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Long-term exposure to new peritoneal dialysis solutions: Effects on the peritoneal membrane

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Long-term exposure to new peritoneal dialysis solutions: Effects on the peritoneal membrane.

Background. Chronic exposure to peritoneal dialysis fluid (PDF) affects the peritoneum, but precise causative factors are incompletely understood. We examined the effects of standard and new PDF on peritoneal function and structure.

Methods. Female Wistar rats received twice daily intraperitoneal infusions of a standard lactate-buffered 3.86% glucose PDF at pH 5.5 (Dianeal®) ($N = 12$), a low glucose degradation product (GDP) containing bicarbonate/lactate-buffered 3.86% glucose PDF at pH 7.4 (Physioneal®) ($N = 12$), a lactate-buffered amino acid–based PDF at pH 6.7 (Nutrineal®) ($N = 12$) or Earle's Balanced Salt Solution at pH 7.4 (EBSS) ($N = 12$) during 12 weeks.

Results. Net ultrafiltration was lower after treatment with standard PDF, but not with low-GDP bicarbonate/lactate-buffered and amino acid–based PDF, compared to EBSS. Peritonea exposed to standard PDF were characterized by an increased expression of vascular endothelial growth factor (VEGF), microvascular proliferation as well as submesothelial fibrosis, which were not observed in other groups. Staining for methylglyoxal adducts was prominent in the standard PDF-exposed group, mild in the low GDP bicarbonate/lactate-buffered group and absent in the other groups. Standard PDF induced accumulation of advanced glycation end products (AGEs) and up-regulation of the receptor for AGE (RAGE). AGEs accumulation was absent and RAGE expression was only modestly increased in low-GDP bicarbonate/lactate-buffered and amino acid–based PDF.

Conclusion. Long-term in vivo exposure to standard PDF adversely affects peritoneal function and structure. A low-GDP bicarbonate/lactate-buffered and amino acid–based PDF better preserved peritoneal integrity and may thus improve the longevity of the peritoneal membrane. GDPs and associated accelerated AGE formation are the main causative factors in PDF-induced peritoneal damage.

Key words: peritoneal membrane, biocompatibility, neoangiogenesis, VEGF, AGE.

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The performance of the peritoneum as a dialyzing membrane can be compromised by the development of various structural and functional alterations occurring in the course of long-term peritoneal dialysis treatment [1]. Morphologically, a chronically exposed peritoneal membrane is characterized by interstitial fibrosis, reduplication of basement membrane of the mesothelium and the blood vessels, hyalinization of the blood vessel media, and neoangiogenesis. Functionally, increased transport of small solutes and a loss of ultrafiltration capacity may develop, for which an increased effective vascular surface area is held responsible.

Evidence is mounting that standard peritoneal dialysis fluids (PDF) play a pathogenic role in the development of peritoneal changes. The relative contribution of the different PDF components in mediating peritoneal changes is, however, incompletely understood. As the described structural alterations resemble those that occur in diabetes, it was hypothesized that high glucose is an important contributing factor. In vitro exposure of endothelial and mesothelial cells to high glucose stimulates the expression of vascular endothelial growth factor (VEGF) [2, 3] and transforming growth factor β (TGF-β) [4, 5]. Chronic in vivo exposure of the peritoneal membrane to high glucose concentrations resulted in microvascular proliferation and submesothelial fibrosis, mediated by VEGF and TGF-β, respectively [6, 7].

Glucose carmelization during heat-sterilization and storage of PDF results in the formation of glucose degradation products (GDPs), such as formaldehyde, acetaldehyde, glyoxal, methylglyoxal (MGO), 3-deoxyglucosone, and 3,4-dideoxyglucosone-3-ene [8]. MGO stimulates VEGF synthesis by peritoneal mesothelial and endothelial cells, which implies that GDPs may contribute to the vascular proliferation in the peritoneal membrane [9]. Glucose and reactive carbonyl compounds, such as MGO, glyoxal, and 3-deoxyglucosone have the potential to bind nonenzymatically to free amino groups on proteins or lipids and form irreversible advanced glycation endproducts (AGEs). AGEs promote in vitro VEGF expression in several cell types in vitro [10, 11]. Vascular

Table 1. Composition of the solutions

	Dianeal®	Physioneal®	Nutrineal®	Earle's Balanced Salt Solution (EBSS)
pH	5.5	7.4	6.7	7.4
Osmolality *mOsmol/L*	483	483	367	290
Osmotic agent *mmol/L*	Glucose (214)	Glucose (214)	Amino acids (87)	Glucose (5.6)
Buffer	Lactate	Lactate/bicarbonate	Lactate	Bicarbonate
Glucose degradation product (GDP) content μ*mol/L*:	High	Low	Low	Low
3-deoxyglucosone	525 ± 51	253 ± 30	<0.1	NA
Methylglyoxal	6.9 ± 0.5	1.1 ± 0.2	0.3 ± 0.2	NA
Glyoxal	9.4 ± 2.2	6.0 ± 1.6	0.8 ± 0.1	NA

Glucose degradation product (GDP) levels (a mean of three different batches) were taken from Schalkwijk, ter Wee, and Teerlink [37]. <, below limit of detection; NA, data not available.

and interstitial AGE content correlated with the extent of vascular sclerosis and interstitial fibrosis in the peritoneal membrane of long-term peritoneal dialysis patients [12]. Functionally, AGE formation was associated with a decrease in ultrafiltration volume [12] and an increased transport of various soluta [13]. The best characterized of the AGE receptors has been termed the receptor for AGE (RAGE), although other AGE-binding structures have been described [14]. Chronic exposure to high glucose concentrations greatly increased peritoneal expression of RAGE, most distinctly in the mesothelium, submesothelial fibrotic tissue, and blood vessel walls. The development of high glucose-induced interstitial fibrosis in the peritoneal membrane was prevented by an anti-RAGE antibody [7].

