SHORT REPORTS

Soluble VCAM-1 and E-Selectin in PD Patients: The Additive Value of the Free Diffusion Coefficient in the Assessment of Local Peritoneal Production

Vascular peritoneal membrane changes may develop with duration of peritoneal dialysis (PD). Patients with end-stage renal disease are already at high risk for the development of atherosclerotic disease, due to the effects of uremia (1). Peritoneal dialysis in combination with the uraemic state may therefore accelerate vascular damage. It is likely that endothelial dysfunction precedes the development of the peritoneal vascular abnormalities. Markers of endothelial dysfunction include soluble forms of the adhesion molecules vascular cell adhesion molecule 1 (VCAM-1) and E-selectin. These molecules are almost exclusively produced by endothelium (2) and are elevated during various chronic inflammatory conditions (2–5). At present, no knowledge is available about their levels in effluent of PD patients and their relationships with other parameters, like solute and fluid transport. The aim of this study was to analyze the presence of soluble (s) VCAM-1 and E-selectin in effluent of PD patients in relationship to serum concentrations. The contribution of local peritoneal production of these markers to the levels observed in effluent was determined to assess their value as markers for endothelial dysfunction in PD patients.

STUDY POPULATION AND METHODS

We performed a cross-sectional study of 20 patients who underwent standard peritoneal permeability analyses (SPA) as part of their routine follow-up. Patients with a previous renal transplant as well as patients with diabetes mellitus were excluded. The patient selection was based on extremes for PD duration and ultrafiltration (UF). Both groups contained an equal number of patients with normal ultrafiltration and with UF failure. Ultrafiltration failure was defined according to the 3 × 4 rule (net UF < 400 mL after a dwell of 4 h with a 3.86% glucose dialysis solution). The 3.86% glucose SPAs were performed as described by Pannekeet *et al.* (6). Effluent and plasma samples were obtained. None of the patients had peritonitis at the time of the SPA or in the 4 preceding weeks.

Soluble VCAM-1 and sE-selectin were measured in 20-fold diluted serum and in a 10-fold concentrated effluent by enzyme-linked immunosorbent assays (R&D Systems Europe Ltd., Abingdon, UK). The concentration procedure of the effluent was achieved by an Amicon ultra-15 centrifugal

filter device with a nominal molecular weight limit of 10 kDa (Millipore, Molsheim, France). The precise concentration factor was thereafter calculated by obtaining the pre- and post- levels of dialysate albumin. The fluid and solute transport characteristics were calculated as described previously (6,7) and appearance rates were established. Residual glomerular filtration rate (rGFR) was estimated by plasma cystatin C. A validated formula for PD patients was used: $rGFR = -0.70 + 22/\text{cystatin C}$ (8). C-reactive protein (CRP) was measured as a marker of systemic inflammation and von Willebrand Factor antigen as a surrogate marker of endothelial function.

Local peritoneal production of sVCAM-1 and sE-selectin was calculated as follows. A first analysis was done by computing a peritoneal transport line for each patient based on dialysate/plasma (D/P) ratio and their molecular weights (MW) (9). With this method no signs of intraperitoneal production of the adhesion molecules was shown. Therefore, free diffusion coefficients ($D_{20,w}$) were determined to account for conformational variability and molecule interactions. The primary step consisted of a technique based on size-exclusion high-performance liquid chromatography (HPLC, Superose 6 10/300 GL column, MW range 5000 – 5.0×10^6 , Tricorn, Amersham Biosciences Limited, UK), equilibrated with NaCl 0.9% buffer pH 7.4 and with the wavelength of an ultraviolet detector set at 280 nm. Aldolase (158 kDa) and chymotrypsinogen A (25 kDa) were used as standards for MW analysis. A sample volume of 300 µl was inserted in 3 distinctive runs at a flow rate of the mobile phase set at 0.5 mL/min. Selection of the fractions, derived from the column, rested on the regions of interest which illustrated the peak of the molecules of interest and the proteins used as standards, estimated by their known MW. All sample types and obtained fractions were centrifuged at $1710 \times g$ for 10 minutes at 4°C and stored at -26°C until assayed. Depending on the distribution of the factors, univariate analyses between 2 groups were performed by an unpaired *t*-test or Mann-Whitney U test. Likewise, possible relationships were studied with Pearson's or Spearman's correlation coefficients.

