A PERITONEAL DIALYSIS REGIMEN LOW IN GLUCOSE AND GLUCOSE DEGRADATION PRODUCTS RESULTS IN INCREASED CANCER ANTIGEN 125 AND PERITONEAL ACTIVATION

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◆ **Background:** Glucose and glucose degradation products (GDPs) in peritoneal dialysis fluids (PDFs) are both thought to mediate progressive peritoneal worsening.

◆ **Methods:** In a multicenter, prospective, randomized crossover study, incident continuous ambulatory peritoneal dialysis patients were treated either with conventional lactate-buffered PDF (sPD regimen) or with a regimen low in glucose and GDPs: Nutrineal×1, Extraneal×1, and Physioneal×2 (NEPP regimen; all solutions: Baxter Healthcare, Utrecht, Netherlands). After 6 months, patients were switched to the alternative regimen for another 6 months. After 6 weeks of run-in, before the switch, and at the end of the study, 4-hour peritoneal equilibration tests were performed, and overnight effluents were analyzed for cells and biomarkers. Differences between the regimens were assessed by multivariate analysis corrected for time and regimen sequence.

◆ **Results:** The 45 patients who completed the study were equally distributed over both groups. During NEPP treatment, D_4/D_0 glucose was lower ($p < 0.01$) and D/P creatinine was higher ($p = 0.04$). In NEPP overnight effluent, mesothelial cells ($p < 0.0001$), cancer antigen 125 ($p < 0.0001$), hyaluronan ($p < 0.0001$), leukocytes ($p < 0.001$), interleukins 6 ($p = 0.001$) and 8 ($p = 0.0001$), and vascular endothelial growth factor (VEGF, $p < 0.0001$) were increased by a factor of 2–3 compared with levels in sPD effluent. The NEPP regimen was associated with higher transport parameters, but that association disappeared after the addition of VEGF to the model. The association between NEPP and higher effluent levels of VEGF could not be attributed to glucose and GDP loads.

◆ **Conclusions:** Study results indicate preservation of the mesothelium and increased peritoneal activation during

NEPP treatment. Whether the increase in VEGF reflects an increase in mesothelial cell mass or whether it points to another, undesirable mechanism cannot be determined from the present study. Longitudinal studies are needed to finally evaluate the usefulness of the NEPP regimen for further clinical use.

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The parietal and visceral peritoneum are both covered with a monolayer of mesothelial cells (MCs). Those cells play a crucial role in homeostasis of the peritoneum and orchestrate serosal repair upon injury (1,2). During peritoneal dialysis (PD), the MCs are chronically exposed to PD fluids (PDFs) that contain high levels of glucose and glucose degradation products (GDPs). Possibly as a consequence of this exposure, MCs are damaged and start a regeneration response (3). In a scrape-wound model, GDPs—especially formaldehyde and 3,4-dideoxyglucosone—strongly retard remesothelialization (4). Damage to the MCs and the serosal healing process may both become chronic upon permanent exposure of the peritoneal membrane to PDF. Over time, the peritoneal membrane becomes progressively more vascularized and shows increased fibrosis, with the presence of inflammatory cells (5). Chronic exposure to the non-physiologic conditions of PDFs (such as high glucose concentrations, hyperosmolarity, lactate buffer, low pH, and GDPs) may contribute to morphology changes (6), ultimately resulting in ultrafiltration failure (7). Because it is not possible in clinical PD studies to use histology to evaluate peritoneal changes over time, a number of effluent

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parameters—including chemokines, cytokines, growth factors, and cell numbers—are used as pseudo-markers for peritoneal inflammation and tissue remodeling.

In recent years, much attention has been paid to the development of new-generation PDFs that use alternative osmotic agents instead of glucose or that are manufactured with advanced sterilization techniques, inducing lesser quantities of GDPs. The main goal of these developments is to preserve the capacity of the peritoneal membrane for PD. In clinical studies, use of new glucose-based solutions with low GDPs and a neutral pH is associated with increased effluent levels of cancer antigen 125 (CA125) (8–10), which is used as indicator for MC mass (11). Higher CA125 levels suggest regeneration of the MC layer (12). Furthermore, in a rat model of PD exposure, better MC morphology was observed with use of a bicarbonate/lactate-buffered PDF than with use of a conventional PDF (13).