Finally, the lactate buffer has been implicated in altering peritoneal structure. In vivo exposure of mice to 40 mmol/L lactate induced mesothelial damage, although not as pronounced as contact with standard PDF [15]. Cultured peritoneal fibroblasts produced more collagen when exposed to increasing concentrations of lactate (10 mmol/L to 40 mmol/L) [16]. Replacement of lactate by pyruvate in PDF prevented the development of both neoangiogenesis and fibrosis during chronic in vivo exposure in rats [abstract; Van Westrheenen R, *Perit Dial Int* 22:154, 2002].

Low-GDP or nonglucose-based PDF and PDF with alternative buffers are currently available. The aim of the present study was to investigate the effects of new PDF on the peritoneal membrane. More, in particular, the relative contribution of glucose, GDPs, and lactate in the development of functional and structural alterations in the peritoneum were examined. To this end, the effects of chronic exposure to a standard acidic lactate-buffered PDF, a double-chambered, physiologic pH bicarbonate/lactate-buffered PDF and a lactate-buffered amino acid–based PDF were compared.

METHODS

Laboratory animals

The studies were performed in 48 female Wistar rats (Iffacredo, Brussels, Belgium) with a mean body weight of 213 ± 1 g, receiving care in accordance with the national guidelines for care and use of laboratory animals. A subcutaneous port (PMINA-CBAS-C30 Soloport) (Instech Solomon, Plymouth Meeting, MA, USA) was implanted in the neck under halothane (Fluothane) (Zeneca, Destelbergen, Belgium) anesthesia in sterile conditions. The attached polyurethane, heparin-coated catheter (Instech Solomon) was tunneled over the left flank to the peritoneal cavity [17]. After surgery, the animals received an intramuscular injection of buprenorphine (0.1 mL/kg) (Temgesic) (Schering Plough NV/SA, Brussels, Belgium). The first week after implantation, catheters were flushed once daily with 1 mL of Earle's Balanced Salt Solution (EBSS) (ICN Biomedicals Inc., Aurora, OH, USA). Thereafter, 10 mL of PDF was administered twice daily during 12 weeks. Oxacilline (2.5 mg/day) (Penstapho) (Bristol-Myers Squibb, Brussels, Belgium) and gentamycine (0.04 mg/day) (Geomycine) (Shering-Plough NV/SA) were added to all solutions [18]. Laboratory technicians wore masks and gloves during manipulations. The area of the port was disinfected with ethanol 97% 20 seconds before puncture.

Study protocol

Animals received twice daily intraperitoneal infusions of 10 mL of a standard lactate-buffered 3.86% glucose PDF at pH 5.5 (Dianeal®) (Baxter, SA, Lessines, Belgium) ($N = 12$), a low GDP bicarbonate/lactate-buffered 3.86% glucose PDF at pH 7.4 (Physioneal®) (Baxter) ($N = 12$), a lactate-buffered amino acid–based PDF at pH 6.7 (Nutrineal®) (Baxter) ($N = 12$) or EBSS at pH 7.4 ($N = 12$) (Table 1). The weight of the rats was recorded weekly. Catheter patency and the integrity of the skin of the abdomen and around the port were evaluated twice daily. In case of catheter obstruction, an attempt was made to infuse fluids under halothane anesthesia. In case of persistent catheter obstruction, skin lesions, or severe weight loss, dialysate and catheter tip cultures as well as dialysate white blood cell counts were obtained and the animal was sacrificed. At 4-week intervals, dialysate cultures and white blood cell counts

were performed on 2 mL of fluid obtained through a sterile abdominal puncture with a silicon catheter (Venflon) (Becton Dickinson, Erembodegem-Aalst, Belgium) under halothane anesthesia 4 hours after the last dialysate injection. White blood cell counts were performed in a Bürker chamber. Infection was arbitrarily defined as a positive dialysate culture with a dialysate white blood cell count higher than 1000/mm^3 [17].

Study of peritoneal function

After 12 weeks of dialysate exposure, rats were anaesthetized with thiobutabarbital (Inactin, RBI, Natick, MA, USA) (100 mg/kg subcutaneously). The trachea was intubated and a jugular vein was cannulated for continuous infusion of isotonic saline. After 30 minutes, a silicone catheter was inserted in the abdomen and 15 mL of 3.86% Dianeal was infused. After 120 minutes, dialysate was recovered through the silicone catheter and samples were obtained for culture and white blood cell counts. The abdomen was opened by midline incision to collect the rest of the dialysate for determination of net ultrafiltration and to sample tissue. The tunneled polyurethane catheter was removed in a sterile way and the tip was cultured.

Study of peritoneal morphology

One sample of visceral and parietal peritoneum was obtained in each experimental animal in a standardized manner. Parietal peritoneum was obtained at the right hand side of the linea alba and visceral peritoneum was taken from the most distal loop of the small bowel. The samples were fixed in 4% neutral buffered formalin and embedded in paraffin. Five micrometer sections were cut for histology and immunohistochemistry.

