Impairment of skin microvascular reactivity in hypertension and uraemia

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Abstract

Background. Uraemia and hypertension are associated with higher risk for cardiovascular complications. Endothelial dysfunction plays an important role in the pathogenesis of cardiovascular diseases. The aim of the present study was to evaluate endothelial function in the forearm skin microcirculation of patients with essential hypertension, in hypertensive haemodialysis patients and in normotensive control subjects.

Methods. We performed laser Doppler flowmetry with iontophoresis of acetylcholine (ACh) and of sodium nitroprusside (SNP) as well as the post-occlusive reactive hyperaemia test (PORH) in 16 normal control subjects (CONT), in 16 patients with essential hypertension (EHT) and in 16 haemodialysis patients with essential hypertension (DHT). Plasma levels of endothelin-1, big-endothelin and von Willebrand factor (vWF) were also measured.

Results. The average hyperaemic response to the higher dose of ACh iontophoresis was 801±110% in CONT, 563±69% in EHT and 308±64% in DHT (P<0.05, between all comparisons). Vasodilation to the higher dose of SNP was 791±79% in CONT, 633±72% in EHT and 355±69% in DHT (NS, P<0.001 compared with controls, respectively). The average peak flow during PORH was significantly lower in both the EHT and DHT groups compared with controls (294±39, 267±59 and 429±45%, respectively, P<0.05). Levels of endothelin-1, big endothelin, vWF and vWF activity were significantly higher in the DHT group (P<0.05, compared with controls).

Conclusions. In hypertensive haemodialysis patients, both endothelium-dependent and -independent vasodilation was impaired. The observed increase in plasma markers of endothelial damage indicated a progression of vascular disease.

Keywords: acetylcholine; chronic renal failure; endothelium; essential hypertension; laser Doppler flowmetry; skin microcirculation

Introduction

Endothelial dysfunction has been linked with the initiation and acceleration of the atherosclerotic process [1]. Endothelial cells play an important role in the local regulation of microcirculation by secreting substances that control both vascular tone and structure [2]. Hypertension is a well-known risk factor for atherosclerosis. Several reports have clearly demonstrated that essential hypertension is associated with endothelial dysfunction both in muscle vascular beds and in the skin microcirculation of the forearm [1]. Endothelial dysfunction may play a role in the pathogenesis of hypertension or may simply be the result of elevated blood pressure. High blood pressure is very common in patients with end-stage renal failure, but it often decreases to normal or hypotensive values after initiation of chronic haemodialysis (HD) [3].

Using the laser Doppler method, Cupisti et al. [1] found that endothelium-dependent vasodilation was impaired in patients with essential hypertension, but preserved in a selected group of normotensive patients with moderate renal failure on conservative therapy. This finding suggests that chronic renal failure alone without arterial hypertension and HD treatment is not associated with endothelial dysfunction of the skin microcirculation [1].

Cardiovascular disease is a major cause of morbidity and mortality in patients maintained by chronic HD [3]. Their cardiovascular mortality is increased >10-fold compared with the general population [4]. Although
a single HD treatment causes a rapid clearance of uraemic toxins and markers of endothelial damage [5], chronic HD therapy appears to lead to aggravation of atherosclerosis and to significantly accelerate pre-existing coronary artery disease [3]. Although chronic uraemia is not associated with endothelial dysfunction of the cutaneous microcirculation, there is an impaired endothelium-independent vasodilatory capacity in HD patients, which can probably be explained by structural vascular changes that are related to the severity of uraemia and/or factors associated with the maintenance of HD treatment [6].

There are both invasive and non-invasive methods to measure endothelial function. These methods generally determine vasodilatation after specific stimuli, or measure plasma levels of specific factors released by the endothelium [7].

The aim of our study was to assess endothelial function in hypertensive patients with normal renal function and with end-stage renal disease by using the non-invasive LD method and by measuring plasma levels of vWF, ET-1 and big-ET.