Extraneal and Nutrineal (Baxter Healthcare, Utrecht, Netherlands) both use an osmotic agent other than glucose. Extraneal, a glucose polymer solution, and Nutrineal, an amino-acid solution, have demonstrated advantages in clinical studies (14,15), but can be administered only once daily. A glucose-free PD regimen based on these new fluids is therefore not feasible.

To be able to study a regimen as low in glucose and GDPs as possible, we designed a PD scheme with two glucose-free dwells and two glucose-containing dwells. In a prospective randomized trial, that regimen was compared with a standard glucose-containing regimen (sPD) in incident PD patients (16,17). In the present report about the same regimen comparison, we focus on peritoneal transport characteristics, cellular parameters, and some biomarkers that are important indicators for MC integrity, peritoneal inflammation, and tissue remodeling during peritoneal repair, such as angiogenesis and fibrosis.

METHODS

STUDY DESIGN

A multicenter prospective randomized crossover study enrolled new PD patients from the VU University Medical Center, Amsterdam; the Red Cross Hospital, The Hague; Leyenburg Hospital, The Hague; and the Medical Center, Alkmaar. The present work is a substudy of that study, executed at 3 of the participating hospitals and involving 69 of the 74 patients in the original study. Those patients were randomized into two treatment groups:

- The NEPP–sPD group started on NEPP and switched to sPD half way through the study period.

- The sPD–NEPP group started on sPD and switched to NEPP.

The first 6 weeks were used to stabilize patients on the first-allocated PD regimen and to teach patients how to perform continuous ambulatory PD. Thereafter, patients were treated using the two PD regimens during two separate study periods of 24 weeks each. The NEPP regimen consisted of 2 exchanges of bicarbonate/lactate-buffered glucose-based PDF (Physioneal: Baxter Healthcare), 1 bag of an amino-acid solution (Nutrineal), and 1 exchange of an icodextrin-based solution (Extraneal) for the overnight dwell. The sPD regimen consisted of 4 exchanges of lactate-buffered glucose-based PD solution (Dianeal: Baxter Healthcare). Figure 1 shows the treatment schedule.

Patients were instructed to determine ultrafiltration at home by subtracting the weight of the drain bag from its corresponding weight before administration. A peritoneal equilibration test (PET) and blood draw were performed at 6 weeks (after the run-in period), 30 weeks (just before the regimen switch), and at 54 weeks (at the end of the study period). Effluents were rendered cell-free by centrifugation, and aliquots were frozen at –80°C until further investigation. A full description of the study design and of its efficacy, safety, and tolerability has been reported elsewhere (16,17).

PERITONEAL EQUILIBRATION TEST

The 4-hour PET administered at 6, 30, and 54 weeks of treatment was done as described by Twardowski *et al.* (18) using a 2.27% Physioneal or Dianeal dwell according to the regimen currently in use by the patient. Urea, creatinine, and protein in effluent were determined using routine laboratory techniques.

Figure 1 — Overview of the study design. NEPP = dialysis regimen using 1 exchange of Nutrineal, 1 exchange of Extraneal, and 2 exchanges of Physioneal (all solutions: Baxter Healthcare, Utrecht, Netherlands).

EFFLUENT CELL ANALYSIS

At 6, 30, and 54 weeks, effluent cells were counted, and cytocentrifuge slides were made for differentiation by May–Grünwald–Giemsa staining. Two independent observers performed the differential counts in a blinded fashion. Computer-automated counts of the T-lymphocyte population (AnalySIS: Soft Imaging System, Münster, Germany) were performed after staining for CD3 (Becton Dickinson, San Jose, CA, USA) and discriminated into CD4+ and CD8+ subsets (Sanquin Reagents, Amsterdam, Netherlands). The percentage of Fc-receptor-positive cells was determined by rosette formation with immunoglobulin G-coated sheep red blood cells (SRBCs) as previously described (19). To assay Fc-receptor-mediated phagocytosis, effluent cells and immunoglobulin G-coated SRBCs were incubated for 1 hour at 37°C. After May–Grünwald–Giemsa staining, the number of phagocytosing cells and their phagocytosed SRBCs were counted. In addition, in patients with a cell count greater than 1.0×10^6 , Fcγ-receptor expression on effluent cells was determined (FACSCalibur flow cytometer: Becton Dickinson) using fluorescent-labeled antibodies: 10.1 for FcγRI (CD64), IV-3 for FcγRIIa (CD32a), and 3G8 for FcγRIII (CD16) (BD Biosciences Pharmingen, San Diego, CA, USA). Results are reported as a mean fluorescence index: the mean fluorescence of stained cells divided by the mean fluorescence of unstained cells (CellQuest: Becton Dickinson).

