Ramipril prevents basal arterial constriction and enhanced myogenic tone in the femoral artery in mildly uraemic normotensive rats

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ABSTRACT

Some aspects of vascular reactivity are altered in mild experimental uraemia, as shown by increased myogenic tone and a reduced lumen diameter in the femoral artery. This study was conducted to investigate the prevention of these uraemia-induced vascular abnormalities by the angiotensin-converting enzyme inhibitor (ACE-I) Ramipril. Ten male Wistar rats were rendered uraemic (U) by 5/6th nephrectomy, and 10 control (C) rats were concurrently sham-operated. After 4 weeks, both groups were given daily subcutaneous injections of 3 μg of Ramipril for a further 4 weeks. Tail-cuff systolic blood pressure was then recorded and the rat was killed. Isolated femoral arteries were mounted on a pressure myograph and pressurized at 40 mmHg for baseline measurements of the lumen internal diameter. Myogenic tone was then assessed over a range of intravascular pressures from 40 to 160 mmHg. Biochemically, serum urea and creatinine were significantly higher in the uraemic (U) group [urea: U, 23±3 mmol/l; C, 6±1 mmol/l (P < 0.001); creatinine: U, 147±17 mmol/l; C, 72±11 mmol/l (P < 0.001)]. Systolic blood pressure was the same in both groups (U, 127±7 mmHg; C, 127±3 mmHg). The mean baseline internal diameter was the same in both groups (U, 756±22 μm; C, 721±34 μm, not significant), as was mean myogenic tone (U, 4.7±1%; C, 3.4±1%). In conclusion, there were no differences in baseline lumen diameter or myogenic tone in uraemic compared with control femoral arteries of rats treated with Ramipril, which suggests that Ramipril may prevent the development of elevated myogenic tone and decreased lumen diameter previously observed in this model of uraemia. These results suggest that these specific vascular abnormalities in uraemia may be mediated by renin or bradykinin, or by the direct action of angiotensin II on vascular smooth muscle.

INTRODUCTION

For over 20 years, cardiovascular disease has persisted as the leading cause of mortality, accounting for over 50% of deaths in patients with end-stage renal disease (ESRD), both in Europe [1] and in the U.S.A. [2]. An important prognostic risk factor is left ventricular hypertrophy, which is found in 60–70% of dialysis patients [3], and independently confers a mortality rate of 70% within 5 years in this population [4]. Changes in the structure and function of large arteries have been found to correlate with changes in left ventricular dimensions in ESRD patients [5], even in those without pre-existing cardiac or vascular disease [6]. Recently we have reported that, in the femoral artery of mildly uraemic rats, myogenic tone is increased and the basal arterial lumen diameter is

Key words: femoral artery, myogenic tone, pressure myography, Ramipril, uraemia.

Abbreviations: ACE-I, angiotensin-converting enzyme inhibitor; ESRD, end-stage renal disease; i.d., internal diameter; PSS, physiological salt solution; KPSS, potassium-substituted PSS; SHR, spontaneously hypertensive rat.

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reduced in the absence of hypertension before structural changes become apparent [7].

Enhanced vasoconstriction and concurrent loss of endothelium-dependent relaxation is regarded as an early marker of endothelial dysfunction in atherosclerosis, even before it becomes angiographically apparent [8,9]. Therefore it is possible that the reduced lumen diameter observed in uraemic rats may indicate endothelial dysfunction in this model of uraemia.

In spontaneously hypertensive rats (SHRs), angiotensin-converting enzyme inhibitors (ACE-Is) both reverse and regress cardiac and vascular hypertrophy [10] and, in hypertensive patients, intervention with the ACE-I Ramipril was shown to induce regression of carotid intima-media thickness [11]. At sub-anti-hypertensive doses (0.01 mg·day⁻¹·kg⁻¹), Ramipril has been shown to halt the progression of atherosclerosis, reduce the amount of surface atherosclerotic lesions present in the aorta and restore impaired endothelial function in cholesterol-fed rabbits [9,12]. Of particular interest was the observation that, in SHRs treated with the same dose, although hypertension and its associated vascular hypertrophy did develop, the response of the aorta to the vasoconstrictor noradrenaline (0.01 μM) was reduced, and relaxation in response to acetylcholine (0.01–1.0 μM) was enhanced [13], suggesting that the observed changes were not related to structural alterations in the vascular wall.

Given these findings, the aim of the present study was to investigate whether intervention with the ACE-I Ramipril might prevent the enhanced basal constriction previously observed in mildly uraemic rats [7]. We also aimed to assess the effects of Ramipril on myogenic tone.