The degree of fibrosis was evaluated using a Picro Sirius Red staining F3B (Klinipath, Geel, Belgium). Sections were deparaffinized, rehydrated, and stained briefly with Giemsa. Subsequently, sections were washed and stained with the Sirius Red solution, resulting in a brick red staining of all fibrillary collagen.

Immunostaining for endothelial nitric oxide synthase (eNOS), VEGF, MGO adducts, AGE, and RAGE were performed. Sections were deparaffinized, rehydrated, incubated in 3% H_2O_2 in phosphate-buffered saline (PBS) for 15 minutes to block endogenous peroxidase and washed in 10% normal horse serum (Sigma, St. Louis, MO, USA) in PBS for 20 minutes to block nonspecific binding. Subsequently, they were incubated with the primary antibody, mouse antihuman eNOS (Transduction Laboratories, Lexington, KY, USA), mouse antihuman VEGF (Santa Cruz Biotechnology, Santa Cruz, CA, USA), mouse antihuman MGO adduct, mouse antihuman AGE (6D12) (Cosmo Bio Ltd., Tokyo, Japan) and goat antihuman RAGE (Research Diagnostics, Flanders, NJ, USA), respectively. Thereafter, a biotinylated IgG (Vector Laboratories, Burlingame, CA, USA) and streptavidine-peroxidase were applied for 45 minutes each. 3,3′ diaminobenzidine (DAB) was used as the chromogenic substrate to visualize immunolabeling, resulting in a brown precipitate.

The mouse monoclonal antibody specific for MGO adducts was developed using MGO-modified keyhole limpet hemocyanin (KLH), that was prepared by the reaction of MGO (10 mmol/L) with KHL for 7 days at 37°C, as antigen for the immunization of mice. Ten days after the final booster, antisera were tested with MGO-albumin and the mouse with the highest titer was used for fusion. One out of 40 clones was further characterized. The monoclonal antibody is an IgG1 and has a tenfold preference for 5-hydro-5-methyl-4-imidazolone and/or tetrahydropyrimidine as compared to agrypyrimidine, which are three of the characterized epitopes that are formed after the reaction of MGO with arginine [19]. The monoclonal anti-AGE antibody 6D12 recognizes carboxymethyllysine (CML)-like structures, as well as carboxyethyllysine (CEL) and several unidentified AGE epitopes [20].

Morphometric analysis

Morphometric measurements of the Picro Sirius Red staining and the eNOS, VEGF, MGO adduct, AGE, and RAGE immunostaining were made by a blinded operator with a Zeiss Axiophot microscope (Zeiss, Oberkochen, Germany) at magnification ×200. For each sample of peritoneum, two sections were analyzed quantitatively with a computerized image analysis system (Zeiss). A camera sampled the image of the stained sections and generated an electronic signal proportional to the intensity of illumination, which was then digitized into picture elements or pixels. The digital representation of the tissue was analyzed with KS400 Software (Zeiss). Each pixel in a color image was divided into three color components (hue, saturation, and intensity). The threshold for each color component of the staining was defined and kept constant throughout the analysis. In a predefined area, the Picro Sirius Red, VEGF, eNOS, MGO adduct, AGE, and RAGE staining was measured and expressed as a percentage. In addition, eNOS-labeled blood vessels were counted as number/field. Mesothelial damage was evaluated on the Picro Sirius Red staining by measuring the length of damaged mesothelium and expressing it as percentage of total mesothelial length.

Statistical analyses

The results are expressed as mean ± SEM. Statistical analysis was performed using analysis of variance (ANOVA) and, where appropriate, the Tukey test was

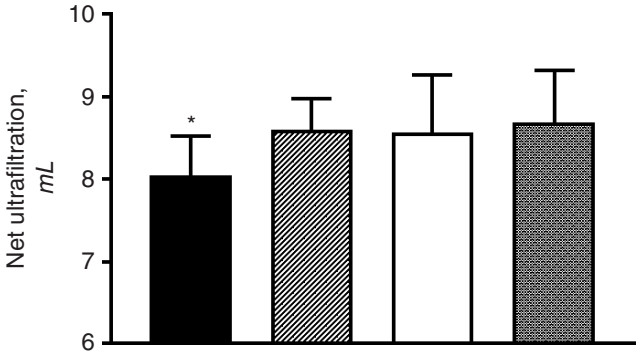

Fig. 1. Net ultrafiltration after a 120-minute dwell of 15 mL of 3.86% glucose dialysate in experimental animals exposed to the standard peritoneal dialysis fluid (PDF) ($N = 10$) (■), low-glucose degradation product (GDP) bicarbonate/lactate-buffered PDF ($N = 10$) (▩), amino acid–based PDF ($N = 9$) (□), and Earle's Balanced Salt Solution (EBSS) ($N = 10$) (■). $^*P < 0.05$ vs. EBSS, low-GDP bicarbonate/lactate-buffered PDF, and amino acid–based PDF.

used as multiple comparison *t* test. The significance level was set at $P \leq 0.05$.

RESULTS

Technique survival and infection rate of laboratory animals

Body weight was similar in the different experimental groups at all time points (data not shown). Technique survival was 83% in the standard PDF, the low-GDP bicarbonate/lactate-buffered PDF and EBSS group and 75% in the amino acid–based PDF group. Drop-out was caused by open abdominal wound with catheter damage ($N = 2$) and leakage around the catheter port ($N = 7$). No episodes of infection, defined as a positive dialysate culture and a dialysate white blood cell count >1000/mm^3, were diagnosed (data not shown).

