use pH-adjusted Dianeal, and, in the treatment of peritonitis, lavage should be carried out with Dianeal containing 0.5% dextrose with the pH adjusted before infusion. In addition, the fluid volume remaining in the peritoneum should be kept small.

ACKNOWLEDGMENT

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LITERATURE CITED


