Endothelium-dependent vasodilatation is impaired in peritoneal dialysis patients

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Abstract

Background. Peritoneal dialysis (PD) patients have a high risk of cardiovascular mortality, which is not completely explained by conventional risk factors. Other factors related to chronic renal failure and/or dialysis treatment might lead to endothelial dysfunction, which is associated with an adverse cardiovascular outcome. One such factor is hyperhomocysteinaemia, which has a high prevalence in PD patients.

Methods. A vessel wall movement detector system was used to investigate endothelium-dependent, flow-mediated, and endothelium-independent, glyceryl trinitrate-induced, vasodilatation of the brachial artery in 29 PD patients and 29 control subjects.

Results. Endothelium-dependent vasodilatation was markedly reduced in the PD group: 5.7 ±1.0% vs 10.4 ±1.3% in the control group (P=0.004). Endothelium-independent vasodilatation was not impaired. Plasma total homocysteine was elevated in the PD patients (45.2 ±6.2 μmol/l), but was not related to endothelium-dependent vasodilatation.

Conclusion. Chronic peritoneal dialysis patients have impaired endothelium-dependent vasodilatation, which may reflect an increased susceptibility for the development of atherosclerosis and thrombosis.

Introduction

Data of the US Renal Data System show that cardiovascular disease is the major cause of the limited life expectancy in patients on maintenance haemodialysis (HD) and peritoneal dialysis (PD) [1]. PD patients older than 55 years may have an even higher 1-year mortality rate than HD patients [1,2], a difference that has been attributed mainly to an increased risk of death due to infection and cardiovascular disease [1,3], although an increased prevalence of comorbidity in PD patients may also play a role [4]. Hypertension, smoking, hyperlipidaemia, and diabetes mellitus are prevalent in end-stage renal disease (ESRD), but cannot fully explain the increased cardiovascular mortality [5]. In the process of atherothrombotic disease, endothelial dysfunction is an important initiating event [6]. Traditional and non-traditional risk factors can adversely influence endothelial function. One of the new cardiovascular risk factors is hyperhomocysteinaemia [7]. High homocysteine levels have been shown to be toxic to the vascular endothelium and to be associated with impaired nitric oxide-mediated vascular responses [8–11]. Hyperhomocysteinaemia is extremely common in ESRD [12–14] and has therefore been implicated in the atherogenesis of these patients [5,14].

Endothelial function as assessed by endothelium-dependent vasodilatation is impaired in patients with hypercholesterolaemia [15], cigarette smoking [16], and essential hypertension [17]. We have recently demonstrated that in chronic haemodialysis patients endothelium-dependent vasodilatation is impaired independently of these factors [18]. We did not find a relation with homocysteine levels, so we proposed that the haemodialysis procedures per se (with concomitant neutrophil and complement activation) and/or other factors intrinsic to the uraemic state could cause endothelial dysfunction.

In order to further address these issues, we determined endothelium-dependent vasodilatation in PD patients and examined its relationship with plasma homocysteine levels.

Subjects and methods

Patients

Consecutive patients from our Nephrology department who were on maintenance peritoneal dialysis for at least 3 months were asked to participate in the study. Twenty-nine PD patients and 29 healthy control subjects were included. The groups were matched for gender and comparable for factors that are known to influence vascular responses: age, body
mass index, blood pressure, serum total cholesterol, and number of smokers (Table 1). Mean duration of dialysis was 2.9 years (median: 2.4 years; range: 0.5–7.4 years). Renal diagnoses were hypertensive nephrosclerosis (n = 11), chronic glomerulonephritis (n = 6), familial poly cystic kidney disease (n = 3), focal glomerulosclerosis (n = 2), diabetic nephropathy, light-chain deposition disease, reflex nephropathy, IgA nephropathy, chronic pyelonephritis, Henoch–Schönlein purpura, and renal failure of unknown origin (all n = 1). Eleven patients were on continuous ambulatory peritoneal dialysis (CAPD) and 18 on continuous cyclic peritoneal dialysis (CCPD). Adequacy of dialysis was assessed by calculation of weekly total Kt/V and normalized creatinine clearance. Nutritional status of the patients was evaluated by serum albumin and protein catabolic rate (PCR). None of the patients used folic acid supplementation. All patients used regular dialysis medication, i.e. phosphate binders, erythropoetin, multivitamin B, but no folic acid or vitamin B6, vitamin C, and vitamin D if indicated. Twenty-two patients were hypertensive and used antihypertensives (calcium-antagonists in 15, ACE-inhibitors in nine, β-blockers in two, and nα-blockers in five). Six patients had a history of cardiovascular disease (stroke in three, myocardial infarction in two, and peripheral artery disease in one). The protocol was approved by the local ethics committee and all participants gave informed consent.