Ultrafiltration

Net ultrafiltration was significantly lower in the animals that were exposed to the standard PDF, as compared to those treated with low-GDP bicarbonate/lactate-buffered PDF, amino acid–based PDF and EBSS (Fig. 1). Net ultrafiltration was not different among the latter three groups.

Peritoneal morphology

Standard PDF exposure caused an upregulation of eNOS expression and increased vascular density, as compared to all other groups (Table 2) (Fig. 2). No differences in eNOS expression and vascular density were observed between animals treated with low-GDP bicarbonate/lactate-buffered PDF, amino acid–based PDF and EBSS. Similarly, VEGF expression was increased in standard PDF-exposed peritonea, most distinctly in the mesothelial cell layer and in the blood vessel wall (Table 2) (Fig. 3). VEGF immunostaining was not different between the other groups (Table 2) (Fig. 3). An inverse correlation was observed between net ultrafiltration and the number of blood vessels/area (Pearson $r = -0.3696$, $P < 0.05$). In addition, vascular density and VEGF expression correlated positively (Pearson $r = 0.6745$, $P < 0.0001$). Submesothelial fibrosis was more pronounced in animals exposed to standard PDF, than in the other experimental groups (Table 2) (Fig. 4). No correlation was observed between net ultrafiltration and the degree of fibrosis in the peritoneal tissue (Pearson $r = -0.1716$, $P = 0.31$).

Long-term exposure to standard PDF was associated with prominent staining for MGO adducts, especially in the vascular wall and mesothelium. A more modest staining for MGO adducts was visible in the low-GDP bicarbonate/lactate-buffered PDF group. No MGO adducts accumulation was present in the peritoneum of rats treated with amino acid–based PDF and EBSS (Table 2) (Fig. 5). The staining for MGO adducts correlated positively with VEGF expression and vascular density (Pearson $r = 0.5043$, $P < 0.001$ and Pearson $r = 0.4089$, $P < 0.01$, respectively).

AGE strongly accumulated in peritoneal tissue exposed to standard PDF, which was most obvious in the mesothelial layer and the areas of neoangiogenesis. Virtually no AGE staining was observed in the other groups (Table 2) (Fig. 6). RAGE expression was higher in all PDF-exposed peritonea, but the most prominent expression was observed in animals treated with standard PDF and was localized in the mesothelial layer, submesothelial fibrotic tissue, and blood vessel walls (Table 2) (Fig. 7). MGO adducts accumulation significantly correlated with the staining for AGE and RAGE (Pearson $r = 0.4471$, $P < 0.005$ and Pearson $r = 0.3751$, $P < 0.05$). In addition, fibrosis correlated positively with the staining for MGO adducts**,** AGE and RAGE (Pearson $r = 0.4809$, $P < 0.005$, Pearson $r = 0.4843$, $P < 0.005$ and Pearson $r = 0.4455$, $P < 0.01$, respectively). The staining for AGE and RAGE also correlated with VEGF expression (Pearson $r = 0.4928$, $P < 0.005$ and Pearson $r = 0.4015$, $P < 0.05$, respectively), but not with vascular density.

Standard PDF caused significant mesothelial damage as compared to all other groups (Table 2).

DISCUSSION

Chronic exposure of the rat peritoneal membrane to a high-glucose standard PDF results in mesothelial damage, development of submesothelial and interstitial fibrosis and pronounced neoangiogenesis, the latter evidenced by an increased eNOS expression and vascular density. Functionally, the membrane is characterized by a prominent loss of ultrafiltration capacity. These functional and structural characteristics of the peritoneal membrane

Table 2. Histologic and immunohistochemical analysis of the peritoneum

	Dianeal® ($N = 10$)	Physioneal® ($N = 10$)	Nutrineal® ($N = 9$)	Earle's Balanced Salt Solution (EBSS) ($N = 10$)
Blood vessels *number/mm*^{2}	174 ± 17^a	97 ± 9	102 ± 2	95 ± 5
eNOS staining %	0.28 ± 0.09^b	0.17 ± 0.09	0.17 ± 0.3	0.18 ± 0.2
VEGF staining %	1.41 ± 0.22^b	0.83 ± 0.15	0.53 ± 0.15	0.49 ± 0.08
Picro Sirius Red staining %	4.11 ± 2.39^a	1.47 ± 1.09	1.44 ± 0.79	0.87 ± 0.52
Methylglyoxal staining %	3.75 ± 0.75^b	2.28 ± 0.72^c	1.40 ± 0.97	1.33 ± 0.32
AGE staining %	1.70 ± 0.23^b	0.92 ± 0.04	0.96 ± 0.13	1.02 ± 0.05
RAGE staining %	2.48 ± 0.31^b	1.58 ± 0.25^d	1.47 ± 0.27^d	0.46 ± 0.11
Mesothelial damage %	8.04 ± 2.04^b	2.24 ± 1.29	0.20 ± 0.13	1.28 ± 0.82

Abbreviations are: eNOS, endothelial nitric oxide synthase; VEGF, vascular endothelial growth factor; AGE, advanced glycation end products; RAGE, receptors of AGE.

[a] $P < 0.005$ vs. EBSS, low-glucose degradation product (GDP) bicarbonate/lactate-buffered peritoneal dialysis fluid (PDF), and amino acid–based PDF.

