Peritoneal Dialysis Fluid Inhibition of Phagocyte Function: Effects of Osmolality and Glucose Concentration

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ABSTRACT

Solutions were formulated to examine, independently, the roles of osmolality and glucose in the reduction of viability and inhibition of phagocyte function by dextrose-containing peritoneal dialysis fluids. The exposure of neutrophils (polymorphonuclear leukocytes) to test fluids containing ≥2.7% (wt/vol) glucose resulted in significant cytotoxicity as assessed by the release of lactate dehydrogenase above control values (7.12 ± 2.65%). At the highest concentration of glucose (4.5%), lactate dehydrogenase release was 15.83 ± 0.49% (P < 0.05). These effects were directly related to the presence of α-glucose in the test fluids. In contrast, phagocytosis and the release of leukotriene B4 from PMN stimulated with serum-treated zymosan were significantly inhibited in an osmolality-, but not glucose-, dependent manner. The inhibition of tumor necrosis factor α and interleukin-6 release from mononuclear leukocytes was inhibited by a combination of osmolality and monosaccharide concentration. Under the same conditions, PMN respiratory burst activation remained unaffected irrespective of glucose concentration or fluid osmolality. These data indicate that, in addition to the low pH of peritoneal dialysis fluid and its high lactate concentration, its glucose content (either directly or as a consequence of the resulting hyperosmolality of the fluid) inhibits cell functional parameters. These findings suggest clinically significant inhibition of host defense mechanisms because, in high-glucose dialysis fluids, osmolality does not reach physiologic values, even during extended intraperitoneal dwell periods.

Key Words: Continuous ambulatory peritoneal dialysis, host defense, osmolality, cytokines, dialysis

The removal of excess body water is an integral part of any dialysis procedure used in the treatment of end-stage renal failure. In peritoneal dialysis, this is achieved by the process of osmotic ultrafiltration. Commercial peritoneal dialysis fluids utilize glucose to form a hypertonic solution and create an osmotic gradient between the plasma and peritoneal fluid.

The disadvantages of using glucose as an osmotic agent in peritoneal dialysate are well recognized, but only recently have alternative iso-osmolar fluids been introduced into clinical practice (1). The adverse metabolic consequences of glucose absorption become especially prominent in the continuous ambulatory peritoneal dialysis (CAPD) setting where patients are exposed to high glucose concentrations for extended periods (2,3).

Increasing interest has recently been focused on the biocompatibility of the peritoneal dialysis solutions, in particular, whether host defense mechanisms in the peritoneal cavity are significantly impaired during the CAPD cycle. Preliminary evidence has been provided that suggests that fluids containing high glucose concentrations are inhibitory to different cell functions in vitro, including phagocytosis, intracellular killing, the generation of leukotriene B4 (LTB4) and respiratory burst activation (4–6). The precise mechanisms of these effects, whether they are related to high glucose concentration or hyperosmolality, have, however, not been fully elucidated. This question may be especially important in vivo, because the osmolality of high glucose-containing fluids, unlike the pH and lactate concentration, although lowered to some extent during the interperitoneal dwell cycle, never reaches physiologic values (7).

In this study, we have formulated laboratory solutions to examine, independently, the effects of high osmolality and glucose concentration upon various
cell functions relevant to peritoneal host defense. Our observations demonstrate that the inhibition of polymorphonuclear leukocytes (PMN) function by high glucose-containing dialysis fluids is related to high osmolality; however, for mononuclear leukocytes (MNC) cytokine release, the combined effect of glucose concentration and osmolality reduces cell function. The reduction in PMN viability, however, appears to be directly related to the concentration of d-glucose.

