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Responses of the skin microcirculation to acetylcholine and to sodium nitroprusside in chronic uremic patients

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Abstract The aim of the present study was to assess the endothelial function of the microcirculation in chronic renal failure. We investigated the responses of the cutaneous blood flow to locally delivered acetylcholine and sodium nitroprusside in uremic patients. The study included 60 chronic uremic patients: 40 patients with a creatinine clearance of 4–25 ml/min were on conservative treatment and 20 patients were on maintenance hemodialysis. The changes in skin blood flow following iontophoretic delivery of acetylcholine (an endothelium-dependent vasodilator) and sodium nitroprusside (an endothelium-independent vasodilator) were measured by laser Doppler flowmetry. Acetylcholine induced a progressive increase in blood flow in both groups, reaching approximately 100% of the maximal hyperemic response obtained by sodium nitroprusside delivery. The percent increase in blood flow from baseline was lower in hemodialysis patients than in patients on conservative treatment, after both acetylcholine (550±44 vs. 718±61%, $P<0.05$) and sodium nitroprusside (553±46 vs. 735±69%, $P<0.05$) delivery. In the hemodialysis group, the hyperemic responses to acetylcholine and sodium nitroprusside did not improve after the hemodialysis session. Hence, the hyperemic responses of the skin microcirculation are lower in hemodialysis patients than in patients on conservative treatment, and did not ameliorate after hemodialysis. It seems to be independent of endothelial dysfunction, and associated with the severity of uremia and with the maintenance hemodialysis treatment. This microcirculatory abnormality is in keeping with the arterial stiffness and vascular wall damages described in dialysis patients, which contribute to the cardiovascular morbidity of chronic uremia.

Key words Acetylcholine · Chronic renal failure · Skin circulation · Vascular endothelium · Laser Doppler flowmetry · Hemodialysis

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Introduction

Cardiovascular morbidity and mortality are frequently reported in chronic uremia, where the high prevalence of arterial hypertension, secondary hyperparathyroidism, hyperlipidemia, diabetes, and hyperhomocysteinemia can contribute to accelerated atherosclerosis [1–3]. Recently, endothelial dysfunction has been proposed as an important factor associated with hypertension and progressive vascular damage [4–6]. Evidence exists that the endothelium-dependent vasodilation of brachial artery is also impaired in adult dialysis patients and in children with chronic renal failure [7–9]. However, few data are available on the endothelial function of small arterial vessels in patients with chronic renal failure.

Some reports have demonstrated arterial stiffening and microangiopathic changes in the skin of patients with chronic uremia [10–12]. The cutaneous vasculature is an example of microcirculation which can be easily studied using non-

invasive procedures. Laser Doppler flowmetry associated with iontophoretic delivery of vasoactive substances represents a validated method that has been applied for studying skin microcirculation in normal individuals and in several pathologies, such as essential hypertension, systemic sclerosis and diabetes [13–17].

Iontophoresis has been used to locally deliver vasoactive charged drugs across the skin, through a small electric current [18]. The current strengths chosen for this study are well tolerated by the majority of subjects, but a mild prickling sensation is reported in a few cases. The effect of the iontophoresis current itself on skin blood flow has been previously evaluated, but only negligible changes are induced by the same duration and strength of electric current used in the present study. The laser Doppler probe is fixed within the drug chamber to explore the same small area of the skin. A laser beam penetrates the skin and it is partially back scattered by moving blood cells: a frequency shift occurs and generates a signal linearly related to the red cell flux, as predicted by theoretical and experimental models [19–21]. The reproducibility of laser Doppler flowmetry has been studied in stable emulsion, with an intra-assay coefficient of variation of about 6% [21], whereas in humans the technique shows a coefficient of variation of 20%–21% [22].