**Procedures**

Blood samples were taken in the fasting state. Serum total cholesterol was measured enzymatically by the CHOD-PAP method (Boehringer, Mannheim, Germany). In the PD patients, plasma total (free plus protein bound) homocysteine (tHcy) was measured by HPLC with fluorescence detection [19] (reference value: 6–15 μmol/l), and serum albumin immunochemically by rate nephelometry (Beckman, Galway, Ireland; reference value: 34–50 g/l). Intra- and interassay coefficients of variation are 2.1 and 5.1% for tHcy [20], and ≤ 5 and ≤ 8% for albumin. PCR was calculated according to the Randerson method [21]. Body mass index was measured as weight (kg)/length (m²). Blood pressure was measured with an automated device (BP-8800, Colin, Japan). Participants were classified as current or non-smoker.

Endothelium-dependent vasodilatation was assessed non-invasively by determination of the flow-mediated vasodilatation by an investigator not blinded to patient/control subject status [22,23]. All subjects refrained from smoking and from use of caffeine-containing drinks for at least 10 h before the start of the measurements. Medication was withdrawn for at least 12 h before the measurement. The PD patients were at their designated dry weight and free of clinical signs of fluid overload. The brachial artery, just above the elbow, was studied by a vessel wall movement detector system (Wall Track System, Neurodata). In M-mode, this system measures the arterial diameter with an accuracy of 0.1 to 0.2 mm [24]. End diastolic brachial artery diameter was measured at baseline after 15 min of supine rest. Reactive hyperaemia was induced by release of a blood pressure tourniquet that had been inflated on the forearm for four min at a pressure of 100 mmHg above the systolic blood pressure. The brachial artery diameter was again measured between 45 and 60 s after the release of the cuff. After 15 min, a second baseline measurement was performed. The final measurement was performed five min after sublingual administration of 0.4 mg of glyceryl trinitrate (GTN). Endothelium-dependent and independent vasodilatation were expressed as the percentage increase of the brachial artery diameter during reactive hyperaemia and after administration of GTN, respectively. Intra-observer within-subjects coefficients of variation for baseline diameter, endothelium-dependent, and endothelium-independent vasodilatation are 4.6, 5.5, and 7.7% respectively [23].

**Statistical methods**

Data are expressed as mean ± SEM. Chi-square and two-sample t tests were used for comparison of variables between groups. Correlations were tested with Pearson’s and Spearman’s test as appropriate. A two-tailed P < 0.05 was considered statistically significant. Univariate and multivariate regression were used to assess determinants of vascular responses. The multivariate analyses were performed after taking the PD patients and control subjects together and adding a factor ‘peritoneal dialysis present or absent’ to the model. We adjusted for baseline diameter as it is known to affect vascular responses [23]. The diameter after endothelium-dependent or -independent vasodilatation was used as the dependent variable, because relating the percentage increase or the absolute increase in diameter to baseline diameter predictably yields a strong correlation [25].

**Results**

Endothelium-dependent vasodilatation was significantly reduced in the PD patients: 5.7 ± 1.0 versus 10.4 ± 1.3% in the control group (P = 0.004) (Figure

| Table 1. Characteristics of the peritoneal dialysis patients and the control subjects |
|----------------------------------------|------------------|------------------|--------|
| PD (n = 29)                            | C (n = 29)       | P                 |
| Gender (male/female)                   | 17/12            | 17/12            | —      |
| Age (years)                            | 52.3 ± 3.1a      | 51.3 ± 1.4b      | 0.77   |
| Body mass index (kg/m²)                | 24.8 ± 0.5       | 25.3 ± 0.6       | 0.55   |
| Smokers (number)                       | 10               | 9                | 0.78   |
| Systolic blood pressure (mmHg)         | 136.2 ± 3.7      | 131.9 ± 2.7      | 0.36   |
| Diastolic blood pressure (mmHg)        | 77.6 ± 2.5       | 78.4 ± 2.0       | 0.81   |
| Mean arterial pressure (mmHg)          | 99.1 ± 3.0       | 96.7 ± 2.3       | 0.52   |
| Serum total cholesterol (mmol/l)       | 6.0 ± 0.2        | 6.6 ± 0.2        | 0.084  |
| Baseline brachial artery diameter (mm) | 3.6 ± 0.1        | 3.4 ± 0.1        | 0.15   |

PD = peritoneal dialysis patients, C = control subjects. a Range 26–81; b range 25–61.
Endothelium-independent vasodilatation did not differ between groups: $9.6 \pm 1.1$ vs $10.5 \pm 1.0\%$ ($P = 0.58$). No significant differences were found between CAPD and CCPD patients for endothelium-dependent vasodilatation ($5.2 \pm 1.6$ vs $6.0 \pm 1.2\%; P = 0.67$), or endothelium-independent vasodilatation ($9.1 \pm 1.8$ vs $9.9 \pm 1.5\%; P = 0.74$). When PD patients with vascular disease were excluded, endothelium-dependent vasodilatation remained decreased ($6.1 \pm 1.3\%$) as compared to the control group ($P = 0.016$). No difference in vascular response was detected between patients with and without intraperitoneal dialysis fluid during the measurements. Brachial artery diameter did not differ significantly between the PD patients and the control group (Table 1). Plasma tHcy was markedly elevated in the PD patients: $45.2 \pm 6.2 \mu mol/l$ (range: 12.4–147.6 \mu mol/l). Serum albumin was $36.0 \pm 0.9 g/l$, weekly $Kt/V$ 1.82 $\pm$ 0.1, creatinine clearance 65.0 $\pm$ 4.5 l/week/1.73 m$^2$, and PCR 0.90 $\pm$ 0.05 g/kg body weight/day.

