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Calcium citrate ameliorates the progression of chronic renal injury

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Calcium citrate ameliorates the progression of chronic renal injury.

Background. Metabolic acidosis is a consequence of chronic renal failure and it may produce bone demineralization, muscle proteolysis, and progression of chronic renal failure. The aim of this study was to evaluate the effects of correction of metabolic acidosis with calcium citrate in an experimental model of renal mass ablation.

Methods. Wistar rats were subjected to 5/6 nephrectomy and were randomly assigned to one of 4 groups: nontreated (NFX); treated with calcium citrate (1.45 g/100 g feed) (NFX-CIT); treated with captopril (500 mg/L water) (NFX-CAP); or treated with both (NFX-CAP-CIT) during 1, 10, or 20 weeks. Body weight, systolic blood pressure, proteinuria, arterial bicarbonate concentration, urine citrate excretion, plasma calcium, and inulin clearance were measured. Histologic glomerular and tubulointerstitial damage scores were measured at 1, 10, and 20 weeks, and glomerular and tubular proliferating cell nuclear antigen (PCNA)-positive cells, α–smooth muscle actin, and desmin staining were studied by immunohistochemistry at 1 and 10 weeks.

Results. The treated groups showed significantly less glomerular and tubulointerstitial cellular proliferation in the first week ($P < 0.05$), less glomerular cell transdifferentiation and higher plasma bicarbonate at 10 weeks ($P < 0.05$), as well as diminished histologic glomerular and tubulointerstitial damage scores at 20 weeks ($P < 0.05$). Inulin clearances were higher ($P < 0.05$), and urine protein excretion rates were lower ($P < 0.05$) than in the NFX non-treated group, but arterial blood pressure was not significantly different in the NFX-CIT group.

Conclusion. Calcium citrate slows the progression of chronic renal injury in the 5/6 NFX model. It improves metabolic acidosis and diminishes cell proliferation and transdifferentiation without changes in systolic blood pressure.

Key words: chronic renal failure, 5/6 nephrectomy, calcium citrate, metabolic acidosis.

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The progression to end-stage renal failure is independent of the initial pathogenetic mechanism [1]. The common destructive mechanism that leads any chronic renal disease to glomerulosclerosis and chronic tubulointerstitial damage has been extensively investigated [2–7], and the experimental model most widely used is the 5/6 nephrectomy [4].

Metabolic acidosis is a common consequence of chronic renal failure [8, 9] that results from an inadequate ammonium excretion and decreased tubular bicarbonate reabsorption [10]. It has severe consequences because it enhances renal bone disease (renal osteodystrophy) [11] and activates muscle proteolysis [12]. There is evidence that acidosis diminishes glomerular filtration rate [13]. Ammonia production by remnant nephrons is increased after partial nephrectomy [14], and it probably produces renal damage. Logan [15] found in normal rat kidney cells that urea stimulates DNA synthesis, and Ling et al [16] observed that ammonia produces mesangial and tubular cell hypertrophy. Nath et al [17] have demonstrated that proteinuria (after 5/6 nephrectomy in rats) decreased when renal ammoniagenesis is reduced by supplementation with sodium bicarbonate. They suggested that ammonia stimulates the alternative complement pathway so it produces tubulointerstitial inflammation and renal injury with altered tubular function [18, 19], and it is also a stimulus to renal growth [19]. Throssell et al [20] were not able to demonstrate the benefits of sodium bicarbonate supplementation. Acidotic rats with normal renal function developed proteinuria and renal hypertrophy and hyperplasia. Throssell et al [21] considered that filtered proteins are not metabolized by tubular cells. Together these data suggest that metabolic acidosis may aggravate progression of chronic renal failure.

In previous experiments we administered $NaHCO_3$ during 20 weeks to 5/6 nephrectomized Wistar rats, and they developed severe arterial hypertension and neither improved glomerular filtration rate nor diminished

histologic lesions compared with nontreated nephrectomized groups (unpublished results).