METHODS

Animals
Male Wistar rats (180–200 g) were housed in the on-site Biological Services Unit individually in holding rooms at a constant temperature of 21 ± 2 °C and humidity of 40%. The 24 h day was fixed in a 12 h light/12 h dark cycle. All procedures had prior approval from the U.K. Home Office (project licence 70/3619) and were performed in accordance with the Animals Scientific Procedures Act 1986.

Induction of uraemia
The 5/6th nephrectomy animal model of uraemia was chosen, as it induces mild uraemia without the confounding factor of hypertension which is present in other uraemic models [14]. All animals underwent two surgical procedures in pairs 1 week apart. Anaesthesia was induced initially by an intramuscular injection into a hind leg of 0.18 ml of Hypnorm® (fentanyl citrate 0.315 mg/ml and fluanisone 10 mg/ml; Janssen-Cilag Ltd., Saunderton, High Wycombe, Bucks., U.K.), followed by 0.06 ml of Diazepem (Phoenix Pharmaceuticals Ltd., Gloucester, U.K.), which was given intraperitoneally. The rat was then shaved in the abdominal area for the first stage of surgery, which involved decapsulation and removal of two-thirds of the left kidney for the uraemic group (n = 10) and decapsulation of the left kidney for the sham-operated control group (n = 10). The following week, the rats were shaved over the area covering the right flank; the uraemic group underwent a total right nephrectomy and the control group was sham-operated as described above. For 8 weeks, the rats were pair-fed to control for appetite suppression associated with uraemia.

Administration of Ramipril
As Ramipril has been shown to exert effects on the vasculature independently of its anti-hypertensive properties [9,12], and since in previous experiments using the same model at 8 weeks of uraemia there was no evidence of hypertension [7], we elected to give a sub-anti-hypertensive dose of Ramipril. Beginning 4 weeks after the second stage of surgery, both groups were given Ramipril (3 μg/day) subcutaneously into the scruff of the necks at the same time each day for the remaining 4 weeks before initiation of the experiment. This dose has been shown to have positive effects on the vasculature in SHRs [13].

Anaesthesia
On each study day, one rat was studied. The rat was given 0.25 ml of Hypnorm into a rear thigh muscle, and a further 1.6 ml of diazepem was then given intraperitoneally to induce deep anaesthesia. This was tested by lifting the rat’s head and checking that its neck was truly relaxed, and then sharply pinching its claw. If there was no physical reaction, it was assumed that the rat was sufficiently anaesthetized to proceed (usually 5–10 min later). As concurrent experiments were being performed by colleagues on cardiac myocytes, the rat was killed following the opening of the thoracic cavity and rapid removal of the whole heart.

Systolic blood pressure
This was measured on three consecutive days using the tail-cuff method (Programmed Electrosphygmomanometer PE-300; Nargo Bio-Systems, Houston, TX, U.S.A.) 1 week before the rat was killed. The rats were placed in restrainers with chocolate at the head end, which kept them still while systolic blood pressure was recorded on their tails held stable by the end of the restrainer. The mean of five readings on each day was recorded, but for analysis the mean from day 3 only was used, as the first 2 days were considered as a training period. Uraemic and control rats were handled identically with respect to the measurement of systolic blood pressure, to reduce the chances of systematic error.
Bloods
Cardiac venepuncture was performed to obtain bloods for urea and electrolytes and a full blood count at the time that the rat was killed.

Dissection of the femoral artery
The femoral artery and surrounding muscle was excised from both legs immediately after the rat was killed, and pinned on to a silastic Petri dish containing cold physiological salt solution (PSS) and left to equilibrate for about 15 min. Two segments of the femoral artery (approx. 5 mm) were dissected under a microscope (Stemi SV6; Zeiss) using microscissors and fine forceps.

Pressure myography
The technique employed for studying the femoral artery was perfusion myography, which has been described extensively elsewhere [15]. Briefly, the vessel was mounted in the myograph chamber, composed of two opposing microglass cannulae (tips ~ 70 μm), and secured at each end with two sutures. The vessel was then perfused with PSS both intraluminally and extraluminally. On either side of the chamber was a series of three-way taps with windkessels to dampen pulsatile flow and solid-state ‘in-line’ pressure transducers. These proximal and distal pressure transducers monitored the respective pressures at each end of the vessel, and the mean intraluminal pressure was calculated and maintained by the pressure servo control pump. The internal diameter (i.d.) was measured continuously by the video dimension analyser, and the vessel was displayed on the video monitor which was connected to a charge-coupled device (CCD) camera positioned on the inverted microscope (× 10).

