Reduced systemic advanced glycation endproducts in children receiving peritoneal dialysis with low glucose degradation product content

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Abstract

Background. Glucose degradation products (GDP) in peritoneal dialysis (PD) solutions are toxic to the peritoneal membrane and promote the formation of advanced glycation end products (AGE), which contribute to accelerated atherosclerosis and amyloidosis. Double chamber PD solutions have a markedly reduced GDP content.

Methods. We analysed GDP and AGE kinetics in 21 children (7 months to 18 years) on automated PD in a prospective multicentre trial with randomized administration of single chamber, high-GDP and double-chamber, low-GDP dialysis solution for 12 weeks each. Total AGE fluorescence, carboxymethyllysine (CML, ELISA) and 3-deoxyglucosone (3-DG, HPLC) were measured in plasma and PD effluent during a 4 h peritoneal equilibration test. Plasma AGE profiles were assessed by size selective gel permeation chromatography and compared with 23 healthy controls.

Results. Initial effluent 3-DG concentrations were 140 ± 55 and 25 ± 4 μmol/l with high- and low-GDP PD fluid, respectively and declined to 53 ± 32 and 7 ± 2 μmol/l within 4 h dwell time (P < 0.001). The ex vivo AGE generating capacity was three times higher with the high-GDP solution and decreased significantly with dwell time. Plasma AGE levels were 1.8–7.4-fold above those of healthy controls; the elevation was most marked for the small molecular fraction (<2 kDa). Plasma AGE and CML levels were significantly higher after 12 weeks exposure to high-GDP solution (20991 ± 4145 AU and 1505 ± 617 ng/ml) than following treatment with low-GDP fluid (17518 ± 4676 AU and 1151 ± 438 ng/ml; both P < 0.05). Four hour AGE clearance was higher with low-GDP solution (0.74 ± 0.3 vs 0.44 ± 0.15 ml/min/1.73 m2, P < 0.01).

Conclusion. GDP are rapidly absorbed from the peritoneal cavity. Administration of PD solutions with low-GDP content reduces plasma AGE levels and may thus improve the cardiovascular risk profile of dialysed children.

Keywords: advanced glycation endproducts; biocompatibility; children; glucose degradation products; peritoneal dialysis

Introduction

Heat sterilization and prolonged storage of single chamber peritoneal dialysis (PD) solutions result in the formation of high amounts of glucose degradation products (GDP), such as formaldehyde, methylglyoxal and 3-deoxyglucosone (3-DG) [1,2]. When infused into the peritoneal cavity, GDP impair mesothelial cell function [3] and modulate the generation of various cytokines including IL-6 [4], TGF-β [5] and VEGF [6]. Long-term exposure of the peritoneal membrane to GDP-rich PD solutions likely results in progressive deterioration of the mesothelial cell layer, fibrosis and neangiogenesis [7], contributing to ultrafiltration and PD technique failure [8,9].

In addition to these direct deleterious effects, GDPs can bind non-enzymatically to proteins and lipids and form advanced glycation endproducts (AGE). AGE accumulate locally [10] and bind to the AGE receptor (RAGE) on cells in the peritoneal layer. Antibody-mediated inhibition of RAGE can prevent fibrotic changes in the peritoneum, suggesting that local AGE action contributes significantly to the morphological

Apart from their local toxicity, GDP in PD fluids may exert important systemic effects. Zeier et al. [12] demonstrated rapid disappearance of GDP from the peritoneal cavity in adult patients. Double-chambered PD solutions contain glucose separate from the buffer substance at a very low pH, which largely prevents GDP formation during heating sterilization and prolonged storage. The use of low GDP dialysate in adults resulted in a significant reduction of circulating AGE levels within 4 weeks of administration [12]. In children, the relative impact of dialysate GDP on systemic AGE load has not been assessed to date. Different GDP and AGE kinetics might be expected in young, growing organisms secondary to differences in tissue turnover rates. Moreover, the expression pattern of various AGE receptors differs in children from adults, and may result not only in different local action but also different scavenger receptor-mediated endocytotic elimination of AGE [13,14]. Hence, in the work presented here, we investigated in children on chronic PD the peritoneal GDP kinetics and the effects of PD fluids with high or low GDP content on the concentrations and molecular weight distribution of AGE in plasma.