Citrate, an alkalinizing agent, is reabsorbed in the renal proximal tubule by a sodium-coupled transporter, the Na^+/dicarboxylate cotransporter, with a broad substrate specificity for Kreb's cycle intermediates [22, 23]. Tanner et al have recently demonstrated [24–26] that citrate salts improve renal function in rats with polycystic kidney disease, mainly by its alkalinizing effect. Toblli et al [27] also demonstrated a beneficial effect of potassium citrate supplementation in uric acid nephropathy. We have observed that calcium citrate supplementation also attenuates tubulointerstitial damage in the rat model of obstructive nephropathy [abstract; Gadola et al, *J Am Soc Nephrol* 12:703A, 2001]. Calcium citrate would be a safer citrate salt to use in a chronic renal failure model to avoid the risks of sodium or potassium overload. As far as we know there are no reports on calcium citrate effects on the ablation model of progression of renal failure.

The aims of this study were to evaluate the effects on chronic renal failure progression and its mechanisms of an early and prolonged alkalinization with calcium citrate in the 5/6 nephrectomy model, with no changes in the diet sodium content or systemic haemodynamics.

METHODS

Male Wistar rats weighing 256 ± 74 g were divided into 5 groups. One of the groups was sham operated (SHAM) ($N = 18$). Five-sixths renal ablation by right nephrectomy, and ablation of two thirds of the left kidney, under anesthesia with intraperitoneal thiopental**,** was performed in 72 rats. Previously, systolic blood pressure in awake rats was measured by tail optical plethysmography (custom-made device). Urine was collected during 24 hours in a metabolic cage (Nalgene; Nalge Nunc International, Rochester, NY, USA) and urinary protein excretion was determined. All rats were placed in individual cages with natural light and controlled room temperature, and they were handled according to Principles of Laboratory Animal Care (National Institutes of Health, 1985). They had free access to water and standard diet with 24% protein. Immediately after nephrectomy (NFX), the rats were randomly assigned to 4 groups (18 rats each): (*1*) nontreated (NFX); (*2*) captopril (NFX-CAP) (Squibb, Darmstadt, Germany) was administered 500 mg/L in drinking water; (*3*) calcium citrate (NFX-CIT) (Mission Pharmacal, San Antonio, TX, USA) was administered 1.45 g/100 g feed; and (*4*) calcium citrate plus captopril (NFX-CAP-CIT). The 5 groups (sham and the 4 NFX groups) were divided into three subgroups according to observation time: 1 week, 10 weeks, and 20 weeks (6 rats each).

At 10 and 20 weeks body weight, systolic blood pressure, 24-hour urinary protein excretion, and arterial bicarbonate (obtained from aorta under anesthesia with thiopental) were measured. At 20 weeks, 24-hour urine citrate excretion, inulin clearance, and plasma calcium were also measured.

Analytical methods

Urinary protein excretion was assayed by the turbidimetric method with sulfosalicylic acid. Plasma calcium was measured by O-cresolphthalein automated assay (Roche Diagnostics, Mannheim, Germany). Inulin concentrations in plasma and urine were measured by colorimetric anthrone method (Shimadzu UV400A; Kyoto, Japan). Arterial bicarbonate was measured with a blood gas analyzer (ABL 700; Radiometer, Copenhagen, Denmark). Urine citrate was determined by the enzymatic citrate lyase method (Nielsen's method).

Inulin clearance

Rats were anesthetized with intraperitoneal sodium thiopental (35 mg/kg body weight) and they were placed on a temperature-regulated table. After tracheostomy, PE-50 catheters were inserted into the left and right internal jugular veins for infusion of normal saline and inulin (10%; Sigma Chemical Co., St. Louis, MO, USA). A PE-50 catheter was inserted in the left femoral artery to measure mean arterial pressure (Nikon Kohden Life Scope 8, Tokyo, Japan) and blood sampling. A PE-10 catheter was inserted into the left ureter for collection of urine (or both ureters in the sham-operated group). Inulin was administered by a continuous flow of 1.2 mL/hour via infusion pump (Harvard Apparatus, Hollinston, MA, USA). Clearance measurements were carried out in two intervals of 20 minutes each, after allowing 50 minutes for equilibration of plasma inulin concentration.