Recently, using this procedure, endothelium-dependent vasodilation has been found to be impaired in patients with essential hypertension, but preserved in a selected group of normotensive patients with moderate renal failure [17]. This finding emphasized the important role of arterial hypertension in endothelial dysfunction, but did not allow definite conclusions to be drawn about the cutaneous microcirculatory responses in chronic uremia.

Hence, the present study aimed to investigate the skin hyperemic responses to the iontophoretic delivery of an endothelium-dependent (acetylcholine, ACh) and an endothelium-independent (sodium nitroprusside, SNP) vasodilating agent in chronic uremic patients with moderate to severe renal failure, including hemodialysis (HD) patients.

Patients and methods

Subjects

The study population included 60 chronic uremic patients: 20 were on maintenance HD (HD group) and 40 were on conservative treatment (CT group). These two groups were comparable for age, sex, hematocrit, serum lipids, and blood pressure values (Table 1). In the patients of the CT group, the residual renal function, expressed as creatinine clearance, was 11.4±0.9 ml/min (range 4–25 ml/min).

Patients older than 65 years, heavy smokers (more than 5 cigarettes daily), or affected by diabetes mellitus or with abnormally elevated plasma cholesterol levels (higher than 240 mg/dl) were excluded. Subjects smoking up to 5 cigarettes daily were requested to refrain from smoking for at least 12 h before examination. No patient was on nitrate treatment, and the antihypertensive drugs (if any) were withdrawn 24 h at least before examination. Informed consent was obtained from all patients. Sixteen normal subjects, age matched (48±3 years) with the patients, served as controls.

Table 1 Age, sex, serum biochemistry and arterial blood pressure (BP) values in the conservative treatment (CT) and in the hemodialysis (HD) groups (mean±SEM)

	CT group	HD group	*P*
M/F	22/18	11/9	NS
Age (years)	50±2	51±3	NS
Hemoglobin (g/dl)	10.8±0.2	11.1±0.4	NS
Hematocrit (%)	32.9±0.7	34.3±1.1	NS
Serum total proteins (g/dl)	6.9±0.1	6.9±0.1	NS
Serum albumin (g/dl)	4.0±0.1	4.0±0.1	NS
Total cholesterol (mg/dl)	191±6	180±12	NS
Triglycerides (mg/dl)	174±13	218±22	NS
Systolic BP (mmHg)	135.4±2.6	135.5±3.5	NS
Diastolic BP (mmHg)	82.3±1.3	82.1±1.8	NS
Mean BP (mmHg)	100.2±1.7	99.9±1.7	NS

The CT group patients were on dietary treatment, with a standard low-protein (0.6 g/kg per day) diet or with a very low-protein (0.3 g/kg per day) diet supplemented with essential amino acids and ketoanalogues [23]. The CT group was divided into two subgroups of 20 patients each, according to the residual renal function: CT1 with creatinine clearance ≥10 ml/min (16.0±1.0 ml/min) and CT2 with creatinine clearance <10 ml/min (6.9±0.4 ml/min).

The HD group patients were on bicarbonate HD (10 patients) or acetate-free biofiltration (10 patients) for 6 months at least (46±11 months, range 6–228 months). Their residual renal function was extremely low (0.9±0.2 ml/min, range 0–3.2 ml/min), and most of the patients were anuric. This group of patients was studied before and after the HD session; serum biochemistry was assessed before starting dialysis.

Procedures

Iontophoresis delivery combined with laser Doppler flowmetry was used to assess the endothelium-dependent or -independent hyperemic responses in the skin of the forearm. In patients with arteriovenous fistula or graft, the procedure was carried out on the contralateral arm. The HD patients were studied 30 min before and within 60 min of completion of the dialysis session. The examinations were performed in a temperature-controlled room (22±1°C), with the subjects lying in the supine position, after 20 min acclimatization. Before starting, the skin of the forearm was carefully cleaned using alcohol.