MATERIALS AND METHODS

Test Fluids

To evaluate the effect of glucose on cell viability and function and to separate the effect of increased osmolality from the effect of glucose per se, three groups of solutions were formulated (Table 1). In the first two, the osmolality of the fluids was increased stepwise by the addition of either glucose or sodium chloride to the basal electrolyte solution, thus creating fluids with osmolalities of 350, 400, 450, 500, and 550 mosM/kg. In the third group of fluids, equal amounts of glucose were added as in the first group, and the osmolality of these fluids was maintained at 300 mosM/kg by preparing them in hypotonic saline solutions (Table 1). In separate experiments, fluid osmolality was increased to 500 mosM/kg by either d-glucose, l-glucose, or 1-mannitol supplement. In addition to sodium chloride, all solutions contained: Ca2+ (1.75 mM) and Mg2+ (0.75 mM). Chemicals used in test fluid preparation were purchased from Merck Ltd (Poole, United Kingdom).

In experiments with human MNC, solutions were prepared at three different osmolalities (300, 400, and 500 mosM/kg). These solutions were prepared either in the absence of glucose or in the presence of 1.36 or 3.86% wt/vol glucose. The osmolality of the solutions was controlled either by the addition of dry powdered RPMI-1640 (Gibco/BRL Life Technologies Ltd, Paisley, United Kingdom) to increase osmolality in the case of control and 1.36% glucose solutions or preparation in hypoosmolar RPMI-1640 in the case of 3.86% glucose solutions. In a separate series of experiments, fluid osmolality was increased in RPMI-1640 by the addition of d-glucose, mannose, or NaCl to give solutions with osmolalities of 300, 400, and 500 mosM/kg. All solutions were used at pH 7.3.

Control Buffers

Krebs-Ringer phosphate buffer (KRPG) contained 12.7 mM Na2HPO4, 11 mM d-glucose, 120 mM NaCl, 4.8 mM KCl, 0.7 mM CaCl2, and 1.2 mM MgSO4·7H2O (Merck Ltd) and was used as the control fluid in most experiments. For LTB4 generation experiments, RPMI medium was used as a control.

Serum-Treated Zymosan

Boiled zymosan (Sigma Chemical Co Ltd, Poole, United Kingdom) (40 mg) was incubated with 75% vol/vol pooled normal human serum in KRPG for 30 min at 37°C, washed three times with KRPG, and resuspended to 4 mL. A stock solution of 10-mg/mL concentration was aliquoted, stored at −70°C, and used as a stimulus at various final concentrations.

Preparation of PMN and MNC

Human PMN and human MNC were prepared from the citrated peripheral blood of healthy volunteers by dextran sedimentation and Ficoll-Hypaque density gradient centrifugation as described previously (8). The polymorphonuclear cell fraction routinely contained >95% PMN and the MNC fraction consisted of 15 to 30% monocytes, 60 to 70% lymphocytes, and <5% granulocytes, as assessed by light microscopy of stained preparations (Neat Stain; Guest Medical Ltd, Sevenoaks, United Kingdom).

PMN Viability (Release of Lactate Dehydrogenase)

Five million PMN were incubated in 5 mL of the test fluid for 30 min at 37°C with constant tube rotation. The cells were removed by low-speed cen-

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<th>TABLE 1. Glucose/osmolality experiment test fluids</th>
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<td>Osmolality (mosM/kg)</td>
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<td>Glucose (g/L)</td>
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<td>NaCl (g/L)</td>
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*The first fluid is identical in all series. All fluids contained Ca2+ (1.75 mM) and Mg2+ (0.75 mM).
Chemiluminescence Assay

PMN (100 μL at a concentration of 5.0 × 10⁷/mL) were incubated with 5 mL of the dialysate fluid to be tested for 30 min at 37°C, with constant tube rotation. The cells were subsequently washed three times with PBS, air dried, fixed with methanol, stained (Neat Stain; Guest Medical Ltd), and examined microscopically. The number of PMN ingesting two or more particles was assessed by counting 200 cells per plate, and the results were expressed as a percent phagocytosis.