Immunohistochemistry

Sections of methyl Carnoy's fixed kidney tissues were processed by indirect immunodetection technique with Biotin streptavidin amplified detection system (BioGenex, San Ramon, CA, USA) using three primary antibodies (Biogenex): mouse monoclonal antibodies to (*1*) proliferating nuclear cell antigen (PCNA) (dilution 1:200), as a marker of cell proliferation; to (*2*) desmin (dilution 1:80); and to (*3*) α–smooth muscle actin (dilution 1:100) as markers of myofibroblast transformation [28–30].

Negative controls consisted of substitution of the primary antibody with equivalent concentrations of a normal rabbit immunoglobulin (Ig)G (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Mean score per biopsy was calculated as follows: glomerular PCNA as the mean number of proliferating (positive) cells in 15 glomeruli (cells/glomerular cross section); PCNA tubular/interstitial score was obtained as the mean number of proliferating cells in 20 fields, 0.1 mm^2 each (cells/fields).

Table 1. Functional measurements on sham-operated rats and 5/6-nephrectomized rats treated with captopril, calcium citrate, or captopril-calcium citrate

	SHAM	NFX	NFX-CAP	NFX-CIT	NFX-CAP-CIT
Body weight 20 w *g*	467 ± 56	318 ± 60[b]	402 ± 25	334 ± 47	387 ± 35
SBP 10 w *mm Hg*	106 ± 12	102 ± 18	105 ± 15	117 ± 18	102 ± 4
SBP 20 w *mm Hg*	111 ± 13	137 ± 20[b]	99 ± 9[a]	129 ± 19	103 ± 9[a]
Prot ur 10 w *mg/day*	9 ± 4	69 ± 6[b]	13 ± 8[a]	23 ± 10[a]	32 ± 14[a]
Prot ur 20 w *mg/day*	15 ± 7	152 ± 71[b]	27 ± 22[a]	28 ± 19[a]	46 ± 21[a]
Art bic 10 w *mEq/L*	25.5 ± 1.6	17.3 ± 2.4[b]	23.8 ± 3.6[a]	25 ± 3.5[a]	25.3 ± 2.8[a]
Art bic 20 w *mEq/L*	20.5 ± 3.3	16.3 ± 3.7	20.5 ± 1.6	20.7 ± 2.3	20.3 ± 1.6
Ca 20 w *mg/dL*	7.6 ± 0.5	7.4 ± 0.9	8.4 ± 0.8[a]	8.3 ± 0.3[a]	9.1 ± 0.4[a]
Ur citrate 20 w *mg/day*	25 ± 7	7 ± 5[b]	5 ± 3	17 ± 8	18 ± 12[a]
Inulin Cl 20 w μ*L/min/100 g*	447 ± 142	22 ± 11[b]	87 ± 47[a]	92 ± 29[a]	103 ± 60[a]

Abbreviations are: SBP, systolic blood pressure; Prot ur, proteinuria; Art bic, arterial bicarbonate concentration; Ca, plasma calcium concentration; Ur citrate, urine citrate excretion; Inulin Cl, inulin clearance; w, weeks; SHAM, sham-operated group; NFX, 5/6 nephrectomized group; NFX-CAP, treated with captopril group; NFX-CIT, treated with calcium citrate group; NFX-CAP-CIT, treated with captopril and calcium citrate group. $N = 6$ in all the subgroups. All data are mean ± SD.

[a] $P < 0.05$ vs. NFX.

[b] $P < 0.05$ vs. SHAM.

For the evaluation of desmin and α−smooth muscle actin, each glomerulus and tubulointerstitial grid field was graded semiquantitatively according to the extent of the staining from 0 (absent) to 4 (more than 75% in the glomerular tuft) or 5 (more than 75% of tubulointerstitial field). Mean scores of 15 glomeruli and 20 fields were calculated in each kidney.

