Morphological and functional characteristics of isolated resistance vessels in advanced uraemia

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Summary

1. In order to obtain direct information on the properties of the resistance vasculature of patients with advanced uraemia, a technique was developed to dissect out small arteries (internal diameter about 165 µm) from biopsies of subcutaneous fat.

2. Such arteries responded in a concentration-dependent manner to noradrenaline and angiotensin II, and the maximal force developed suggested that the vessels were fully viable.

3. Although the biopsies were normally taken during operations under general anaesthesia, biopsies taken under local anaesthesia also appeared to be fully viable, suggesting that this technique may prove useful as a general method for studying the intrinsic vascular properties of humans.

4. Biopsies were taken from 20 patients with uraemia, all of whom were treated with chronic intermittent dialysis, and 11 control subjects; up to three vessels were examined per biopsy.

5. The uraemic state was not associated with changes in vascular morphology, or in vascular reactivity or sensitivity to noradrenaline, angiotensin II, potassium or calcium. However, for the uraemic patients and for the controls there was a positive correlation between mean blood pressure and the ratio of vessel media thickness to lumen diameter, as well as a negative correlation between mean blood pressure and vessel active media stress.

6. The results suggest that uraemia treated with dialysis may not be associated with altered properties of the resistance vasculature. However, it appears that uraemic hypertension is associated

with both morphological and functional abnormalities of the resistance vasculature.

Key words: angiotensin II, hypertension, morphology, noradrenaline, resistance vessels, uraemia.

Abbreviation: ANG II, angiotensin II.

Introduction

In uraemia the possibility that an altered intrinsic responsiveness of the vasculature is of importance for the haemodynamic changes is still a matter of debate. In humans, evidence for an increased pressor reactivity to infused noradrenaline has been reported in mild renal disease [1] as well as a decreased or unchanged reactivity in more severe renal disease [2]. In rats with experimentally induced renal failure, conflicting conclusions have been reached [3, 4]. Some of the differences may be ascribed to differences in the extent of the renal failure, to whether the patients were dialysed or not, or to differences in the animal models studied. Furthermore, since the human studies are based on measurements of haemodynamic parameters in response to infusion of noradrenaline or to induced changes in sympathetic activity, an evaluation of the importance of altered intrinsic properties of the resistance vasculature itself has been difficult to obtain.

A more direct way of obtaining information on the intrinsic properties of the resistance vasculature is to investigate isolated small arteries. We have previously described a human omental resistance vessel preparation [5]. However, since these omental biopsies require a laparotomy it is difficult to obtain such biopsies from patients with uraemia. Due to these difficulties we have developed a new

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human vascular preparation, where small arteries (internal diameter about 165 μm) are dissected from small subcutaneous biopsies. Using this preparation and a myograph technique [6], we have looked at functional and morphological characteristics of vessels from 20 patients with uraemia while on dialysis and from 11 control subjects.

Methods

Subjects

Twenty patients with end-stage kidney disease (10 men, 10 women; mean age 41 years, range 9–67 years) were studied. All patients had been treated with chronic intermittent haemodialysis for a mean period of 24 months (range 1–113 months). The primary kidney disease was chronic glomerulonephritis in nine patients, hereditary adult polycystic renal disease in two patients, chronic interstitial nephritis in two patients, renal hypoplasia in one patient and obstructive nephropathy in one patient; in five patients the nature of the primary renal disease could not be established due to advanced chronic renal failure. In 18 patients the native kidneys were in situ. In two patients bilateral nephrectomy had been performed; they had both received a renal graft previously and they were uraemic due to late renal graft failure; their grafts were in situ at the time of examination. For purpose of analysis, patients were subdivided according to the existence of arterial hypertension in the period before dialysis treatment was given and during the period of chronic intermittent dialysis. All blood pressures were measured with a random sphygmomanometer after 15 min in the recumbent position taking phase 5 of the Korotkoff sounds as diastolic pressure. Group 1 comprised eight patients (four men, four women; mean age 40 years, range 9–63 years) who had been hypertensive both before and during the dialysis period. The blood pressure on the day of study was 169/99 mmHg (range 200–135/75–120 mmHg) and the average of 10–15 determinations of blood pressure during the preceding month was 164/97 mmHg (range 115–200/75–120 mmHg). All patients received antihypertensive drugs in various combinations (five patients on propranolol, one on pindolol, one on captopril, two on minoxidil and one on verapamil); however, blood pressure regulation was difficult in three patients, who had diastolic levels higher than 100 mmHg in spite of therapy with drugs and dialysis to dry weight. Seven patients had signs of left ventricular hypertrophy and three of strain on the electrocardiogram. The cardiothoracic ratio was increased in five patients on X-ray examination. The time from the last drug was taken until the biopsy was taken ranged from 12 to 24 h, and a further 3–4 h elapsed after the biopsy was taken before the mechanical experiments were started. Group 2 comprised seven patients (four men, three women; mean age 41 years, range 18–55 years) who had been hypertensive and received antihypertensive drugs before dialysis treatment was started, but were normotensive in the dialysis period. The blood pressure on the day of study was 127/80 mmHg (range 105–140/60–90 mmHg) and the average of 10–15 determinations of blood pressure during the preceding month was 118/76 mmHg (range 90–130/59–90 mmHg). None had received antihypertensive drugs for the last 3 months. Group 3 comprised five patients (two men, three women; mean age 42 years, range 18–66 years), who had been normotensive both before and during the dialysis period. Blood pressure on the day of study was 130/71 mmHg (range 110–140/60–80 mmHg) and the average of 10–15 determinations of blood pressure during the preceding month was 129/74 mmHg (range 114–145/65–85 mmHg).

