No Relation Between Peritoneal Fibrosis and Free Water Transport in a Rat Model

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Free water transport (FWT) during peritoneal dialysis (PD) can easily be measured by Na^+ kinetics. In long-term PD, FWT might reflect peritoneal fibrosis, but morphologic or functional relationships have not been investigated. Nonconventional dialysis solutions might be associated with better preservation of peritoneal tissues and function. We developed a long-term peritoneal exposure model in rats with impaired kidney function and investigated peritoneal morphology and function in that model after exposure to conventional and nonconventional solutions.

Two studies were reanalyzed. Transport was assessed using a standard peritoneal permeability analysis adapted for the rat. Omental tissue was stained with picro-sirius red (PSR) for uniform quantification of fibrosis. A semiquantitative fibrosis score was also calculated.

Rats (n = 9) exposed to a conventional solution for 16 weeks were compared with rats (n = 9) exposed to other solutions. Peritoneal transport parameters were similar, but the degree of fibrosis tended to be more severe in the conventional-solution group. Compared with the situation in humans, the contribution of FWT to ultrafiltration in rats was larger than that of small-pore fluid transport. No correlation between the percentage PSR positivity and FWT was observed. A marked difference in PSR positivity was found between the two studies.

The long-term exposure model is not suitable for the study of relationships between FWT and peritoneal fibrosis. Quantitative assessment of the fibrosis is difficult.

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Key words
Free water transport, peritoneal fibrosis, long-term peritoneal exposure, rats with kidney failure, biocompatibility

Introduction

Ultrafiltration in peritoneal dialysis (PD) patients occurs from the circulation to the dialysate-filled peritoneal cavity by dialysate glucose–induced osmosis, partly through small interendothelial pores (SPFT) and as free water transport (FWT) through the endothelial water channel aquaporin-1 (AQP1). We and others found that FWT, estimated by Na^+ removal, is a predictor for encapsulating peritoneal sclerosis in long-term PD patients (1,2). In those patients, AQP1 expression is normal, which led to the theory that filtered water is bound to collagen fibers in the peritoneal interstitial tissue without binding of Na.

It is known that FWT is often low in long-term PD patients with late ultrafiltration failure (3,4) and that FWT declines, especially after a PD duration of more than 4 years (5). No longitudinal data about human peritoneal morphology have been published, but peritoneal fibrosis is a feature of long-term PD (6). We therefore hypothesized that the magnitude of FWT in long-term PD patients might represent peritoneal fibrosis (7).

The use of "biocompatible" rather than conventional dialysis solutions is associated with less peritoneal fibrosis, especially in patients without any peritonitis episodes (8,9), but no information on FWT is available from those studies. Functional–morphologic relationships can be assessed in long-term models using animals with renal failure. Our group developed a long-term peritoneal exposure model in rats with chronic renal failure induced by 70% nephrectomy (10). The aim of the present study was to analyze possible relationships between peritoneal fibrosis and FWT in that experimental model.

Methods

Two previously published studies were used for the analyses (11,12). The models used in both studies were identical. In study A, a conventional 3.86% glucose solution (Dianeal: Baxter, Nivelles, Belgium) was compared with a mixture of low concentrations of glycerol, amino acids, and dextrose (GLAD), which had a similar osmolarity and normal pH (11). Study B compared Dianeal with a "biocompatible" solution (Physioneal: Baxter), both with 3.86% glucose (12). Table I shows the solution comparison.

In both of the foregoing studies, male Wistar rats with a body weight of 260–300 g underwent implantation of a peritoneal catheter with an attached vascular access port after an acclimatization period of 1 week. A 70% nephrectomy was performed 1 week later. After a recovery period of 2 weeks, daily intraperitoneal infusions through the catheter were performed for a period of 16 weeks. The infused solutions were not drained, but were allowed to be reabsorbed. After the infusion period, before humane sacrifice, a standard peritoneal permeability analysis adapted for the rat (SPARa) was performed. The methodology has been extensively described (10,11).