Subjects and methods

Subjects

The study population included 16 patients with essential hypertension (EHT), 16 haemodialysed hypertensive patients (DHT) and 16 normotensive control subjects (CONT). The EHT patients had a well-established history of chronically elevated blood pressure in the moderate stage (systolic pressure, 160–179 mmHg; diastolic pressure, 100–109 mmHg) without any apparent underlying cause. They were referred to the out-patient clinic of the St Imre Teaching Hospital because of uncontrolled hypertension, meaning that blood pressure was >145/95 mmHg despite antihypertensive therapy. LD measurements were performed within 2 weeks after their appearance at the outpatient department. At 72 h before the examinations, antihypertensive drugs were withdrawn. None of the patients were on nitrate treatment. Two patients were excluded from the study because withdrawal of antihypertensive medication led to profound increases in blood pressure (≥190/110 mmHg). Previous antihypertensive treatment lasted 13.3±2.95 years. The antihypertensive medications of the patients are summarized in Table 1.

The DHT patients were on bicarbonate HD. They had a Cimino fistula and all measurements were performed on the contralateral forearm. Causes of renal insufficiency included polycystic disease and chronic tubulointerstitial nephritis each in four cases, chronic glomerulonephritis and renal tumour each in two cases, and hydronephrosis, Wegener granulomatosis, nephrotic syndrome and lymphoid leukaemia, each in one case. In the renal tumour patients, the tumorous kidney was removed and the insufficiency of the remaining kidney led to chronic renal failure. In 10 uraemic patients, residual renal function was extremely low (average glomerular filtration rate was 5.56 ml/min, range 6.1–13), and six DHT patients were anuric. Half of the DHT patients had mild hypertension (140–159/90–99 mmHg) and half had moderate hypertension (160–179/100–109 mmHg) with
a well-established history. The average time on antihypertensive therapy was 14.88 ± 2.29 years. Their medication was withdrawn 72 h before the measurements. This group of patients was studied on the day of their HD session; LD and serum biochemistry were assessed 2 h before starting dialysis.

In both groups, patients were free of concomitant diseases predisposing to alterations in endothelium-dependent vasomotion, such as diabetes mellitus, coronary or peripheral artery disease, or elevated plasma levels of uric acid. Patients with a serum cholesterol level >7 mmol/l were excluded from the study. Secondary hypertension was identified using abdominal ultrasound combined with measurements of serum renin, aldosterone, cortisol, thyroid-stimulating hormone and urine vanillic acid levels, and these patients were excluded from the study. Patients with suspicion of secondary hypertension underwent phentolamine or glucagon tests (intravenous administration), as well as renal angiography or abdominal computed tomography (CT) examinations.

The normal volunteers were screened by clinical history, physical examination, electrocardiography at rest and routine chemical analysis. None of these subjects showed present or past evidence of diabetes mellitus, hypercholesterolaemia, chronic renal failure, peripheral artery disease or cardiovascular diseases known to affect vasomotion, such as hypertension (blood pressure levels >135/85 mmHg) or chronic heart failure.

Patients and subjects older than 67 years or heavy smokers (>5 cigarettes daily) were excluded. All subjects were given a light breakfast 3 h before the start of the measurements. They were asked to refrain from smoking on the day of the study and not to consume caffeine-containing drinks at least 2 h before the start of the measurements. Informed consent was obtained from each patient and the local ethics committee approved the study. Subject characteristics are summarized in Table 2. Subject characteristics are summarized in Table 2.

### Study protocol

LD measurements were carried out after 20 min of acclimatization in a temperature-controlled room (24 ± 1°C) with the subjects in the supine position. Before starting, the flexor surface of the measured forearm was gently cleaned using alcohol. We determined pulse rate and blood pressure using a validated mercury sphygmomanometer.