ANALYSIS OF EFFLUENT CYTOKINES AND BIOMARKERS

Assays were performed in cell-free supernatants of the overnight effluents—that is, Dianeal during SPD treatment and Extraneal during NEPP treatment. In all assays and on every microtiter plate, 2 standard curves were produced: the first in Dianeal and the second in Extraneal (to calculate the Dianeal and Extraneal samples respectively).

Levels of CA125 were determined in overnight effluents using the Centaur OV assay (Bayer Diagnostics, Tarrytown, NY, USA), a 2-side sandwich immunoassay using direct chemiluminometric technology. Detection limit of the assay is 5 U/mL.

Levels of VEGF were determined in effluent and plasma using sandwich ELISA (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Detection limit was 5 pg/mL.

A sandwich ELISA with matching antibody pairs (BioSource International, Camarillo, CA, USA) was also used to detect interleukin 6 (IL-6) in effluent and plasma and CXCL8 (IL-8) in effluent. The detection level was 10 pg/mL for both assays.

As described elsewhere, CCL2 [monocyte chemotactic protein 1 (MCP-1)] was determined by ELISA (20), with a detection limit of 30 pg/mL. Hyaluronan was determined in an ELISA-based assay according to the technique of Fosang *et al.* (21), using immobilized hyaluronan and competition for the binding of biotinylated hyaluronan-binding protein by hyaluronan-containing samples.

In a random subset of patients, levels of syndecan 1 and basic fibroblast growth factor (bFGF) were determined. Syndecan 1 is a heparan sulfate proteoglycan present on the surface of MCs (22,23). Upon activation of the cell, shedding occurs by means of a metalloprotease (24,25), and that soluble ectodomain can be measured by ELISA in effluent (Diacclone Research, Besançon, France) with a sensitivity of 2.0 ng/mL. The bFGF assay used a third-generation Quantikine HS ELISA kit (R&D Systems), with a minimal detectable dose of 1 pg/mL.

The effluents at 6, 30, and 54 weeks were concentrated 5–8 times by centrifugation through a 5 kD cut-off filter (Amicon Ultra: Millipore Corporation, Bedford, MA, USA) to determine VEGF, IL-6, IL-8, syndecan 1, and bFGF. The standard curves were determined in concentrated Dianeal and Extraneal according to the patient's current PD regimen. Data were normalized for the concentration factor (volume before concentration divided by volume after concentration).

Effluent markers are expressed as amounts per overnight bag, calculated by multiplying measured concentrations by the drained volume.

CONTAMINATED ICODEXTRIN

During the present study, a small number of patients were treated for a limited period with icodextrin from batches contaminated with peptidoglycans (26,27). Retrospectively, in collaboration with Baxter Healthcare, we were able to determine which patients had been treated with possibly contaminated bags and, if so, for how long.

STATISTICAL ANALYSIS

Using Stata 7 for Windows (StataCorp LP, College Station, TX, USA), data were analyzed by the “generalized estimating equations” (GEE) longitudinal data analysis technique. The GEE method is a sophisticated approach suitable for longitudinal data analysis between a continuous variable and several time-dependent and time-independent covariates (28). In the GEE model, the studied variable [dialysate-to-plasma ratio (D/P), end-to-initial dialysate ratio (D/D₀), or effluent marker] is analyzed as dependent variable, with the PD regimen

(NEPP = 1, sPD = 0) as the independent variable. In this analysis, additional independent variables were used to make adjustments for time, for the regimen sequence (to deal with carryover effects), and if possible, for previous observations. The effect of the icodextrin contamination was analyzed using an additional independent dichotomous variable indicating contamination at a specific time point. In cases of skewed data, analyses were performed after log-transformation.