The parameters analyzed in peritoneal effluent were dextran 70 by high-performance liquid chromatography for determination of the intraperitoneal volume at various time points (13), Na^+ by the indirect ion selective electrode method, and creatinine by an enzymatic method. Creatinine and Na^+ were also measured in plasma. During the first hour of the dwell time, $FWT_{0–60}$ was calculated by Na^+ kinetics, based on the intraperitoneal volume and the dialysate and plasma Na^+ (14). A simpler method, in which the difference between the dialysate Na^+ before inflow and at 60 minutes was calculated, was also used. The ratio between FWT and SPFT was calculated as well.

Omental tissue was obtained directly after sacrifice, preserved in 4% formaldehyde, and embedded in paraffin. After sectioning, the tissues were stained with picro-sirius red [PSR (Gurr: BDH Chemicals, Poole, U.K.)], which stains all fibrillary collagen.

Fibrosis was blindly assessed in 3 different areas of omentum (submesothelial, perivascular, and intersegmental), using a 3-grade scoring system that adds to a maximum score of 9 (10). Additionally, PSR-stained sections were scanned, and TIFF (tagged image file format) images were acquired using a BX61VS microscope and a dotSlide imaging system (Olympus, Zoeterwoude, Netherlands). The area of PSR-positive staining was measured (ImagePro Premier 9.1: Media Cybernetics, Rockville, MD, U.S.A.) and expressed as percentage of the total surface area, calculated using ImageJ 1.44 (National Institutes of Health, Rockville, MD, U.S.A.).

Results are presented as mean values with standard deviation, or as medians with range, depending on the data distribution. A Mann–Whitney U-test was used to detect statistical differences. A logarithmic transformation was done for the calculation of the Pearson correlation coefficient between the parameters of FWT and the amount of fibrosis.

TABLE I Characteristics of the three solutions[a]

Characteristic	*Conventional*	*GLAD*	*Biocompatible*
Osmotic agent or agents	3.86% Dextrose	1.4% Glycerol 0.5% Amino acids 1.1% Dextrose	3.86% Dextrose
Buffer (mmol/L)	Lactate (35)	Lactate (15) Bicarbonate (25)	Lactate (15) Bicarbonate (25)
Glucose degradation products	High	Very low	Low
pH	5.2	7.4	7.4
Na^+ (mmol/L)	132	132	132
Osmolarity (mOsm/L)	486	512	486

[a] All solutions provided by Baxter, Nivelles, Belgium.
GLAD = glycerol, amino acids, dextrose.

Results

Of the 18 rats available for the analyses, 2×5 came from study A, and 2×4 came from study B. Table II summarizes the associated transport and morphology data. The results for dialysate-to-plasma (D/P) creatinine were similar in all groups, and no statistically significant differences in FWT parameters were found. The FWT/SPFT ratio averaged 0.85 in the rats exposed to conventional solutions and 1.11 in those exposed to biocompatible solutions. No correlation between FWT and SPFT was evident. An FWT/SPFT ratio less than 0.6 was present only in 3 rats from the conventional-exposure group and in 3 rats from the other groups.

The semiquantitative fibrosis scores for rats exposed to conventional solutions were in the same order of magnitude. Compared with the conventional solutions, the GLAD and biocompatible solutions were both associated with less fibrosis. However, the areas identified as positive for PSR, expressed as percentage of the total surface area, were much smaller in study B than in study A for all solutions. Nevertheless, and in accordance with the semiquantitative scoring, the percentage PSR remained lower in the GLAD group than in the conventional-solution group in study A. In study B, the same trend was present, but statistical significance for PSR between the two solutions was not reached. No relation between the parameters of FWT and the PSR-positive areas was evident in either study A or study B. Also, when the PSR areas were normalized to the mean of all PSR values obtained in the conventional-exposure studies, no trace of an association was detected.

Discussion

The present analysis has shown convincingly that the long-term peritoneal exposure model in rats with chronic kidney failure is unsuitable to mimic the human pre–encapsulating peritoneal sclerosis stage, in which a very severe degree of fibrosis is associated with a reduction in FWT (1,2).