Statistical Analysis

All statistical analysis was performed by use of the Wilcoxon signed rank test (Statview 512+; Apple Macintosh Inc. Cupertino, CA) for nonparametric data. All data are presented as mean (± standard error).

RESULTS

Cytotoxicity of the Fluids

The level of cytotoxicity, as assessed by LDH release, of the basal solution was 7.12 ± 2.65%, which did not differ significantly from the LDH release induced by the control medium RPMI (3.74 ± 2.35% release). Increasing the osmolality of the basal solution with sodium chloride supplement did not signif-
significantly increase the cytotoxicity of the fluids, although at the highest osmolality tested (550 mosM/kg), LDH release was 13.25 ± 4.41%. In intermediate hyperosmolar fluids (400 to 500 mosM/kg), the presence of glucose resulted in significant elevation of LDH release compared with NaCl-supplemented fluids (Figure 1b).

The release of LDH from PMN incubated in iso-osmolar fluids containing rising glucose concentrations increased in a dose-dependent manner. This release, however, was only significantly above control levels (basal solution) in fluids containing 4.5% glucose, when the release of LDH reached 15.84 ± 3.20% (P < 0.05) (Figure 1a). In fluids with the osmolality increased by glucose supplementation, LDH release was significantly higher than control in all fluids containing ≥2.7% glucose (osmolality ≥450 mosM/kg). The levels of LDH released under these conditions were 15.15 ± 1.6 and 15.83 ± 0.49% for solutions containing 2.7 and 4.5% glucose, respectively (P < 0.05 for both) (Figure 1). In separate experiments, LDH release from PMN incubated in the fluids containing L-glucose or D-mannitol were 0.96 ± 0.86 and 9.74 ± 0.94, respectively; these levels were significantly lower compared with the basal release after exposure to fluids containing an equal amount of D-glucose (13.32 ± 1.77; P < 0.05 for both).

**Phagocytosis**

Increasing the osmolality of fluids with either sodium chloride or glucose resulted in a significant, osmolality-dependent decrease of phagocytosis of STZ by PMN. In the fluids with osmolality increased with sodium chloride supplement, the percentage of cells ingesting two or more particles was reduced from 64.0 ± 15.7% in the basal solution to 26.2 ± 6.9 at 400 mosM/kg and 3.8 ± 1.3% in 550 mosM/kg fluid (P < 0.05 for both) (Figure 2a).

The phagocytosis of STZ in the fluids with the osmolality increased by glucose supplementation was also significantly reduced with respect to the basal solution (64.0 ± 15.7% at 300 mosM/kg versus 22.0 ± 9.0 at 550 mosM/kg (P < 0.05)). The degree of inhibition of phagocytosis in fluids supplemented with NaCl was, however, greater for all osmolalities above 400 mosM/kg than the inhibition of phagocytosis in the glucose-supplemented fluids of equal osmolality (Figure 2a). In iso-osmolar test fluids supplemented with the increasing amounts of glucose, no inhibition of STZ phagocytosis was observed even at the highest concentration (4.5% wt/vol) tested (Figure 2b).

**CL Response**

The integrated PMN CL response stimulated by STZ was 3.3 ± 0.12 × 10^7 counts over a 40-min period after pretreatment with basal fluids and 3.4 ± 0.87 × 10^7 after treatment with RPMI (control). For each experiment, integrated counts were compared with those of control and were expressed as percentage of control response. Increasing the osmolality of the test fluids, either with sodium chloride or with glucose, did not significantly alter the level of the respiratory burst activation as measured by the PMN CL response, even at the highest osmolality tested (550 mosM/kg) when the responses were 90 ± 10 and 75 ± 12% of control in sodium chloride- and glucose-supplemented fluids, respectively. Neither was the PMN CL response affected after pretreatment of PMN.
Osomolality (mOsm/kg)

Phagocyte Function in Dialysis Fluids

Figure 2. (a) PMN phagocytosis of STZ in test fluids with osmolality increased with either glucose or sodium chloride supplement. Data presented are the means (±SE) of five separate experiments performed with cells from different donors. *Statistically significant difference from basal electrolyte solution (300 mosM/kg). (b) PMN phagocytosis of STZ in test fluids with increasing glucose concentration and either rising or unchanged osmolality. Data presented are the means (±SE) of five separate experiments performed with cells from different donors. *Statistically significant difference from basal electrolyte solution (no glucose, 300 mosM/kg).

with iso-osmolar fluids containing up to 4.5% wt/vol glucose (90 ± 12% of control).