A drug delivery electrode (PF 383, Perimed, Jarfallan, Sweden) and an indifferent electrode (PF 384, Perimed, Jarfallan, Sweden) were used. The delivery electrode, as well as the laser probe, was fixed inside the drug delivery chamber. Before starting the examination, the drug delivery chamber was filled with 62.5 μl of 1%

ACh solution and with 62.5 µl of 1% SNP solution, and then attached on the volar surface of the forearm by double-sided adhesive disc, at 10 mm distance from the drug delivery electrode. A battery-powered iontophoresis controller (Perilout 328, Power Supply) was used to provide the current for drug administration. ACh was delivered using an anodal current: six doses (0.1 mA for 20 s each) followed by another two doses (0.2 mA for 20 s and 0.3 mA for 20 s) were delivered with a 60-s interval between each single dose. The 60-s interval was needed to achieve the plateau of the hyperemia induced by each ACh delivery.

After a 1-min recovery period, SNP was delivered using a cathodal current: two doses (0.1 mA for 20 s each) followed by one dose (0.2 mA for 20 s) with a 180-s interval between two successive doses. This interval was needed to achieve the steady state of the flux response following each SNP dose.

Before and during ACh or SNP delivery, the skin erythrocyte flux was measured by a laser Doppler flowmeter (Periflux PF4001, standard probe PF408, Perimed, Jarfallan, Sweden). The laser Doppler outputs were recorded continuously by an interfaced computer equipped with acquisition software (PC Notebook, Zenith Data Systems, Taiwan), and given as arbitrary units (perfusion units). Calibration was performed by a device composed of colloidal latex particles whose Brownian motion provides the standard value. The skin blood flux was determined as the mean value recorded during 60 s at each delivery step. The absolute maximal response was defined as the flux reached after the last drug delivery.

In order to eliminate baseline variability, the blood flux responses to ACh and SNP delivery were expressed as percent change from the baseline [20, 21, 24]. Similarly, the flux increment following each one of the eight iontophoretically delivered ACh doses was expressed as percentage of the maximal endothelium-independent hyperemic response to SNP, as follows: 100 ACh-induced flux/maximum SNP-induced flux.

Statistical analysis

All data are expressed as mean±SEM. Statistical evaluation was performed using Student's *t*-test for unpaired data. The hyperemic responses following the eight ACh delivery steps were analyzed by ANOVA for repeated measures; Scheffè's test was applied for multiple comparison testing. Differences were considered as statistically significant when $P<0.05$.

Results

The Fig. 1 shows the hyperemic responses caused by the eight doses of ACh. The iontophoretic delivery of ACh was followed by a progressive increment of cutaneous blood flow in the CT and HD groups, as in normals (Fig. 1), with no statistical difference among the studied groups. The blood flow curves in response to ACh finally reached nearly 100% of the maximal endothelium-independent response elicited by SNP delivery (Fig. 1).

The HD group showed a lower maximal hyperemic response than the CT group, both after ACh and SNP delivery (Fig. 2). Values of the CT group were very similar to those of normal controls (Fig. 2). The SNP-induced hyperemia was quite similar in the CT1 and the CT2 groups (Fig. 3) despite the difference in the residual renal function. Furthermore, in the HD group, the HD procedure did not increase the maximal vasodilation induced both by ACh and by SNP delivery (Fig. 3).

