Biocompatibility of a bicarbonate-buffered amino-acid-based solution for peritoneal dialysis

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Abstract Amino-acid-based peritoneal dialysis (PD) fluids have been developed to improve the nutritional status of PD patients. As they may potentially exacerbate acidosis, an amino-acid-containing solution buffered with bicarbonate (Aminobic) has been proposed to effectively maintain acid-base balance. The aim of this study was to evaluate the mesothelial biocompatibility profile of this solution in comparison with a conventional low-glucose-based fluid. Omentum-derived human peritoneal mesothelial cells (HPMC) were preexposed to test PD solutions for up to 120 min, then allowed to recover in control medium for 24 h, and assessed for heat-shock response, viability, and basal and stimulated cytokine [interleukin (IL)-6] and prostaglandin (PGE₂) release. Acute exposure of HPMC to conventional low-glucose-based PD solution resulted in a time-dependent increase in heat-shock protein (HSP-72) expression, impaired viability, and reduced ability to release IL-6 in response to stimulation. In contrast, in cells treated with Aminobic, the expression of HSP-72 was significantly lower, and viability and cytokine-producing capacity were preserved and did not differ from those seen in control cells. In addition, exposure to Aminobic increased basal release of IL-6 and PGE₂. These data point to a favorable biocompatibility profile of the amino-acid-based bicarbonate-buffered PD solution toward HPMC.

Keywords Peritoneal dialysis · Human peritoneal mesothelial cells · Amino-acid-based peritoneal dialysis solutions · Heat-shock protein 72 · Biocompatibility

Introduction

Conventional glucose-based peritoneal dialysis (PD) solutions have extensively been shown to have limited biocompatibility. Relative toxicity of PD fluid components, as measured by growth inhibition of L929 cells, has been attributed to low pH, the presence of glucose degradation products (GDP), high osmolality, and high lactate concentrations [1]. Newer multi-chambered-bag PD solutions with markedly reduced GDP contents and near-neutral pH display improved biocompatibility both in vitro and in experimental animals and possibly also in PD patients (reviewed in [2]).

Amino-acid-based PD solutions were originally developed to improve the nutritional status of PD patients who...
lose significant quantities of proteins into the dialysate and frequently have poor appetite and inadequate dietary protein intake. In several short-term clinical studies, amino-acid-based solutions have been found to improve nutritional parameters in malnourished patients, although the long-term benefits are less clear (see [3] for a recent review). Additional advantage of amino-acid-based fluids is the avoidance of hyperglycemia and hyperinsulinemia, which may contribute to improved systemic hemodynamics [4]. Although Martikainen et al. did not find changes in insulinemia in patients using one daily exchange of amino-acid-based PD solution for 8 weeks, they recorded favorable decreases in serum cholesterol, free fatty acids, and triglycerides [5]. Furthermore, the elimination of GDP from PD fluids has been shown to improve food intake by experimental rats [6]. On the other hand, the exposure to methionine-containing amino-acid dialysate may impair acute endothelial-dependent vasodilation [7], possibly through a rise in plasma homocysteine [8]. Another potential disadvantage of amino-acid-based solutions could be an increase in serum urea and an exacerbation of acidosis.

The only commercially available amino-acid-containing PD solution (Nutrineal®, Baxter) is buffered with lactate, which may have some biocompatibility limitations. As bicarbonate-buffered PD solutions have been shown to offer the possibility of tailoring PD therapy to individual acid-base status [9], a new amino-acid/bicarbonate-based PD formulation has been proposed (Aminobic®, Fresenius Medical Care). The aim of this study was to evaluate the mesothelial biocompatibility profile of this solution in comparison with a conventional low-glucose-based fluid.

### Materials and methods

**Materials**

All chemicals, unless otherwise stated, were purchased from Sigma-Aldrich. All tissue-culture plastics were Falcon® from Becton Dickinson. PD solutions were from Fresenius Medical Care (Table 1).

