Proximal renal tubular peptide catabolism, ammonia excretion
and tubular injury in patients with proteinuria: before and after
lisinopril

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INTRODUCTION

Proteinuria and progression of renal disease may be linked at the tubular level [1]. As well as tubular hypercatabolism itself being damaging [2, 3], ammonia may also be toxic within the kidney [4-6]. We developed a method to measure the renal handling of filtered small polypeptides using the catabolism of 99mTc-labelled aprotinin (Trasylol), a 6500 Da polypeptide, radiolabelled with technetium, as a physiological probe in renal patients with variable function and proteinuria [7-9]. We showed that heavy proteinuria (>5.0 g/24 h) is linked to hypercatabolism of aprotinin (Apr) in the proximal tubular cell, and this was associated with higher ammonia excretion compared with patients with similar function but less proteinuria [10].

Markers of renal tubular injury are not perfect, especially in proteinuric states. However, we have separated isoenzymes of N-acetyl-β-D-glucosaminidase (NAG), an index of proximal renal tubular injury (or hyperfunction) [11]. The previously known ‘A’ isoenzyme was further separated into ‘A1’ and ‘A2’ isoenzymes, not previously described in man, and ‘A2’ was the predominant fraction in both human kidney homogenates and urine, rather than plasma.

Angiotensin-converting enzyme (ACE) inhibitors are increasingly being used to reduce proteinuria, separate from any blood-pressure-lowering effects [12-16]. No data are available in man on the kinetics of proximal renal tubular protein catabolism or markers of tubular injury before and after ACE inhibition. In this study we investigate 10 renal patients with heavy proteinuria and measure the kinetics of renal tubular degradation of Apr, as well as urinary ammonia excretion, urinary total NAG and the NAG ‘A2’ isoenzyme before and after lisinopril. We do not address renal disease progres-
sion nor the renoprotective mechanisms of action of ACE inhibitors.

METHODS AND MATERIALS

Patients

Ten patients with normal function or mild renal impairment were studied [two females and eight males, mean age 40.6 (23–58) years]. All had biopsy proven glomerulonephritis except one patient with diabetic nephropathy of > 10 years duration, with no evidence of other renal disease based on radiological, biochemical and immunological data. Mean corrected glomerular filtration rate ($^{51}$Cr-EDTA clearance corrected for height, weight and surface area) was 63.7 ± 8.3 (33.1–105.6) ml·min$^{-1}$·1.73 m$^{-2}$. Proteinuria varied, averaging 9.5 g/24 h, and of these five >5.0 g/24 h; seven patients >5.0 g/24 h, and of these five >10.0 g/24 h.

None of the patients had oedema, were on dietary protein restriction, immunosuppressive therapy, ACE inhibitors or sodium bicarbonate supplements. Blood pressure in one patient was stabilized with doxazosin. Loop diuretics were also used in two patients. All patients were continually encouraged to reduce their dietary sodium, aiming for an intake of 80 mmol/24 h. This was quite difficult to achieve despite each patient being seen by a senior dietician with written instructions to follow and frequent monitoring. The study had the approval of both the local Ethics Committee and the Administration of Radioactive Substances Advisory Committee. All patients gave their written informed consent to participation in the study.

Radiolabelled aprotinin

Aprotinin (Bayer), 0.5 mg, was radiolabelled with $^{99m}$Tc (approximately 80 MBq) by the stannous chloride method [7]. The radiochemical purity was routinely >97%.

Urinary pH, ammonia and titratable acidity

Fresh timed (over 2–3 h) morning urine samples were collected and analysed immediately (in duplicate). Urinary pH was measured using a Pye model (292 meter, Pye Unicam). Titratable acidity was measured by standard techniques. Urinary ammonia measurements were made using a validated modified Boehringer–Mannheim diagnostic kit with standards (Ammonia UV-system and Preciset Ammonia standards). The standard coefficient of variation is 3.7 ± 0.3 (0–6.7)%.

Urinary total NAG and the NAG 'A2' isoenzyme

Each fraction was analysed for total NAG activity by measuring the conversion of para-nitrophenol N-acetyl-$eta$-D-glucosaminide to para-nitrophenol, as described by Marauhn [17]. The SCV is 8% and the detection limit is 0.1 nmol of para-nitrophenol. Values for total urinary NAG activity in 21 control subjects were 46 (16–92) µmol/24 h [median (range)].

The methodology used [11] to separate the NAG isoenzymes is a modification of the fast protein liquid chromatography procedure of Shibasaki et al. [18].