The control group comprised 11 subjects (six men, five women; mean age 38 years, range 18–67 years). All had normal kidney function with a mean serum creatinine of 86 μmol/l (range 58–105 μmol/l) and a mean blood pressure on admission of 125/80 mmHg (range 90–160/60–90 mmHg).

All patients and controls were informed of the nature and purpose of the study according to the regulations of the local ethics committee, and the study was approved by the local ethics committee.

Protocol

On the day of study, biopsies (about 1 cm³) of the deep part of the subcutaneous fat were taken from the abdominal wall. For the uraemic patients, the biopsies were taken during renal transplant operations and insertion of catheters for peritoneal dialysis. For the controls, the biopsies were taken during surgery performed either for non-malignant gastrointestinal or urological diseases (nine patients) or kidney donation (two patients). In a few preliminary experiments, subcutaneous biopsies were taken from the gluteal region under local anaesthesia; vessels from these biopsies were not used in the comparison between uraemic patients and controls.

The biopsies were immediately immersed in cold physiological saline solution (defined below) and brought to the laboratory. Here, one, two or three (mean 1.84) artery segments (2 mm long) were dissected out from each biopsy and mounted on a myograph as ring preparations by threading them on to two stainless steel wires which were fastened to a force transducer and a micrometer, respec-
vessels. After a 1 h rest period at 37°C, the vessels were, on the basis of the passive tension-length characteristic, set to a normalized circumference \( L_0 \) estimated to be 0.9 times the circumference the vessels would have had if relaxed and exposed to 100 mmHg in vivo \([6]\). In preliminary experiments the force development was found to be maximal at this circumference in the vessels taken from both uraemic patients and controls. The vessel wall thickness to lumen diameter and a very small active wall force \( \Delta T < 0.5 \) N/m. It was, however, possible from 34 out of 36 biopsies studied to find one or two and in rare cases three or more artery segments which had a length of at least 3 mm. The ease with which these segments were found and dissected depended to a great extent on the experience of the person who did the dissection. In contrast to the omental bed \([5]\), the arteries did not normally run alongside the veins. When seen under the microscope the arteries had a well-defined tunica media and tunica adventitia. Regardless of whether the vessels had been dissected from subcutaneous biopsies taken from the abdominal wall under general anaesthesia or from the gluteal region under local anaesthesia, when the vessels were stimulated either with \( K^+ \) physiological saline or with control activating solution there was a rapid force development (Fig. 1). The force development normally reached a plateau within 1 min, while the relaxation in physiological saline was much slower. The responses of such vessels to noradrenaline were concentration dependent (Fig. 2), as they were for responses to ANG II, to potassium and to calcium.

**Comparison of vessels from uraemic patients and controls**

As indicated in Table 1, we were not able to detect any significant difference in the mean values of the parameters which we investigated in the vessels from uraemic patients and from controls. Thus uraemia did not appear to be associated with alterations in vascular parameters, as regards either their morphological or their functional properties. Furthermore, there was no significant difference in any of the parameters between the three groups of uraemic patients (groups 1, 2 and 3). We also investigated whether there was any correlation between the length of dialysis and any of the parameters, including blood pressure, but none was found.

**Correlation of mean blood pressure and vascular parameters**

**Morphology.** In the group of patients with uraemia there was a significant correlation between mean blood pressure (average of diastolic + one-third pulse pressure during the month preceding the day of study) and media thickness to lumen diameter ratio (Fig. 3), while there was no significant correlation between mean blood pressure and either lumen diameter \( (r = -0.37) \) or media thick-
FIG. 1. Tracing of the force response to K⁺ physiological saline of resistance vessels dissected from a subcutaneous biopsy taken either (a) under general anaesthesia or (b) under local anaesthesia. Normalized internal diameters were 139 μm and 96 μm, respectively. At the arrows K⁺ physiological saline was added and the double arrows indicate wash with physiological saline.