Data are presented in graphs showing mean $\pm$ standard error (for normally distributed data) or median with 25% and 75% percentiles (for skewed data). Values of p less than 0.05 were considered significant.

RESULTS

PATIENTS

In the present substudy, 24 of the 69 enrolled patients dropped out because of clinical conditions, at a rate that was similar in both groups (16). The study evaluation therefore includes 45 patients, 24 in the sPD-NEPP group, and 21 in the NEPP-sPD group.

In 4 NEPP-sPD patients and 2 sPD-NEPP patients, icodextrin had to be discontinued because of an allergic skin reaction ($n = 1$ and 0), sterile peritonitis ($n = 2$ and 2), or nausea ($n = 1$ and 0). Retrospectively, we discovered that 9 patients (5 NEPP-sPD and 4 sPD-NEPP) had used contaminated icodextrin during part of their NEPP treatment period [mean: 92 days (range: 35 – 191 days)]. In only 1 of those patients was treatment with icodextrin discontinued (because of sterile peritonitis). Amino-acid solution was stopped in 1 NEPP-sPD patient because of skin eruptions.

Demographic data and the percentage of patients with diabetes (sPD-NEPP, 26%; NEPP-sPD, 20%) were not different between the groups.

SOLUTE TRANSPORT

Figure 2 shows the results of the PETs. During NEPP treatment, small-solute transport appeared to be higher, with a higher D/P creatinine ($p = 0.04$) and a lower D/D₀ glucose ($p = 0.01$). When the first PET was omitted from the analysis [because of reported unstable results within the first weeks of treatment (29)], the foregoing p values changed to 0.14 and 0.02 respectively. Ultrafiltration during PETs did not differ between NEPP and sPD treatment ($p = 0.3$). Daily protein loss in effluents was higher during NEPP treatment (7.2 ± 2.6 g vs 6.4 ± 2.7 g). Adjusting for time and carryover effects, that difference was statistically significant in the multivariate analysis ($p = 0.03$).

MESOTHELIAL CELLS AND MARKERS

During NEPP treatment, numbers of free-floating mesothelial cells and amounts of CA125 were both higher per overnight bag than they were during sPD treatment [both $p < 0.0001$ for NEPP vs sPD, Figure 3(A,B)]. We also observed low but detectable levels of syndecan 1 (range: 2.0 – 7.7 ng/mL) in 4 of 33 samples during sPD treatment and in 12 of 41 samples during NEPP treatment (chi-square $p = 0.07$).

IMMUNE PARAMETERS

Leukocytes: Counts of total peritoneal leukocytes and monocytes/macrophages were lower in overnight

Figure 2 — Peritoneal transport characteristics after a 4-hour peritoneal equilibration test (PET), comparing NEPP→sPD (filled circles) with sPD→NEPP (filled squares). (A) Dialysate-to-plasma (D/P) creatinine. (B) End-to-initial dialysate concentration (D₄/D₀) of glucose. (C) 4-Hour ultrafiltration. Crossover takes place after 30 weeks; the second period is indicated by the gray background. Data are reported as mean $\pm$ standard error. NEPP = dialysis regimen using 1 exchange of Nutrineal, 1 exchange of Extraneal, and 2 exchanges of Physioneal (all solutions: Baxter Healthcare, Utrecht, Netherlands); sPD = dialysis regimen using conventional lactate-buffered glucose solutions.

bags during sPD treatment than during NEPP treatment [Figure 4(A,B)]. After adjustments for time and sequence of treatment, that finding appeared to be statistically significant (NEPP vs sPD: $p < 0.0001$). Omitting the first time point (effluents at 6 weeks) from the analysis did not change that result ($p < 0.0001$ for total cells and for monocytes). The count of peritoneal neutrophils did not differ between the treatment regimens ($p = 0.18$), although the same trend to lower numbers during sPD treatment was observed [Figure 4(C)].

In general, the composition of the peritoneal cell population was independent of the PD regimen being used and contained predominantly monocytes/macrophages (approximately 65% – 80%), which differed neither between the patient groups, nor over time. The

lymphocyte population appeared to be mainly of T-cell origin, because 15% – 20% of the peritoneal cell population was CD3+. Of those cells, about 35% – 40% were of the CD8+ subtype. We observed no variation in the percentage of CD3+ and CD8+ cells, neither over time, nor with respect to the current PD regimen.