**Subjects and methods**

**Patients**

The study was performed in five paediatric dialysis units in Germany, Austria and France in full compliance with the Declaration of Helsinki and the EU Good Clinical Practice guidelines for clinical trials. Ethical committee approval for the study protocol was obtained in each centre. Written informed consent was obtained from the parents, and assent from the patients. Patients were eligible for the trial if they were 18 years or younger, received chronic automated peritoneal dialysis (APD) treatment with an average peritoneal fill volume of 1000–1100 ml/m² body surface area, and had no severe chronic pulmonary, cardiac, liver or malignant disease, no history of peritonitis within the last 3 weeks and no clinical evidence of major peritoneal adhesions.

Results on solute and water transport of this prospective, randomized crossover trial, comparing lactate-buffered high-GDP and bicarbonate-buffered low-GDP PD fluids, have been published previously [15]. Data for the present analysis were obtained in 21 children (13 boys, 8 girls); intraindividual comparisons from low- and high-GDP dialysis were available in 15 children. Reasons for drop out were transplantation [3] and wish of the family [3]. Median age was 6.8 years (range 7 months to 18 years), duration of PD, 8 (1–78 months) months. Five patients had experienced at least one episode of peritonitis. All episodes were cured at least 8 weeks prior to study entry. Six peritonitis episodes occurred during the study, three with low- and three with high-GDP fluid. The biochemical profile of the patients at study entry is given in Table 1. Residual GFR did not differ in the low- and high-GDP group.

<table>
<thead>
<tr>
<th><strong>Table 1.</strong> Biochemical characteristics of patients at study entry</th>
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<tr>
<td><strong>Mean ± SD</strong></td>
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<tr>
<td>Serum creatinine (mg/dl)</td>
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<tr>
<td>6.8 ± 2.4</td>
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<tr>
<td>Blood urea nitrogen (mg/dl)</td>
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<td>93 ± 18</td>
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<tr>
<td>Haemocrit (%)</td>
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<td>31.5 ± 3.9</td>
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<tr>
<td>Serum albumin (g/l)</td>
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<td>33.8 ± 4.7</td>
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<tr>
<td>Serum alkaline phosphatase (U/l)</td>
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<tr>
<td>534 ± 416</td>
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<tr>
<td>Serum phosphate (mmol/l)</td>
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<td>1.85 ± 0.40</td>
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<tr>
<td>Residual GFR (ml/min/1.73 m²)</td>
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<td>0.84 (0–6.9)</td>
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Control plasma for analysis of AGE levels was obtained from 23 healthy children (10 boys), aged 0.1–15 (median 5) years, with normal findings on physical examination, blood and urine biochemical analyses.

**Study design**

Stable dialysis conditions were assured during a 4-week run-in period. Subsequently, the patients were randomized into two consecutive 12-week crossover study periods separated by a 4-week wash-out period. APD was performed with either a pH-neutral PD fluid containing 34 mM bicarbonate (BicaVera 170/180/190, Fresenius Medical Care, Bad Homburg, Germany) or a conventional PD fluid with 35 mM lactate and pH 5.5 (continuous ambulatory peritoneal dialysis (CAPD) 17/18/19, Fresenius Medical Care, Bad Homburg, Germany). Both solutions contained identical electrolyte concentrations, glucose content was 15, 23 or 42.5 g/l. During the study, all patients maintained their previous APD prescription, which was adapted according to clinical needs.

Every 4 weeks, a physical examination and a laboratory workup were performed. Monthly plasma carboxymethyllysine (CML) concentrations were measured during low-GDP dialysis in 10 patients. At the start of the study and at the end of each 12-week period a standardized peritoneal equilibration test (PET) (using 1000 ml fill volume per square meter of body surface area and 2.4% glucose), dialytic and urine 24 h clearance studies were performed.

**Laboratory analyses**

Blood and dialysate concentrations of glucose, creatinine, urea, electrolytes, inorganic phosphate, albumin, and β2-microglobulin were measured centrally at the laboratory of Heidelberg University Medical Center using standard analytical methods. Dialysate creatinine measurements were corrected for the presence of glucose as described previously [16].