Morphologic techniques

All remnant kidneys were fixed with Bouin's solution for histologic study and those from the 1 and 10 weeks subgroups were divided in halves, and one half was fixed with Carnoy to perform immunohistochemistry. Glomerulosclerosis (GS) index was assessed in 100 glomeruli per animal, according to El Nahas et al [31] on periodic-acid Schiff (PAS)-stained paraffin sections at a magnification of ×400. Glomerulosclerosis was graded from 0 to 4 by a semiquantitative score: grade *0 normal, *1 mesangial expansion/sclerosis involving less than 25% of the tuft, *2 moderate GS (25%-50%), *3 severe GS (50%-75%), and grade *4 (diffuse GS involving more than 75% of the glomerular tuft). Glomerulosclerosis index for each animal was calculated as a mean value of all glomerular scores obtained. Tubulointerstitial indexes were determined using a semiquantitative scoring system according to Veniant et al [32] on PAS-stained sections at a magnification of ×100. Ten fields per kidney were examined and lesions were graded from 0 to 3 according to the area with tubulointerstitial changes (tubular atrophy, casts, interstitial inflammation, and fibrosis). The score index in each rat was expressed as a mean value of all scores obtained.

All the histologic analyses were performed by an observer unaware of the treatment received by each group.

STATISTICS

Values are reported as mean ± SD. Groups were compared by one-way analysis of variance (ANOVA) test, followed by post hoc pair-wise comparisons using Student-Newman–Keuls test, or linear regression and correlation test. P values < 0.05 were considered statistically significant.

RESULTS

Values for body weight, systolic blood pressure, urinary protein excretion, arterial bicarbonate, plasma calcium, urine citrate, and inulin clearance are shown in Table 1. The NFX group has less final weight, significantly higher systolic blood pressure ($P < 0.05$), and urinary protein excretion ($P < 0.05$) versus the sham-operated group. Arterial bicarbonate and inulin clearance were lower than the sham-operated group ($P < 0.05$).

The group treated with calcium citrate alone (NFX-CIT) for 20 weeks had significantly lower proteinuria (28 ± 18 mg/day) than the NFX group (152 ± 71 mg/day) ($P < 0.05$), but no difference in systolic blood pressure was observed. The groups treated with captopril alone or with captopril and calcium citrate showed significantly lower blood pressure and urinary protein excretion versus the control group ($P < 0.05$).

Plasma calcium concentration was higher in the groups treated with calcium citrate alone (8.3 ± 0.3 mg/dL) or with captopril (9.1 ± 0.4 mg/dL) than that in the NFX group (7.4 ± 0.9 mg/dL) ($P < 0.05$). At 10 weeks the arterial bicarbonate was significantly lower in the NFX group (17.3 ± 2.4 mEq/L) versus the sham-operated (25.5 ± 1.6 mEq/L) and the treated groups (NFX-CIT 25 ± 3.5 mEq/L, NFX-CAP 23.8 ± 3.6 mEq/L, and NFX-CAP-CIT 25.3 ± 2.8 mEq/L) ($P < 0.05$). At 20 weeks the arterial bicarbonate was low in all groups (because it was obtained after the performance of the inulin clearance), and it was lowest in the NFX group (NS). Urine citrate excretion was lower in the NFX group (7 ± 5 mg/day) versus the sham-operated (25 ± 7 mg/day) ($P < 0.05$), and higher in the NFX-CIT (NS) and NFX-CAP-CIT ($P < 0.05$) groups.