We analyzed the expression of Fcγ receptors, important in phagocytosis of opsonized bacteria, on effluent cells from a subset of patients. We observed that most peritoneal macrophages express FcγRI (92%) and FcγRIIa (95%); FcγRIII was expressed on 50% of the macrophages. The percentage of neutrophils that express those FcRs was lower: FcγRI, 20%; FcγRIIa, 64%; and FcγRIII, 27%. For FcγRI and FcγRIII, expression levels were similar in the treatment groups. However,

Figure 3 — Mesothelial characteristics in overnight effluent, comparing NEPP→sPD (filled circles) with sPD→NEPP (filled squares). (A) Total number of free-floating mesothelial cells per overnight bag. (B) Cancer antigen 125 (CA125: units per overnight bag). Crossover takes place after 30 weeks; the second period is indicated by the gray background. Data are reported as medians, with 25th and 75th percentiles. NEPP = dialysis regimen using 1 exchange of Nutrineal, 1 exchange of Extraneal, and 2 exchanges of Physioneal (all solutions: Baxter Healthcare, Utrecht, Netherlands); sPD = dialysis regimen using conventional lactate-buffered glucose solutions.

Figure 4 — Overnight leukocyte numbers (from a count of cells in overnight effluent), comparing NEPP→sPD (filled circles) with sPD→NEPP (filled squares). (A) Total leukocyte numbers per overnight bag. (B) Total monocyte numbers per overnight bag. (C) Total neutrophil numbers per overnight bag. Crossover takes place after 30 weeks; the second period is indicated by the gray background. Data are reported as medians, with 25th and 75th percentiles. NEPP = dialysis regimen using 1 exchange of Nutrineal, 1 exchange of Extraneal, and 2 exchanges of Physioneal (all solutions: Baxter Healthcare, Utrecht, Netherlands); sPD = dialysis regimen using conventional lactate-buffered glucose solutions.

for FcγRIIa, macrophages and neutrophils both showed a lower expression level during NEPP treatment, with a lower mean fluorescence intensity in macrophages (16.7 in sPD vs 8.4 in NEPP, $p < 0.001$) and also in neutrophils (3.7 vs 2.7, $p < 0.0001$). The number of FcγRIIa-positive cells did not change. The expression profile of these Fcγ receptors, analyzed by rosette formation assay, and the capacity for phagocytosis via these Fcγ receptors were not statistically different between the groups (rosette formation, NEPP vs sPD: $p = 0.28$). We found that 72% – 84% of the effluent cells expressed Fcγ receptors (rosette-positive). Only 21% – 44% of those cells had the capacity to phagocytose, without any differences in the degree of phagocytosis indicated by the number of SRBCs per cell (3.0 – 3.8). Taken together, these data indicate increased cell influx into the PD fluid under the NEPP regimen, with some minor changes in leukocyte differentiation and phenotype.

Cytokines: Increased monocyte influx under NEPP was observed, with significant differences in the amounts of CCL2 [Figure 5(A), NEPP vs sPD: $p = 0.002$]. During treatment with NEPP (compared with treatment with sPD), CXCL8 appeared at higher levels [Figure 5(B), NEPP vs sPD: $p = 0.0001$]. Relatively high levels of IL-6 were detectable in effluent from all patients [Figure 5(C)]. Levels of IL-6 were higher during NEPP treatment than during sPD treatment ($p = 0.01$). If effluent concentrations of MCP-1, IL-8, and IL-6 had been analyzed instead of amounts per overnight bag, the results would have been similar.

To control for systemic inflammation, we determined levels of IL-6 in serum. In both groups, IL-6 was not detectable in 80% of serum samples. Levels above the

detection limit of 10 pg/mL were measured in a few patients. The range was 11.5 pg/mL – 141.0 pg/mL in the sPD–NEPP group and 10.9 pg/mL – 34.0 pg/mL in the NEPP–sPD group. We observed no statistical differences in the number of patients or in the levels of serum IL-6 over time or with respect to the PD regimen in use.