Fluorescent AGE were measured with a spectrofluorimeter (LS 50B, Perkin Elmer, Überlingen, Germany) at 350 nm excitation and 420 nm emission wavelengths. In previous analyses a maximum of absorption and emission has been obtained from both plasma and dialysate samples [17]. Plasma samples were diluted 1:25–1:50 with phosphate-buffered saline (PBS).

Size-selective gel permeation chromatography (GPC) of plasma and dialysate was performed using an isocratic HLC system (Waters, Eschborn, Germany) equipped with a
The relative amount of 3-DG absorbed was similar to the standard peritoneal dialysis fluid (PDF) (<0.001) and to 15% vs 9% compared with control, whereas the absolute amount of 3-DG absorbed with standard GDP solution was more than 4-fold higher (P < 0.05 vs initial dialysate 3-DG concentration).

The concentration of 3-DG was analysed by measuring the specific fluorescence of unused and spent PD fluids that was mirrored the changes in 3-DG in standard PDF. After 1 and 4 h of dwell time, the capacity to generate AGE decreases with dwell time, while AGE generating capacity of low-GDP dialysate is small and independent of dwell time. (P < 0.05 vs unused dialysate).

For the statistical analysis, paired Student’s t-test or by Mann–Whitney rank sum test, in case of non-Gaussian distribution. Paired Student’s t-test and Wilcoxon signed rank test, in case of non-Gaussian distribution, were applied to assess intra-individual changes in patients who had both low and standard GDP dialysis. Associations were examined using Spearman rank order coefficient. To assess the overall effect of the dialysate type on appearance of AGE subgroups in the dialysate, a two-way repeated measure ANOVA was performed.

Results

Peritoneal kinetics of 3-DG

Unused standard PD solution contained 140 ± 55 μmol/l, low-GDP fluid 25 ± 4 μmol/l of 3-DG. After 1 and 4 h of dwell time, 3-DG concentrations were reduced to 128 ± 58 and 53 ± 32 μmol/l in the standard peritoneal dialysis fluid (PDF) (P = ns/ <0.001) and to 15 ± 4/7 ± 2 μmol/l in the low-GDP PDF (P < 0.001, Figure 1). The absolute amount of 3-DG absorbed within 4 h with standard PDF was much greater than with low-GDP fluid (14.2 ± 6.2 vs 2.9 ± 0.7 mg/m²/4h, P < 0.001), the relative amount of 3-DG absorbed was similar (37 ± 15% vs 27 ± 9%, P = ns).

Dialysate ex vivo capacity to generate AGE

AGE generation capacity of spent dialysate, a surrogate parameter of reactive carbonyl compound content, mirrored the changes in 3-DG in standard PDF. Unused standard PDF incubated with albumin for 10 days increased the concentration of fluorescent AGE levels by 35.2 ± 10.7% compared with control, whereas after 1 and 4 h of dwell time the capacity to generate AGE was reduced by 26.4 ± 11.3% and 38.4 ± 8.4% (P < 0.05/0.01 vs unused dialysate). Similar findings were obtained for 3-day incubation intervals. The AGE generating capacity of the low-GDP solution was low (unused solution + 9.9 ± 10% compared with control solution) and independent of dwell time (Figure 2).
Effect of low-GDP PD solution on plasma AGE

In healthy children, total fluorescent plasma AGE concentrations were 391 ± 1008 AU, plasma concentrations of the non-fluorescent AGE CML 281 ± 109 ng/ml and independent of age. In contrast, in children on PD with high-GDP dialysate plasma AGE and CML levels were markedly increased (20 991 ± 4145 AU and 1505 ± 617 ng/ml, each \( P < 0.001 \) vs controls). After 12 weeks of PD with low-GDP dialysate, both plasma AGE and plasma CML concentrations were significantly lower (17 518 ± 4676 AU and 1151 ± 438 ng/ml, each \( P < 0.05 \)). The intra-individual changes in AGE and CML levels are shown in Figure 3. In the patients who returned to high-GDP fluid after 3 months treatment with low-GDP fluid, AGE levels increased by 24% \((-2\sim -29\) and CML by 13% \((-14\sim -30\)\). Changes in plasma AGE and CML levels were highly correlated \((r = 0.81, P < 0.01)\) but independent of dialysis regime, glucose exposure and body surface area. Daily glucose exposure was 155 ± 61 g with standard and 150 ± 53 g with low-GDP dialysate and unchanged after 12 weeks \((152 ± 72 and 150 ± 56 g with standard and low-GDP solution; p = ns)\). Likewise, daily ultrafiltration was similar with both solutions and did not change throughout the study.