An LD instrument (PeriFlux 5001, wavelength 780 nm) and a micropharmacology system (PeriIont) were used for non-invasive and continuous measurement of perfusion changes during vascular provocations in the skin (Perimed AB, Järfalla, Sweden). A drug delivery electrode was incorporated into the head of the laser probe. The probe temperature was standardized to 32°C during drug tests. The drug delivery electrode was filled with 140 μl of 1% Ach (Clinalfa AG, Switzerland) and was attached with the laser probe to the volar surface of the forearm. The position of the probe was chosen to avoid hair, freckles and broken skin. The dispersive electrode was attached to the volar aspect of the wrist to complete the circuit. We placed a control standard probe 4 cm laterally from the drug delivery electrode. After registration of baseline flow (60 s), two doses of Ach were delivered using an anodal current (0.1 mA for 30 s and 0.16 mA for 30 s) with a 120 s interval. Using a new delivery electrode applied to a different location on the same forearm, two doses of sodium nitroprusside 1% (Nitropress, ABBOTT, USA) were delivered using a cathodal current (0.1 mA for 20 s and 0.1 mA for 30 s) with a 120 s interval. During the PORH test, and after registration of baseline flow (60 s), arterial occlusion was performed with a suprasystolic pressure using a pneumatic cuff of a sphygmomanometer for 3 min (biological zero). Following the release of pressure, we measured skin hyperaemia on the volar surface of the forearm contralateral to the Cimino fistula at 10 cm below the elbow with a standard LD probe. Another standard probe was placed on the skin of the contralateral forearm as a control.

The LD signal is proportional to the number and velocity of moving blood cells in illuminated superficial skin microvessels. The laser beam penetrates the skin and it is partially backscattered by moving blood cells. According to the Doppler principle, a frequency shift occurs, generating a signal that is linearly related to red blood cell flow, as predicted by theoretical and experimental models. The LD output is semi-quantitative and expressed in PU of output voltage (1 PU = 10 mV) in accordance with general consensus (European Laser Doppler Users Groups, London 1992). The LD outputs were recorded continuously by an interfaced computer with acquisition software (Perisoft). For calibration, we used a device composed of colloidal latex particles (Brownian motion provides the standard value). Because the output cannot easily be translated into absolute values of blood flow, the magnitude of the changes in skin perfusion was calculated as the ratio between peak and mean baseline perfusions.

According to previous measurements in our laboratory, the day to day variability of this system was 16–21% [13], which is comparable with other studies [14]. The plasma vWF level was determined by the immune-turbidimetric method (STA-LIATEST vWF), and ristocetin cofactor activity was assessed by agglutination method (Von Willebrand Reagent, Dade Behring). Plasma ET-1 and big-ET levels were determined by quantitative enzyme immune assay (Biomedica).

### Statistical evaluation

Descriptive characteristics and LD results from the three groups were compared by analysis of variance (ANOVA;
factor = treatment, Scheffe post hoc test). Differences were considered statistically significant when \( P < 0.05 \). Statistical analysis was performed by Statistica for Windows software. All results are expressed as means ± SEM.

Results

Basal forearm skin perfusion

Basal forearm skin perfusion was not significantly different between the three groups before ACh (6.16 ± 0.72; 7.92 ± 0.83; 8.96 ± 0.82) or SNP (7.01 ± 0.92; 8.87 ± 1.10; 10.50 ± 1.04) administration, or before the PORH (8.58 ± 0.56; 12.01 ± 2.8; 10.56 ± 1.51) test.

Responses to acetylcholine

Iontophoresis of ACh produced significant dose-dependent increases in cutaneous blood flow in CONT, EHT and DHT patients, with the values of 430.35 ± 82.43, 208.81 ± 49.55 and 136.69 ± 25.37% for the first dose of ACh, and 801.35 ± 110.07, 563.25 ± 69.03 and 308.94 ± 64.95% for the second dose of ACh, respectively. Vasodilatative responses for the two doses of ACh were significantly smaller in the EHT and DHT groups than in the CONT group (\( P < 0.05 \) for the first dose and \( P < 0.001 \) for the second dose), and responses were smaller in DHT than in EHT during the second dose of ACh (\( P < 0.05 \), Figure 1).