Other laboratory analyses

Plasma and urinary creatinine were measured with a Hitachi 747 and 911 analyser using Boehringer Mannheim Chemistries (Hitachi, Lewes, U.K.). Creatinine clearance was measured on 24 h urine collections. Twenty-four hour urinary protein content was measured using a Biotrol urine protein kit [19]. Urinary transferrin and IgG were measured by immuno-turbidometric procedures on the Hitachi 911 analyser and reagents supplied by Boehringer Mannheim Chemistries.

Procedure

Studies were performed on outpatients over at least 5 months. In the control period (0–6 weeks) patients were monitored weekly or fortnightly to stabilize their weight, blood pressure and therapy. Plasma and 24 h urines were collected for standard biochemistries, measurement of creatinine clearance and protein excretion as well as 24 h urinary NAG and the NAG 'A2' isoenzyme. Fresh timed urines were also analysed for pH, titratable acidity and ammonia.

Lisinopril was then given (5 mg daily for 1 week, and increased to 10–20 mg depending on reduction in proteinuria, aiming for the maximum reduction without a significant or symptomatic drop in blood pressure), with similar monitoring on a weekly or fortnightly basis as before. After 6 weeks of treatment lisinopril was stopped but clinical review and urinary and plasma biochemical monitoring continued for a further 6 weeks.

At the end of the control period, and 6 weeks after starting lisinopril, 0.5 mg of Apr (approximately 80 MBq) was injected intravenously. A test dose of Apr (200 inhibitory units in 0.2 ml) was given to every patient before each Apr injection.

Urine was collected over 26 h after each injection, and aliquots were taken at intervals for counting the free pertechnetate excretion rate (with a gamma counter). Plasma was sampled at 2, 4, 8, 12, 20, 40 and 60 min, and at 1.5, 3, 18 and 26 h.

Renal activity was measured at 18 and 26 h using a large field-of-view gamma camera (Siemens 75 Digitrac, linked to a Nodencrest V77 digital computer) [7]. Regions of interest were drawn over the kidneys on the anterior and posterior images. Over this period, renal activity is stable, as is fractional...
degradation since losses from catabolism are equal to uptake from tubular fluid after filtration from plasma [7, 8]. Geometric mean counts were calculated (and corrected for background activity) [7]. One patient was unable to attend for the second kidney scan, and thus data with respect to Apr metabolism relate only to the control period.

Glomerular haemodynamics were also measured at the end of the control period and after the sixth week of treatment, 5 days after the Apr study, using isotopic $^{51}$Cr-EDTA (3 MBq) and, as a measure of effective renal plasma flow, $^{99m}$Tc-mercaptoacetyltriglycine tubular excretion rate $^{[99m}$Tc-MAG3 TER (5 MBq)], injected simultaneously with blood samples taken at 20 and 40 min and at 2 and 4 h. Calculations were made using a volume dilution technique, and the data corrected for height, weight and surface area. MAG3 is excreted predominantly by the renal tubules. The values obtained represent 85% of the effective renal plasma flow.

**Data analysis**

All data are expressed as means and S.E.M. and paired Student’s t-test was used to compare the effects of treatment with lisinopril. Correlation coefficients were also applied.

**Renal catabolism of Apr**

The data were handled as previously described with two parameters measured [kidney uptake, representing undegraded Apr in the proximal tubular cells (% of dose), and free urinary pertechnetate representing metabolism (% of dose/h) and one calculated (fractional degradation/h) [8]. Chromatography (Sephadex-G-25-M) had previously established the nature of the radioactivity recovered in urine, and extra-renal catabolism was excluded [7, 8].

Metabolism of Apr was measured over the entire duration of study (26 h) as well as between 18 and 26 h after Apr injection. Kidney uptake was measured between 18 and 26 h after Apr injection. Fractional degradation between 18 and 26 h was thus derived from the latter two measurements.

**Total urinary NAG and the NAG 'A2' isoenzyme**

For urinary excretion rates, statistical analyses were performed after log transformation to normalize the distribution.

**Plasma Apr curves**

The data were handled as described previously [7], from which volumes of distribution and clearance rates were then derived.