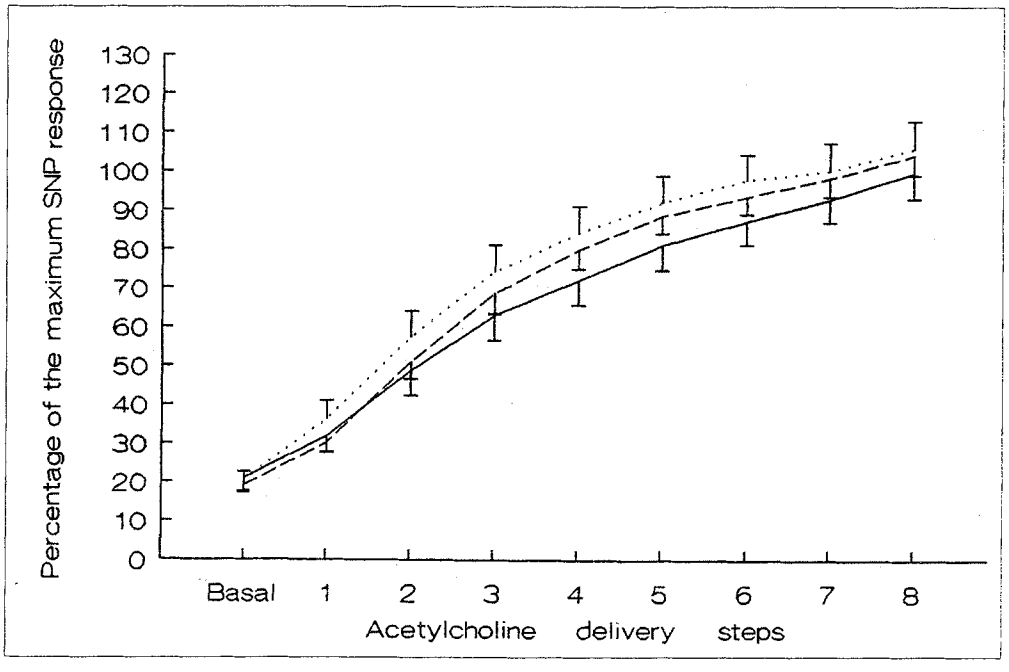


Fig. 1 Skin blood flux changes following the eight doses of acetylcholine (ACh) via iontophoretic delivery in the hemodialysis (HD) group (*dotted line*), in the conservative treatment (CT) group (*dashed line*), and in normal controls (*solid line*). The values are given as percentage of the maximal flux obtained with sodium nitroprusside (*SNP*) delivery (mean±SEM)