Responses to sodium nitroprusside

Following iontophoretic administration of SNP, cutaneous blood flow significantly increased (\( P < 0.001 \)) in all groups. Although vasodilation to SNP was smaller in the EHT group than in the CONT group, this difference was not significant (356.87 ± 63.31 and 408.58 ± 65.63% for the first dose of SNP and 633.75 ± 72.24 and 791.17 ± 79.10% for the second dose of SNP, respectively). Vasodilation was smaller in the DHT group than in the CONT and EHT groups (150.81 ± 38.15% for the first dose and 355 ± 69.77% for the second dose of SNP, \( P < 0.05 \) at both doses, Figure 2).

Post-occlusive reactive hyperaemia

A 3 min occlusion of the brachial artery by a pneumatic cuff produced a significant (\( P < 0.001 \)) increase in the cutaneous blood flow after cuff release. Mean biological zero was not significantly different among the three groups (3.69 ± 0.14 PU in the CONT group, 3.63 ± 0.21 PU in the EHT group and 3.75 ± 0.16 in the DHT group, \( P = 0.81 \)). The percentage changes in peak flow were significantly smaller in the EHT and DHT groups than in the CONT group (294 ± 39, 267 ± 59 and 429 ± 45%, respectively, \( P < 0.01 \), Figure 3). For the analysis of time to peak, there was a tendency for decreases in the EHT and DHT groups compared with the CONT group (with the values of 8.97 ± 1.02, 7.68 ± 1.07 and 10.79 ± 1.14 s, respectively) but the difference was not significant (\( P = 0.13 \)). The rise of the curve to peak was more marked in the EHT and DHT groups but the difference was again not significant (3.26 ± 0.85 and 4.48 ± 1.55 vs 3.01 ± 0.43, respectively, \( P = 0.36 \)).

von Willebrand factor level and activity

The vWF level and the ristocetin cofactor activity were significantly higher (149 ± 22 and 92.4 ± 11.84%) in the

![Fig. 1. Changes in skin blood flow in response to iontophoresis of two doses of acetylcholine (ACh). Significant differences between controls (CONT), essential hypertensive (EHT) and haemodialysed hypertensive (DHT) patient groups: * \( P < 0.05 \). Significant difference between hypertensives and haemodialysed patients: # \( P < 0.05 \). ACh1 CONT and ACh2 CONT: maximal skin blood flow percentage increase after the first and second dose of ACh in controls. ACh1 EHT and ACh2 EHT: maximal skin blood flow percentage increase after the first and second dose of ACh in patients with essential hypertension. ACh1 DHT and ACh2 DHT: maximal skin blood flow percentage increase after the first and second dose of ACh in haemodialysed hypertensive patients.](https://academic.oup.com/ndt/article-abstract/20/9/1821/1850256)

![Fig. 2. Changes in skin blood flow in response to iontophoresis of two doses of sodium nitroprusside (SNP). Significant difference between controls (CONT), essential hypertensive (EHT) and haemodialysed hypertensive (DHT) patient groups: * \( P < 0.05 \). SNP1 CONT and SNP2 CONT: maximal skin blood flow percentage increase after the first and second dose of SNP in controls. SNP1 EHT and SNP2 EHT: maximal skin blood flow percentage increase after the first and second dose of SNP in patients with essential hypertension. SNP1 DHT and SNP2 DHT: maximal skin blood flow percentage increase after the first and second dose of SNP in haemodialysed hypertensive patients.](https://academic.oup.com/ndt/article-abstract/20/9/1821/1850256)
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The ET-1 and big-ET levels were in the normal range in the CONT group (0.46 ± 0.55 and 0.75 ± 0.48 fmol/l). The ET-1 level was above the normal range in both the EHT (0.80 ± 2.04 fmol/l) and DHT (1.4 ± 2.87 fmol/l) groups. The big-ET level was above the normal range in the EHT group (1.29 ± 1.2 fmol/l). The elevated big-ET level (2.67 ± 1.61 fmol/l) in the DHT group was significantly higher compared with the CONT (P < 0.001) and EHT (P < 0.01) groups.

\[ P < 0.05 \]

DHT group than in the CONT group (95 ± 4 and 66.5 ± 7.37%) (P < 0.05), but neither of these were different from the EHT group (116 ± 26 and 84 ± 7.23%).