Table 2. Immunohistochemistry

	SHAM	NFX	NFX-CAP	NFX-CIT	NFX-CAP-CIT
PCNA glom 1 w	0.35 ± 0.25	0.92 ± 0.6[b]	0.17 ± 0.25[a]	0.22 ± 0.21[a]	0.4 ± 0.3[a]
PCNA glom 10 w	0.13 ± 0.06	1.58 ± 0.98[b]	0.08 ± 0.18[a]	0.19 ± 0.22[a]	0.07 ± 0.13[a]
PCNA tub 1 w	1.8 ± 0.8	15.1 ± 14.6[b]	3.98 ± 1.11[a]	4.83 ± 2.29[a]	2.36 ± 1.36[a]
PCNA tub 10 w	0.27 ± 0.11	1.57 ± 0.16[b]	0.38 ± 0.23[a]	1.48 ± 1	1.07 ± 0.63
PCNA int 1 w	1.0 ± 0.93	3.27 ± 2.3[b]	0.89 ± 0.23[a]	1.23 ± 0.57[a]	0.87 ± 0.25[a]
PCNA int 10 w	0.11 ± 0.04	0.66 ± 0.5	0.21 ± 0.16	0.59 ± 0.33	0.4 ± 0.35
Desmin glom 1 w	0.18 ± 0.18	0.43 ± 0.15	0.19 ± 0.2	0.23 ± 0.29	0.3 ± 0.18
Desmin glom 10 w	0.13 ± 0.15	0.85 ± 0.34[b]	0.48 ± 0.41[a]	0.28 ± 0.21[a]	0.14 ± 0.13[a]
Desmin T-int 1 w	0.23 ± 0.19	0.67 ± 0.67	0.29 ± 0.31	0.2 ± 0.26	0.14 ± 0.1
Desmin T-int 10 w	0.19 ± 0.08	0.34 ± 0.19	0.18 ± 0.15	0.27 ± 0.18	0.09 ± 0.07
α–SMA glom 1 w	0 ± 0	0.33 ± 0.5	0 ± 0	0.14 ± 0.11	0 ± 0
α–SMA glom 10 w	0 ± 0	1.62 ± 1.17[b]	0.65 ± 0.88[a]	0.13 ± 0.13[a]	0.12 ± 0.14[a]
α–SMA T-int 1 w	0.03 ± 0.05	0 ± 0	0 ± 0	0.45 ± 0.46	0 ± 0
α–SMA T-int 10 w	0 ± 0	1.34 ± 1.6	0.83 ± 0.65	0.25 ± 0.42	0.06 ± 0.13

Abbreviations are: PCNA, proliferating cell nuclear antigen; glom, glomerular, tub, tubular; T-int, tubulointerstitial, α–SMA, α–smooth muscle actin; w, weeks. $N = 6$ in all the subgroups. All data are mean ± SD.

[a] $P < 0.05$ vs. NFX.

[b] $P < 0.05$ vs. SHAM.

The inulin clearances were significantly higher in the groups treated with calcium citrate (NFX-CIT) (92 ± 29 uL/min/100 g), with captopril (NFX-CAP) (87 ± 47 uL/min/100 g), and both (NFX-CAP-CIT) (103 ± 60 uL/min/100 g) than in the NFX group (22 ± 11 uL/min/100 g) ($P < 0.05$). There were no significant differences in urine protein excretion, arterial bicarbonate, inulin clearance between NFX-CIT, NFX-CAP, and NFX-CAP-CIT.

Thus, calcium citrate diminished proteinuria and enhanced plasma calcium concentration, arterial bicarbonate, and inulin clearances in spite of persistent high systolic blood pressure.

Immunohistochemistry

The immunohistochemistry data at 1 and 10 weeks are shown in Table 2 and Figure 1A-F. At 1 and 10 weeks glomerular and tubulointerstitial cell proliferation (PCNA-positive cells) are significantly lower in the sham-operated or the treated groups versus the NFX group ($P < 0.05$). At 10 weeks, desmin and α–smooth muscle actin staining were significantly more extensive at the glomerular tufts in the NFX group than in the sham-operated or the treated groups ($P < 0.05$).

Renal morphology

The histologic glomerular and tubulointerstitial scores are shown in Table 3 and Figure 1G-H. At 20 weeks the groups treated with calcium citrate and/or captopril had significantly less glomerular and tubulointerstitial damage than the NFX nontreated group ($P < 0.05$). The urine protein excretion had a significantly positive correlation with tubulointerstitial damage ($r^2 = 0.6, P < 0.05$) (Fig. 2), and a weak correlation with glomerulosclerosis ($r^2 = 0.25$, $P < 0.05$).

DISCUSSION

In the present study, calcium citrate supplementation to rats with 5/6 nephrectomy improved arterial plasma bicarbonate, diminished proteinuria, and enhanced inulin clearance, and it ameliorated glomerular and tubulointerstitial damage. Calcium citrate also diminished glomerular and tubular cell proliferation and glomerular cell transdifferentiation (myofibroblast transformation) at early stages as shown by PCNA, desmin, and α−smooth muscle actin staining.

It is well known that captopril also diminishes proteinuria, enhances inulin clearance [4, 7, 33], and, in this study, also elevates plasma bicarbonate, probably as it improves renal function. Although calcium citrate has similar effects on proteinuria, plasma bicarbonate, and inulin clearance, there was no additive effect of calcium citrate and captopril.