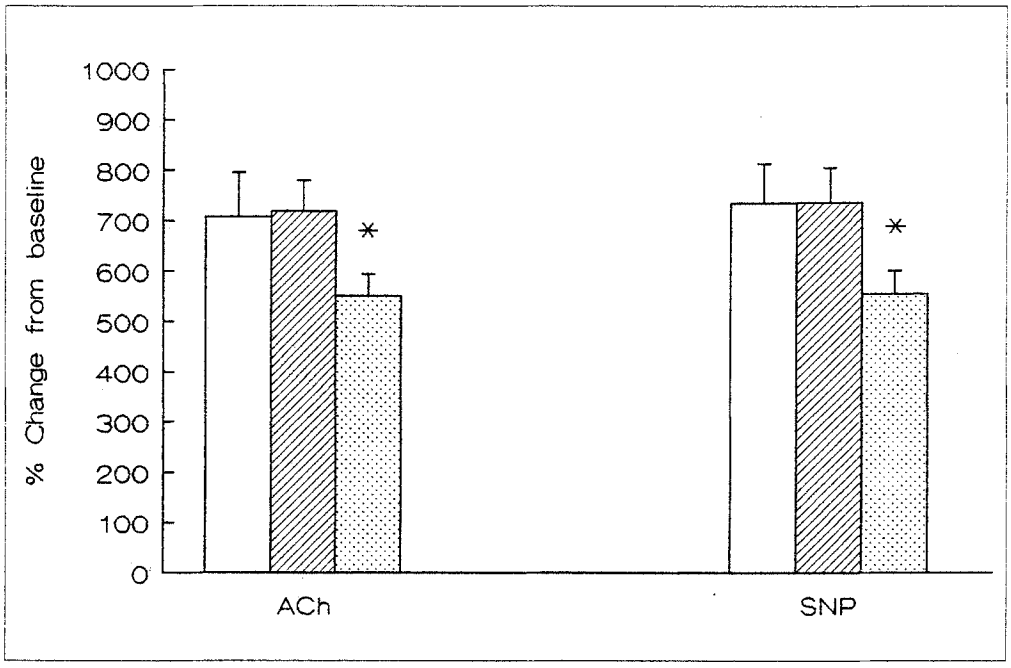
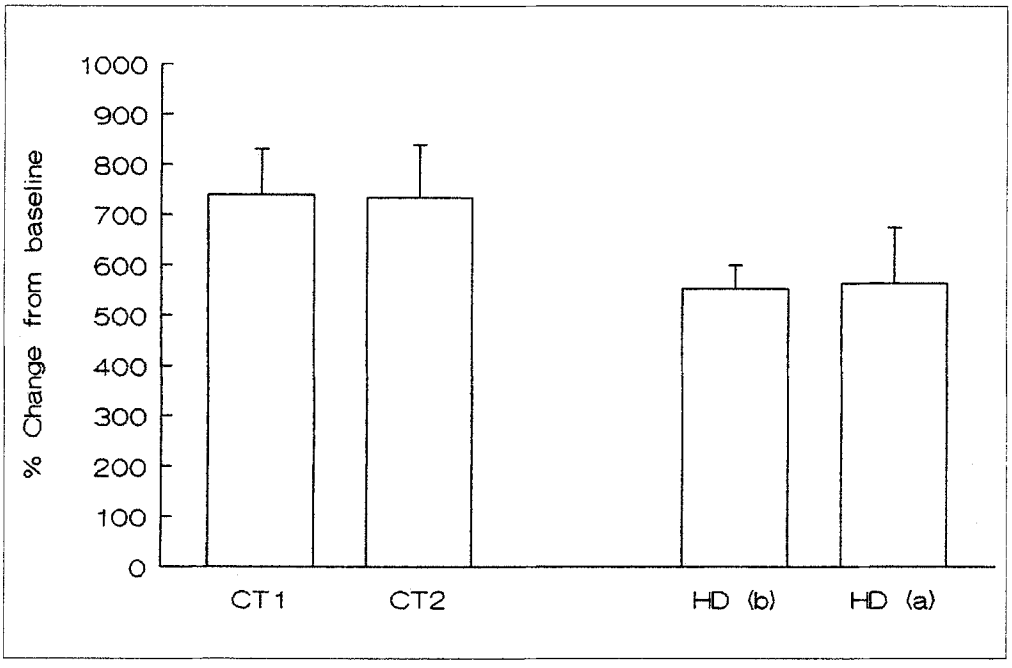


Fig. 2 Percent change from baseline of the maximal skin blood flux following ACh or SNP iontophoretic delivery in the CT group (*stripped bars*), in the HD group (*dotted bars*), and in normals (*empty bars*) (mean±SEM). *P <0.05 vs. CT group

Fig. 3 Maximal endothelium-independent hyperemic response to SNP delivery in the CT1 and CT2 groups and in the patients of the HD group before (*b*) and after (*a*) the HD session (mean±SEM)



Discussion

Endothelium can be considered as an organ that regulates vascular tone and structure, producing and releasing vasodilatory substances, such as nitric oxide (NO) and dilator prostanoids, and vasoconstricting substances, such as constrictor prostanoids and endothelins [25]. The study of endothelium is important in chronic uremia, because endothelial dysfunction is linked to the initiation and acceleration of the atherosclerosis process [26], which is a very important determinant of the progression of renal failure and of the morbidity and mortality rate of uremic patients. Endothelial function, especially of larger vessels, can be affected by changes of NO metabolism and production [27–29] in chronic uremia. However, in the skin circulation, the vasodilatory response to ACh seems to be mainly mediated by endothelium-derived prostanoids rather than NO production [30, 31].