**Human peritoneal mesothelial cells**

Human peritoneal mesothelial cells (HPMC) were isolated from specimens of omentum obtained from consenting nonuremic patients undergoing elective abdominal surgery. Cells were isolated and characterized as described previously [10]. HPMC were propagated in the M199 culture medium supplemented with L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μg/ml), hydrocortisone (0.4 μg/ml), and 10% v/v fetal calf serum (FCS; Invitrogen). All experiments were performed using cells only from the first three passages, as later subcultures contained an increasing number of senescent cells [10, 11]. HPMC were plated into multiwell clusters and grown until confluence. The standard medium containing 10% FCS was replaced with medium supplemented with 0.1% FCS for 48 h prior to experiments to render the cells quiescent.

**Exposure to PD solutions**

Previous studies have clearly demonstrated that preexposure of HPMC to PD solutions for periods as short as 1–4 h allows registration of significant changes in HPMC viability and function [12, 13]. In contrast, prolonged exposure to neat PD solutions resulted in massive HPMC damage. Therefore, in this study, confluent cultures were exposed either to standard low-glucose heat-sterilized PD solution (Stay Safe CAPD2) or to amino-acid-based bicarbonate-buffered PD solution (Aminobic) for up to 120 min. After that the solutions were removed, and cells were allowed to recover for 24 h in regular culture medium containing 0.1% FCS. The same medium was used to treat control cultures both in the treatment and recovery phases. At the end of the recovery period, the cells were examined for expression of heat-shock proteins, viability, and production of interleukin-6 (IL-6) and prostaglandin E2 (PGE2). These mediators have previously been used as markers of HPMC function [12].

### Table 1 Composition of peritoneal dialysis (PD) fluids studied

<table>
<thead>
<tr>
<th></th>
<th>Aminobic (Manufacturer: Fresenius Medical Care)</th>
<th>CAPD2 (Manufacturer: Fresenius Medical Care)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na+ (mmol/l)</td>
<td>137</td>
<td>134</td>
</tr>
<tr>
<td>Cl− (mmol/l)</td>
<td>103.5</td>
<td>103.5</td>
</tr>
<tr>
<td>Ca++ (mmol/l)</td>
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<td>1.75</td>
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<tr>
<td>Mg++ (mmol/l)</td>
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<td>0.5</td>
</tr>
<tr>
<td>Lactate (mmol/l)</td>
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<tr>
<td>HCO3− (mmol/l)</td>
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<tr>
<td>Glucose (g/l)</td>
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<td>15</td>
</tr>
<tr>
<td>Amino acidsa (g/L)</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>pH</td>
<td>7.2–7.6</td>
<td>5.5</td>
</tr>
<tr>
<td>Osmolality (mosmol/kg)</td>
<td>369</td>
<td>358</td>
</tr>
</tbody>
</table>

*a Amino acids composition of Aminobic:

Essential amino acids (mmol/l): L-histidine 3.222, L-isoleucine 5.72, L-leucine 8.61, L-lysine 7.181, L-methionine 2.681, L-phenylalanine 3.33, L-threonine 7.05, L-tryptophan 2.01, L-valine 14.51.

Nonessential amino acids (mmol/l): L-alanine 6.73, L-arginine 2.698, L-glutamine 5.99, Proline 3.91, L-serine 4.76, L-taurine 0.799, L-tyrosine 0.552.
Heat-shock-protein (HSP) expression

The expression of HSP-72 in HPMC extracts was assessed by Western blotting, as previously described [14]. Briefly: cells were washed twice in phosphate-buffered saline (PBS) and lysed in buffer containing 0.1% Triton X-100, 60 mM piperazine-1,4-bis-2-ethanesulfonic acid (PIPES), 2 mM 1,2-cyclohexane diamine tetraacetate (CDTA), 1 mM ethylenediaminetetraacetate (EDTA), 1 mM ethyleneglycoltetaacetic acid (EGTA), 100 mM sodium chloride (NaCl), 0.5 mM phenylmethylsulfonyl fluoride (PMSF), and 0.75 mg/l Leupeptin. Protein contents was determined with the Bradford assay (BioRad), and equal amounts of protein samples (5 μg/lane) were separated by standard sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using a Pharmacia Multiphore II unit. Size-fractionated proteins were transferred to polyvinylidene fluoride (PVDF) membranes by semidry transfer in a Pharmacia Multiphore II Novablot unit. Membranes were blocked in 5% dry milk in Tris-buffered saline (TBS)-TWEEN (10 mM Tris, 150 mM NaCl, 0.05% Tween 20, pH 8.0). Membranes were incubated with the primary antibody against HSP-72 (SPA 810, Stressgen, Biotechnologies Corp., Victoria, BC, Canada). Detection was accomplished by incubation with secondary, peroxidase-coupled antibodies (Sigma Chemical Company, St. Louis, MO, USA) and enhanced chemiluminescence (ECL) using ECL Western blotting analysis system (Renaissance, NEN-Life Science Products, Boston, MA, USA).

