In vivo and in vitro effects of amino-acid-based and bicarbonate-buffered peritoneal dialysis solutions with regard to peritoneal transport and cytokines/prostanoids dialysate concentrations

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Abstract

Background. Increasing evidence suggests that conventional PD solutions are detrimental to host defence mechanisms of peritoneal cells. We tested a new amino-acid-based and bicarbonate-buffered PD solution under in vivo and in vitro conditions.

Methods. During a prospective, cross-over randomized, intraindividual study 10 CAPD patients were investigated with three different solutions: Amino/Bic, 1% amino acid, 34 mmol/l bicarbonate; Glu/Bic, 1.5% glucose, 34 mmol/l bicarbonate; and Glu/Lac, 1.5% glucose, 35 mmol/l lactate. A PET was performed and transport properties (clearance, D/P ratio, MTAC) were calculated. Prostanoid and cytokine concentrations were measured in serum and the 6 h effluent. Using an in vitro model, mononuclear leukocytes of healthy donors were also incubated with the test fluids.

In vivo results. Peritoneal clearance and MTAC of small solutes (creatinine, urea) were not significantly altered by amino acids or bicarbonate. Peritoneal permeability and transperitoneal excretion of higher-weight protein molecules (β2-microglobulin, albumin, IgG) were increased with Amino/Bic compared to Glu/Lac (P<0.05) (D/P ratio albumin: Amino/Bic, 0.027 ± 0.003; Glu/Bic, 0.023 ± 0.003; Glu/Lac, 0.022 ± 0.002). Application of Amino/Bic was accompanied by an increased effluent concentration of IL-6, IL-8, TNFα, PGE2, and 6-keto-PGF1α (P<0.05). Dialysate nitrite/nitrate and cGMP concentrations (as indicators of NO generation) did not differ between the solutions.

In vitro results. Both bicarbonate fluids demonstrated a better preservation of the mitochondrial dehydrogenases activity (MTT assay) compared to Glu/Lac (P<0.01) (Amino/Bic: 80.6 ± 3.2%; Glu/Bic: 86.0 ± 1.8%; Glu/Lac, 64.9 ± 2.3%, referred to RPMI as control). Constitutive and LPS stimulated release of II-1β and II-6 was less suppressed with both bicarbonate fluids (P<0.05) (LPS-stim. IL-6 release: Amino/Bic, 33.0 ± 6.6%; Glu/Bic, 65.5 ± 10.3%; Glu/Lac, 1.5 ± 0.7% referred to RPMI).

Conclusion. Application of an amino-acid/bicarbonate solution resulted in a small but significant increase in peritoneal permeability. Also increased concentrations of various cytokines/prostanoids were measured in the effluent. According to in vitro testing with mononuclear phagocytes both bicarbonate-buffered fluids were to the same extent less inhibitory to certain cell functions than lactate-buffered solution.

Key words: peritoneal dialysis fluids; peritoneal transport; bicarbonate; lactate; cytokines; prostanoids

Introduction

Despite the fact that continuous ambulatory peritoneal dialysis (CAPD) is a safe and well-established treatment method for patients with end-stage renal failure, the question of bioincompatibility of conventional dialysis solutions has stimulated increasing interest in recent years. In vitro experiments gave evidence that conventional glucose/lactate-containing dialysis fluids have an adverse effect on peritoneal host defence [1] while clinical findings demonstrated a negative influence on patients’ glucose, lipid, and protein metabolism [2–4].

In terms of peritoneal host defence all resident (mesothelial cells, fibroblasts) and transient (macrophages, neutrophils) cell populations in the peritoneal cavity interact by a network of immune mediators, which seem to be decisive for the potential prevention or successful limitation of peritonitis [5,6]. The inhibitory effects of dialysis solutions on peritoneal host defence have been recognized as their low pH especially in combination with lactate as a buffer [7,8], their hyperosmolality [9,10], and their high glucose concentration [4,11]. Earlier in vitro studies underlined the cytotoxic and inhibitory effects of conventional fluids.

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on fundamental cell functions of macrophages and polymorphonuclear granulocytes concerning their phagocytic capacity [9,12], the oxidative burst mechanism [13], and their ability to secrete inflammatory cytokines and prostanooids [11,14–15]. More recently research has focused on the role of peritoneal mesothelial cells and fibroblasts [16–18], and in vitro investigations have demonstrated a harmful effect on these cells, too [19–21].

The clinical important issues are metabolic alterations due to the use of glucose as an osmotic agent. The high glucose absorption from the dialysate is associated with hyperinsulinaemia [4] and hypertriglyceridaemia [2], which are common findings of the so-called metabolic syndrome that could be defined as a risk factor for cardiovascular morbidity and mortality. A loss of appetite and reduced protein intake leading to malnutrition [1] is another negative aspect of the high glucose load.

For these reasons it seemed to be rational consequence to replace glucose as the osmotic agent and in a second step to replace the buffer lactate (35–40 mmol/l) to avoid an acidic dialysate pH (about 5.5). In vivo studies demonstrated that bicarbonate provided a physiological pH, was well tolerated, and guaranteed adequate dialysis [22]. It was superior to lactate-buffered fluids in terms of biocompatibility as demonstrated by in vitro cell culture experiments [11,23].

