NEW INSIGHTS INTO THE EFFECTS OF CHRONIC KIDNEY FAILURE AND DIALYSATE EXPOSURE ON THE PERITONEUM

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◆ **Introduction:** Chronic uremia and the exposure to dialysis solutions during peritoneal dialysis (PD) induce peritoneal alterations. Using a long-term peritoneal exposure model, we compared the effects of chronic kidney failure (CKD) itself and exposure to either a 'conventional' or a 'biocompatible' dialysis solution on peritoneal morphology and function.

◆ **Methods:** Wistar rats (Harlan, Zeist, the Netherlands) were grouped into: normal kidney function (NKF), CKD induced by 70% nephrectomy, CKD receiving daily peritoneal infusions with 3.86% glucose Dianeal (CKDD), or Physioneal (both solutions from Baxter Healthcare, Castlebar, Ireland) (CKDP). At 16 weeks, a peritoneal function test was performed, and histology, ultrastructure, and hydroxyproline content of peritoneal tissue were assessed.

◆ **Results:** Comparing CKD with NKF, peritoneal transport rates were higher, mesothelial cells (MC) displayed increased number of microvilli, blood and lymph vasculature expanded, vascular basal lamina appeared thicker, with limited areas of duplication, and fibrosis had developed. All alterations, except lymphangiogenesis, were enhanced by exposure to both dialysis fluids. Distinct MC alterations were observed in CKDD and CKDP, the latter displaying prominent basolateral protrusions. In addition, CKDP was associated with a trend towards less fibrosis compared to CKDD.

◆ **Conclusions:** Chronic kidney failure itself induced peritoneal alterations, which were in part augmented by exposure to glucose-based dialysis solutions. Overall, the conventional and biocompatible solutions had similar long-term effects on the peritoneum. Importantly, the latter may attenuate the development of fibrosis.

Advanced knowledge is required to optimize peritoneal dialysis (PD) for long-term renal replacement therapy, especially in view of the continuing shortage of kidneys for transplantation. Long-term PD can be associated with alterations of peritoneal morphology and function (1,2). Angiogenesis, lymphangiogenesis, hyalinising vasculopathy and fibrosis can develop and lead to changes in peritoneal solute transport and decreased ultrafiltration. Various mechanisms have been hypothesized, of which the combination of glucose with a high dialysate lactate concentration (3), the formation of glucose degradation products (GDPs), and the associated formation of advanced glycation end-products (AGEs) are likely the main culprits (4,5).

New peritoneal dialysis fluids (PDF) have been developed to prevent PD-related peritoneal damage and thereby allow a longer duration for treatment. These 'biocompatible' solutions have low GDP content, neutral or normal pH, and are either lactate-, bicarbonate- or bicarbonate/lactate-buffered. Since peritoneal biopsies are not readily available from clinical studies, the effects of biocompatible PDF on peritoneal morphology have been studied in animal models (6–14). All of these showed a better morphologic status, when biocompatible solutions were compared with conventional ones. However, these studies were either done in rats with normal kidney function (NKF), or peritoneal function measurements were absent or limited, or the duration of exposure was relatively short. Kidney failure itself induces morphological and functional alterations of the peritoneum (15,16), and it should therefore be included in peritoneal exposure models. Moreover, most studies did not extensively assess the morphological and ultrastructural alterations induced by various types of dialysis solutions, especially in long-term exposure models. Presently, specific markers and new methodologies are available and provide valuable tools for improved accuracy (17–20).

The goal of the present study was to perform an extensive comparison between the functional, morphologic, and ultrastructural changes in the peritoneum induced by long-term peritoneal exposure to a conventional and a biocompatible dialysis solution in a rat model with CKD.