FACTORS INVOLVED IN TISSUE REMODELING

Hyaluronan levels in effluents were clearly elevated during NEPP treatment compared with sPD treatment [Figure 6(A)]. The same holds true for VEGF [Figure 6(B); both parameters, NEPP vs sPD: $p < 0.0001$]. Serum VEGF levels were constant over time and not different between the study groups or the treatment regimens, with medians ranging from 177 pg/mL to 212 pg/mL. Effluents contained trace amounts of bFGF, the highest value being 2.7 pg/mL. Results in a limited number of patients (11 in each group) indicated elevated levels of bFGF during NEPP treatment ($p < 0.0001$).

INTERRELATIONSHIPS

To unravel physiologic relationships between the study variables, we performed multivariate GEE analyses. First, we tried to uncover determinants of transport parameters. In univariate analysis, D/P creatinine and D_4/D_0 glucose were statistically significantly related to the presence of NEPP, the effluent level of VEGF, the number of MCs, and the concentration of CA125. Table 1 shows the results of multivariate analyses combining those variables. The models show that D/P creatinine is positively associated with VEGF, and VEGF itself is associated with NEPP and CA125. Determinants of D_4/D_0 glucose were

Figure 5 — Cytokine quantities per overnight bag, comparing NEPP→sPD (filled circles) with sPD→NEPP (filled squares). (A) Chemokine (C–C motif) ligand 2 [CCL-2 (also known as monocyte chemotactic protein, MCP-1)]. (B) CXCL8 [also known as interleukin 8 (IL-8)]. (C) Interleukin 6 (IL-6). Crossover takes place after 30 weeks; the second period is indicated by the gray background. Data are reported as concentrations per milliliter, giving medians, with 25th and 75th percentile. NEPP = dialysis regimen using 1 exchange of Nutrineal, 1 exchange of Extraneal, and 2 exchanges of Physioneal (all solutions: Baxter Healthcare, Utrecht, Netherlands); sPD = dialysis regimen using conventional lactate-buffered glucose solutions.

Figure 6 — Markers of tissue remodeling in overnight effluent (quantity per overnight bag), comparing NEPP→sPD (filled circles) with sPD→NEPP (filled squares). (A) Hyaluronan. (B) Vascular endothelial growth factor (VEGF). (C) Basic fibroblast growth factor (b-FGF). Crossover takes place after 30 weeks; the second period is indicated by the gray background. Data are reported as medians, with 25th and 75th percentiles. NEPP = dialysis regimen using 1 exchange of Nutrineal, 1 exchange of Extraneal, and 2 exchanges of Physioneal (all solutions: Baxter Healthcare, Utrecht, Netherlands); sPD = dialysis regimen using conventional lactate-buffered glucose solutions.

TABLE 1

Multivariate Analyses: Determinants of Peritoneal Transport, Vascular Endothelial Growth Factor (VEGF), and Cancer Antigen 125 (CA125)

Model and variables	Regression coefficient	<i>p</i> Value (regression coefficient)	Variance explained (model)
D/P creatinine			
NEPP (yes vs no)	−0.031	0.45	0.18
VEGF ^a	0.071	0.04	
Mesothelial cells ^a (<i>n</i>)	0.015	0.11	
CA125 ^a	0.003	0.88	
VEGF ^a			
NEPP (yes vs no)	0.429	<0.0001	0.65
CA125 ^a	0.250	<0.0001	
Mesothelial cells ^a (<i>n</i>)	0.041	0.08	
CA125 ^a			
NEPP (yes vs no)	0.586	<0.0001	0.28
Mesothelial cells ^a (<i>n</i>)	0.068	0.15	

NEPP = dialysis regimen using 1 exchange of Nutrineal, 1 exchange of Extraneal, and 2 exchanges of Physioneal (all solutions: Baxter Healthcare, Utrecht, Netherlands).

^a Log-transformed values were used in the model for these variables.

similar (VEGF: *p* = 0.04). The higher protein loss during NEPP was also associated with VEGF (*p* = 0.005).

To unravel the NEPP constituent that determines those relationships, we defined NEPP by

- the estimated glucose load,
- the estimated GDP-load (3-deoxyglucosone, glyoxal, methylglyoxal), and

- other NEPP-associated characteristics (such as the use of solutions with amino acids or icodextrin, and the alternation of various PDFs).