Amino acids are reported to be well tolerated and to behave similarly to glucose with regard to ultrafiltration rates and clearance capacities [4,24,25]. They have been shown to improve the nutritional status of malnourished CAPD patients, compensating for protein and amino acid losses during CAPD, and to have a positive effect on hypertriglyceridaemia [2,3]. However, clinical studies gave conflicting results about changes in peritoneal permeability [2,24,27,28] This point remains questionable and has not been investigated so far with regard to bicarbonate-buffered (pH 7.4) amino-acid solutions. Little is also known about biocompatibility patterns of amino acids. In vitro studies with amino acid solutions gave some divergent results, either with a negative impact on macrophage function and metabolism [29] or even showing superiority with regard to conventional solutions [30].

We report about a new amino-acid-based and bicarbonate-buffered PD solution with regard to its influence on peritoneal transport tested by an intradividual, randomized, cross-over study design in a clinical application phase. Additionally the solutions were exposed to isolated mononuclear cells in an in vitro model. Under both conditions the release of proinflammatory/vasodilatory factors (cytokines and prostanooids) was monitored.

Subjects and methods

Dialysis solutions

The following dialysis solutions were used: Amino/Bic, 1% amino acid solution with 34 mmol/l bicarbonate; Glu/Bic, 1.5% glucose solution with 34 mmol/l bicarbonate; Glu/Lac, conventional 1.5% glucose solution with 35 mmol/l lactate (Table 1).

Osmolarity of all solutions ranged within narrow limits (358–369 mosmol/l), the composition of electrolytes was identical. pH was low in the conventional lactate-buffered solution (pH 5.2–5.5) and near to physiological levels in both bicarbonate-buffered solutions (pH 7.2–7.6). The bicarbonate fluids were provided in a double-chamber bag to avoid calcium–carbonate precipitation. The two components were mixed immediately prior to use.

In vivo study

Patients

Ten non-diabetic CAPD patients participated in three 6-h dwell sessions with each test solution. All patients (4 females/6 males) were stable on CAPD and had been free of peritonitis for more than 6 weeks. Mean age was 46.8 ±11.5, BMI 21.9 ±3.4 (kg/m²). The residual diuresis was less than 1000 ml/day. All patients had a Kt/V of more than 1.7 and a weekly creatinine clearance of more than 50 l/week.

Study design and analytics

The trial was designed as a prospective, cross-over randomized, intraindividual study with three different PD solutions. Patients were studied once with each solution after an overnight fast for a 6-h dwell in randomized order. The intervals between the test phases with each solution ranged from 1 to 3 weeks, during which patients used their conventional dialysate. After a 60 min dwell time patients received a standard breakfast of 730 kcal with 74 g carbohydrates. Venuous blood and dialysate samples were taken immediately after the dwell time.

Table 1. Composition of the different PD solutions with amino-acid composition in Amino/Bic

<table>
<thead>
<tr>
<th>Composition</th>
<th>Amino/Bic</th>
<th>Glucose/Bic</th>
<th>Glucose/Lac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids (g/l)</td>
<td>10.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Glucose (g/l)</td>
<td>15.0</td>
<td>15.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Lactate (g/l)</td>
<td>—</td>
<td>34.0</td>
<td>34.0</td>
</tr>
<tr>
<td>Bicarbonate (g/l)</td>
<td>34.0</td>
<td>34.0</td>
<td>34.0</td>
</tr>
<tr>
<td>Sodium (mmol/l)</td>
<td>137.0</td>
<td>134.0</td>
<td>134.0</td>
</tr>
<tr>
<td>Calcium (mmol/l)</td>
<td>1.25</td>
<td>1.25</td>
<td>1.25</td>
</tr>
<tr>
<td>Magnesium (mmol/l)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Chloride (mmol/l)</td>
<td>103.5</td>
<td>103.5</td>
<td>103.5</td>
</tr>
<tr>
<td>Osmolarity (mosmol/l)</td>
<td>369.0</td>
<td>361.0</td>
<td>359.0</td>
</tr>
<tr>
<td>pH</td>
<td>7.2–7.6</td>
<td>7.2–7.4</td>
<td>5.2–5.5</td>
</tr>
</tbody>
</table>

Amino/Bic

Non-essential amino acids (mmol/l) | Essential amino acids (mmol/l)
|——|——|
| L-Alanine | 6.73 | L-Histidine | 3.222 |
| L-Arginine | 2.698 | L-Isoleucine | 5.72 |
| L-Glycine | 5.99 | L-Leucine | 8.61 |
| Prolin | 3.91 | L-Lysine | 7.18 |
| L-Serine | 4.76 | L-Methionine | 2.681 |
| L-Taurin | 0.799 | L-Phenylalanine | 3.33 |
| L-Tyrosin | 0.552 | L-Threonine | 7.05 |
| L-Tryptophan | 2.01 | L-Valine | 14.51 |

Amino-acid-based bicarbonate PD solution
before and during the dwell at various time points (0, 30, 60, 120, 240, 360 min).