FIG. 2. Record of the force development of a 201 μm subcutaneous resistance vessel after stimulation with cumulative doses of noradrenaline (from 0.04 to 5 μmol/l). At the arrows the noradrenaline concentration was doubled and at the double arrow the noradrenaline was washed out.

ness \( (r = 0.02) \). Similar findings were made as regards the vessels from the controls (Fig. 3), although here the correlation coefficient between mean blood pressure and lumen diameter reached significance \( (r = -0.64, P < 0.05) \).

Functional parameters. Both in the vessels from the uraemic patients and from the controls, there was a negative correlation between mean blood pressure and active media stress in response to control activating solution (Fig. 4). However, no such correlations with blood pressure were seen with any of the other contractile parameters (active wall tension, effective active pressure), or with any of the sensitivity parameters (pD₂ values for noradrenaline, potassium, ANG II and calcium). It might be added that in the control group the correlation is very dependent on one point and since for both groups this analysis was made for a number of different parameters the findings of a significant correlation with media stress is only suggestive although likely.

Discussion

The aim of the present study has been twofold. First, to develop a technique which makes it possible to study isolated human resistance vessels that are easily obtainable (possibly with local anaesthesia) without ethical problems. Second, to investigate the morphology and functional characteristics of such vessels in advanced renal failure treated with dialysis.

The subcutaneous vessels used in the uraemic study were dissected out from subcutaneous biopsies taken under general anaesthesia. These vessels developed an effective pressure which had the same magnitude as we have seen previously in rat vessels of similar size [9], and although the force production expressed as media stress was less than in the rat vessels [9] it was still in the same range and not much less than that we found in human omental resistance vessels [5]. Also, the vessels responded in a concentration dependent way to two different agonists (noradrenaline and ANG II). Finally, we have in similar vessels found a very pronounced relaxing effect of acetylcholine which disappears after rubbing the endothelium with a stainless steel wire (C. Aalkjær, A. M. Heagarty, J. D. Swales & H. Thurston, unpublished work), suggesting that the preparation has an intact endothelium [10]. The vessels obtained from these biopsies thus appeared to be intact and viable when mounted in the myograph. Furthermore, since the experiments with subcutaneous biopsies taken under local anaesthetic gave similar results, this suggests that in future it will be possible to investigate such resistance vessels on a more routine basis than hitherto achieved [5, 11-13]. We therefore believe that this
TABLE 1. Functional and morphological characteristics of subcutaneous resistance vessels from patients with uraemia treated with dialysis

The values are means ± SE obtained from 20 patients with uraemia and 11 controls. None of the parameters was significantly different (P > 0.05).

<table>
<thead>
<tr>
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<th>Uraemia</th>
<th>Control</th>
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<tbody>
<tr>
<td>Lumen diameter (µm)</td>
<td>164 ± 10</td>
<td>166 ± 21</td>
</tr>
<tr>
<td>Media thickness (µm)</td>
<td>14.8 ± 0.56</td>
<td>12.5 ± 1.2</td>
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<tr>
<td>Media thickness/lumen diameter (%)</td>
<td>9.71 ± 0.61</td>
<td>8.74 ± 1.02</td>
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<tr>
<td>Active wall tension* (mN/mm)</td>
<td>2.04 ± 0.21</td>
<td>1.72 ± 0.29</td>
</tr>
<tr>
<td>Active media stress* (kPa)</td>
<td>14.0 ± 13</td>
<td>15.7 ± 27</td>
</tr>
<tr>
<td>Effective pressure* (kPa)</td>
<td>24.7 ± 1.5</td>
<td>22.5 ± 1.2</td>
</tr>
<tr>
<td>Active wall tension (Na⁺) (mN/mm)</td>
<td>1.74 ± 0.17</td>
<td>1.66 ± 0.30</td>
</tr>
<tr>
<td>Active wall tension (ANG II) (mN/mm)</td>
<td>1.65 ± 0.25</td>
<td>1.24 ± 0.22</td>
</tr>
<tr>
<td>Active wall tension (potassium⁺) (mN/mm)</td>
<td>1.61 ± 0.21</td>
<td>1.29 ± 0.30</td>
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<tr>
<td>pD₂ (Na⁺)</td>
<td>6.40 ± 0.08</td>
<td>6.55 ± 0.08</td>
</tr>
<tr>
<td>pD₂ (ANG II)</td>
<td>7.89 ± 0.09</td>
<td>7.99 ± 0.16</td>
</tr>
<tr>
<td>pD₂ (potassium⁺)</td>
<td>1.48 ± 0.01</td>
<td>1.44 ± 0.03</td>
</tr>
<tr>
<td>pD₂ (calcium)</td>
<td>4.05 ± 0.07</td>
<td>4.23 ± 0.05</td>
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*Stimulation with K⁺ physiological saline + 5 µmol/l noradrenaline.
†Noradrenaline.
‡K⁺ physiological saline.
§pD₂ = -log (EC₅₀), where EC₅₀ is the concentration (mol/l) which gives half-maximal response.