[b] $P < 0.05$ vs. EBSS, low-GDP bicarbonate/lactate-buffered PDF, and amino acid–based PDF.

[c] $P < 0.01$ vs. EBSS and amino acid–based PDF.

[d] $P < 0.005$ vs. EBSS.

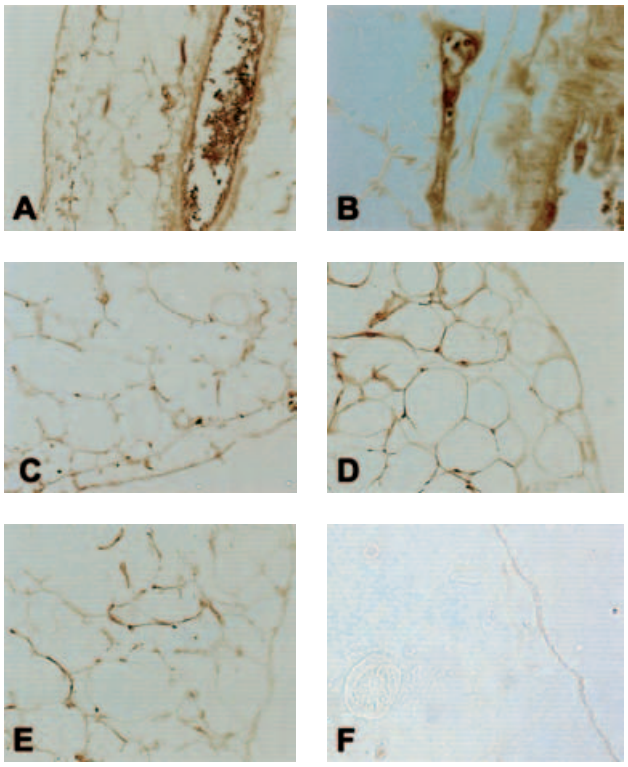

Fig. 2. Immunostaining for endothelial nitric oxide synthase (eNOS) of the visceral peritoneum. (*A*) Prominent eNOS staining was present after 12 weeks' exposure to standard peritoneal dialysis fluid (PDF) (×200). (*B*) Detail of the eNOS staining in the vascular endothelium of a standard PDF-treated rat (×630). Animals exposed to low-glucose degradation product (GDP) bicarbonate/lactate-buffered PDF (*C*), and amino acid–based PDF (*D*) showed an eNOS staining comparable to the Earle's Balanced Salt Solution (EBSS)-exposed group (*E*) (×200). No specific staining is observed when sections are incubated without primary antibody (*F*) (×200).

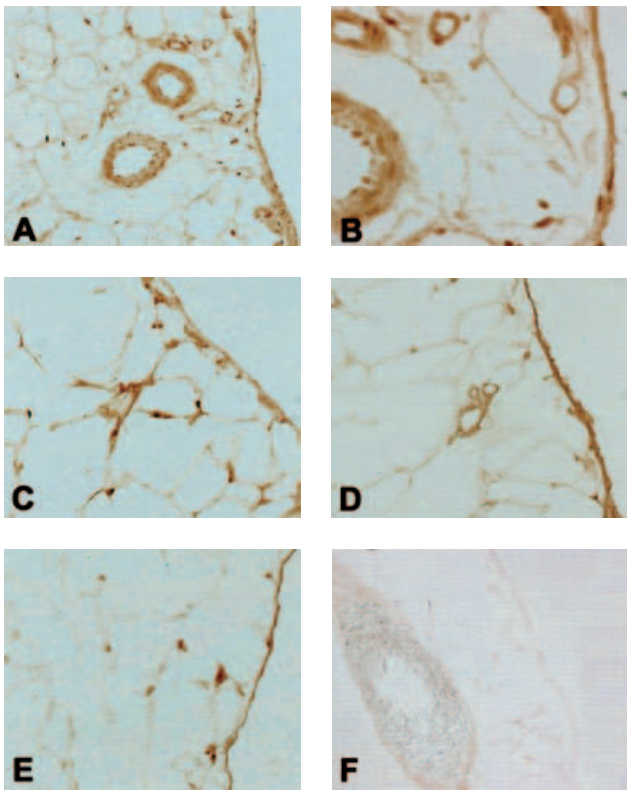

Fig. 3. Immunostaining for vascular endothelial growth factor (VEGF) staining of the visceral peritoneum. (*A*) VEGF staining was prominent in the mesothelial cells and vascular wall of standard peritoneal dialysis fluid (PDF)-exposed rats (×200). (*B*) Detail of mesothelium and blood vessel of a rat exposed to standard PDF (×630). No pronounced staining was observed in rats exposed to low-glucose degradation product (GDP) bicarbonate/lactate-buffered PDF (*C*), amino acid–based PDF (*D*), and Earle's Balanced Salt Solution (EBSS) (*E*) (×200). No specific staining is observed when sections are incubated without primary antibody (*F*) (×200).

are similar of those found in patients on long-term peritoneal dialysis [21, 22], thus underlining the relevance of our experimental model. The salient observation of the present study is that the ultrafiltration failure as well as the diverse structural alterations were absent or much less pronounced with an amino acid–based PDF or a bicarbonate/lactate-buffered PDF with a low GDP content.