Osmolarity, pH, creatinine (corrected for glucose interference) and urea were determined in dialysate and plasma by standard laboratory methods. Proteins (β2-microglobulin, albumin, IgG) were determined by immunonephelometry (Behring Nephelometer 100, Behring, Marburg, Germany). Blood and dialysate samples for cytokine and prostanoid measurement were cooled after collection and immediately centrifuged at 3000 g for 10 min at 4 °C to remove cellular components. Blood samples for prostaglandin measurements were stabilized adding EDTA and diclofenac-sodium. Supernatants were stored at −70 °C for further analysis.

The arachidonic acid metabolites PGE₂, 6-keto-PGF₁α and thromboxane B₂ were determined by enzyme immunoassays after sample extraction (C₂, C₁₈ solid phase columns) (Biotrak-Enzyme immunoassay, Amersham, Buckinghamshire, UK); 5 ml plasma and 15 ml dialysate were extracted (lower detection limit 1.0, 0.5, 0.5 pg/well resp.). The samples for cytokine measurement were centrifuged immediately and stored at −70 °C. The cytokines II-1β (Quantikine High Sensitivity, R&D Systems Europe, Abingdon, UK), TNFα, II-6 (Cytoscreen ELISA UltraSensitiv, Laboserv GmbH, Giessen, Germany), II-8 (Cytoscreen ELISA, Laboserv GmbH, Giessen, Germany), II-10 (Sigma, St Louis, USA) and II-12 were determined using sandwich ELISAs (lower detection limit 0.125, 0.5, 0.156, 15.6 pg/ml resp.). cGMP in the dialysate was measured by a [3H]RIA after acetylation using a second antibody with magnetic beads (lower detection limit 0.5 pmol/ml) (Amersham Buchler, Braunschweig, Germany). The specificity of all assays including potential matrix effects (dialysates) was tested for by sample spiking and dilution. As a parameter of potential NO generation nitrite/nitrate was measured in the dialysate using the Griess reagent.

Ultrafiltration rate was evaluated subtracting the inflow dialysate volume from the dialysate effluent for the 6-h dwell and were corrected for the cumulative sample volumes taken from the dialysate for laboratory analysis (235 ml). One patient had low ultrafiltration during Glu/Bic application due to an incomplete drainage. This data and derived results were classified as drop out. Clearance rates as well as D/P ratios were calculated for creatinine, urea and for protein markers with different molecular weight (β₂-microglobulin, albumin, IgG). Clearance rates were corrected for the solute removal by the dialysate samples taken. Mass transfer area coefficient (MTAC) was determined for small solutes (creatinine, urea) using the following equation [31]:

\[
\text{MTAC} = \frac{V_1 + V_2}{2t_{12}} \times \ln \left( \frac{C_b - C_{D1}}{C_b - C_{D2}} \right)
\]

\[
V_{1,2} = \text{dialysate volume at time point 0 and 6 h}
\]

\[
V = \frac{V_2}{t_2 - t_1} \times \ln \left( \frac{C_b - C_{D1}}{C_b - C_{D2}} \right)
\]

\[
C_b = \text{blood concentration of solute}
\]

\[
C_{D1,2} = \text{dialysate concentration at time point 0 and 6 h}
\]

In vitro study

Preparation of cells. Monocytes were isolated from buffy coats of healthy volunteers (n = 11). Citrated blood was diluted 1:1 with PBS-Bufet (pH: 7.3, 288 mosmol/kg) and centrifuged on a Ficoll Paque (Pharmacia, Uppsala, Sweden) density gradient. The interphase cells were washed with PBS-buffer and resuspended in RPMI 1640 (Biochrom, Berlin, Germany) with supplements. At a final concentration of 2 × 10⁶ cells/ml the cell suspension was incubated for 90 min at 37 °C in a humidified 5% CO₂ atmosphere. After washing with prewarmed PBS buffer the cells were incubated overnight at 4°C to detach the adherent monocytes. After gently shaking off the monocytes and again washing with PBS the cells were resuspended in PBS buffer and adjusted to a concentration of about 3000 cells/μl. Viability was assessed by the trypan blue exclusion test. Less than 5% trypan-blue-positive cells were judged mandatory.

38.0 ± 3.7% of the isolated cell were monocytes according to a naphthyl-acetate-esterase staining (Sigma, Deisenhofen, Germany).

Test procedure. The monocytes (200 000 cells/ml) were incubated with the test fluids for 45 min in 24-well plates (cytokine assay) or 96-well plates (MTT assay). Response curves for cytokine release and formazan generation were established during initial time-course experiments with incubation periods between 0 and 60 min. Forty-five minutes of incubation turned out to be adequate to register both the influence of pH and of osmotic agents in the dialysate. Exposure times to PDF vary from study to study trying to simulate the intraperitoneal in vivo situation. In general either 15 to 30 min for assessing the effect of buffers [7,13] or even up to 2 h prior to assessment of cell function when testing osmotic agents and/or buffers were reported [32]. The same volume of supplemented RPMI 1640 was added for a further 24 h or 4 h (MTT) incubation period at 37 °C, 5% CO₂ (humidified) to imitate the conditions in the peritoneal cavity with subsequent equilibration of pH and osmolarity and with increasing concentration of nutritional factors [33]. After addition of RPMI 1640 pH levels ranged between 7.3 and 7.8 and osmolarity was between 290 and 330 mosmol/l.