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MATERIALS AND METHODS

STUDY DESIGN

Forty-four male Wistar rats (Harlan, Zeist, the Netherlands), with a body weight (BW) of 260 – 280 grams, were randomly assigned to 4 groups: normal kidney function (NKF, $n = 8$), chronic kidney failure (CKD, $n = 12$, 70% nephrectomy, no peritoneal exposure), CKD and daily peritoneal exposure to either a conventional lactate-buffered dialysis solution, 3.86% glucose, Dianeal (CKDD, $n = 12$), or a bicarbonate/lactate-buffered dialysis solution, 3.86% glucose, Physioneal (CKDP, $n = 12$). The solutions were produced by Baxter Healthcare (Castlebar, Ireland) and provided by Baxter (Utrecht, The Netherlands). The exposure groups were infused daily for 16 weeks with the appropriate solution via a catheter. Before instillation, the dialysis fluids were preheated to 37°C and heparinized (5 IU/mL). The rats received 6 mL/100 g BW dialysis fluid intraperitoneally, and the infused volume was set at 20 mL/day in case their BW exceeded 420 g. The fluid was not removed from the peritoneal cavity but allowed to be absorbed. After 16 weeks, a standard peritoneal permeability analysis adapted for the rat (SPARa) and morphologic assessment of the peritoneum, both by light- and electron microscopy were performed. The presence of peritonitis was excluded in all rats at the end of the experiments (results not shown) (21). The protocol was approved by the Committee for Animal Experiments of the Academic Medical Center, University of Amsterdam.

HOUSING CONDITIONS, CATHETER IMPLANTATION, NEPHRECTOMY AND PERITONEAL FUNCTION TEST

Housing conditions, catheter implantation, 70% subtotal nephrectomy, blood collection and standard peritoneal permeability analysis adjusted for rats (SPARa), were all performed as described previously (16), with the following exceptions: the 70% subtotal nephrectomy was performed under anesthesia with isoflurane (4% induction and 2% maintenance) and analgesics were administered both pre- and postoperatively. In short, a vascular access port (Rat-o-Port, Access Technologies, Norfolk Medical, Skokie, IL, USA) was implanted subcutaneously in the neck, and the attached catheter was tunneled subcutaneously and inserted into the peritoneal cavity. After 1 week allocated for recovery, 3 groups underwent 70% subtotal nephrectomy (the right kidney and the upper and lower pole of the left kidney were resected) during a single procedure. Two weeks were allowed for recovery with daily infusions of 1 mL heparinized buffer (5 UI/mL).

PERITONEAL TRANSPORT PARAMETERS

The SPARa was performed in at least 2 animals in each group, as previously described (16). Dextran 70 (5 g/L Hyskon, Medisan Pharmaceuticals AB, Uppsala, Sweden) was added to the solution as a volume marker for calculation of fluid

kinetics. Peritoneal small solute transport was expressed as mass transfer area coefficients (MTAC) of urea and creatinine. Glucose absorption, peritoneal fluid kinetics (transcapillary ultrafiltration rate, net ultrafiltration rate), and the peritoneal clearances of albumin and IgG, were calculated as previously described (22,23). Because of the small number of animals undergoing a SPARa in the NKF group, we did not include this group in the statistical analysis.

RENAL FUNCTION

Twenty-four-hour urine was collected in metabolic cages and used for the calculation of renal function expressed as the mean of creatinine and urea clearances.

IMMUNOHISTOCHEMISTRY

Omentum was collected, fixed in 4% buffered formaldehyde, and embedded in paraffin. Sections were deparaffinized and rehydrated, followed by heat-induced epitope retrieval in sodium citrate, pH = 6. To detect blood vessel endothelial cells, monoclonal mouse IgG1 anti-rat aminopeptidase P 1:200 (ReliaTech GmbH, Wolfenbuttel, Germany) was applied, followed by alkaline phosphatase (AP)-conjugated goat anti-mouse IgG₁ 1:50 (Southern Biotech, Birmingham, AL, USA). Bound antibodies were visualized using the Vector Blue Substrate kit (Vector Laboratories, Inc., Burlingame, CA, USA). For double staining, to distinguish lymphatic endothelial cells, sections were washed, heated in citrate pH = 6, and incubated with monoclonal mouse anti-rat Podoplanin 1:10000 (ReliaTech GmbH, Wolfenbuttel, Germany) as first antibody, followed by AP-conjugated goat anti-mouse and the Vector Red Substrate kit (Vector Laboratories, Inc., Burlingame, CA, USA) for visualization.