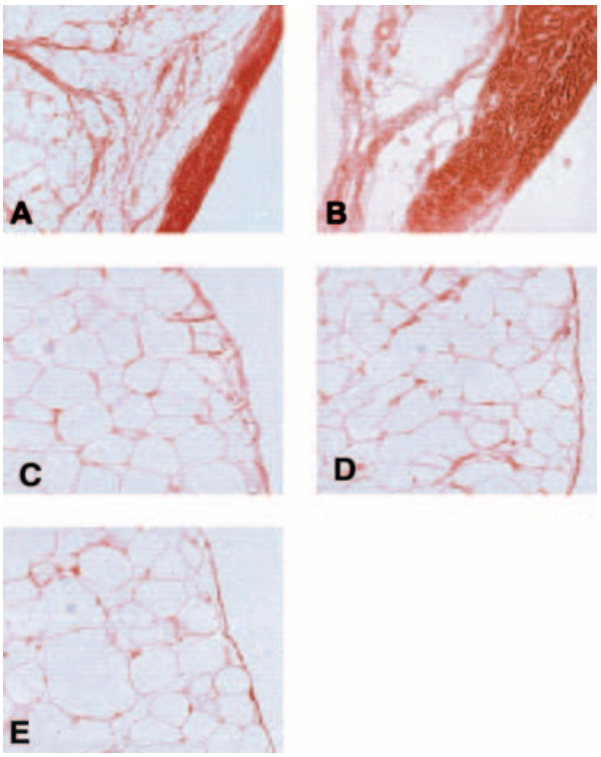

Fig. 4. Picro Sirius Red staining of the visceral peritoneum. (*A*) Animals exposed to standard peritoneal dialysis fluid (PDF) for 12 weeks showed a prominent deposition of fibrous tissue (×200). Detail of the submesothelial fibrotic tissue in a standard PDF-treated animal (×630) (*B*). Almost no fibrosis was present in the animals exposed to low-glucose degradation product (GDP) bicarbonate/lactate-buffered PDF (*C*), amino acid–based PDF (*D*), and Earle's Balanced Salt Solution (EBSS) (*E*) (×200).

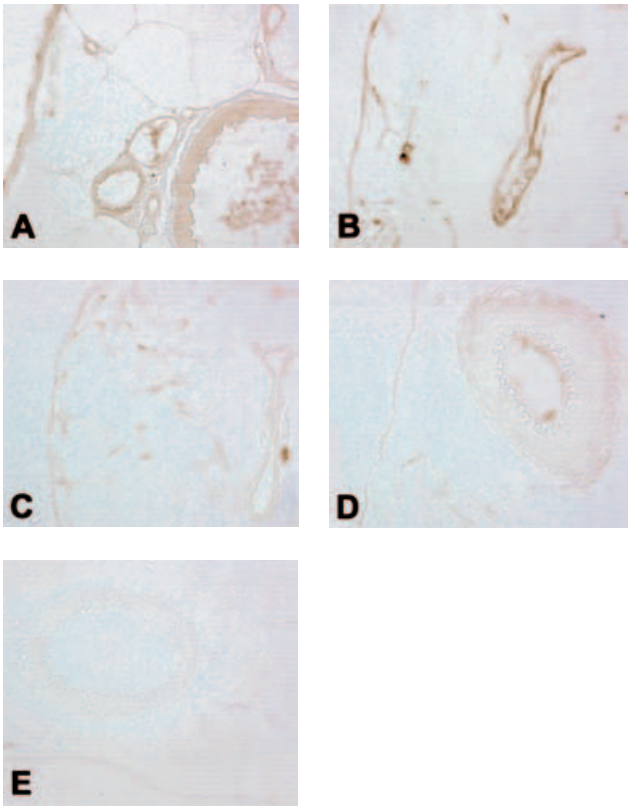

Fig. 5. Immunostaining for methylglyoxal (MGO) adducts of the visceral peritoneum. (*A*) MGO adducts strongly accumulated in peritoneal tissue exposed to standard peritoneal dialysis fluid (PDF) (×200) and to a lesser extent in low-glucose degradation product (GDP) bicarbonate/lactate-buffered PDF (*B*) (×200). No MGO adduct accumulation was observed in animals exposed to amino acid–based PDF (*C*) (×200) or Earle's Balanced Salt Solution (EBSS) (*D*) (×200). No specific staining is observed when sections are incubated without primary antibody (*E*) (×200).

Both the standard PDF and the bicarbonate/lactate-buffered PDF with low GDP content use glucose as the osmotic agent and both the standard PDF and the amino acid–based PDF are characterized by a nonphysiologic pH and use lactate as the buffer. Only the standard PDF, however, contains a high level of GDPs. The double chamber design of the bicarbonate/lactate-buffered PDF allows glucose to be sterilized at a very low pH, substantially reducing GDP formation. As the amino acid–based PDF is nonglucose based, it contains no GDPs at all. Taken together, the present results point to GDPs as the principal causative factor for peritoneal damage.

The causality of GDPs in disturbing viability and normal function in cultured mesothelial cells is a well-established phenomenon. A better preservation of the integrity of the mesothelial cell layer with the bicarbonate/lactate-buffered PDF than with standard PDF has previously been observed [23, 24], but the results could not be ascribed with certainty to differences in GDP content, as high lactate concentrations are also known to affect mesothelial cell viability in vitro [25]. The amino acid–based PDF did not convey prominent mesothelial damage, although it also contains high lactate concentrations. The present findings thus strongly incriminate GDPs as responsible for PDF-induced in vivo mesothelial cell loss.