The present investigation indicates that the maximal hyperemic response following local delivery of endothelium-independent vasodilator (i.e., SNP) is impaired in dialysis patients, suggesting a reduced vasodilatory capacity of

skin microcirculation. In addition, the endothelium-dependent vasodilation in response to ACh delivery is similar to that induced by SNP delivery, suggesting a preserved endothelium-dependent hyperemic response in the CT and HD groups. Taken together, these data are in accordance with an impaired vasodilatory capacity in the HD group, not related to endothelial dysfunction. It must be stressed that the vasodilatory response in uremic patients on CT was very similar to normal controls, and that the patients on HD and on CT were comparable for age, sex, serum levels of cholesterol, and arterial blood pressure. Hence the greater severity of uremia and the maintenance HD treatment are the major distinctive factors of the HD group that could account for our results. Chronic uremia is considered as a vasculopathic state [3], and hence it is conceivable that the more severe the uremia, the more severe the vascular damage. In addition, the HD treatment per se induces oxidant stress [32] and inflammatory response [33, 34] that in turn can contribute to accelerated atherosclerosis.

The present study, demonstrating a preserved endothelium-dependent hyperemic response to ACh in chronic uremic patients, confirms our previous preliminary data on patients with moderate to severe renal failure, with no arterial hypertension and without dialysis treatment, in whom the endothelium-mediated skin vasodilation was not impaired [17]. Similarly, our results are in accordance with those of Thuraisingham and Raine [35] who demonstrated a preserved endothelium-dependent vasodilation in the mesenteric vessels of uremic rats. Different conclusions have been reached when the responsiveness of the brachial artery was studied in uremic patients. Van Guldener et al. [7, 8] reported a lower than normal endothelial-dependent vasodilation of the brachial artery in chronic uremic patients either on HD or on peritoneal dialysis. However, the structural difference between the brachial artery and cutaneous vessels might also reflect different mechanisms of endothelium-dependent vasodilation [30, 31]. Hence, a preserved endothelium-derived dilatory prostanoid production in the skin vasculature of patients with chronic renal failure, independent of changes in NO generation, could partly explain this discrepancy.

Our data also indicate a reduced maximal vasodilatory capacity of the skin microcirculation in HD patients. This seems to be unrelated to endothelial dysfunction, because it also occurred with an endothelium-independent hyperemic stimulus, suggesting the presence of structural abnormalities of skin vessels. This is in agreement with several reports demonstrating vascular structural damage and accelerated atherosclerosis in end-stage renal disease, namely an abnormal peripheral microvasculature consisting of arterial stiffening [10], thickening of the basement membrane, and chronic inflammatory cell infiltration in arterial vessels and subepidermal capillaries [11].

Laser Doppler flowmetry is an inexpensive non-invasive method, which is useful for assessing the real-time changes in skin blood flow [14, 36]. Iontophoresis delivery in combination with laser Doppler flowmetry has been used for studying blood flow changes of skin microcirculation in several diseases [15–17]. Recently, this method has been further validated in patients with essential hypertension, by infusing ACh and SNP into the brachial artery [37]. The blunted endothelium-dependent vasodilation observed by plethysmography in muscle vascular bed was also detected by laser Doppler flowmetry in the cutaneous microcirculation. This confirms that the skin vascular bed is a good "window" for evaluating endothelial function, and validates laser Doppler flowmetry as useful non-invasive tool for the measurement of the blood flux changes [14]. However this method has quite a high variability because of the many variables affecting cutaneous blood flow and of the intrinsic rapid and frequent fluctuations of skin microcirculation. Data processing methods have been suggested to increase day-to-day reproducibility and to eliminate baseline variability [14, 24]; these include change from baseline and percentage of the maximal response obtained by the endothelium-independent SNP vasodilation.

In conclusion, our results demonstrate a preserved endothelium-dependent hyperemic response in the skin of chronic uremic patients and a reduced maximal vasodilation in HD patients only. This suggests that chronic uremia is not associated with endothelial dysfunction of the cutaneous microcirculation, whereas there is an impaired vasodilatory capacity in HD patients, which can probably be explained by the structural vascular changes related to the severity of uremia and/or factors associated with maintenance HD treatment.

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