IMAGE ACQUISITION

Slides were scanned and TIFF images acquired (1 pixel = 648 nm × 648 nm) using a BX61VS microscope with 10×/0.40 UPlanSApo objective and an Olympus dotSlide imaging system (Olympus, Zoeterwoude, The Netherlands). TIFFs were exported as 10-megabyte files for analysis of blood vessels (BV) and lymph vessel (LV) profile densities.

ANALYSIS OF BLOOD AND LYMPH VESSEL PROFILE DENSITY

The stereology tool STEPanizer version 1 (Bern, Switzerland) was used to quantify the number of vascular profiles and the surface area (15). We analyzed 8 images in each rat, selected after applying the systematic uniform random sampling (SURS) of the images. The vascular profiles were counted in a counting frame with an area of 924 × 924 pixels if they were located inside the counting frame, but not touching the exclusion line or its extensions. The profile density was expressed as ratio of number of vessel profiles per tissue surface area.

HYDROXYPROLINE (OH-PROLINE) CONTENT IN TISSUE SPECIMENS

Omental tissue was collected in Tissue-Tek and frozen at -80°C. Hydroxyproline content was determined as described in detail elsewhere (16), with some modifications. In short, 2 samples in each rat were processed and left to hydrolyze overnight at 85°C, followed by quantitation by high performance liquid chromatography (HPLC) on an Ultimate 3000 LC system (Dionex, Sunnyvale, CA, USA) and fluorescence detection (FP920, Jasco Corporation, Tokyo, Japan). In 3 rats, frozen omental tissue was not available.

ANALYSIS OF FIBROSIS

Omental sections were cut and stained with picro-sirius red, PSR (Gurr, BDH, United Kingdom). In addition, omental tissue from the groups exposed to PDF was stained for type I collagen, using rabbit polyclonal IgG anti-collagen type I $\alpha 1$ at 1:400 (GTX41286, GeneTex Inc., Irvine, CA, USA) as primary antibody, followed by poly alkaline phosphase-conjugated goat IgG anti-rabbit IgG (BrightVision, ImmunoLogic, Duiven, The Netherlands) as secondary antibody. Bound antibody was detected using Vector Blue Substrate Kit.

SEMI-QUANTITATIVE ANALYSIS

Fibrosis was assessed blindly in 3 different areas of omentum (submesothelial, perivascular, and intersegmental), using a 4-graded scoring system as described previously (16). Maximum overall fibrosis score was 9.

IMAGE ANALYSIS

Picro-sirius red-stained sections were scanned as described above, and images were exported as 20-megabyte files. The area of PSR-positive staining was measured (ImagePro premier 9.1, Media Cybernetics, Inc., Rockville, MD, USA), and expressed as percentage of the total surface area. The latter was calculated using ImageJ 1.44 (National Institutes of Health, USA).

Collagen I immunostained sections were scanned as described above and images were exported as 40-megabyte files. After SURS of the files was applied, 5 images were selected in each rat for analysis.

ELECTRON MICROSCOPY

Mesenteric tissue collected in 3 rats from each group was fixed overnight in 1% glutaraldehyde and 4% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH = 7.4) and further processed for transmission electron microscopy (TEM) (see Supplemental data). Sections were examined with a FEI Technai-12 G2 Spirit Biotwin electron microscope and micrographs were taken with a Veleta camera (Olympus SIS).

MESOTHELIAL CELL LAYER

A TEM operator took micrographs of the mesothelial cell (MC) layer, which were blindly analyzed using Leica Qwin software. We analyzed the number of all visible microvilli attached to the membrane and expressed it per length of the membrane surface. No statistical analysis was attempted because of the limited number of rats in which the MC layer was assessed.