The involvement of GDPs in peritoneal neoangiogenesis has been suggested repeatedly, though direct evidence has not been provided yet. So far, only one study demonstrated up-regulation of VEGF expression by MGO in mesothelial and endothelial cells in vitro, as well as in the peritoneal membrane in vivo [9]. VEGF is a known promotor of peritoneal neoangiogenesis [6]. Several lines of recent evidence have supported a strong causative link between peritoneal neoangiogenesis and loss of ultrafiltration capacity [7, 26]. In the present study, exposure to standard PDF resulted in an increased staining for VEGF, an increased vascular density, and a lower ultrafiltration rate, with correlations between these four parameters. While these findings are descriptive and do not demonstrate causality, they support the previously formulated hypothesis that VEGF mediates neoangiogenesis and subsequent ultrafiltration failure [6]. Although the

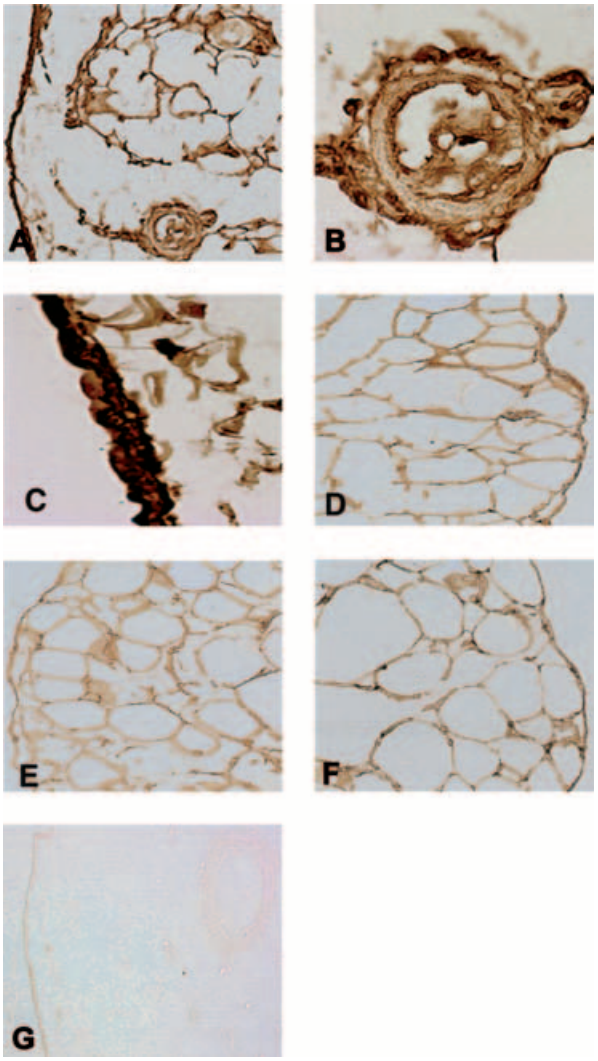

Fig. 6. Immunostaining for advanced glycation end products (AGE) of the visceral peritoneum. (*A*) Standard peritoneal dialysis fluid (PDF) exposure resulted in AGE accumulation in peritoneal tissue (×200). Detail of AGE localization in the standard PDF-exposed blood vessels (*B*) and mesothelium (*C*) (×630). In contrast, almost no AGE accumulation was present in tissue exposed to low-glucose degradation product (GDP) bicarbonate/lactate-buffered PDF (*D*), amino acid-based PDF (*E*), and Earle's Balanced Salt Solution (EBSS) (*F*) (×200). No specific staining is observed when sections are incubated without primary antibody (*G*) (×200).