LTB₄ Generation

Unstimulated PMN released 0.012 ± 0.004 ng/10⁶ cells after 30 min of incubation in control fluids at 37°C. PMN pretreated with the basal solution generated 4.4 ± 0.66 ng/10⁶ cells after A23187 challenge. In fluids with osmolality increased to 550 mosM/kg, with either sodium chloride or glucose, levels of LTB₄ release were not significantly different from control (5.1 ± 0.9 and 3.9 ± 1.05 ng/mL, respectively). Pretreatment with iso-osmolar fluids supplemented with increasing glucose concentrations did not have any effect on the amount of LTB₄ generated by PMN in response to A23187, e.g., at 4.5% glucose, when LTB₄ release was 4.4 ± 1.3 ng/10⁶ cells.

In contrast, fluids with high osmolality were significantly inhibitory to LTB₄ synthesis from PMN stimulated by STZ, irrespective of whether the fluids were supplemented with either sodium chloride or glucose. In basal fluids, PMN generated 0.175 ± 0.007 ng of LTB₄/10⁶ cells after 30 min of STZ stimulation; this release was significantly suppressed in both sodium chloride- and glucose-supplemented fluids at 400 mosM/kg to 0.021 ± 0.007 and to 0.015 ± 0.007 ng of LTB₄/10⁶ cells, respectively (P < 0.05 for both). The degree of suppression did not differ significantly between these two series of fluids when compared at corresponding osmolality (Figure 3a). No similar inhibitory effect of glucose was observed in iso-osmolar fluids with increasing glucose concentration, where LTB₄ generation was not suppressed when compared with the basal solution (Figure 3b).

Figure 3. (a) LTB₄ synthesis by PMN stimulated with STZ in test fluids with osmolality increased with glucose or sodium chloride supplement. Data presented are the means (±SE) of five separate experiments performed with cells from different donors. *Statistically significant difference from basal electrolyte solution (300 mosM/kg). (b) LTB₄ synthesis from PMN stimulated with STZ in test fluids with increasing glucose concentration and either rising or unchanged osmolality. Data presented are the means (±SE) of five separate experiments performed with cells from different donors. *Statistically significant difference from basal electrolyte solution (no glucose, 300 mosM/kg).
TNF-α and IL-6 Release From MNC

Exposure of MNC to LPS in the presence of RPMI or laboratory-prepared fluids of increasing osmolality and/or glucose content resulted in an osmolality-dependent inhibition of TNF-α release (Figure 4a). This inhibition was independent of the type of monosaccharide utilized, because the pattern of release was identical in glucose- and mannose-supplemented fluids. The combined effect of glucose and osmolality on cytokine release from MNC solutions with increasing osmolality in the presence or absence of 1.36 and 3.86% wt/vol glucose was investigated. These experiments demonstrated a significant and dose-dependent cytokine inhibitory effect of glucose in a mildly hyperosmolar (400 mosM/kg) environment. In fluids of osmolality of 400 mosM/kg, the release of TNF-α in RPMI was 1062 ± 205 pg/mL. This level was reduced to 783 ± 194 and 401 ± 165 pg/mL in 1.36 and 3.86% glucose solutions (P < 0.05 and P < 0.01, respectively). At 500 mosM/kg, the same pattern of inhibition was evident, although the total inhibition of TNF-α, in some cases, did not allow statistical comparison between groups. MNC IL-6 release was also reduced in an osmolality- and glucose-dependent manner; at 400 mosM/kg, the IL-6 release was significantly inhibited compared with control in fluids containing 3.86% glucose (P < 0.05) (Table 2) but not in fluids containing 1.36% glucose.