VASCULAR BASEMENT MEMBRANE

At least 25 BV cross-sections were analyzed in each group. Micrographs were taken at a 23,000 $\times$ magnification and the analysis was done blindly. The alterations of the vascular basement membrane (VBM) were defined based on an adaptation of a previously published method (24) as: (1) normal: monolayered, < 200 nm thickness; (2) thick: monolayered, > 200 nm thickness; (3) mild alteration: 2 – 3 layers, duplication present on at least 30% of the visible blood vessel circumference; (4) moderate alteration: 4 layers or more, duplication present on at least 30% of the visible BV circumference. Vascular basement membrane thickness was measured as follows: measurement sites were placed every 1 μ m along the vessel circumference. Only measurement sites where the endothelial cell membrane was clearly delineated as a thin sharp black line were taken into account for the analysis to correct for oblique sections. To avoid error, no measurements were done in areas with pericytes. For analysis, we used Qwin (Leica Microsystems Imaging Solutions Ltd., Cambridge, UK) software. Non-homogeneity, defined as variability in electron density throughout the lamina basalis, was assessed as present or absent. No statistical analysis was attempted because of the limited number of rats in which the vascular basal lamina was assessed.

STATISTICS

The data are presented as median and interquartile range. Differences between the groups were assessed with an ANOVA test with Games-Howell post-hoc analysis or Mann-Whitney U test for continuous variables, and the Pearson chi-square test for categorical variables. When these showed significant differences, further comparisons were made. These included NKF vs all other groups, CKD vs all rats exposed to dialysis solutions (CKD+exposure), and CKDD vs CKDP. For the analysis, we used the IBM SPSS statistics software, version 21.

RESULTS

DEVELOPMENT OF CHRONIC KIDNEY DISEASE

Glomerular filtration rate was uniformly decreased at 16 weeks after 70% nephrectomy (Table 1). Five rats (2 CKDD, 3 CKDP) were excluded from further analysis, because of complications due to excessive bleeding post-nephrectomy ($n = 2$), catheter obstruction ($n = 1$), and peritonitis ($n = 2$).

PERITONEAL TRANSPORT

The rats with kidney failure had higher small solute transport rates than the NKF animals (Table 2). Exposure to PDF augmented this effect, showing it also for glucose absorption. Also, peritoneal clearance of IgG was higher in the exposed rats. No difference was present between CKDD and CKDP. No difference was found for fluid transport parameters.

MESOTHELIAL CELL ALTERATIONS

Mesothelial cells in NKF rats appeared as a flattened monolayer (Figure 1). Mesothelial cells in CKD showed a reactive state, being enlarged with a significant increase in the number of microvilli (NKF: 0.26 microvilli/ μm , CKD: 0.38 microvilli/ μm), and many intracytoplasmic vesicles. Areas of detachment from the basal lamina were present. The exposure to both dialysis fluids led to further enlargement of MCs and alterations of their plasma membrane resulting in increased numbers of microvilli (CKDD: 0.44 microvilli/ μm , CKDP: 0.51 microvilli/ μm). Mesothelial cells in CKDD contained numerous intracytoplasmic vesicles but maintained extensive

cell-to-cell lateral contact. In contrast, MC in CKDP displayed prominent cytoplasmic protrusions forming bridged intercellular spaces. In addition, similar processes were present at the basal membrane, occasionally traversing the lamina basalis and protruding into the submesothelial compartment (Figure 1). Occasional necrotic MC were found in CKDD, in association with areas of denudation.

BLOOD AND LYMPH VESSEL PROFILE DENSITIES

All groups with kidney failure had higher blood and lymph vessel profile densities compared with NKF, as shown in Table 3. Angiogenesis, but not lymphangiogenesis, was more extensive in the exposed groups compared with CKD (Figure 2, upper panel, and Supplemental Figure 1 for a higher magnification). Lymphatic vessel and BV profile densities were similar in CKDD and CKDP (see also Supplemental Figure 2).

VASCULAR BASEMENT MEMBRANE ALTERATIONS

Chronic kidney failure rats tended to have a thicker VBM compared with the NKF group, with areas of duplication

TABLE 1
Renal Function at 16 Weeks after 70% Nephrectomy^a

	NKF N = 8	CKD N = 12	CKDD N = 10	CKDP N = 9
Removed kidney mass (%)	0	69.6 (68.3–70.4) ^b	69.5 (68.4–70.2) ^b	68.8 (68.7–69.6) ^b
GFR (mL/min)	2.9 (2.7–3.5)	1.5 (1.2–1.6) ^b	1.7 (1.4–1.9) ^b	1.4 (1.0–1.7) ^b

NKF = normal kidney function; CKD = chronic kidney failure; CKDD = CKD and exposure to Dianeal; CKDP = CKD and exposure to Physioneal; GFR = glomerular filtration rate.