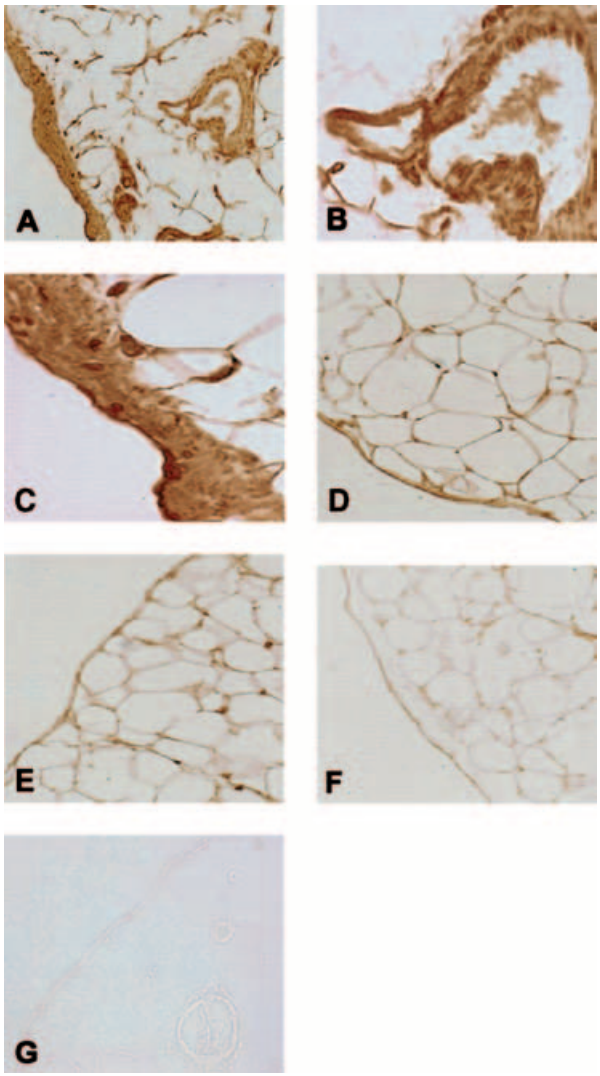

Fig. 7. Immunostaining for receptor for advanced glycation end products (RAGE) of the visceral peritoneum. (*A*) RAGE staining was elevated in the standard peritoneal dialysis fluid (PDF)-exposed animals compared to the other PDF-treated groups (×200). Detail of RAGE-stained vascular tissue (*B*) and mesothelium (*C*) in a standard PDF-treated rat (×630). Low-grade staining was detected in animals treated with low-glucose degradation product (GDP) bicarbonate/lactate-buffered PDF (*D*) and amino acid–based PDF (*E*) which was significantly higher than those exposed to Earle's Balanced Salt Solution (EBSS) (*F*) (×200). No specific staining is observed when sections are incubated without primary antibody (*G*) (×200).

potential of high glucose concentrations to up-regulate VEGF expression in several cell types is well-established [2, 3, 27] and to cause angiogenesis in vivo [28], exposure to high glucose bicarbonate/lactate-buffered PDF was counter-intuitively not associated with increased VEGF expression or vascular density. In contrast with our findings, chronic exposure to a bicarbonate/lactate-buffered PDF was associated with a higher vascular density compared to the control group, though not to the same extent as a standard PDF. It should be noted that the control group was left untreated [23]. The present results, however, suggest that GDPs may be the principal mediators of peritoneal neoangiogenesis. Comparison of heat-sterilized versus filter-sterilized PDF could further strengthen this hypothesis.

GDPs are known to promote AGE generation in vitro [29]. MGO adducts strongly accumulated in the peritonea exposed to standard PDF. Only mild staining for MGO adducts was present in the low-GDP bicarbonate/lactate-buffered PDF group, corresponding with low MGO levels in this PDF. No staining for MGO adducts was observed in the amino acid–based PDF and EBSS group. AGE

accumulation was indeed prominent in the peritonea exposed to standard PDF, mainly in the mesothelial layer where contact with the PDF is most intense, but also in areas of neoangiogenesis and correlated with MGO adduct accumulation. The results are commensurate with clinical studies demonstrating progressive AGE accumulation in the peritoneal membrane of long-term peritoneal dialysis patients [30, 31]. Virtually no AGE formation was seen in the low-GDP PDF groups, suggesting that high glucose exposure by itself is not sufficient to produce AGEs. The pathogenicity of AGEs hinges on their ability to form cross-links with matrix proteins, resulting in an increased tissue thickness and rigidity. Moreover, they trigger a variety of signal transduction pathways through interaction with specific cellular receptors. RAGE, defined as the receptor for AGE, is best characterized of these AGE-binding structures [14]. Human peritoneal mesothelial cells are known to express functional RAGE in vitro [32]. In a normal peritoneal membrane, RAGE expression is low-grade [7], as it is in other tissues in the absence of disease processes [14]. Conditions characterized by cellular activation, including diabetes, uremia, and inflammation, are associated with strong up-regulation of RAGE expression. In addition, AGE ligands themselves are potent inducers of RAGE gene transcription [33]. RAGE expression was profoundly enhanced in the peritonea exposed to standard PDF, but a less pronounced expression was also found in the low-GDP bicarbonate/lactate-buffered and the amino acids–based PDF groups. The etiology nor the consequences of the latter observation are presently clear.

Both AGE accumulation and RAGE expression correlated strongly with the degree of fibrosis in the peritoneal membrane. The fibrotic properties of AGEs are well-known [34]. We have previously demonstrated that binding of AGE to RAGE results in peritoneal fibrosis with TGF-β as downstream mediator [7].

AGEs have been implicated in PDF-induced neoangiogenesis, by virtue of their ability to up-regulate VEGF expression in diverse cell types [11, 35]. The correlation between AGE accumulation and VEGF expression makes it tempting to speculate that they function as upstream mediators of VEGF-induced neoangiogenesis, though no causality was demonstrated. Inhibition of AGE-RAGE interaction did not prevent peritoneal angiogenesis [7]. AGEs may, however, up-regulate VEGF expression through binding with other receptors or nonreceptor-mediated mechanisms.

Although GDPs and the associated accelerated AGE formation appear as the principal causative factors of peritoneal dialysis–related membrane alterations, a role for combined low pH and high lactate concentrations is not entirely ruled out. Equilibration of PDF during the dialysis procedure results in a neutralization of the pH within 30 minutes and a reduction of the lactate concentration to 12.5 mmol/L within the first hour of dialysis [36], thus weakening their potential to provoke persevering membrane damage. Chronic exposure to an acidic lactate buffer without glucose, however, was associated with peritoneal angiogenesis, but the control group was left untreated [28]. It thus remains unclear to what extent these observations were related to the combined effect of high lactate concentrations and low pH or to the repeated fluid instillation, mechanical trauma, and potential introduction of infection.

CONCLUSION

Long-term exposure of the peritoneal membrane to low-GDP or non-GDP PDF was not associated with the development of structural and functional alterations that were prominently present after exposure to standard PDF. As GDPs and associated accelerated AGE formation appear to be the main pathogenetic factors in the progressive membrane deterioration, technologic and pharmacologic strategies reducing GDP and/or AGE formation have the potential to better preserve long-term peritoneal membrane integrity.

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