Both FWT and SPFT depend on the crystalloid osmotic pressure gradient, and so a relationship between the two is often present, except in conditions with markedly reduced FWT, such as long-term ultrafiltration failure (15) and encapsulating peritoneal sclerosis (1,2). The contribution of FWT to transcapillary ultrafiltration is assumed to average 40% in studies using kinetic modeling (16). In PD patients, the $FWT_{0–60}$ has a median value of 38%, with a range between 8% and 82% (4). Consequently, the ratio between FWT and SPFT averaged 61%, with a maximum of 5%. The mean ratio of 0.85 in the present analysis is therefore much higher and suggests either the presence of an inappropriately low SPFT or a high FWT rate. The explanation for the phenomenon is unknown.

Intraperitoneal dextran was used for measurement of the intraperitoneal volume after correction for incomplete recovery. The calculated dextran disappearance rate during the 4-hour dwell was 0.001% of the instilled dialysate volume, which is similar to the 0.0008% observed in PD patients. That observation

TABLE II Peritoneal transport and fibrosis parameters[a] in two studies

	Study A solutions		*Study B solutions*	
Parameter	*Conventional*	*GLAD*	*Conventional*	*Biocompatible*
D/P creatinine	0.63±0.2	0.64±0.1	0.60±0.1	0.63±0.1
$FWT_{0–60}$ (mL)	3.6±0.5	4.3±2.0	3.4±0.1	3.1±0.5
$\Delta Na^{+}_{0–60}$ (mmol/L)	9.4±1.5	9.6±2.9	10.3±1.3	9.5±2.4
Fibrosis score	7 (6–9)	4 (3–5)[b]	6 (5–7)	4 (4–5)[b]
PSR staining (% per slide)	33.1±19.0[c]	17.3±5.5[d]	9.2±5.1[c]	6.2±3.0

[a] Presented as mean ± standard deviation or median (range).
[d] $p < 0.1$ for GLAD compared with conventional solution.
[b] $p < 0.05$ for GLAD compared with conventional solution, and for biocompatible compared with conventional solution.
[c] $p < 0.05$ for study A compared with study B.
GLAD = glycerol, amino acids, and dextrose; D/P = dialysate-to-plasma concentration ratio after 4 h; $FWT_{0–60}$ = free water transport during the first 60 minutes; $\Delta Na^{+}_{0–60}$ = difference in the dialysate Na+ concentration before inflow and after 60 minutes; fibrosis score = semiquantitative score consisting of submesothelial, perivascular, and intersegmental fibrosis; PSR = picro-sirius red.

makes it unlikely that a marked fluid reabsorption rate explains the low SPFT rate.

The presence of only a small number of rats with low rates of FWT argues against the development of extensive peritoneal fibrosis in any of the groups. That hypothesis was confirmed in the morphology assessments. Exposure to the conventional solution was associated with semiquantitative scores of 6 and 7 of the maximum 9; scores of 4 were found for the other solutions. The latter scores are similar to those obtained with a hypotonic glucose-free solution (11) and were confirmed by PSR staining, in which only a maximum of one third of the total surface area was stained positive for PSR. Staining was less than 25% in nearly all rats. The PSR-positive area tended to be larger in the two conventional-exposure groups than in the other groups.

Positivity for PSR was markedly higher in study A than in study B. That discrepancy is likely attributable to the method used and the accuracy between the versions of the imaging programs. Nevertheless, the results from both studies showed similar trends. The findings suggest that the staining results in various studies cannot be compared despite initial efforts to standardize the experimental conditions, illustrating the difficulties involved in quantifying peritoneal fibrosis, which is not uniformly localized in the peritoneal tissues. The often-used thickness of the submesothelial compact zone is also unreliable, because it is markedly influenced by the peritoneal hydration state (17).

Conclusions

The current analysis shows that the long-term peritoneal exposure model in rats with kidney failure is not useful to test the hypothesis that FWT reflects peritoneal fibrosis in long-term PD patients. It could be that the duration of the exposure is too short, that kidney function is still too good, or that the amount of induced fibrosis is insufficient. Given that FWT in humans depends on both the number of perfused peritoneal vessels and the severity of fibrosis, species differences in angiogenesis and fibrogenesis could also be the reason for the results in the present analyses.