Chemicals and solutions
All other chemicals were purchased from Sigma–Aldrich Co. (Poole, Dorset, U.K.), and all solutions were made up on the day of experimentation. PSS consisted of 5 litres of AnaLR water to which was added (mmol/l): 119 NaCl, 4.7 KCl, 1.17 MgSO₄, 25 NaHCO₃, 1.18 NaH₂PO₄, 0.026 EDTA and 5.5 glucose; PSS was kept refrigerated until use. To each 1 litre of PSS was added in PSS, which constantly perfused the vessel extra-

Experimental protocol
At the start of the experiment, intraluminal perfusion of the vessel was stopped; the intraluminal pressure was raised to 40 mmHg and the vessel was left to equilibrate in PSS, which constantly perfused the vessel extra-
Figure 1  i.d. of the femoral artery pressurized at 40 mmHg in uraemic (U; n = 10) and control (C; n = 9) rats

Figure 2  Mean myogenic tone (at 40–160 mmHg) in the femoral arteries of uraemic (U; n = 10) and control (C; n = 9) rats

not differ significantly at the time of experiments, indicating that the uraemic group was not volume-expanded. Serum urea was raised 3-fold and serum creatinine was significantly lower in the uraemics, although still within the normal range for male Wistar rats (11–18 g/dl) [17]. Systolic blood pressure was the same and normotensive in both groups (U, 127 ± 7 mmHg; C, 127 ± 3 mmHg), as was expected using the model of uraemia [14].

Pressure myography
Following the 40 min equilibration period when the vessel was pressurized at 40 mmHg, the lumen i.d. was the same in both groups (U, 756 ± 22 μm; C, 721 ± 34 μm; not significant) (Figure 1). Moreover, passive diameters obtained in calcium-free PSS were also similar in the two groups (U, 779 ± 27 μm; C, 747 ± 22 μm).

The mean myogenic tone for all intravascular pressures did not differ between the two groups (U, 4.7 ± 1%; C, 3.4 ± 1%) (Figure 2), and there was no significant difference at any single intraluminal pressure.

DISCUSSION

The principle findings of this study are that there were no differences in baseline lumen diameter or myogenic tone in the femoral arteries of uraemic compared with control rats treated with low-dose Ramipril. This differs from a previous study in which we observed a significantly reduced lumen diameter and elevated myogenic tone in vessels from untreated uraemic rats compared with those from control rats [7]. This suggests that Ramipril may prevent the development of these specific vascular abnormalities in this model of uraemia, and that the abnormalities might be mediated by renin or bradykinin, or by the direct action of angiotensin II on vascular smooth muscle.

The use of ACE-Is has been shown to exert a protective renal effect by slowing down the progression of renal disease in both diabetic [18] and non-diabetic [19] populations, and clinical studies of patients with pre-ESRD have demonstrated a 19–20% regression in left ventricular mass index with a substantial improvement in diastolic left ventricle function after treatment with enalapril or captopril for 12 months [20]. As changes in large-artery function and structure have been associated with increased left ventricular mass in ESRD patients [5], it is possible that our results may be of potential clinical vascular benefit to patients before reaching ESRD.

We deliberately elected to use Ramipril at a sub-antihypertensive dose, as hypertension is not usually a feature of this model of experimental uraemia [14], and this dose has been shown to reduce both cardiac and vascular hypertrophy in other experimental models [10]. Ramipril is an ACE-I which results in reduced angiotensin II formation, accumulation of bradykinins and even increased angiotensin-(1–7) formation. It may be that rendering angiotensin II production below normal with Ramipril contributed to the prevention of the elevation of myogenic tone. Several experimental studies have demonstrated that there are many beneficial cardiovascular effects of ACE-Is and the resulting specific AT₂-receptor antagonism. These include a reduction in neointima formation following carotid balloon denudation in rats [21–23], a decrease in blood pressure and vascular hypertrophy in SHRs [13,23,24], an improvement in endothelial function and cessation of atherosclerosis in rabbits [9], a decrease in the size of myocardial infarcts in rabbits, and regression of left ventricular hypertrophy in rats following aortic banding [12]. In our study, we cannot comment on the relative contributions of angiotensin II and bradykinin activities to account for our results, as Ramipril blocks all ACE activity. Thus further study is required to elucidate the precise mechanisms underlying our results.

To summarize, there were no differences in baseline lumen diameter or myogenic tone in the femoral arteries of uraemic compared with control rats treated with Ramipril. This suggests that Ramipril may prevent the development of elevated myogenic tone and decreased lumen diameter observed previously in uraemic vessels compared with control vessels using this model of experimental uraemia. These observations suggest that the use of an ACE-I (Ramipril) in uraemic patients may confer a cardiovascular benefit as well as a renoprotective advantage.
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REFERENCES


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