Monthly plasma CML levels were obtained in 10 patients during low-GDP dialysis. CML levels declined by 18 ± 36%, 21 ± 21% and 27 ± 21% after 4, 8 and 12 weeks (each \( P < 0.05 \) compared with baseline). Residual GFR remained stable and was not correlated with plasma AGE or CML levels during the observation period.

Peritoneal clearance of AGE

The D/P ratios and clearance rates of small, middle and large molecules during the 4 h PET did not differ between standard and low-GDP fluid. The clearance of phosphate was 4.2 ± 1.2 and 4.4 ± 1.1 ml/min*1.73 m², of creatinine 5.9 ± 1.6 and 5.6 ± 0.8 ml/min*1.73 m², of β2-microglobulin 1.0 ± 0.7 and 1.0 ± 0.6 ml/min*1.73 m² and of albumin 0.11 ± 0.06 and 0.14 ± 0.05 ml/min*1.73 m² with standard and low-GDP dialysate fluid, respectively (all \( P = ns \)).

In contrast, the clearance of fluorescent AGE was significantly less with the standard compared with the low-GDP solution \((0.44 ± 0.15 vs 0.74 ± 0.3 ml/min*1.73 m², P < 0.01)\).

Molecular weight-specific AGE distribution

Size selective gel permeation chromatography was performed in plasma and dialysate in nine patients. Mean plasma levels of 75, 70, 14 and <2 kDa AGE were 1.8, 3, 4.7 and 7.4-fold higher compared with healthy children \((75 \text{kDa}: 7.8 ± 2.2 AU, 70 \text{kDa}: 24.3 ± 8.5 \text{AU}, 14 \text{kDa}: 11.8 ± 3.5, <2 \text{kDa}: 3.6 ± 1.8 \text{AU, all } P < 0.001 \text{ vs children on PD})\). The appearance rate of AGE in the peritoneal dialysate fluid was molecular weight dependent, with a lower clearance of large AGE compounds (Figure 4). Dialysate AGE concentrations in the 14 kDa range and below 2 kDa increased significantly faster during the PET when low-GDP solution was used \((P < 0.05)\). No difference was observed for the 70 kDa AGE group with low and standard GDP PD fluid. The effluent concentration of the >75 kDa AGE fraction remained low (Figure 4). Peritoneal AGE removal did not correlate with time on PD.

The plasma AGE composition was not correlated with the peritoneal clearance of AGE subgroups in the dialysate. The reduction in plasma AGE concentrations after 12 weeks with low-GDP dialysis solution appeared to be accounted for mainly by decreases of the high molecular weight fractions...
AGE are a class of compounds resulting from glycation and oxidation of proteins, lipids or nucleic acids. They accumulate in patients with chronic renal failure, irreversibly cross link proteins, stimulate specific receptors and contribute essentially to the development of severe complications of uraemia, e.g. accelerated vasculopathy and amyloidosis. The salient feature of the present study is a significant reduction in plasma concentrations of total fluorescent AGE and of the non-fluorescent AGE/CML in paediatric PD patients switched from a standard PD solution, containing high amounts of GDP, to a double-chamber solution with a reduced GDP content. In addition, we demonstrate an increased peritoneal removal of small AGE compounds with the low-GDP solution.

Discussion

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Single chamber PD solutions contain high amounts of GDP. The large differences in 3-DG concentrations observed in the low and standard GDP solutions in this study are in line with previous findings [1,2]. Three-DG rapidly disappeared from the PD fluid and this was accompanied by a reduced capacity to form AGE in vitro, reflecting a decline in reactive carbonyl compound concentrations. In contrast, the AGE generating capacity of the low GDP solution was low and did not change with dwell time.