^a Comparison of renal function of the groups at the end of the experimental period. No difference was present between CKDP and CKDD.

^b NKF vs CKD, CKDD, CKDP all $p < 0.001$

Results are expressed as median and interquartile range.

ANOVA with Games-Howell post-hoc analysis.

TABLE 2
Peritoneal Transport Parameters as Measured by the Standard Peritoneal Permeability Analysis Adapted for Rats

Parameter	NKF N = 2	CKD N = 3	CKD+exposure N = 8	CKDD N = 4	CKDP N = 4
MTAC urea 240 ($\mu\text{L}/\text{min}$)	105.4 (104–107)	160.1 (129–196)	188.4 (145–496)	185.4 (148–206)	190.6 (145–496)
MTAC creatinine 240 ($\mu\text{L}/\text{min}$)	41.4 (40–43)	72.9 (49–82)	115.7 (89–150) ^a	96.0 (89–150)	134.2 (89–137)
Glucose absorption (%)	58.2 (54.0–62.3)	58.1 (57.9–59.8)	68.4 (61.9–71.1) ^a	68.5 (67.8–71.1)	67.0 (62–69)
Albumin clearance ($\mu\text{L}/\text{min}$)	1.3 (1.1–1.5)	1.6 (1.3–3.1)	2.7 (2.4–3.8)	2.7 (2.4–2.9)	2.7 (2.6–3.8)
IgG clearance ($\mu\text{L}/\text{min}$)	0.4 (0.3–0.5)	0.6 (0.5–0.6)	0.8 (0.7–0.9) ^a	0.7 (0.7–0.9)	0.77 (0.76–0.78)
TCUFR ($\mu\text{L}/\text{min}$)	74.2 (73.6–74.9)	78.2 (75.4–79.6)	87.1 (63.2–167.2)	89.8 (68.4–124)	85.2 (63.2–167.2)
NUFR ($\mu\text{L}/\text{min}$)	72.6 (63.6–81.6)	60.5 (58.4–103.0)	61.5 (29.2–77.5)	62.4 (60.2–64.4)	57.9 (29.2–77.5)

NKF = normal kidney function; CKD = chronic kidney failure; CKDD = CKD and exposure to Dianeal; CKDP = CKD and exposure to Physioneal; CKD+exposure = CKDD and CKDP analyzed together; MTAC 240 = mass transfer area coefficient at 240 min; IgG = immunoglobulin G; TCUFR = transcapillary ultrafiltration rate; NUFR = net ultrafiltration rate.

Mann-Whitney test: ^a $p < 0.05$, CKD versus CKD+exposure

Results are expressed as median (range).

Figure 1 — Mesothelial cell ultrastructure is differently modified in CKD and after exposure to dialysis solutions. Compared with MC in NKF, MC in CKD rats are enlarged, with more microvilli (black arrowhead), and higher number of cytoplasmic vesicles. Mesothelial cells in both exposed groups are further enlarged and occasionally contain lamellar bodies (white arrowhead). In CKDD, both microvilli and intracytoplasmic vesicles (inset) are more numerous. In CKDP, cytoplasmic processes are bridging widened inter-cellular gaps (open arrow). At the basal side of the plasma membrane, invadopodia-like structures are present. Occasionally these penetrate through the mesothelial basal lamina into the sub-mesothelial stroma (inset). CKD = chronic kidney failure; MC = mesothelial cells; NKF = normal kidney function; CKDD = chronic kidney failure and exposure to Dianeal; CKDP = chronic kidney failure and exposure to Physioneal. Scale bars represent 2 μ m, in insets 0.4 μ m.