Interestingly, in those multivariate analyses (Table 2), D/P creatinine, VEGF, and hyaluronan were not determined by glucose load or GDP load, but by the other NEPP-associated characteristics, suggesting that it is not the restriction of glucose and GDPs, but other characteristics of the NEPP regimen (the use of amino acids and icodextrin, or the alternation of various PDFs) that are responsible for the observed increase in the measured parameters during NEPP. A clear association between CA125 and one of the NEPP characteristics could not be found.

After the addition to these analyses of a dichotomous variable representing the possible presence of contaminated icodextrin at a specific time point, the relationship between NEPP and the previously described effluent levels persisted, indicating that the observed relationships were not a consequence of the contaminating constituents of icodextrin.

DISCUSSION

In the present study, we showed that, compared with a standard PD regimen, a low-glucose low-GDP regimen (NEPP) is associated with higher levels of CA125 and higher levels of inflammation and tissue remodeling parameters in peritoneal effluent, in association with increased small-solute transport. Most parameters already differed between the study groups after 6 weeks of treatment, and they remained different during the first study period. Most of the differences were reversible and were not affected by the order in which the treatment

TABLE 2

Multivariate Analyses: Effects of NEPP Determinant on Peritoneal Transport, Vascular Endothelial Growth Factor (VEGF), and Hyaluronic Acid

Model and variables	Regression coefficient	<i>p</i> Value (regression coefficient)	Variance explained (model)
D/P creatinine			
NEPP (yes vs no)	0.130	0.034	0.09
Glucose load ^a	-0.057	0.49	
3DG load ^a	0.178	0.18	
Glyoxal load ^a	-0.054	0.54	
Methylglyoxal load ^a	-0.008	0.33	
VEGF ^a			
NEPP (yes vs no)	0.619	0.009	0.44
Glucose load ^a	0.149	0.51	
3DG load ^a	0.375	0.35	
Glyoxal load ^a	-0.393	0.12	
Methylglyoxal load	-0.003	0.57	
Hyaluronic acid ^a			
NEPP (yes vs no)	1.812	0.003	0.34
Glucose load ^a	-0.477	0.25	
3DG load ^a	0.184	0.79	
Glyoxal load ^a	0.703	0.16	
Methylglyoxal load	0.009	0.61	

NEPP = dialysis regimen using 1 exchange of Nutrineal, 1 exchange of Extraneal, and 2 exchanges of Physioneal (all solutions: Baxter Healthcare, Utrecht, Netherlands); D/P = dialysate-to-plasma ratio; 3DG = 3-deoxyglucosone.

^a Log-transformed values were used in the model for these variables.

regimens were administered, as revealed by conversion of the data upon patient crossover.

At first glance, our study is limited by two factors: striking differences between the groups at 6 weeks, the starting point of the study; and the use of icodextrin batches contaminated with peptidoglycans.

Concerning the first point, we think that the differences at 6 weeks are a consequence of the PDFs used during the run-in period and are not related to inappropriate randomization. That hypothesis is strongly confirmed by conversion of the results upon crossover of the patients to their second treatment regimen. In the design phase of the study, we considered running in all patients on the same fluids—for example, standard glucose-containing fluids. However, at that time, we considered the possibility that carryover effects would be so important that run-in using a standard regimen in the first 6 weeks would disturb the possible beneficial

effects of the new regimen in the subsequent treatment period (6–24 weeks). We therefore decided that, during the run-in period, we would have patients use the PDFs of the first regimen to which they had been randomized.

Concerning the second point (that is, the contaminated icodextrin), we were able to determine the use of possibly contaminated bags in the present study, and we performed a multivariate analysis with an additional independent variable for contamination. In those analyses, contamination of icodextrin did not affect the study outcomes. Therefore, the results of the present study must be explained by regular differences in the dialysis regimens studied.

We interpret the higher effluent CA125 levels during NEPP as indicative of a higher MC mass and, therefore, a consequence of the improved biocompatibility of the fluids of the NEPP regimen (30). At the same time, however, the NEPP regimen is associated with increased values of syndecan 1, a cell-surface heparan sulfate proteoglycan that is highly upregulated in regenerating cells and that is shed from the cell upon cellular activation (24,25,31). This finding suggests that, during NEPP treatment, the MCs become activated. The increased numbers of free-floating MCs in NEPP dialysates provide additional corroboration for that suggestion.