GDPs are small molecules and GDP-dependent advanced glycation is a slow process [19]. In vitro, neither peritoneal mesothelial cells nor peritoneal fibroblasts, collagen, soluble proteins or other components of spent dialysate reduce GDP levels in the medium [20]. Thus, GDPs do not appear to be locally retained or metabolized to a major extent, but most likely diffuse from the peritoneal cavity into the circulation. Patients dialysed with standard PD solutions are exposed to large amounts of exogenous GDP, which can be substantially reduced by administration of low-GDP solutions. In our study the 3-DG load was reduced by 80%.
In this cohort of children with ESRD, plasma AGE levels were elevated several fold compared with healthy controls, in whom AGE are cleared enzymatically and via the kidney. The molecular weight-specific AGE analysis revealed preferential accumulation of small AGE. Switching the patients to the low-GDP solution however resulted in a 17% and 23% reduction in plasma AGE and CML levels, respectively. The decline in CML levels occurred to a major part within the first 4 weeks. A similar degree of reduction in plasma AGE concentrations has recently been described in adult CAPD patients on low-GDP dialysis fluid [12,21]. Our molecular weight-specific analysis of fluorescent AGE revealed a preferential reduction of compounds in the high molecular weight range with low-GDP PD solution, which are poorly removed by peritoneal and haemodialysis [16].

An impact of dietary delivery of AGE on plasma levels is unlikely. In patients with ESRD, endogenous production of AGE is high and enzymatic and renal clearances are persistently reduced [22,23]. In contrast, the bioavailability of AGE ingested with food is low [24,25] and the contribution of nutritional precursors such as methylglyoxal is small relative to the high endogenous formation rate [26]. Hence, it appears reasonable to assume that the plasma AGE profile observed in the patients on low-GDP dialysate was mainly due to the reduced peritoneal GDP resorption and subsequent AGE formation. Thus, the improved AGE plasma profile observed may be due to the markedly reduced GDP exposure of the patients.

In addition, the peritoneal clearance of total AGE was significantly increased with the low-compared with the high-GDP solution. This finding was supported by markedly increased concentrations of the small AGE fraction in low-GDP effluents, as determined by GPC. Whether the improved AGE clearance contributes to a reduction in systemic AGE levels or whether it reflects increased elimination of locally accumulated AGE remains open. In previous studies using standard, high-GDP solutions, dialysate concentrations of albumin-linked pentosidine exceeded serum levels by >30%, the clearance of albumin-linked pentosidine and of glycated proteins were 50% and 30% higher than the clearance of albumin and of non-glycated proteins, respectively [27,28]. Facilitated diffusion or an active transport mechanism was suggested. However, significant correlations of pentosidine and Amadori albumin effluent concentrations with the duration of PD and an Amadori albumin clearance 90-fold higher compared with albumin clearance argue in favour of a local wash-out [28,29]. Peritoneal AGE deposition is markedly increased in PD patients compared with other tissues [30]. Switching from standard to low-GDP PD fluids may shift the balance from local AGE deposition towards an increased removal. Differences in pH or buffer composition of the PD solutions may play a role as well. Previous short-term studies demonstrated a small reduction in pore area available for transport and in clearance of creatinine and phosphate with biocompatible, low-GDP solutions [31,32]. These differences, however, were not reconfirmed with extended administration [15]. In the present analysis, the clearances of creatinine, phosphate and albumin were not different with low- compared with high-GDP solution, arguing against specific changes in peritoneal transport characteristics.

Peritoneal clearance of AGE was low and did not have an impact on the composition of plasma AGE. Despite a high peritoneal removal of AGE < 2 kDa, their accumulation was most pronounced. Switching the patients to low-GDP fluid did not reduce plasma concentrations of AGE < 2 kDa but those of 70 kDa AGE. Peritoneal removal, however, was only increased for AGE < 2 kDa. Thus, a local wash-out of accumulated AGE is likely, although dialytic AGE removal did not correlate with time on PD in the limited number of patients in this study.

In conclusion, low-GDP PD solutions significantly reduce total AGE and CML plasma levels in paediatric patients on APD, most likely due to a markedly reduced systemic GDP load. The long-term impact on sequelae of ESRD awaits clarification in extended clinical trials.

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Conflict of interest statement. None declared.

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