TABLE 3
Morphological Alterations of the Peritoneal Membrane^a

	NKF N = 8	CKD N = 12	CKD+exposure N = 19	CKDD N = 10	CKDP N = 9
Vessel densities (profiles/mm ²)					
Blood vessels	2.9 (2.2–3.8)	5.5 (4.4–6.9) ^b	7.0 (5.7–8.5) ^{b,c}	7.3 (5.6–9.0)	7.0 (5.7–8.3)
Lymph vessels	0.3 (0.1–0.5)	0.6 (0.5–1.2) ^b	0.9 (0.6–1.2) ^b	0.7 (0.5–1.7)	0.9 (0.6–1.2)
Analysis of fibrosis					
Fibrosis score	0 (0–1)	3 (1–3) ^b	5 (4–6) ^{b,c}	6 (5–7)	4 (4–5) ^d
PSR staining (%)	1.3 (0.9–2.2)	2.8 (2.0–3.5) ^b	7.9 (4.4–11.8) ^{b,c}	7.1 (5.3–16.6)	8.7 (4.1–11.2)
OH-proline (μ g/mg tissue)	0.3 (0.2–0.5)	0.5 (0.4–1.0)	0.8 (0.4–1.3)	1.0 (0.4–1.3)	0.6 (0.2–1.1)
Collagen I (%)	—	—	14.2 (9.7–18.8)	14.8 (13.1–25.8)	9.8 (5.9–18.5) ^e

NKF = normal kidney function; CKD = chronic kidney failure; CKDD = CKD and exposure to Dianeal; CKDP = CKD and exposure to Physioneal; CKD+exposure = CKDD and CKDP analyzed together; PSR = picro-sirius red; OH-proline = hydroxyproline.

^a We assessed blood and lymph vessel profile densities, fibrosis by a semi-quantitative method (fibrosis score), percentage of the picro-sirius red positive area from the total tissue section (PSR staining), OH-proline content in omentum, and fraction of collagen I positive area from the total tissue section.

^b $p < 0.05$ CKD, CKD+exposure vs NKF.

^c $p < 0.05$ CKD+exposure versus CKD.

^d $p < 0.05$ and ^e $p = 0.09$ CKDP versus CKDD.

Results are expressed as median and interquartile range.

(Figure 3 and Supplemental Figure 3). The severity of these alterations was further increased by exposure to PDF, and no difference was evident between CKDD and CKDP. In the majority of vessels, however, the duplication was mostly focally distributed. In addition, variability in electron density of VBM was observed in CKD compared with NKF, and this alteration was more evident in the exposed groups.

PERITONEAL FIBROSIS

Both the semi-quantitative analysis and whole-slide image analysis of PSR staining (Figure 2, lower panel) showed more

fibrosis in CKD compared with NKF (Table 3, Supplemental Figure 2). The exposure to PDF further increased the amount of fibrosis. Although not statistically significant, the hydroxyproline content of peritoneal tissue was higher in CKD than in NKF rats and further increased in those exposed to dialysis solutions. The semi-quantitative analysis showed less fibrosis in the CKDP rats compared with the CKDD ones. In agreement, the whole-slide image analysis, the OH-proline content of homogenized peritoneal tissues, and collagen I immunohistology (Supplemental Figure 4) showed a trend toward less fibrosis in the CKDP rats (Table 3, Supplemental Figure 2).

Figure 2 — Upper panel. Both angiogenesis and lymphangiogenesis are induced in CKD and only the former is further enhanced after exposure to both dialysis solutions. These alterations occurred to a similar extent in CKDD and CKDP. Double immunostaining was performed to detect endothelial cells in blood vessels using anti-aminopeptidase P and in lymph vessels using anti-podoplanin. Mesothelial cells also stain for podoplanin. Lower panel. Fibrosis is increased in CKD and further enhanced in CKDD and CKDP. Omental tissue was stained with picro sirius red to detect collagen. NKF = normal kidney function; CKD = chronic kidney failure; CKDD = chronic kidney failure and exposure to Dianeal; CKDP = chronic kidney failure and exposure to Physioneal. Scale bar represents 200 μ m.