Cell viability

Cell viability was assessed by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) conversion assay. The test is based on metabolic conversion of the MTT salt by mitochondrial dehydrogenases of living cells. After the recovery period, HPMC were treated with MTT (1.25 mg/ml in culture medium) for 4 h at 37°C. The formazan product generated was solubilized by the addition of acidic solution of 20% (wt/vol) SDS and 50% (vol/vol) N,N-dimethylformamide. Absorbance of the converted dye was recorded at 595 nm with a reference wavelength of 690 nm [15].

IL-6 and PGE2 measurements

HPMC-derived supernatants were collected, centrifuged to remove cellular debris, and stored at −70°C until assayed. IL-6 concentrations were measured with an enzyme-linked immunosorbed assay (ELISA) using an ELISA-matched antibody pair (R&D Systems). The test was performed according to manufacturer’s instructions. Sensitivity of the system was 2 pg/ml. PGE2 concentrations were determined with the use of PGE2 EIA Kit (Cayman Chemical). Sensitivity of the system was 20 pg/ml.

Cell protein

Total cellular protein in solubilized HPMC monolayers was analyzed with biecinchonic acid (BCA) protein assay (Pierce, Rockford, IL, USA) using bovine serum albumin (BSA) as the standard. Repeated cell counts revealed that 1 μg of HPMC protein corresponded to mean ± standard deviation (SD) 2.08 ± 0.89 × 10^3 cells under control conditions. The concentrations of released IL-6 and PGE2 were normalized per 1 μg of cell protein.

Statistical analysis

All statistical analyses were performed using GraphPad Prism™ 4.00 software (GraphPad Software Inc., San Diego, CA, USA). The n number (as indicated in figure legends) represents the number of experiments performed with cells from different donors. Comparisons were made with either repeated measures or two-way analysis of variance (ANOVA), where appropriate. A p value < 0.05 was considered significant. Results are presented as means ± standard error of the mean (SEM).

Results

Effect of Aminobic on heat-shock response in HPMC

As described previously [16], acute exposure of HPMC to conventional low-glucose-based PD solution resulted in a time-dependent increase in HSP-72 expression. In contrast, the expression of HSP-72 in Aminobic-treated cells was much lower and did not differ from that seen in control cells (Fig. 1).

Effect of Aminobic on HPMC viability

Pretreatment of HPMC with Aminobic for up to 120 min did not significantly impair cell viability when assessed after a 24-h recovery period. The exposure to conventional 1.5% glucose-based solution resulted in a significant time-dependent decrease in HPMC viability (Fig. 2).

Effect of Aminobic on stimulated cytokine release by HPMC

Preexposure of HPMC to Aminobic for up to 120 min did not reduce the ability of cells to release IL-6 in response to IL-1β (1,000 pg/ml). In contrast, pretreatment of HPMC with a low-glucose-based solution resulted in a significant time-dependent reduction in IL-1β-driven IL-6 secretion (Fig. 3).
Effect of Aminobic on basal IL-6 and PGE2 release by HPMC

Pretreatment of HPMC with Aminobic resulted in significant time-dependent increases in constitutive releases of both IL-6 (Fig. 4a) and PGE2 (Fig. 4b). Preexposure to low-

Fig. 1 Effect of amino-acid- and low-glucose-based peritoneal dialysis (PD) solutions on heat-shock response. Human peritoneal mesothelial cells (HPMC) were preexposed for up to 120 min to PD solutions, then allowed to recover in control medium for 24 h and analyzed for heat-shock-protein (HSP) expression. Data were derived from six experiments with HPMC isolated from separate donors. The results are expressed as a percentage of the HSP expression detected in control cells. The statistics shown refer to the difference between the curves as analyzed by two-way analysis of variance (ANOVA).