RESULTS

Patient characteristics and their peritoneal transport parameters are presented in Table 1. Serum CRP was significantly higher in long-term PD patients ($p < 0.05$). As expected, these patients had lower values of rGFR ($p < 0.05$). Serum and effluent levels of sVCAM-1 and sE-selectin are depicted in Figure 1, where the dotted line represents the reported serum values in healthy individuals (3). Significantly higher serum

TABLE 1
Clinical and Functional Characteristics of the Patients

	Short-term PD (n=10)	Long-term PD (n=10)	Normal UF (n=10)	UF failure (n=10)
Gender (% male)	40	60	60	40
Age at SPA (years)	53 (36–66)	53 (23–87)	53 (36–87)	54 (23–84)
PD duration (months)	4 (3–6)	57 (36–71)	25 (4–71)	20 (3–69)
Davies comorbidity score (%)				
- Low	0	40	20	20
- Intermediate	100	60	80	80
Serum CRP (mg/L)	1.7 (1.0–5.2)	7.4 (1.0–62.0) ^a	2.5 (1.0–27.6)	2.6 (1.0–62.0)
rGFR (mL/min)	3.7 (2.9–6.0)	2.9 (1.9–6.4) ^a	3.6 (2.1–6.4)	3.2 (1.9–4.9)
MTAC _{creatinine} (mL/min)	11.5±4.2	9.7±3.2	9.1±3.0	12.2±4.0
Net UF at 240 min (mL)	442.5±262.1	588.8±392.9	793.8±236.0	237.4±85.6 ^c
Glucose absorption (%)	69.0±10.5	59.0±11.5	56.9±10.9	71.1±8.2 ^c
FWT at 60 min (mL)	100.5±57.0	124.8±88.4	166.0±55.3	59.4±45.0 ^c
FWT at 60 min (%)	23.2±11.6	29.4±18.1	33.6±8.2	19.0±17.3 ^b
TCUF at 240 min (mL)	875.3±241.4	968.3±523.7	1181.1±388.2	662.5±192.8 ^c

PD = peritoneal dialysis; UF = ultrafiltration; SPA = standard peritoneal permeability analysis; CRP = C-reactive protein; rGFR = residual glomerular filtration rate; MTAC = mass transfer area coefficients; FWT = free water transport; TCUF = total transcapillary UF.

According to the distributions, values are expressed as mean±standard deviation or as median and ranges.

^a $p < 0.05$ short term vs long term.

^b $p < 0.05$.

^c $p \leq 0.001$ normal UF vs UF failure.

sVCAM-1 levels were present in the long-term compared to the short-term patients (Panel A). No differences in serum sVCAM-1, sE-selectin, CRP, and rGFR were found between patients with normal UF and UF failure (data not shown). Elevated levels of von Willebrand Factor antigen were present in all patients, but no significant differences between the groups were observed. Correlations between serum and effluent levels of sVCAM-1, sE-selectin, and CRP or rGFR were absent. Also, no relationships were present between CRP and rGFR. Peritoneal dialysis duration correlated modestly with serum sVCAM-1 ($r = 0.48$, $p = 0.03$), but not with serum sE-selectin.

From the size-exclusion HPLC R-I, where only the serum sample was injected into the column, the proteins albumin ($D_{20,w} = 6.1$), immunoglobulin G (IgG) ($D_{20,w} = 4.0$), and $\alpha 2$ -macroglobulin ($D_{20,w} = 2.4$) were detected. With these data, we were able to set up a free diffusion coefficient calibration graph applying linear regression. R-II comprised the injection of PD patient serum combined with standards of sE-selectin and sVCAM-1. After interpolation of the data, the $D_{20,w}$ of sE-selectin and sVCAM-1 were 3.0 and 3.2 $\text{cm}^2\text{sec}^{-1}10^{-7}$ respectively.