Figure 3 — Lamina basalis of blood vessels is thickened in CKD and duplicated after exposure to both dialysis solutions. Lamina basalis is indicated by arrow heads. NKF = normal kidney function; CKD = chronic kidney failure; CKDD = chronic kidney failure and exposure to Dianeal; CKDP = chronic kidney failure and exposure to Physioneal. Scale bars represent 1 μ m.

DISCUSSION

This comprehensive analysis of the effects of long-term exposure to PDF on the peritoneum in a rat model with CKD, similar to stage 3 to 4 in humans, shows that CKD itself induced MC alterations, new BV and LV formation, duplication of the vascular basal lamina, peritoneal fibrosis, and high peritoneal transport rates. Long-term exposure to PDF further increased these transport rates and enhanced the ultrastructural alterations, angiogenesis, and the extent of fibrosis, but had no effect on new LV formation. The biocompatible PDF was associated with increased MC membrane surface area and a trend toward less fibrosis, compared with the conventional solution.

Mesothelial cells are at the interface between peritoneum and PDF during PD. They are metabolically active, constitute a

source of growth factors, extracellular matrix molecules, and various immunomodulatory mediators (25), and contribute to the structural and functional integrity of the peritoneum. Chronic kidney failure induced MC alterations, which is in line with data in patients (26,27). An additional effect of exposure to PDF was present. The increased number of microvilli found in our study seems at odds with some studies in which exposure to PDF induced a decrease in the number of microvilli (28,29). It is likely that mesothelial degeneration prevailed in those studies, while in others (30) and here, activation and regeneration were more prominent. Importantly, an influence of the kind of dialysis solution administered could be seen. Whereas in CKDD, the increased number of vesicles was predominant, in CKDP, cytoplasmic protrusions were present at the basal side, causing an additional increase in the MC plasma membrane

surface area. The occasional extension of these structures into the submesothelial stroma is consistent with the migratory phenotype of MC during epithelial-to-mesenchymal transition, as described previously by Yanez-Mo *et al.* (31). Of note, in patient studies, the switch from a conventional to a biocompatible solution is associated with an increase in effluent cancer antigen 125 (CA125), a mucinous glycoprotein mainly located at the cell membrane of MC (4,32). The higher levels of this biomarker are thought to reflect the increased MC mass and turnover in this condition, consistent with less damage to the peritoneum (32–34). Little is known about the mechanisms which lead to increased CA125 in peritoneal effluent. Both increased production by mesothelial cells and increased shedding from the cell membrane may be involved. Mesothelial cells with enlarged cell membrane area, as we describe in CKDP rats, may be the source of higher CA125 effluent levels, as found in PD patients after the switch to a biocompatible PDF. Unfortunately, due to the unavailability of a suitable antibody, we were unable to determine effluent CA125 levels in our rats.

Chronic kidney failure was associated with higher peritoneal blood and lymph profile densities than NKF. Previous experimental studies investigating angiogenesis in PD used endothelial markers such as CD31, von Willebrand factor, or eNOS, which are expressed by both blood and lymphatic endothelium. We used specific staining to distinguish BV from LV and applied unbiased stereological methods for the analysis. Aminopeptidase P, a glycosyl-phosphatidylinositol membrane-linked aminoacylproline aminopeptidase, is expressed specifically by endothelial cells of BV only (20). Using this methodology allowed us to confirm that exposure to PDF causes a further increase in new BV formation. There was no difference between CKDD and CKDP in this respect. This contrasts with the reduction in vessel density reported previously using a non-specific endothelial staining, after exposure to a biocompatible PDF for various periods of time (6,8,9,11).