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**Table 1. Effects of lisinopril on creatinine and $^{51}$Cr-EDTA clearances, $^{99m}$Tc-MAG 3 TER, urinary transferrin and IgG (means ± SEM). No significant differences shown in data before and after lisinopril (paired Student’s t-test).**

<table>
<thead>
<tr>
<th></th>
<th>Pre-lisinopril</th>
<th>Post-lisinopril</th>
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</thead>
<tbody>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>90.8 ± 12.0</td>
<td>83.0 ± 11.9</td>
</tr>
<tr>
<td>$^{51}$Cr-EDTA clearance (ml·min$^{-1}$·1.73 m$^{-2}$)</td>
<td>63.7 ± 8.3</td>
<td>57.8 ± 8.2</td>
</tr>
<tr>
<td>$^{99m}$Tc-MAG3 TER (ml·min$^{-1}$·1.73 m$^{-2}$)</td>
<td>346.5 ± 112.7</td>
<td>272.7 ± 51.0</td>
</tr>
<tr>
<td>Filtration fraction</td>
<td>0.21 ± 0.07</td>
<td>0.20 ± 0.06</td>
</tr>
<tr>
<td>Urinary transferrin (g/l)</td>
<td>0.30 ± 0.06</td>
<td>0.22 ± 0.05</td>
</tr>
<tr>
<td>Urinary IgG (g/l)</td>
<td>0.060 ± 0.008</td>
<td>0.067 ± 0.02</td>
</tr>
<tr>
<td>IgG/transferrin clearance ratio</td>
<td>0.23 ± 0.08</td>
<td>0.18 ± 0.05</td>
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**RESULTS**

**Urinary protein**

Proteinuria fell significantly from $9.5 ± 1.6$ (3.7–20.3) g/24 h before lisinopril to $4.5 ± 1.0$ (0.8–10.7) g/24 h 6 weeks after lisinopril ($P<0.001$). However, there was quite a variation in the response between patients, the mean percentage reduction in proteinuria being $52.6 ± 6.3$ (22–84)%.

Urinary transferrin and IgG concentrations were unchanged, as were the IgG/transferrin clearance ratios after lisinopril treatment (Table 1).

**Renal tubular uptake and metabolism**

The rate of appearance of the free urinary pertechnetate over the 26 h, representing renal tubular metabolism of Apr, fell significantly from $1.7 ± 0.1$ to $1.2 ± 0.1$% dose/h after lisinopril ($P<0.01$). Metabolism fell in all patients, and individual values for the 18–26 h interval (from which the fractional degradation of Apr was calculated) are shown in Figure 1.

Kidney uptake, however, was unchanged, either at 18 h ($23.9 ± 3.1$ and $25.6 ± 4.3$)% of dose before and after Lisinopril.
after lisinopril respectively) or at 26 h (25.7 ± 2.8 and 23.6 ± 3.3% of dose before and after lisinopril respectively).

Fractional degradation of Apr, a measure of the turnover rate, calculated from the free urinary per-technetate excretion rate and kidney uptake between 18 and 26 h, did fall significantly from 0.08 ± 0.02/h before lisinopril to 0.04 ± 0.007/h after (P < 0.04). Data for the individual patients are shown in Figure 2. Fractional degradation was also correlated with proteinuria when all the data were taken together (y = 0.03 + 0.04x; r = 0.55, P < 0.02).

**Urinary pH, ammonia and titratable acidity**

After lisinopril ammonia excretion fell significantly from 1.2 ± 0.1 to 0.6 ± 0.1 mmol/h (P < 0.0001). In contrast, urinary pH was unchanged (6.1 ± 0.3 before lisinopril and 5.9 ± 0.2 afterwards). Likewise, titratable acidity excretion was also unaffected (1.0 ± 0.1 mmol/h before and 0.9 ± 0.2 mmol/h after).

Figures 3 and 4 illustrate the relationship in the individual patients between proteinuria and urinary ammonia excretion (y = 0.496 ± 0.046x; r = 0.50, P < 0.02), and in all but one patient, between metabolism of Apr and urinary ammonia (y = 0.194 ± 0.54x; r = 0.49, P < 0.05), before and after lisinopril. As proteinuria is reduced after treatment with lisinopril urinary ammonia excretion fell in all of the individual patients (Figure 3). Likewise, as metabolism of Apr is also suppressed after lisinopril, ammonia excretion was reduced (Figure 4).

**Total urinary NAG and NAG ‘A2’ isoenzyme**

Total urinary NAG fell from a median (range) of 176 (86–1003) μmol/24 h before lisinopril to 108 (46–343) μmol/24 h after lisinopril (P < 0.0001). The NAG ‘A2’ isoenzyme also fell significantly from 56 (16–642) μmol/24 h before lisinopril to 20.5 (6–393) μmol/24 h after lisinopril (P < 0.02). There was also a significant fall in the NAG A2/A1 ratio,
after lisinopril respectively), measured glomerular filtration rate (of both creatinine clearance and $^{51}$Cr-EDTA clearance) or effective renal plasma flow (MAG3 TER), nor the calculated filtration fraction ($^{51}$Cr-EDTA clearance/MAG3 TER) (Table 1).