Figure 2 shows the mean transport line for all patients and the measured D/P ratios of sVCAM-1 and sE-selectin for each patient. Local production of sE-selectin and sVCAM-1 accounted for 80% and 35% of effluent concentration respectively. No significant correlation between transcapillary UF and sE-selectin attributed to local production was found ($r = 0.43$, $p = 0.07$). However, there was a relationship between the percentage of free water transport and sE-selectin attributed to local production ($r = 0.47$, $p = 0.04$). Even though

not statistically significant, locally produced sE-selectin was higher in long-term PD patients compared to patients with a short duration of PD treatment (Figure 3). These results were not observed for sVCAM-1.

DISCUSSION

Serum sVCAM-1 was higher in patients with a long PD duration than in short-term patients, which were more in line with healthy controls (3). High serum concentrations in the long-term patients can be explained by enhanced synthesis/release or decreased renal clearance. Although a relationship between serum sVCAM-1 and rGFR has been reported in 1 study, the explained variance of this correlation was only 16% (4). Our cystatin C results did not suggest such a relationship. Similarly, another study in chronic renal failure patients also showed an absent relation between plasma creatinine levels and serum levels of sE-selectin and sVCAM-1 (5). Therefore, increased synthesis of serum sVCAM-1 due to endothelial dysfunction is likely in long-term PD patients.

As the diffusion of a molecule is not only determined by its MW, but also by its shape and density, the free diffusion coefficient of sVCAM-1 and sE-selectin was determined. Using this diffusion coefficient, the apparent MW of both sVCAM-1 and sE-selectin were larger than expected. This explains the seeming absence of local production when the MW was used. Also, for effluent VCAM-1, no local production was found in the majority of patients. The presence of locally produced sE-selectin might be explained by its exclusive expression on endothelial cells after activation by inflammatory cytokines

Figure 1 — Serum levels and appearance rates of sVCAM-1 and sE-selectin. sVCAM = soluble vascular cell adhesion molecule; sE-selectin = soluble E-selectin; UF = ultrafiltration.

Figure 2 — Peritoneal transport line of sVCAM-1 and sE-selectin based on $D_{20,w}$. $\alpha_2\text{M}$ = α 2-macroglobulin; sVCAM = soluble vascular cell adhesion molecule; IgG = immunoglobulin G; sE-selectin = soluble E-selectin.

Figure 3 — Local peritoneal production of dialysate sE-selectin. sE-selectin = soluble E-selectin; UF = ultrafiltration.

or endotoxins (10). Although statistical significance was not reached, higher levels of locally produced sE-selectin suggest a more dysfunctional peritoneal endothelium in long-term PD patients. Also, a positive correlation was found between locally produced sE-selectin and the percentage of free water transport.

The explorative character of this study, as reflected by the small number of patients, as well as the use of von Willebrand Factor as a surrogate marker for endothelial function due to the absence of flow-mediated dilation measurements, can be considered study limitations. However, at present no direct method is available for the assessment of peritoneal endothelial dysfunction. In conclusion, we could detect sVCAM-1 and sE-selectin in the effluent of 20 non-diabetic PD patients with local production of sE-selectin in the peritoneal cavity and report their $D_{20,w}$. Serum sVCAM-1 was higher in long-term PD patients, pointing to the presence of overall endothelial dysfunction in PD patients. Effluent sE-selectin appeared to be involved in endothelial activation of the peritoneal vasculature. Longitudinal research is required to elucidate whether effluent sE-selectin is valuable as a diagnostic marker of endothelial changes of the peritoneal membrane. Furthermore, one should be aware of the potential underestimation of local peritoneal production levels of macromolecules by means of MW and consider the additive merit of $D_{20,w}$ determinations.

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DISCLOSURES

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