The peritoneal alterations are time-dependent (9). It may be that the difference between the 2 solutions is important at the start of the treatment and becomes less important over time. Limited data are available in patients, but a recent study reported an increased BV density in patients treated with a neutral dialysis solution compared with those treated with acidic ones (35). Importantly, the similar BV densities in the CKDD and CKDP rats are in line with the lack of a difference in peritoneal solute transport in both rats and patients. Chronic kidney failure was associated with some increase in small solute transport, confirming previous reports (16). This is in line with the observation that patients with normal renal function had lower small solute transport rates during PD than uremic patients (36). Exposure to PDF augmented these effects in the present study, without any difference in peritoneal transport between the 2 solutions. This is in accordance with the situation in patients, where most studies have shown no effect of biocompatible PDF on peritoneal solute transport after short to median time exposure (37,38). A recent randomized controlled trial showed a small difference in the time-course of peritoneal

transport with lower D/P creatinine values in patients treated with a biocompatible solution (39). The difference between these clinical studies may have been caused either by the use of different solutions or by the duration of follow-up.

At the ultrastructural level, uremia itself causes some increase in thickness, reduced homogeneity, and duplication of the VBM. Although hypertension may have been the cause, vascular abnormalities are present as well in patients with renal failure without hypertension (40). The exposure to PDF augmented these 'diabetiform' alterations without any evidence for a difference between the 2 glucose-based solutions. The fact that these solutions differ only in pH and GDP content strongly suggests that glucose itself, possibly enhanced by hypertension, is by far the most important factor in the genesis of the vascular alterations associated with long-term PD.

Limited data are available on new LV formation induced by PD. Lymphangiogenesis develops after short-term exposure to PDF in both non-uremic and uremic rats (41–43), but data are lacking in long-term exposure models. In the present study, CKD induced an increase in LV density. Of note, lymphangiogenesis was not further enhanced by exposure to PDF, which is in line with the clinical data. In patients, no difference was found between those exposed to either a neutral or an acidic dialysis solution and pre-dialysis patients (35). Our findings are also in agreement with the functional data which showed that no time trend is present for the effective peritoneal lymphatic absorption rate in stable PD patients (44).

Chronic kidney failure itself induced peritoneal fibrosis, which was further enhanced in the groups exposed to PDF. The presence of a catheter in the peritoneal cavity of the exposed animals may itself have caused inflammation and induced structural and functional alterations of the peritoneum (45). However, these reaction patterns tend to occur predominantly during the initial phase of PD and decrease significantly after 8 weeks of exposure (45). Also, in the present study, an initial effect of the catheter may have been present, but an exact contribution to peritoneal alterations cannot be established. Importantly, the long duration of exposure to PDF in the present study allowed us to focus on the long-term effects of the dialysis solutions on the peritoneum. The semi-quantitative analysis of the PSR staining indicated that the biocompatible solution induced less fibrosis than the conventional one. In agreement, the whole-slide analysis, OH-proline determinations, and collagen I staining analysis also showed a trend toward less fibrosis for the biocompatible PDF. This is in line with findings in non-uremic rats (8,11) and in patients, showing a thinner submesothelial compact zone after exposure to a biocompatible solution compared with the conventional one (35). It is speculative whether this can be explained by a lower GDP content, causing reduced formation and accumulation of AGEs. In order to gain more insight into the organization of the collagen fibers, we considered using polarization microscopy. Although extensively used in literature for the characterization of collagen fibers (46), recent data show its limited value (47).

The particular strength of the present study is the application of long-term daily peritoneal exposure to dialysis solutions

in the setting of advanced CKD, thereby truly modeling clinical PD. Using this model, this study is the first to compare exposure of the peritoneal cavity to either conventional or biocompatible dialysis solutions, combining functional peritoneal transport characteristics with detailed morphological and structural analyses. Systematic uniform random sampling, followed by computer-assisted stereology for the quantification of vascular profile densities, ensured accurate estimates for these parameters. Our model may further deepen insight into the pathogenesis of peritoneal fibrosis and offer reliable exploration of potential therapeutic interventions.

In summary, chronic renal failure itself induces peritoneal alterations, which are overall comparably augmented by long-term exposure to both conventional and biocompatible dialysis solutions. Interestingly, however, in contrast to angiogenesis, lymphangiogenesis was not further enhanced by exposure to either of the glucose-based PDFs. Distinct MC phenotypes were induced by each of the dialysis solutions. Importantly, the use of a biocompatible solution tends to be beneficial with respect to the development of peritoneal fibrosis.

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DISCLOSURE

The authors have no financial conflict of interest to declare.

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