Disclosures

We understand that that *Advances in Peritoneal Dialysis* requires disclosure of any conflicts of interest, and we declare that we have no conflicts to disclose.

References

1 Sampimon DE, Coester AM, Struijk DG, Krediet RT. The time course of peritoneal transport parameters in peritoneal dialysis patients who develop encapsulating peritoneal sclerosis. Nephrol Dial Transplant 2011;26:291–8.

2 Morelle J, Sow A, Hautem N, *et al.* Interstitial fibrosis restricts osmotic water transport in encapsulating peritoneal sclerosis. J Am Soc Nephrol 2015;26:2521–33.

3 Smit W, Parikova A, Struijk DG, Krediet RT. The difference in causes of early and late ultrafiltration failure in peritoneal dialysis. Perit Dial Int 2005;25(suppl 3):S41–5.

4 Parikova A, Smit W, Struijk DG, Krediet RT. Analysis of fluid transport pathways and their determinants in peritoneal dialysis patients with ultrafiltration failure. Kidney Int 2006;70:1988–94.

5 Coester AM, Smit W, Struijk DG, Parikova A, Krediet RT. Longitudinal analysis of fluid transport and their determinants in PD patients. Perit Dial Int 2014;34:195–203.

6 Taranu T, Florea L, Păduraru D, Georgescu SO, Frâncu LL, Stan CI. Morphological changes of the peritoneal membrane in patients with long-term dialysis. Rom J Morphol Embryol 2014;55:927–32.

7 Krediet RT, Lopes Barreto D, Struijk DG. Can free water transport be used as a clinical parameter for peritoneal fibrosis in long-term PD patients? Perit Dial Int 2016;36:124–8.

8 Kawanishi K, Honda K, Tsukada M, Oda H, Nitta K. Neutral solution low in glucose degradation products is associated with less peritoneal fibrosis and vascular sclerosis in patients receiving peritoneal dialysis. Perit Dial Int 2013;33:242–51.

9 del Peso G, Jimenez-Heffernan JA, Selgas R, *et al.* Biocompatible dialysis solutions preserve mesothelial cell and vessel wall integrity. A case–control study on human biopsies. Perit Dial Int 2016;36:129–34.

10 Vrovsnik F, Coester AM, Lopes Barreto D, *et al.* The induction of chronic kidney failure in a long-term peritoneal exposure model in the rat: effects on functional and structural peritoneal alterations. Perit Dial Int 2010;30:558–69.

11 De Graaff M, Zegwaard AH, Zweers MM, *et al.* The effects of a dialysis solution with a combination of glycerol/amino acids/dextrose on the peritoneal membrane in chronic renal failure. Perit Dial Int 2010;30:192–200.

12 Vlahu CA, Aten J, de Graaff M, *et al.* New insights into the effects of chronic kidney failure and dialysate exposure on the peritoneum. Perit Dial Int 2016;36:614–22.

13 Koomen GC, Krediet RT, Leegwater AC, Struijk DG, Arisz L, Hoek FJ. A fast reliable method for the measurement of intraperitoneal dextran 70, used to calculate lymphatic absorption. Adv Perit Dial 1991;7:10–14.

14 Smit W, Struijk DG, Ho-Dac-Pannekeet MM, Krediet RT. Quantification of free water transport in peritoneal dialysis. Kidney Int 2004;66:849–54.
15 Bernardo AP, Bajo MA, Santos O, *et al.* Two-in-one protocol: simultaneous small-pore and ultrasmall-pore peritoneal transport quantification. Perit Dial Int 2012;32:537–44.
16 Rippe B, Stelin G, Haraldsson B. Computer simulations of peritoneal fluid transport in CAPD. Kidney Int 1991;40:315–25.
17 Du C, Mendelson AA, Guan Q, *et al.* The size-dependent efficacy and biocompatibility of hyperbranched polyglycerol in peritoneal dialysis. Biomaterials 2014;35:1378–89.

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