**DISCUSSION**

This study investigates the relative roles of high glucose concentration and osmolality on phagocyte function. The results clearly indicate that the principal inhibitory effect on phagocytosis and LT generation from PMN is related to fluid osmolality and not to its glucose content. In the case of cytokine release from MNC and fluid cytotoxicity towards PMN, however, the inhibitory effects appear to be at least partly related to the glucose content of the solutions. Evidence for inhibitory phenomena associated with high glucose–containing peritoneal dialysis fluids was first reported by Duwe et al. (4), who demonstrated inhibition of PMN phagocytosis, intracellular killing of bacteria, and luminol-dependent CL responses. These observations are extended by this study, and it has been further demonstrated that the phagocytosis and LTB4 generation by PMN stimulated with STZ is dose dependently suppressed in laboratory-prepared dialysis fluids when osmolality is increased with either glucose or sodium chloride. High glucose peritoneal dialysis fluids have also been reported to inhibit the phagocytosis of preopsonized E. coli (5). However, in that study, no attempt was made to differentiate between high glucose and osmolality and the inhibition was only significant at high glucose concentrations (>4%), which are above those present in commercial dialysis fluids.

We were unable to demonstrate any significant suppression of respiratory burst activation, as meas-

**TABLE 2. IL-6 release from peripheral MNC**

<table>
<thead>
<tr>
<th>Osmolality</th>
<th>RPMI</th>
<th>Glucose (1.36%)</th>
<th>Glucose (3.86%)</th>
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<tr>
<td>300</td>
<td>361 ± 70</td>
<td>324 ± 111</td>
<td>259 ± 99b</td>
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<tr>
<td>400</td>
<td>179 ± 64b</td>
<td>145 ± 68b</td>
<td>94 ± 44b</td>
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<tr>
<td>500</td>
<td>12 ± 40b</td>
<td>13 ± 60b</td>
<td>5 ± 30b</td>
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* Values are in picograms per milliliter. IL-6 release from MNC stimulated with LPS in test fluids with modified osmolality. The means (±SE) of five separate experiments performed with cells from different donors.

b A statistically significant difference with P < 0.05.

Significantly different from RPMI control at 400 mosM/kg.
ured by luminol-dependent CL either in hyperosmolar or iso-osmolar high glucose fluids at neutral pH. These data confirm our previous observations [8] but appear to be in contrast to those presented by other workers [4,5]. This discrepancy is possibly due to methodologic differences. In order to avoid the problem of nonspecific quenching of CL by fluids containing high glucose concentrations, cells were pretreated in test fluids, washed, and subsequently stimulated in control buffer. This experimental approach has previously allowed us to demonstrate CL inhibition caused by high-lactate/low-pH fluids [6,8].

We have reported previously that high glucose-containing fluids (3.86%) were inhibitory to LTB₄ synthesis after PMN stimulation with STZ [6,10]. In this study, we have demonstrated that this effect is directly related to the osmolality of the fluid but not to its glucose content. This concurrence of inhibition of phagocytosis and LTB₄ synthesis in the presence of maintained CL response suggests that particle cell interaction must be intact; however, the signal transduction mechanisms for phagocytosis and for activation of the 5-lipoxygenase pathway are specifically inhibited. These data confirm our previous observations that PMN effector functions may be independently regulated after particulate stimulation [12]. The fact that the osmolality-mediated inhibition of LTB₄ synthesis is independent of 5-lipoxygenase function is indicated by the lack of osmolality effect on A23 187-stimulated LTB₄ release from these cells. Taken together, these results suggest that a possible site for inhibition may be either at or proximal to the level of phospholipase activation, resulting in reduction of the level of released arachidonic acid, the precursor of eicosanoid synthesis. Experiments are currently underway to examine this hypothesis.