In univariate analyses of the total group, the percentage endothelium-dependent vasodilatation was related only to the mean arterial pressure ($r = -0.39; P = 0.003$; Figure 2) and not to serum total cholesterol level, age, gender or smoking status (Figure 3). Multiple regression analyses showed baseline diameter to be the most important predictor of diameter after endothelium-dependent and -independent vasodilatation (standardized regression coefficient (stand. $r$) = 0.95; $P < 0.001$ and stand. $r$ = 0.93; $P < 0.001$). The only other factor that was significantly related to diameter after endothelium-dependent vasodilatation was the presence of peritoneal dialysis (stand. $r$ = -0.13; $P = 0.014$). PD was not related to endothelium-independent vasodilatation.

In the PD group, univariable and multivariate analyses showed that endothelium-dependent vasodilatation was neither related to plasma tHcy, nor to duration of dialysis treatment, serum albumin, creatinine clearance, $Kt/V$ or PCR. No significant difference in endothelium-dependent vasodilatation was found between PD patients with and without antihypertensive therapy ($P = 0.82$; Figure 4).

**Discussion**

This is the first study to show that endothelium-dependent vasodilatation is impaired in peritoneal dialysis patients when compared to gender-matched control subjects of comparable age. Endothelium-dependent vasodilatation in conduit and resistance vessels can be induced by an increase in blood flow which increases shear stress on the endothelium. Nitric oxide mediates this response [26,27]. Endothelium-mediated vasodilatation in the brachial artery correlates well with the endothelium-dependent response of [28], and the extent of atherosclerotic disease in coronary arteries [29]. The impairment in endothelium-dependent brachial vasodilatation in the...
present study therefore may reflect generalized increased atherogenic and thrombogenic endothelial properties in PD patients.

We have shown previously that endothelium-dependent vasodilatation is impaired in chronic HD patients [18]. Factors which, in general, negatively influence the response to reactive hyperaemia are hypercholesterolaemia [15], smoking [16], age [30], and increased vessel size [23,31]. Hypertension has been associated with impaired endothelium-dependent vasodilatation in most [17,32], but not all studies [33]. As in the HD patients [18], however, the impairment in endothelium-dependent vasodilatation in our PD patients was not related to these factors. Many PD patients had a history of hypertension, but this was unlikely to have been a major determinant of the endothelial vasodilatory response. The reduction in flow-mediated vasodilatation of hypertensive patients is reported to be 30% [17], which is less than the 45% reduction in our PD patients. Furthermore, hypertension was adequately controlled in our patients, who used calcium-antagonists and ACE-inhibitors in most cases. These antihypertensives have been shown to normalize impaired endothelium-dependent vasodilatation in hypertensive patients [34,35].

Plasma tHcy varied from to 12.4 to 147.6 μmol/l in our PD patients. In this range, plasma tHcy levels were not correlated with endothelium-dependent vasodilatation on both univariate and multivariate analyses. In addition, we have found that 1 year of homocysteine-lowering treatment did not improve endothelial function in HD patients [36]. Therefore, endothelial dysfunction in dialysis patients could be related to other factors intrinsic to uraemia. One such factor might be an elevated plasma level of asymmetric dimethylarginine, a potent inhibitor of nitric oxide synthesis [37]. However, Kari et al. did not find a relationship between the impairment of flow-mediated vasodilatation and plasma levels of symmetric and asymmetric dimethylarginine in children with pre-dialysis chronic renal failure [38]. We could not demonstrate a significant difference between the endothelium-dependent vasodilatation in HD patients [18] as compared to PD patients in the present study: 3.8 ± 0.1 (HD) vs 5.7 ± 1.0% (PD; P = 0.19). The mode of dialysis, therefore, does not seem to have a major influence on the impairment in flow-mediated vasodilatation. Finally, the reduction in endothelium-dependent vasodilatation in both PD and HD patients may, at least partially, be caused by the presence of subclinical atherosclerosis. Considerable coronary artery stenosis has been demonstrated in asymptomatic patients starting dialysis treatment [39].

We conclude that brachial artery endothelium-dependent vasodilatation is markedly reduced in PD patients, which may reflect a generalized increase in atherogenic and thrombogenic properties of the endothelium. This reduction does not seem to be related to known cardiovascular risk factors. Fasting plasma tHcy is elevated in PD patients, but is not related to the reduced endothelium-dependent vasodilatation. Whether such a relation is present in patients with moderate renal failure, who have less markedly elevated tHcy levels, remains to be investigated.

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References


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