Fig. 2 Effect of amino-acid- and low-glucose-based peritoneal dialysis (PD) solutions on human peritoneal mesothelial cell (HPMC) viability. HPMC were preexposed to PD solutions for up to 120 min, then allowed to recover in control medium for 24 h and assessed for viability. Data were derived from six experiments with HPMC isolated from separate donors and are expressed as a percentage of control values. The statistics shown refer to the difference between the curves as analyzed by two-way analysis of variance (ANOVA).

Fig. 3 Effect of amino-acid- and low-glucose-based peritoneal dialysis (PD) solutions on interleukin (IL)-1β-induced IL-6 synthesis. Human peritoneal mesothelial cells (HPMC) were preexposed to PD solutions for up to 120 min and then allowed to recover in control medium for 24 h in the presence of IL-1β (1,000 pg/ml). After that, IL-6 release was measured as described in “Methods”. The results were obtained from four experiments with HPMC from different donors. Asterisks represent a significant difference compared with the control at the same time point.

Fig. 4 A+B. Effect of amino-acid- and low-glucose-based peritoneal dialysis (PD) solutions on basal interleukin (IL)-6 and prostaglandin E2 (PGE2) release by human peritoneal mesothelial cells (HPMC). HPMC were preexposed to PD solutions for up to 120 min and then allowed to recover in control medium for 24 h. After that, the release of IL-6 (a) and prostaglandin E2 (PGE2) (b) was measured as described in “Methods”. The results were obtained from nine (a) and 11 (b) experiments with HPMC from different donors. Asterisks represent a significant difference compared with the control at the same time point.
glucose PD solution did not affect basal IL-6 release but increased basal PGE₂ secretion. However, whereas in Aminobic-treated cells a significant rise in PGE₂ was seen after preexposure as short as 30 min, the increase induced by a glucose-based PD solution occurred only after 120 min of preexposure.

Discussion

Biocompatibility of PD solutions is the issue of particular importance for pediatric patients with end-stage renal disease (ESRD) because of the potential long-term dependence of children on the function of peritoneal membrane as a dialyzing organ [17]. Furthermore, children are frequently treated with automated PD with dwell times that may be too short for the acidic pH of lactate PD solutions to fully equilibrate [18]. Moreover, persistent metabolic acidosis is reported to occur commonly in growing children with ESRD and to contribute to uremic complications more significantly than in adults [19]. In addition, hepatic conversion of lactate to bicarbonate in young children may be rather limited, which may reduce the effectiveness of lactate as a buffer [20]. Therefore, bicarbonate-buffered PD solutions were introduced into the pediatric PD setting and showed promising clinical results [21]. The use of bicarbonate may be of potential benefit in amino-acid-containing PD solutions, as these fluids tend to worsen acidosis. Whereas enteral nutrition is a preferred route for correcting malnutrition in ESRD children, the amino-acid-based solutions may be a valuable source of nitrogen without concomitant phosphorus burden [22]. In this study, we assessed acute effects of amino-acid-based bicarbonate-buffered PD solution toward HPMC. For comparison, we chose a conventional solution of similar osmotic strength that contained 1.5% glucose and lactate as a buffer.