The NEPP regimen is also associated with increased hyaluronan values. The bulk of hyaluronan is derived from synthesis by peritoneal MCs; the amount synthesized by macrophages is trivial (32). Hyaluronan is predominantly produced by migrating MCs. It positively influences MC wound healing *in vitro* (33,34), and it may preserve peritoneal membrane transport properties (35). On the other hand, increased hyaluronan is also associated with peritoneal inflammation, which clearly hampers interpretation of this biomarker. In line with our observations, previous studies showed that high concentrations of glucose and GDPs suppress MC wound healing. High glucose inhibits integrin-mediated cell migration along the basal membrane (36), and GDPs decrease the velocity of MC wound closure and impair MC viability *in vitro* (37). Furthermore, compared with MCs taken from glucose effluents, those taken from icodextrin effluents show greater proliferation *ex vivo* (38). As shown by electron microscopy, MC morphology is better preserved under bicarbonate/lactate-buffered (13) and amino-acid-containing solutions (39) than under SPD solutions.

Given all the foregoing considerations, we suggest that the NEPP regimen leads to increased mesothelial regeneration, together with MC activation. Activation of the peritoneum is also highly suggested because of increased cell numbers; increased chemokines, cytokines, and growth factors; and increased peritoneal transport.

Other authors have compared new multi-chamber lactate or bicarbonate/lactate solutions with conventional PD solutions (8–10,12). Those studies showed increased levels of CA125, suggesting increased MC mass, or regeneration, or both. They also described a decrease in hyaluronan levels. However, effects on growth factors and transport are mixed and not uniform. Compared with the aforementioned clinical trials, the findings from our NEPP regimen are more uniform in one direction, with all parameters being increased during NEPP. That result might be related to the fact that, in addition to a lower glucose and GDP load, two non-glucose-based fluids (icodextrin- and amino-acid-based PDFs) were used in our study.

An important issue is whether the reduced glucose or GDP load (or both) under NEPP explains all the differences in the comparison with SPD treatment. Interestingly, the multivariate GEE analysis clearly demonstrates that, after adjustments for glucose and GDP loads, most parameters remain significantly associated with NEPP characteristics, including amino acids and icodextrin or the alternation of various PDFs. It could be that, although each new PD solution has been shown to be more biocompatible on its own, an alternating regimen such as NEPP may have negative side effects, because, compared with chronically high glucose concentrations, intermittently high concentrations may induce more oxidative stress and apoptosis (40).

It could also be that the parameters most increased under NEPP are related to the use of icodextrin. The use of icodextrin for the overnight dwell as an adjunct to daytime SPD treatment has been documented to be associated with increased dialysate cell numbers and free-floating MC numbers; increased levels of hyaluronan, IL-6, and tumor necrosis factor α ; and low molecular solute transport—but without a change in CA125 (41–43). In a small control experiment, we indeed found that total cell numbers were increased by a factor of about 4 in 6 stable PD patients who used icodextrin for the long dwell compared with 7 stable PD patients who used SPD alone (data not shown). We therefore speculate that the use of bicarbonate/lactate PDF, eventually in combination with amino-acid-containing PDF, leads to improved MC mass (based on CA125 level). The inclusion of icodextrin-based PDF into the NEPP scheme might be responsible for peritoneal activation, leading to increased production of chemokines coupled with increased cell influx. Activated MCs also secrete more growth factors (VEGF among them), resulting in increased microvascular permeability and increased transport of small and large molecules. We cannot exclude the possibility that increased transport parameters during the NEPP regimen might be related

to the overnight icodextrin dwell just before 4-hour PET (44).

CONCLUSIONS

In comparison with a standard glucose-containing PD regimen, the NEPP regimen, low in glucose and GDPs, is associated with higher effluent levels of CA125, probably reflecting a higher MC mass. However, NEPP is also associated with increases in effluent biomarkers such as VEGF and in cells, suggesting at least mesothelial activation. Whether the assumed positive effects compensate for the assumed negative effects has to be established in studies of peritoneal tissues and of hard endpoints such as time to loss of ultrafiltration capacity, peritonitis incidence, and rates of mortality and morbidity. Wider clinical use of NEPP should wait on those studies.

DISCLOSURES

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