**ET-1 and big-ET level**

Discussion

In the current study, we demonstrated impaired endothelial function of the forearm skin microcirculation in hypertensive patients. Specifically, vasodilatory responses to iontophoretically applied ACh and to the release of transient arterial occlusion were decreased. With these findings, we were able to reproduce our previous results [13].

In our DHT patients, endothelium-dependent vasodilation following the higher dose of ACh was decreased not only compared with CONT but also compared with EHT. In addition to reduced vasodilatory responses to ACh, this group also showed decreased responses to SNP. Responses to PORH were reduced to similar degrees in the DHT and EHT groups.

We used iontophoretic administration of ACh combined with LD flowmetry to measure microvascular endothelial function. ACh elicits vasodilation through a complex sequence of events. In addition to eliciting NO production, ACh also stimulates the release of the vasodilator prostacyclin and the endothelium-derived hyperpolarizing factor (EDHF). The relative contribution of these factors to cutaneous vasodilation is unclear. Berghoff et al. [9] demonstrated that a prostanoid-dependent mechanism did not significantly contribute to endothelium-dependent vasodilatory responses to ACh, whereas Khan et al. [15] suggested that forearm cutaneous vasodilation is mediated mainly by a prostanoid-dependent mechanism.

As in our previous study [13], we iontophorized two single doses of ACh and of SNP to measure endothelium-dependent and -independent vasodilation. Compared with other studies that used a greater number of doses, the current protocol used a simple and time-sparing method to evaluate endothelial function with the same reproducibility. Although PORH in skeletal muscle is partly mediated by endothelium, its mechanisms have not been studied specifically in dermal microvessels. We found significantly lower responses in the EHT and DHT groups than in the CONT group, which had an average peak flow of ~400%. Decreases in peak flow are observed in many pathological conditions, such as hyperlipidaemia or diabetes mellitus [16]. In contrast to the literature, we found a tendency towards shortening of time to peak and a steeper slope of the curve to the peak in the EHT and DHT groups compared with the control group. The mechanism that produces the elevated pulse pressure in hypertensive patients may explain this difference, such that reflected pressure waves propagate more rapidly and are attenuated less in stiff arteries compared with compliant arteries [17].

Endothelial dysfunction in hypertension was observed in many other studies. Impaired responses to metacholine, bradykinin and substance P have been documented in the forearm microvasculature of essential hypertensive patients compared with normal controls, which reflects a reduction in NO production in the hypertensive population [2]. There is probably an association between higher cardiovascular risk and endothelial dysfunction. NO, a vasodilator product of endothelial cells, and endothelium-derived vasoconstrictor factors (including thromboxane A2, prostaglandin H2, oxygen free radicals and ET-1) not only exert opposite effects on vascular tone, but also inhibit (NO) or activate (vasoconstrictor factors) platelet aggregation, vascular smooth cell proliferation and migration, monocyte adhesion and adhesion molecule expression, which are mechanisms that play important roles in the genesis of thrombosis and atherosclerotic plaque [2].

In non-hypertensive uraemic patients on conservative therapy, preserved ACh- and SNP-mediated vasodilation was reported in the forearm skin with the LD iontophoresis method [1,6]. These data suggest that conservatively treated chronic renal failure by itself and independent of arterial hypertension is not associated with endothelial dysfunction of the skin microcirculation. Morris et al. [18] found an impaired response to carbachol, an endothelium-derived vasodilator, but a preserved response to SNP while using
forearm plethysmography. In their study, most of the patients had hypertension (nine of 16 patients). Bolton et al. [19] found decreased flow-mediated dilatation measured by vascular ultrasound, and increased inflammatory cytokine (interleukin-6 and tumour necrosis factor-α) and C-reactive protein levels, but normal vWF levels in hypertensive patients with chronic renal failure on conservative therapy.