Initially, we assessed cellular stress response by measuring the induction of HSP-72, the main effector in cell-protein repair machinery. We previously demonstrated that out several HSP species, HSP-72 was particularly affected by exposure of HPMC to PD solutions and the pattern of HSP-72 expression could identify bioincompatible components of PD solutions [23]. The finding of the study reported here that exposure to Aminobic did not affect HSP-72 response and did not acutely impair HPMC viability points to improved biocompatible profile of the solution. At first glance, these data appear to be in line with those of Chang et al. [24], who also found no up-regulation of HSP in response to amino-acid solution. Surprisingly, however, these authors also found no significantly increased expression of HSP in cells exposed to the conventional PD solution with acidic pH and high GDP contents. In sharp contrast, we previously described a clear induction of mesothelial HSP-72 in either in vitro, ex vivo, or in vivo models of PD, with low pH and GDP being the most potent HSP inducers [16, 23]. These differences may be related to details of the experimental design. Chang et al. [24] used PD-effluent-derived HPMC obtained from only few uremic patients. Such cells are primed in vivo both by uremia and PD fluids and may be at various stages of epithelial-to-mesenchymal conversion and display different functional properties [25]. In contrast, we used more homogenous populations of omentum-derived HPMC obtained from at least six nonuremic donors.

In addition to reduced stress response, Aminobic-treated HPMC showed better viability as assessed by the MTT assay. Similarly, beneficial effects of amino-acid-based PD solutions have been demonstrated in HPMC [24] and HepG2 cells [26] with the use of the lactate dehydrogenase (LDH) test. Interestingly, Plum et al. used the MTT test to detect improved biocompatibility of HPMC after short-term exposure to a bicarbonate-buffered amino-acid PD solution compared with the same solution buffered with lactate [27]. In turn, Chan et al. used the MTT test to assess HPMC proliferation in response to spent dialysate [28]. They found better proliferation of HPMC in the presence of effluent derived from patients treated with the amino-acid-based PD solution compared with low-glucose-based PD fluid. Few in vivo studies assessed biocompatibility of amino-acid-based PD fluids. Zareie et al. have shown less neangiogenesis, fibrosis, and mesothelial damage in rats treated with amino-acid-based solution compared with animals treated with a conventional glucose-based PDF [29]. Similar effects have been demonstrated in rabbits by Garosi et al. [30].

IL-6 production in response to IL-1β stimulation has been shown to serve as a good parameter of HPMC function following exposure to PD solutions [12]. In our study, the conventional PD solution indeed produced a time-dependent decrease in stimulated IL-6 release. In contrast, the magnitude of IL-1β-stimulated IL-6 release by Aminobic-treated HPMC was similar to that in control cells. Interestingly, the incubation of cells with Aminobic resulted in a significant increase in unstimulated IL-6 secretion. A similar effect has been reported by Chang et al. [24]. Plum et al. [31] and Martikainen et al. [32] who detected increased intraperitoneal levels of IL-6 in patients receiving amino-acid-based solutions and linked this effect to better preservation of HPMC. Moreover, it has been demonstrated that the dialysate from patients treated with amino-acid-based solutions contained increased concentrations of PGE₂ [31, 33]. One may hypothesize that those increased PGE₂ levels were of mesothelial origin. In the context of other data presented here, increased dialysate PGE₂ and IL-6 levels could be interpreted as reflecting better biocompatibility of amino-acid-based PD solution.
toward HPMC rather than inducing intraperitoneal inflammatory response. Interestingly, we could show that HPMC exposed to Aminobic secreted constitutively more PGE2 not only than HPMC treated with low-glucose solution, but also significantly more than control cells. The precise mechanism by which Aminobic stimulates PGE2 secretion remains to be determined. It may be related both to the presence of an individual amino acid and to an altered ratio between various amino acids. In this respect, Reimann et al. demonstrated that amino-acid-based PD solutions stimulate nitric oxide (NO) production by HPMC, and this effect is primarily mediated by arginine [34]. This observation is interesting given that there is a close molecular link between NO and prostaglandin pathways. For example, it has been demonstrated that NO can regulate the activity of cyclooxygenase, a key enzyme in the synthesis of prostaglandins [35].

Taken together, our data suggest an excellent in vitro mesothelial biocompatibility profile of the amino-acid-based PD solution, as visualized by sustained viability and cytokine biosynthetic capacity, as well as by negligible cellular stress response. One may hope that future studies will show similar benefits in the clinical setting.

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Reference