Cell viability was judged again after 4 h or 24 h with the trypan blue test, indicating a survival rate of > 90%.

MTT assay. MTT (Sigma, St Louis, USA) was dissolved in PBS buffer at 8 mg/ml and filtered to sterilize and remove insoluble residue; 10 μl of the cell suspension (3000 cells/μl in PBS) was incubated with 75 μl test sample. After 45 min, 10 μl of the MTT reagent and 75 μl RPMI 1640 (including supplements) was added. After a 4-h incubation SDS plus 0.01N HCl was added and the culture plates were incubated overnight to dissolve the formazan product [34]. Extinctions were measured with an ELISA reader.

Cytokines. For this measurement, 1130 μl of the test sample was added to 80 μl cell suspension, 20 μl diluted Pen/Strep (final concentration 1% v/v) and 20 μl of PBS buffer with or without LPS (Sigma, St Louis, USA) from E. coli serotype 055:B5 (final concentration of 10 ng/ml; pilot experiments were done to establish a dose-response curves for LPS, 0–100 ng/ml). After 45 min incubation, 1250 μl supplemented RPMI 1640 was added and the cells were incubated for 24 h. Supernatants were centrifuged at 0°C and stored at −70 °C for further analysis.

The endotoxin concentration in all solutions was measured with the limulus amoebocyte lysate QCL-1000 assay from Bio Whittaker (Boehringer, Ingelheim, Germany). In all PD test solutions and media the endotoxin level was determined to be low (≤ 2 U/l).

Statistics

Results are expressed as mean ± SEM. Statistical analysis was performed by Student’s paired t test and by Wilcoxon
test for prostanoids and cytokines due to a non-parametric distribution. Bonferroni correction was applied. Linear regression analysis was performed according to Spearman. Statistical significance was accepted if $P < 0.05$.

## Results

### In vivo study

#### Clearance and permeability

Ultrafiltration rates after a 6-h dwell were not significantly different between the three fluids (range $-68 \pm 68$ ml/h to $51 \pm 30$ ml/h), neither did peritoneal clearance rates for urea and creatinine significantly differ between solutions. The effect of the tested PD solutions on small-solute diffusive transport measured by the mass transfer area coefficient (MTAC), also showed comparable efficacy (Table 2). Concerning the peritoneal permeability of higher weight protein molecules, however, the Amino/Bic solution induced an increased permeability (D/P ratio for $\beta_2$-microglobulin, albumin, IgG: Amino/Bic > Glu/Lac, $P < 0.05$; and D/P ratio for $\beta_2$-microglobulin: Amino/Bic > Glu/Bic, $P < 0.05$). The absolute excretion rates of total protein and protein marker molecules showed a comparable pattern with a total protein excretion rate per exchange of $3.05 \pm 0.33$ g in Amino/Bic, $2.77 \pm 0.36$ g in Glu/Bic, and $2.70 \pm 0.25$ g in Glu/Lac (Amino/Bic vs Glu/Bic $P < 0.05$) (Table 3).

#### Prostanoids and cytokines

Whereas serum levels of all measured prostanoids and cytokines did not significantly differ between all solutions (Figure 1), Amino/Bic instillation was accompanied by a higher concentrations of various prostanoids and cytokines in the dialysate. The concentrations of 6-keto-PGF$_{1\alpha}$ and PGE$_2$ in the dialysate after the 6-h dwell were significantly higher with Amino/Bic compared to both glucose dialysates. Due to high interindividual variance a statistical difference between Glu/Bic and Glu/Lac could only be found for TxB2, even though prostanoid release with Glu/Bic also tended to be somewhat higher compared to Glu/Lac (Figure 1).

The release of IL-1β into the dialysate was similar for all solutions (about 0.8 pg/ml, Figure 1). The dialysate levels of TNF$_2$ were significantly increased in the Amino/Bic effluent compared to the bicarbonate or lactate buffered glucose solution ($P < 0.05$). However, dialysate levels for both cytokines did not surpass the corresponding serum concentrations.

The differences in cytokine release were even more marked for IL-6 and IL-8. After the 6-h dwell Amino/Bic had a significantly higher IL-8 effluent concentration ($172.7 \pm 65.7$ pg/ml) than Glu/Bic and Glu/Lac, that fell near or below the detection limit of the assay ($<15$ pg/ml). IL-6 levels in the amino-acid dialysate (Amino/Bic, $492.9 \pm 121.9$ pg/ml) were also significantly increased after the 6-h dwell compared to the glucose-based fluids (Glu/Bic, $143.8 \pm 27.3$; Glu/Lac, $104.5 \pm 23.5$; Table 5). The dialysate concentrations of IL-8 and IL-6 far exceeded the serum levels (Figure 1).

Some correlations could be found between the peritoneal permeability to different molecular weight proteins ($\beta_2$-microglobulin, albumin, IgG) and the dialysate concentration of the measured mediators, especially with regard to Amino/Bic. However, the correlations were weak and did not reach statistical significance in most cases due to the high variance of the prostanoid and cytokine concentrations. The D/P ratio of IgG and the 6-h effluent concentration of IL-6 with Amino/Bic were correlated best ($r = 0.571$; $P < 0.05$).