The observed effects of calcium citrate may be produced by different mechanisms: correction of metabolic acidosis, changes in intracellular pH, calcium, and/or citrate intracellular concentrations.

Citrate effects

Citrate is selectively taken up by the kidneys, and as its metabolism produces bicarbonate, may be a good way to target bases to the kidney cells [25]. Wright et al [34] and Hering-Smith et al [35] have demonstrated on cultured cells with acute and chronic acidosis that a low extravesicular pH stimulated Na-dependent citrate transport in renal brush border membrane vesicles. During metabolic acidosis citrate reabsorption and cytosol concentration would be increased. Tanner and Tanner [25] have recently demonstrated that different citrate salts improved renal function in rats with polycystic kidney disease probably due to its alkalinizing effect, although the exact mechanism is still unknown [26].

Citrate synthase activity is elevated in the remnant glomeruli. Experimental models with reduced glomerular citrate synthase activity are protected from remnant nephropathy [36]. It can be hypothesized that changes in

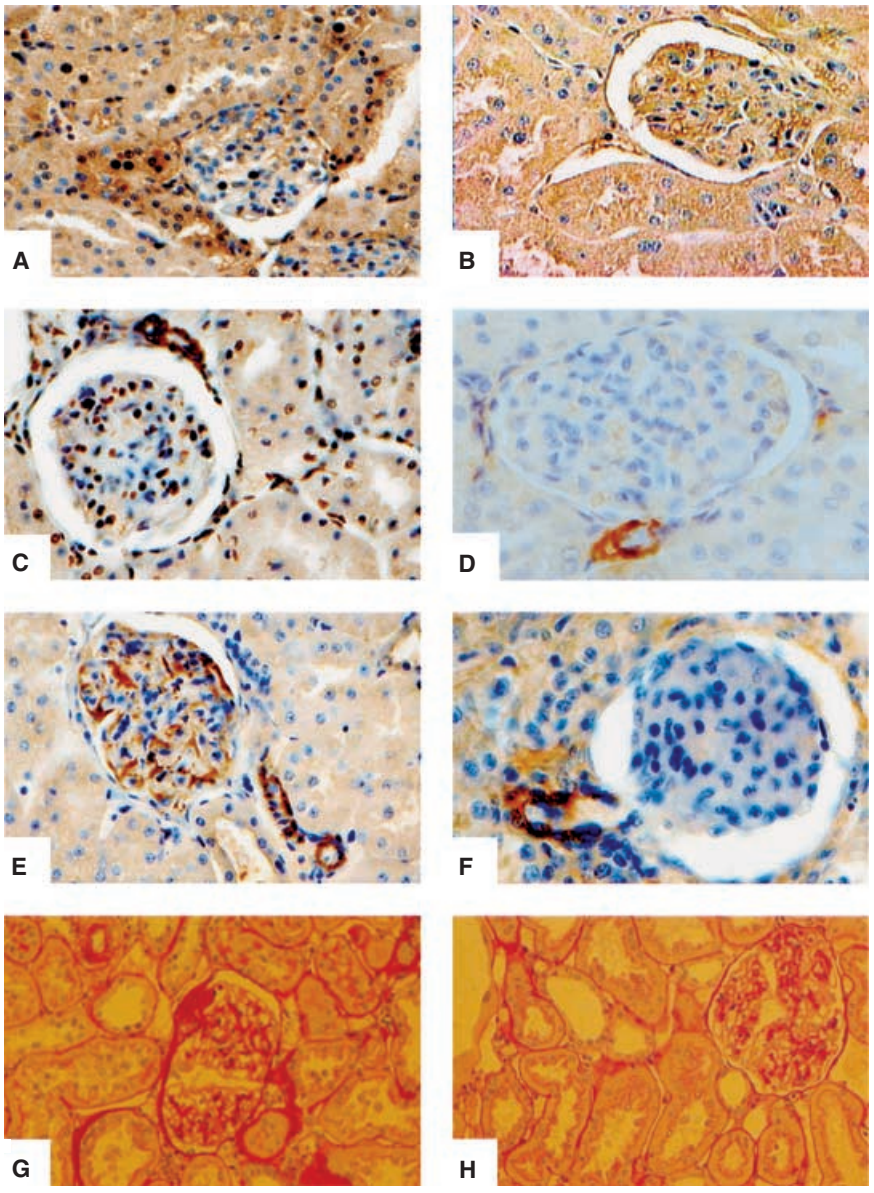

Fig. 1. Histologic sections of kidney from 5/6 nephrectomized Wistar rat. Immunohistochemistry staining. Representative results are shown. (*A*) Glomerular and tubulointerstitial proliferating cell nuclear antigen (PCNA) immunostaining in rats 1 week after 5/6 nephrectomy (NFX). Sections were counterstained with eosin (original magnification ×200), and (*B*) from a calcium citrate–treated animal (NFX-CIT). Sections were counterstained with eosin (original magnification ×200). (*C*) Immunohistochemical detection of α–smooth muscle actin in rats 10 weeks after 5/6 nephrectomy (original magnification ×400), and (*D*) from a calcium citrate–treated animal (NFX-CIT) that shows positive staining only in arterioles (original magnification ×400). (*E*) Immunohistochemical detection of desmin in rats 10 weeks after 5/6 nephrectomy (original magnification ×400), and (*F*) from a calcium citrate–treated animal (NFX-CIT) that shows positive staining only in arterioles (original magnification ×400). (*G*) Periodic-acid Schiff (PAS) staining in rats 20 weeks after nephrectomy showing glomerulosclerosis, tubular atrophy, and interstitial infiltration (original magnification ×200), and (*H*) from a calcium citrate–treated animal (NFX-CIT) (original magnification ×200).