Plasma concentrations of albumin, potassium and bicarbonate were also unchanged (before lisinopril: 30.6 ± 2.1 g/l, 4.3 ± 0.2 mmol/l and 25.7 ± 0.8 mmol/l respectively; after lisinopril: 32.8 ± 2.4 g/l, 4.5 ± 0.2 mmol/l and 23.1 ± 1.2 mmol/l respectively). Likewise, there was no change in urinary urea or in urinary sodium excretion (before lisinopril: 314.7 ± 22.4 and 156.8 ± 13.7 mmol/24 h respectively; after lisinopril: 320.7 ± 31.3 and 136.7 ± 13.1 mmol/24 h respectively).

Figure 5 illustrates the relationship between proteinuria and log total urinary NAG excretion in the individual patients. In all patients NAG excretion fell as proteinuria was reduced after treatment ($y = 1.9 + 0.033x; r = 0.57, P < 0.01$).

Effects of lisinopril withdrawal

Six weeks after stopping lisinopril treatment proteinuria increased significantly [to 7.0 ± 0.9 (2.3-10.7) g/24 h ($P < 0.03$)] compared with proteinuria during treatment with lisinopril. These data were calculated from eight patients since two patients refused to discontinue therapy. However, proteinuria was still significantly lower than before commencing lisinopril treatment ($P < 0.03$).

Ammonia excretion, however, did increase back to pretreatment levels (1.2 ± 0.2 mmol/h), as did total urinary NAG [305 (112–690) μmol/24 h, $P < 0.0001$ compared with levels during lisinopril treatment]. Urinary pH and titratable acidity were not significantly affected (5.9 ± 0.3 and 1.2 ± 0.2 mmol/h respectively). Likewise there were no significant changes in blood pressure (99.9 ± 5.7 mmHg) or in creatinine clearance (82.9 ± 10.9 ml/min).

DISCUSSION

There are no data whatsoever addressing the possible changes in renal tubular protein catabolism, ammonia excretion or markers of tubular injury (or hyperfunction) after treatment with an ACE inhibitor in patients. The purpose of this study was to see whether lisinopril could reverse potentially harmful events (hypercatabolism and/or ammonia excretion) and indices of tubular injury along with the expected reduction in proteinuria.
Proteinuria fell after lisinopril treatment to approximately half its pretreatment value, in keeping with observations by others [12–14]. As previously shown there was considerable variation between individuals. This could arise from differences in compliance, as well from a variety of small differences, for example in salt balance, blood pressure control, use of diuretics, or in genetic factors especially with respect to angiotensin II receptors [20]. However, there was no change in blood pressure control overall, or in glomerular haemodynamics, although there were non-significant reductions in blood pressure, $^{51}$Cr-EDTA clearance, $^{99m}$Tc-MAG3 TER and filtration fraction. Even 6 weeks after withdrawing lisinopril treatment there still seemed to be a significant reduction in proteinuria, compared with levels before treatment began.

Tubular uptake of Apr was unchanged, but tubular peptide metabolism and fractional degradation both fell in parallel to the reduction in proteinuria. Likewise a reduction in proteinuria induced by lisinopril was accompanied by a fall in ammonia excretion, but no change in pH, titratable acidity, or in plasma bicarbonate. This has not been described previously. There was no suggestion that the ACE inhibitor affected ammonia excretion other than by its effect on proteinuria. However, angiotensin II has been shown, in canine proximal tubules, to inhibit ammonia excretion other than by its effect on proteinuria. However, angiotensin II is also associated with increased renal ammoniagenesis [5], and ACE inhibition can cause hyperkalaemia but there were no significant differences in potassium levels either.

Although the reduction in ammonium excretion after ACE inhibition could interfere with the elimination of hydrogen ions from the body, and result in acidosis, this was not apparent in our study. Further observations would be needed over a longer period to identify the timing of the changes and their ultimate effects. However, the possibility that subclinical acidosis might contribute to the hyperkalaemia seen after ACE inhibition, especially in patients with severe renal impairment, has not been considered before.

After lisinopril indices of tubular damage were reduced, in terms of total urinary NAG and the excretion of the more tubulo-specific NAG ‘A2’ iso-enzyme. Again the changes in NAG excretion fell in parallel to the reduction in proteinuria. This relationship between proteinuria and NAG excretion closely resembled that found in other patients with proteinuria over a wide range, and with dissimilar disease [22].

Lisinopril treatment for 6 weeks in patients with proteinuria led to a reduction in proteinuria as expected, but we were able to quantify the changes in tubular kinetics of Apr catabolism, as well as the decreased excretion of ammonia, urinary total NAG and the NAG A2 isoenzyme. These changes after lisinopril treatment in patients have not been measured previously.

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REFERENCES