#### Table 2. Ultrafiltration rates, peritoneal creatinine clearances and mass transfer area coefficients (MTAC) for creatinine after a 6-h dwell using Amino/Bic, Glu/Bic and Glu/Lac

<table>
<thead>
<tr>
<th></th>
<th>Amino/Bic</th>
<th>Glu/Bic</th>
<th>Glu/Lac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrafiltration (ml/min)</td>
<td>$-68 \pm 68$</td>
<td>$51 \pm 30$</td>
<td>$-2 \pm 42$</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>$4.9 \pm 0.6$</td>
<td>$5.1 \pm 0.6$</td>
<td>$4.8 \pm 0.5$</td>
</tr>
<tr>
<td>MTAC for creatinine (ml/min)</td>
<td>$13.8 \pm 1.2$</td>
<td>$12.8 \pm 1.4$</td>
<td>$12.5 \pm 4.0$</td>
</tr>
</tbody>
</table>

Mean values ± SEM are shown ($n = 10$). No significance.

#### Table 3. Effect of Amino/Bic, Glu/Bic and Glu/Lac on the peritoneal protein loss (protein marker molecules with increasing MW) and on D/P ratios during a 6-h dwell

<table>
<thead>
<tr>
<th></th>
<th>Amino/Bic</th>
<th>Glu/Bic</th>
<th>Glu/Lac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (mg/exchange)</td>
<td>$3050 \pm 330^*$</td>
<td>$2770 \pm 360$</td>
<td>$2700 \pm 250$</td>
</tr>
<tr>
<td>$\beta_2$-microglobulin (mg/exchange)</td>
<td>$13.6 \pm 1.0^*$</td>
<td>$11.2 \pm 1.1$</td>
<td>$11.0 \pm 0.8$</td>
</tr>
<tr>
<td>D/P ratio</td>
<td>$0.233 \pm 0.025^*$</td>
<td>$0.199 \pm 0.026$</td>
<td>$0.192 \pm 0.016$</td>
</tr>
<tr>
<td>Albumin (mg/exchange)</td>
<td>$1638.9 \pm 146.6^*$</td>
<td>$1533.9 \pm 160.6$</td>
<td>$1368.2 \pm 97.1$</td>
</tr>
<tr>
<td>D/P ratio</td>
<td>$0.027 \pm 0.003^*$</td>
<td>$0.023 \pm 0.003$</td>
<td>$0.022 \pm 0.002$</td>
</tr>
<tr>
<td>IgG (mg/exchange)</td>
<td>$310.3 \pm 37.8^*$</td>
<td>$291.5 \pm 42.4$</td>
<td>$252.8 \pm 33.4$</td>
</tr>
<tr>
<td>D/P ratio</td>
<td>$0.016 \pm 0.002^*$</td>
<td>$0.013 \pm 0.002$</td>
<td>$0.012 \pm 0.001$</td>
</tr>
</tbody>
</table>

$^*P < 0.05$ for Amino/Bic vs Glu/Lac; $^P < 0.05$ for Amino/Bic vs Glu/Bic.

Mean values ± SEM are shown ($n = 10$).

### Fig. 1. The concentrations of prostanoids (PGE$_2$, TxB2 and 6-keto-PGF$_{1\alpha}$) and of cytokines [IL-1β, TNF$_2$, IL-6, and IL-8] in the 6-h effluent and in the serum when using Amino/Bic, Glu/Bic, and Glu/Lac. The concentrations of vasoactive and potentially permeability-modulating prostanoids and cytokines with high dialysate levels are shown in the figure. Mean values ± SEM are shown ($n = 10$).
### Prostanoids:

<table>
<thead>
<tr>
<th>Prostanoid</th>
<th>Amino/Bic</th>
<th>Glu/Bic</th>
<th>Glu/Lac</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{PGE}_2 ) (pg/ml)</td>
<td>96.8 ± 25.8</td>
<td>112.3 ± 31.1</td>
<td>134.9 ± 32.1</td>
</tr>
<tr>
<td>Serum</td>
<td>59.4 ± 19.4**</td>
<td>31.9 ± 13.5</td>
<td>4.8 ± 2.6</td>
</tr>
<tr>
<td>Dialysate</td>
<td>45.5 ± 8.7</td>
<td>45.2 ± 8.5</td>
<td>34.7 ± 5.6</td>
</tr>
<tr>
<td>( \text{TxB}_2 ) (pg/ml)</td>
<td>13.7 ± 3.5*</td>
<td>8.9 ± 2.4*</td>
<td>1.6 ± 0.7</td>
</tr>
<tr>
<td>Serum</td>
<td>12.1 ± 2.5</td>
<td>14.0 ± 2.5</td>
<td>14.4 ± 2.7</td>
</tr>
<tr>
<td>Dialysate</td>
<td>38.6 ± 16.2**</td>
<td>20.8 ± 10.7</td>
<td>7.8 ± 2.5</td>
</tr>
</tbody>
</table>