HD treatment appears to accelerate the progression of atherosclerosis. In non-hypertensive HD patients with chronic renal failure, Cupisti et al. [6] found a decreased vasodilation in the skin microcirculation in response to ACh and SNP, but a preserved response in patients on conservative therapy [6]. Because endothelium-dependent vasodilation to ACh was similar to that induced by SNP, they concluded that conservatively treated uraemic patients have a preserved endothelium-dependent hyperaemic response. Bolton et al. [19] found increased plasma concentrations of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), vWF and fibrinogen in haemodialysed uraemic patients. In these patients, there was no reduction in cytokine levels because these molecules are too large to be dialysed. This process may contribute to the development of a more severe atherosclerotic lesion formation.

In our previous study, we repeatedly demonstrated that decreased endothelium-dependent vasodilation may play a role in the maintenance of essential hypertension [13]. Our haemodialysed patients in the present study showed similar but significantly decreased hyperaemic responses to ACh and SNP compared with controls. The response to the higher dose of ACh was also significantly decreased compared with the essential hypertensives. There are many possible reasons for the decreased endothelium-dependent and -independent vasodilator capacity of the haemodialysed patients. The greater severity of uraemia is accompanied by higher levels of toxins that can deteriorate endothelial function. These toxins may include dimethylarginine, an endogenous inhibitor of NO synthase, or end-products of protein catabolism that are able to inhibit NO release [1]. An additional mechanism, using detectable endothelial function that does not involve blockage of the NO system, is endothelium-derived dilator prostanoid production of the skin vasculature. The impaired response to both substances could also be the result of reduced NO bioactivity. Animal and human studies have shown enhanced NO production and high plasma NO levels in chronic uraemia, which can lead to NO refractoriness of vascular smooth muscle cells. Beside the impaired endothelium-independent vasodilation, it is impossible to confirm whether reduced responses to ACh are the consequence of endothelial dysfunction or simply a dysfunctional NO response in vascular smooth muscle cells.

As in several previous studies, we demonstrated significant increases in plasma concentrations of ET-1 and big-ET in HD patients compared with healthy control subjects. The increased plasma levels of ET-1 and big-ET are partly due to impaired plasma clearance, but also to a stimulated synthesis caused by hypoxia, shear stress and cytokines present in uraemia. Ottosson-Seeberger et al. [20] demonstrated that plasma concentrations of ET-1 and big-ET were lowered during HD, whereas dialysis-induced ET-1 production may have counteracted that and may have even at times exceed the fall in plasma ET achieved by dialysis. Vascular reactivity to ET-1 and big-ET was preserved in their HD patients [20], and consequently an increased plasma levels of ET-1 and big-ET may have played a role in the impaired microvascular reactivity observed in our study. The significantly elevated level of vWF was a result of different stimuli and, as a marker of dysfunctional endothelium, demonstrated that the normal balance of the coagulation system was shifted to the prothrombotic state.

Structural changes to the vessel wall have also been observed in HD patients. Intrinsic degenerative changes of the arterial wall are likely to cause reduced vessel wall distensibility. There are data about a more pronounced intimal thickening and enhanced calcification of the internal elastic lamella and the media ground substance in uraemic patients. The observed histological changes in the arterial wall may be associated with reduced arterial distensibility [3].

In summary, although hypertension was associated with endothelial dysfunction, endothelium-independent vasodilation of the forearm skin microvessels was preserved. Thus, hypertensive patients with chronic renal failure on HD therapy experience not only a progression of endothelial dysfunction but also a decrease in total dilatative capacity of the forearm skin microvessels. These effects may generate a more severe vascular damage and may be associated with a more pronounced atherosclerotic process.

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