intracellular citrate concentration may also have direct actions.

Calcium effects

Calcium carbonate improved progressive renal damage in deoxycorticosterone acetate (DOCA)-salt model in rats mainly by its phosphate binding action [37], but acid-base status was not determined, and carbonate may also have a mild alkalinizing effect. Hatton and McCarron [38] postulated that calcium has antihypertensive actions mediated by many different possible mechanisms. In the present study the NFX-CIT group has persistent systemic hypertension, although some calcium effect upon microcirculation cannot be ruled out. Plasma calcium levels are significantly higher in the NFX-CIT group and also in the NFX-CAP group (Table 1). Probably as captopril improves renal function, phosphorus and calcium metabolism is improved. Simultaneous supplementation of calcium and citrate may have complementary beneficial effects in a chronic renal failure model.

Metabolic acidosis-glomerular filtration rate

It has been postulated that acidosis is a "uremic toxin" [39] because it enhances protein degradation, stimulates the ubiquitin-proteasome proteolytic complex, produces muscle wasting and malnutrition [40], and inhibits normal bone formation. Furthermore, Farber et al [13] have demonstrated that metabolic acidosis depressed glomerular filtration rate and effective renal plasma flow, perhaps through a hormone-mediated mechanism [41]. Protein catabolic end products (urea and ammonia) [12] may also contribute to renal damage [12, 14–19].

Table 3. Glomerulosclerosis and tubulointerstitial damage score

	SHAM	NFX	NFX-CAP	NFX-CIT	NFX-CAP-CIT
Glom score					
1 week	0.27 ± 0.2	0.42 ± 0.4	0.15 ± 0.13	0.18 ± 0.1	0.24 ± 0.1
10 week	0.18 ± 0.1	0.83 ± 0.54	0.24 ± 0.1	0.37 ± 0.34	0.27 ± 0.2
20 week	0.17 ± 0.09	0.82 ± 0.28[b]	0.39 ± 0.13[a]	0.44 ± 0.43[a]	0.12 ± 0.07[a]
T-int score					
1 week	0.03 ± 0	0 ± 0	0.14 ± 0.2	0.1 ± 0.2	0 ± 0
10 week	0 ± 0	0.1 ± 0.13	0.08 ± 0.15	0.04 ± 0.05	0.25 ± 0.29
20 week	0.4 ± 0.21	1.18 ± 0.56[b]	0.36 ± 0.1[a]	0.52 ± 0.27[a]	0.48 ± 0.25[a]

See Table 1 for abbreviations. Glom score, glomerulosclerosis damage score; T-int score, tubulointerstitial damage score. $N = 6$ in all the subgroups. All data are mean ± SD.