### Cytokines:

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Amino/Bic</th>
<th>Glu/Bic</th>
<th>Glu/Lac</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{II-1ß} ) (pg/ml)</td>
<td>0.78 ± 0.16</td>
<td>1.22 ± 0.33</td>
<td>0.77 ± 0.16</td>
</tr>
<tr>
<td>Serum</td>
<td>0.81 ± 0.11</td>
<td>0.81 ± 0.16</td>
<td>0.86 ± 0.27</td>
</tr>
<tr>
<td>Dialysate</td>
<td>1.74 ± 0.79</td>
<td>1.32 ± 0.30</td>
<td>1.18 ± 0.27</td>
</tr>
<tr>
<td>( \text{TNF}_x ) (pg/ml)</td>
<td>1.29 ± 0.13**</td>
<td>0.45 ± 0.05</td>
<td>0.38 ± 0.07</td>
</tr>
<tr>
<td>Serum</td>
<td>4.9 ± 0.9</td>
<td>4.8 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Dialysate</td>
<td>492.9 ± 121.9**</td>
<td>143.8 ± 27.3</td>
<td>104.5 ± 23.5</td>
</tr>
<tr>
<td>( \text{II-8} ) (pg/ml)</td>
<td>18.0 ± 14.6</td>
<td>19.1 ± 12.2</td>
<td>&lt; 15</td>
</tr>
<tr>
<td>Serum</td>
<td>172.7 ± 65.7**</td>
<td>&lt; 15</td>
<td>&lt; 15</td>
</tr>
</tbody>
</table>

\* * \* \* \* \( P < 0.05, P < 0.01 \) for Amino/Bic vs Glu/Lac.

\( \# \# \# \: P < 0.05, P < 0.01 \) for Amino/Bic vs Glu/Bic.

\( ^{-} P < 0.05 \) for Glu/Bic vs Glu/Lac.
cGMP after a 6-h dwell were 25.7 ± 4.1 pmol/ml in Amino/Bic, 20.8 ± 3.3 pmol/ml in Glu/Bic, and 20.3 ± 2.5 in Glu/Lac (n.s.). With serum concentrations ranging higher and being at equal levels in all test phases (26.1–30.3 pmol/ml) there was no clear indication of an active intraperitoneal cGMP secretion or differences in transperitoneal transport (D/P ratios, MTAC). Nitrite/nitrate concentrations in the dialysates were comparable, with 0.46 ± 0.12 μg/ml for Amino/Bic, 0.34 ± 0.11 μg/ml for Glu/Bic, and 0.39 ± 0.08 μg/ml for Glu/Lac (n.s.).

**In vitro study**

**IL-1β.** Constitutive and LPS stimulated release of IL-1β from mononuclear phagocytes was highest in the RPMI control medium (Table 4). Unstimulated release of IL-1β was less suppressed after incubation with both bicarbonate-buffered fluids compared to the lactate fluid. LPS stimulated release of IL-1β was significantly higher in both bicarbonate-buffered fluids compared to Glu/Lac: RPMI, 2015 ± 329; Amino/Bic, 1166 ± 192; Glu/Bic, 752 ± 107; Glu/Lac, 174 ± 51 pg/ml (Table 4). No difference was found between the bicarbonate solutions.

**IL-6.** Again constitutive and stimulated release of IL-6 was highest in RPMI control medium (Table 4). Constitutive and LPS stimulated release of IL-6 into the cell supernatant was significantly less suppressed in both bicarbonate solutions compared to Glu/Lac (LPS stimulation: RPMI, 1312 ± 180; Amino/Bic, 409 ± 88; Glu/Bic, 827 ± 196; Glu/Lac, 12 ± 3 pg/ml).

**MTT test.** Incubation of monocytes in RPMI medium resulted in the highest cellular formazan generation and was used as internal control (100% reference value, Table 4). Both bicarbonate-buffered CAPD fluids demonstrated a significantly better preserved (P < 0.01) activity of the mitochondrial dehydrogenases compared to Glu/Lac (Amino/Bic, 80.6 ± 3.2%; Glu/Bic, 86.0 ± 1.8%; Glu/Lac, 64.9 ± 2.3%). No significant difference was observed between Amino/Bic and Glu/Bic.

**Discussion**

The present study confirms that an amino-acid solution guarantees adequate dialysis efficacy. Clearance capacities, ultrafiltration rates, and mass transfer coefficients for small solutes were similar in all three solutions. Ultrafiltration tended to be only slightly (n.s.) reduced with amino acids which appeared to be clinically irrelevant. Peritoneal small-solute equilibration was also not affected when comparing bicarbonate and lactate as a buffer (Glu/Lac vs Glu/Bic).

However, in accordance with some earlier reports [3,27] we found that the instillation of the amino-acid solution was accompanied by a small but significantly increased peritoneal permeability to higher-weight molecules. The D/P ratios of β₂-microglobulin, albumin, and IgG were increased. The absolute excretion rates per exchange of these marker proteins were also enhanced especially when comparing Amino/Bic with Glu/Lac. Significance for Amino/Bic against Glu/Bic was only found for β₂-microglobulin, due to higher variances in the latter group. From the clinical point of view, with regard to nutritional aspects, the small increase of transperitoneal protein loss was not relevant in the light of a clearly positive protein nitrogen balance that was achieved by amino acid absorption from the dialysate (data not shown).