Fig. 3. Correlations between mean blood pressure (MBP) and the ratio of media thickness to lumen diameter of subcutaneous resistance vessels of patients with uraemia (○) and of control subjects (●). The correlation coefficients of the regression lines are 0.50 (U, uraemic group, P < 0.05) and 0.55 (C, control group, P < 0.05), respectively.

Fig. 4. Correlations between mean blood pressure (MBP) and the active media stress of subcutaneous resistance vessels from patients with uraemia (○) and from controls (●). The correlation coefficients of the regression lines are −0.49 (U, uraemic group, P < 0.05), −0.71 (C, control group, P < 0.01), respectively.

The morphology of the subcutaneous vessels was qualitatively similar to that which we have previously seen in normotensive human omental resistance vessels [5] and also in rat resistance vessels [9]. However, the media thickness/lumen diameter ratio of the subcutaneous vessels was about 81% higher than that of the normotensive human omental vessels [5], and indeed about 58% greater than that of omental vessels from women with pre-eclampsia [14]. Furthermore, since the subcutaneous vessels and the omental vessels were taken from the same level with respect to the heart and since these vessels with a diameter between 100 and 200 µm probably belong to the larger resistance vessels [15], it is likely that they are exposed to the same blood pressure in vivo. The difference in media thickness/lumen diameter ratio may therefore represent a difference in the quantitative structure which is independent of blood pressure levels. This is interesting in relation to the
discussion about whether the increased media thickness seen in hypertension is merely a consequence of the increased blood pressure or whether it could be a primary factor in the pathogenesis of hypertension [16] and it supports the notion that vascular structure may not alone be determined by the blood pressure level.

The comparison of resistance vessels from control subjects with resistance vessels from patients with uraemia revealed in the present study no significant differences in neither resistance vessel morphology nor intrinsic reactivity assessed in different ways. This does of course not exclude the possibility that small differences may still be present, which were not detected given the method used and the number of individuals investigated. Eight of the patients with uraemia had different types of antihypertensive drug therapy. The extent to which these drugs could still be present in the vessel wall and influence the contractile responses is difficult to predict. The observation that the noradrenaline sensitivity was not significantly different in the group of patients with uraemia has perhaps special interest. This finding is in agreement with that of Campese et al. [2], who found that there was no difference in blood pressure response to noradrenaline infusions in patients with chronic uraemia treated with dialysis. However, our observation that there was no difference in sensitivity to ANG II differs from recent clinical findings that the dose of ANG II required to produce a given increase in blood pressure was greater for uraemic patients than for controls (S. S. Sørensen, H. Danielsen, B. Jespersen & E. B. Pedersen, personal communication).

In contrast to these negative observations, our results showed that both for the group of patients with uraemia and for the control group, the mean blood pressure was related to both morphological and functional vascular parameters. The finding that mean blood pressure and the ratio of media thickness to lumen diameter were positively correlated indicates that structural resetting of the vascular wall, whether primary or secondary in nature, contributes to the increased peripheral resistance seen in this condition [17]. The negative correlation between media stress (force production per unit area of smooth muscle) and mean blood pressure suggests that the function of the smooth muscle is depressed with increasing blood pressure. Similar findings have been made in mesenteric resistance vessels from two-kidney, one-clip renal hypertensive rats [7]. It is difficult to imagine that such a reduced maximal response should be causative in the pathogenesis of the increased blood pressure. A more likely possibility is that it is a consequence of the increase in blood pressure. Whether this decreased media stress of vessels from patients with high blood pressure is caused by an intracellular or extracellular production of non-contractile tissue has not been looked at in the present investigation.

In the present study the noradrenaline stimulated calcium sensitivity was assessed using a protocol previously developed to look at calcium-sensitivity of vessels from the spontaneously hypertensive rat [8]. In mesenteric vessels from the spontaneously hypertensive rat the calcium sensitivity has been found to be increased but recent experiments have suggested that this increased calcium sensitivity may not be a causative abnormality [18]. In the present study we found no evidence for an association of an increased calcium sensitivity and an increased blood pressure, indeed the trend was negative ($r = -0.33, 0.2 > P > 0.1$) between mean blood pressure and the calcium sensitivity. Although this was not significant, inspection of the data indicates that this was mainly due to a low calcium sensitivity of the group of people who before dialysis had been hypertensive but were now normotensive. So the results do not therefore support the possibility that an increased calcium sensitivity of the smooth muscle cells is of importance for the increased peripheral resistance in uraemia.

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References