The exposure of MNC to LPS in iso-osmolar or hypotonic solutions demonstrated an osmolality-dependent inhibition of both TNF-α and IL-6 release, which was observed even in the absence of elevated glucose concentrations. At normal osmolality, high glucose concentrations did not show inhibitory capacity; however, an additive effect of glucose was observed, most clearly when increasing glucose concentrations were present in a moderately hyperosmolar environment. The osmolality effect was, however, not glucose specific because the same degree of inhibition was observed with fluids containing mannose (a monosaccharide of identical molecular weight to glucose and identical osmolality in solution) instead of glucose. The mechanism by which monosaccharides interfere with cytokine production are at present unknown but do not appear to be related to modification of the LPS molecule by test fluids. In this respect, we have previously demonstrated that preexposure of MNC to dialysis fluids in the absence of LPS and subsequent stimulation in control buffer do not result in a restoration of normal cell responsiveness [10].

It has previously been demonstrated that peritoneal dialysis fluids are cytotoxic to PMN, MNC, and mesothelial cells [6,10,13-15]. As in these studies, we were able to demonstrate significant toxicity, as assessed by the release of LDH, in fluids containing high glucose concentrations (≥2.7% wt/vol)—an effect that was at least partly independent of osmolality and related directly to glucose concentration. The specificity of this effect and its possible relationship to α-glucose metabolism was confirmed by the fact that the addition of L-glucose and D-mannitol, neither of which is transported into the cell, did not significantly increase fluid cytotoxicity. Cytotoxicity of the fluids, however, does not appear to be responsible for the observed inhibitory effects on cell function. This is confirmed by the fact that, under the same conditions, STZ-induced CL and A23187-stimulated LTB₄ release were not suppressed even at the highest glucose concentration/osmolality tested.

In the clinical situation of CAPD, it is assumed that the nonphysiologic composition of the instilled dialysis fluids is progressively equilibrated with increasing dwell time. In this respect, it is well known that the initial low pH and the lactate concentration of the solution, both of which are inhibitory to cell function [8,10,11], are normalized within the first hour of dwell time. In contrast, however, the hyperosmolality and excessive glucose content of high-glucose dialysis fluids do not return to their physiologic values even during a 4-h CAPD cycle. The potential thus exists for peritoneal dialysis fluids to inhibit cell function throughout the whole CAPD cycle. In this respect, we have recently demonstrated that spent dialysate collected after 4 h ip dwell time significantly reduces cytokine synthesis by activated leukocytes [11], indicating that despite some degree of intraperitoneal equilibration inhibitory activity is still present in vivo.

In conclusion, our observations demonstrate that the inhibition of cell function in terms of phagocytosis, STZ-induced LTB₄ generation, and cytokine release by high-glucose-concentration dialysis fluids is mediated primarily as a direct result of high osmolality. The in vitro techniques for the multiparametric laboratory evaluation of dialysis fluid toxicity, including the assessment of important functional aspects of cellular host defense, represent an important tool for preclinical evaluation of alternative osmotic agents and CAPD solutions. The results presented in this article, together with our previous findings with regard to pH and lactate effects on cell functions [6,8,10,11,16], appear to indicate that the design of potentially biocompatible CAPD fluids should meet the following requirements: (1) moderate hyperosmolality (≤400 mosM/kg); (2) monosaccha-
ride concentration ≤ 2 g/dL; and (3) neutral pH (or use of an alternative bicarbonate-generating anion).

These data, taken together with our previous observations and with the recent data of Wieslander et al. (15) that heat-sterilized glucose-containing fluids are inhibitory to cell function, suggest that more attention must be invested in the search for alternative osmotic agents and fluid formulations for routine use in CAPD.

ACKNOWLEDGMENTS

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