Steinhauer et al. also found enhanced D/P ratios for protein markers when using a combined 2.6% amino acid/0.55% glucose solution buffered with lactate. They attributed his finding to an increased effluent concentration of PGE₂ [27]. But other studies did not confirm

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**Table 4.** Constitutive and LPS stimulated IL-1β and IL-6 release in the supernatants of mononuclear phagocytes (200 000/ml) after 45 min incubation with Amino/Bic, Glu/Bic, and Glu/Lac. MTT-formazan formation is tested as a global parameter of cell viability (mitochondrial aerobic metabolism). RPMI 1640 is serving as the internal reference control.

<table>
<thead>
<tr>
<th>Reference medium</th>
<th>Test solutions</th>
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<td>RPMI</td>
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**Blood PMN cytokine release (pg/ml)**

IL-1β

- Constitutive: 720 ± 194 vs 449 ± 128** (Amino/Bic) vs 297 ± 60 (Glu/Bic) vs 169 ± 46 (Glu/Lac).
- LPS stimulation: 2015 ± 329 vs 1166 ± 192** (Amino/Bic) vs 752 ± 107** (Glu/Bic) vs 174 ± 51 (Glu/Lac).

IL-6

- Constitutive: 674 ± 229 vs 282 ± 117** (Amino/Bic) vs 441 ± 143 (Glu/Bic) vs 14 ± 2 (Glu/Lac).
- LPS stimulation: 1312 ± 180 vs 409 ± 88** (Amino/Bic) vs 827 ± 196 (Glu/Bic) vs 12 ± 3 (Glu/Lac).

**Mitochondrial aerobic metabolism**

- MTT assay: Reference, 100% vs 80.6 ± 3.2%** (Amino/Bic) vs 86.0 ± 1.8%** (Glu/Bic) vs 64.9 ± 2.3% (Glu/Lac).

* P < 0.05; ** P < 0.01 for Amino/Bic vs Glu/Lac.
* P < 0.05, ** P < 0.01 for Amino/Bic vs Glu/Bic.
* P < 0.05, ** P < 0.01 for Glu/Bic vs Glu/Lac.

Mean values ± SEM are shown (n = 11).
these results [24,25]. The study designs used, however, were not always well suited to answer this question. In this study the increase in peritoneal permeability was associated with a marked elevation of potentially permeability increasing mediators (PGE$_2$, 6-keto-PGF$_{1α}$, TNF$_{α}$, IL-6 and II-8) in the dialysate after a 6-h dwell with Amino/Bic [36–38]. Therefore a causal relation seems probable. However, the statistical correlations between the effluent concentrations of the mentioned mediators and the peritoneal permeability markers were often only at the edge of significance. The best correlation could be found for IL-6. This might be due to the low number of patients ($n=10$) and the high inter- and intraindividual variance of prostanoid and cytokine concentrations [39]. The peritoneal transport is influenced by two distinct properties: the effective surface area of the peritoneum, which is dependent on peritoneal blood flow, and the intrinsic permeability to macromolecules. As we did not observe a significant change in the MTAC of low-molecular-weight solutes (creatinine) an influence of Amino/Bic less suppression of cell function, or are the expression and permeability to macromolecules. As we did not observe a significant change in the MTAC of low-molecular-weight solutes (creatinine) an influence of Amino/Bic less suppression of cell function, or are the expression of prostanoids and cytokines appeared to be prevailing. Mican et al. [36] demonstrated a role of II-6 for increased vasopermeability during anaphylactic reactions in the skin of mice and Colletti et al. [37] showed the same properties for TNF$_{α}$ in the human capillary system of the lung. Interestingly Zemel et al. [39] also observed a significant positive correlation between dialysate II-6 concentrations and the intrinsic permeability of the peritoneum in stable CAPD patients without peritonitis. The permeability-increasing effects of prostanoids and cytokines appear to be prevailing. In this study the increase in peritoneal permeability was associated with an elevated permeability to higher-molecular-weight molecules most probably because of an increased intraperitoneal generation of vasoactive mediators. In the case of Amino/Bic the II-8, II-6, and 6-keto-PGF$_{1α}$ effluent concentrations exceeded serum levels indicating active peritoneal synthesis rather than passive diffusion. The dialysate/serum ratios of II-1β and PGE$_2$ and the D/P ratios of protein markers (e.g. β$_2$-microglobulin or albumin which are not synthesized within the peritoneal cavity) did not show any correlation (data not shown), thereby indicating intraperitoneal secretion of these mediators, too, rather than mere diffusive transport [39].

The amino acid solution contained L-arginine which is a substrate for nitric-oxide synthesis. NO (or endothelium-derived relaxing factor) is involved in many vasodilating processes. This may suggest that nitric oxide may also play a role in the regulation of peritoneal permeability. NO activates the intracellular soluble guanylate cyclase, resulting in an increase of cGMP formation. The latter shows a rapid equilibrium with the extracellular space. Thereby cGMP can be measured as an indicator of NO activity. No differences in nitrite/nitrate concentrations (the stable end products of oxidized NO) could be found between the test solutions. Also cGMP dialysate concentrations did not differ between the amino acid and glucose-containing solutions. As cGMP is a relatively small solute it easily diffuses from the blood space into the dialysate. We could not find increased dialysate levels compared to plasma values as an indicator of active intraperitoneal secretion. These findings are in agreement with Douma et al. who also did not find significant intraperitoneal NO or cGMP formation when using an amino acid/lactate dialysate (Nutrineal®). However, they showed an increase in small solute MTAC with this solution with an unchanged restriction coefficient [28]. Unfortunately it is not possible to get a ‘positive’ or ‘physiological’ control in CAPD (since PD is an unphysiological situation per se) indicating whether higher levels of the mentioned mediators with Amino/Bic indicate a more physiological situation, i.e. less suppression of cell function, or are the expression of an underlying inflammatory activity compared to the situation with Glu/Lac. For this reason we have done some further in vitro experiments on monocytes/macrophages.