[a] $P < 0.05$ vs. NFX.

[b] $P < 0.05$ vs. SHAM.

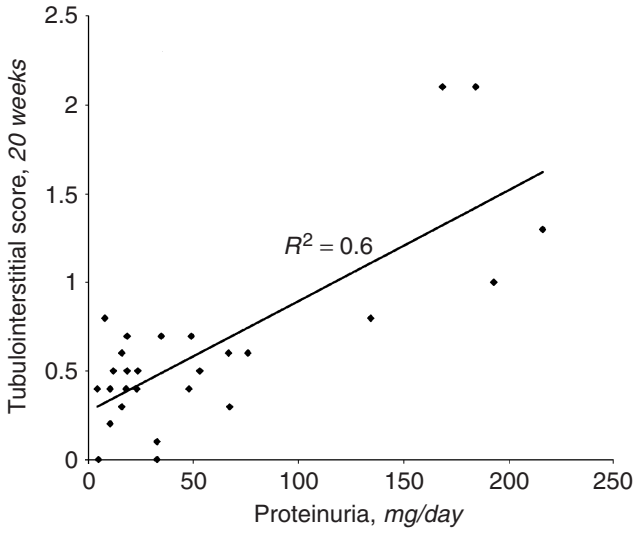

Fig. 2. Correlation between proteinuria and tubulointerstitial score at 20 weeks. ($R^2 = 0.6$, $P < 0.05$).

Enhanced renal ammoniagenesis observed in the reduced renal mass model may determine renal hypertrophy [16, 42]. In the present study plasma bicarbonate and glomerular filtration rate are significantly elevated in the NFX-CIT group, probably due to citrate alkalinizing effect.

Glomerular and tubulointerstitial damage

Floege et al [28] and Kliem et al [29] observed that glomerular and tubulointerstitial cell proliferation began within 5–7 days of renal ablation and peaked at 2 weeks, and the expression of myofibroblast markers such as α−smooth muscle actin and desmin were also increased (phenotypic changes) [43]. The cellular proliferation and transdifferentiation in this model may be mediated by several mechanisms. The tubulointerstitial proliferation was found to correlate with caloric intake, angiotensin II, several growth factors, and ammonia [44]. In the present study calcium citrate administration (Table 2) significantly diminished glomerular cell proliferation and transdifferentiation, and the correction of metabolic acidosis may have participated in this effect because plasma bicarbonate was higher at 10 weeks in the NFX-CIT versus NFX group. The higher inulin clearance in the NFX-CIT group may be explained by the diminished glomerular cell proliferation and transdifferentiation.

Proteinuria

Urinary protein excretion diminished in the group treated with calcium citrate probably as a consequence of the observed decreased glomerular damage (Table 3). Systolic blood pressure did not change in this group (Table 1), suggesting that this effect may be independent of a systemic hemodynamic action.

It has been demonstrated that acidosis may also produce proteinuria of tubular origin [45, 46]. Chronic acidosis increased ammonia production and may have contributed to the tubulointerstitial inflammation by complement activation [17]. It has been observed that NH_4Cl produces swelling of lysosomes in proximal tubular cells in culture [47] and a reduction in lysosomal activity [48]. Calcium citrate, as an alkalinizing agent, would diminish NH_4^+ concentration in the proximal tubular cells and cortical interstitium, improve intracellular protein metabolism, and as a consequence, diminish tubulointerstitial damage (Table 3) and a possible tubular component of proteinuria. The exact composition of proteinuria was not determined in the present study, but we can hypothesize that both a glomerular and tubular component would be diminished by the treatment.

CONCLUSION

The administration of calcium citrate after 5/6 nephrectomy: (*1*) ameliorates metabolic acidosis and diminishes proteinuria without changes in systolic blood pressure; (*2*) diminishes cell proliferation and transdifferentiation at early stages and glomerular and tubulointerstitial damage at 20 weeks; and (*3*) improves glomerular filtration rate.

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