Incubation of mononuclear phagocytes with both bicarbonate-buffered fluids resulted in higher LPS-stimulated release of II-1β and II-6 compared to Glu/Lac. The constitutive and LPS stimulated cytokine release after incubation of monocytes with both bicarbonate fluids, however, was still lower than in the optimum reference culture medium RPMI 1640. We therefore conclude that Amino/Bic and Glu/Lac did not induce a specific cellular stimulation but led to less inhibition of monocyte response compared to Glu/Lac. Constitutive release of II-1β and II-6 (related to cell number or cell protein concentration) was higher in this study than in other reports [32]. We attribute this finding to the fact that not only PBMC were isolated from the blood, but specifically monocytes, which represent the first line of defence in infection-free PD patients. Adhesion of phagocytes to polystyrol and subsequent detachment was performed, which may have induced a higher basal cellular activation. Nevertheless a manifold cytokine stimulation by LPS could be induced, and revealed marked differences with a clearly suppressed II-1β and II-6 release under incubation with Glu/Lac. Oxidative cell metabolism as assessed with an MTT assay via measurement of mitochondrial dehydrogenases activity showed less cytotoxicity with both bicarbonate-buffered solutions. In contrast to the in vivo results both bicarbonate-buffered fluids, whether glucose or amino acid based, did not differ so much in terms of in vitro IL-6 release (with Glu/Bic even slightly higher) and were to the same extent superior to the lactate-buffered dialysate with regard to stimulated II-1β release.

Taken together our in vitro experiments clearly confirmed the superiority of bicarbonate as a buffer in combination with glucose as well as with amino acids. There are only few reports about in vitro testing of
1–1.5% amino-acid solutions. Schenk et al. [29] found a compromised oxidative metabolism and killing capacity of PMNs with 1% and 1.5% amino acids compared to glucose-based fluids. Jörres et al. [30], however, found a better-preserved TNFα response after LPS stimulation with amino acid/bicarbonate.

Summarizing our in vitro and in vivo results, it is evident that lactate in combination with a low pH seems to be the most relevant inhibitory factor of in vivo and in vitro cytokine and prostanoïd release. In vitro, Amino/Bic and Glu/Bic did not greatly differ in terms of II-1β and II-6 release, while in vivo Amino/Bic was accompanied by a significantly higher release of PGE₂, 6-keto-PGF₁α, II-6, and II-8 into patients' effluent compared to Glu/Bic. This finding does not imply a different behaviour of mononuclear phagocytes in vivo and in vitro. Since most of the mentioned mediators are also of mesothelial origin [16–18], this source of additional release of cytokines and prostanoïds has to be taken into account.

Further in vitro studies are needed to evaluate the effect of amino acid/bicarbonate solutions on mesothelial cells and fibroblasts. Cell culture investigations can only assess the biocompatibility patterns of PD solutions with regard to a special cell line and certain aspects of the peritoneal system depending on the in vitro conditions. In general, they do not implicate the interactions between different cell lines. Efforts to establish three-dimensional cell culture systems or coculture designs may help to overcome this problem [40].

It is very difficult to decide whether increased concentrations of potentially inflammatory mediators can be a sign of local non-bacterial inflammation induced by amino acids, as Steinhauer et al. suggested [27]. With a ‘physiological standard’ missing it may also be a sign of less cellular inhibition and a better-preserved constitutive cytokine/prostanoïd release. Besides it is known that the cellular effects of most cytokines are predominantly regulated on the basis of receptor expression and intracellular signal transmission. The physiological relevance of increased intra-peritoneal cytokine and prostanoïd levels has to be further investigated on this background, focusing on cytokine receptor expression, soluble receptors (receptor shedding), and the cytokine-induced intracellular ‘inflammatory activity’ (NO, oxygen radicals etc.) The concentrations of known anti-inflammatory cytokines (e.g. II-4, II-10) are of future interest, also. Nevertheless it cannot be denied that an upregulation (or even missing suppression) of potentially proinflammatory mediators may bear a risk of enhanced fibrotic mechanisms within the peritoneum [41,42]. However, some available long-term clinical studies with amino acid solutions (lactate buffered) give evidence for stable transport properties of the peritoneum using amino acid solutions for up to 6 months [2,35]. So in our opinion clinical considerations and in vitro data justify a more widespread application of an amino-acid/bicarbonate solution especially in malnourished patients. In vitro and in vivo results are indicative of advantages of bicarbonate instead of a lactate buffer, both with glucose and amino-acid-based solutions. Further basic research on the influence of amino acids and other osmotic agents on the cytokine/prostanoïd network within the peritoneum appears necessary, but should be accompanied by prospective clinical trials.

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