Amelioration of metabolic acidosis in patients with low GFR reduced kidney endothelin production and kidney injury, and better preserved GFR

Sorot Phisitkul[1], Apurv Khanna[1], Jan Simoni[2], Kristine Broglio[3,4], Simon Sheather[3], M. Hasan Rajab[4] and Donald E. Wesson[5,6]

[1]Department of Internal Medicine, Texas Tech University Health Sciences Center, Lubbock, Texas, USA; [2]Department of Surgery, Texas Tech University Health Sciences Center, Lubbock, Texas, USA; [3]Department of Statistics, Texas A&M University, College Station, Texas, USA; [4]Department of Biostatistics, Scott & White Healthcare, Temple, Texas, USA; [5]Department of Internal Medicine, Scott & White Healthcare, Temple, Texas, USA and [6]Department of Internal Medicine, Texas A&M College of Medicine, Temple, Texas, USA

Metabolic acidosis often accompanies low glomerular filtration rate and induces secretion of endothelin, which in turn might mediate kidney injury. Here we tested whether treatment of metabolic acidosis in patients with low glomerular filtration rate reduced the progression of kidney disease. Fifty-nine patients with hypertensive nephropathy and metabolic acidosis had their blood pressure reduced with regimens that included angiotensin-converting enzyme inhibition. Thirty patients were then prescribed sodium citrate, and the remaining 29, unable or unwilling to take sodium citrate, served as controls. All were followed for 24 months with maintenance of their blood pressure reduction. Urine endothelin-1 excretion, a surrogate of kidney endothelin production, and N-acetyl-β-D-glucosaminidase, a marker of kidney tubulointerstitial injury, were each significantly lower, while the rate of estimated glomerular filtration rate decline was significantly slower. The estimated glomerular filtration rate was statistically higher after 24 months of sodium citrate treatment compared to the control group. Hence it appears that sodium citrate is an effective kidney-protective adjunct to blood pressure reduction and angiotensin-converting enzyme inhibition.

Correspondence: *Donald E. Wesson, Department of Internal Medicine, Texas A&M College of Medicine, Scott & White Healthcare, 2401 South 31st Street, Temple, Texas 76508, USA. E-mail: dwesson@swmail.sw.org*

Subjects with low glomerular filtration rate (GFR) can have metabolic acidosis[1] and treatment guidelines recommend alkali treatment for those with serum total CO_2 (TCO$_2$) <22 mM.[2] Metabolic acidosis mediates GFR decline in the five-sixths nephrectomy model of low GFR and its amelioration with oral alkali slows GFR decline in most[3–5] but not all[6] studies. Daily oral NaHCO$_3$ administration for 2 years slowed the decline rate of creatinine clearance in subjects with low GFR for various causes[7] but no mechanisms for this alkali effect were reported. Metabolic acidosis-induced GFR decline in the five-sixths nephrectomy model is mediated by tubulointerstitial injury.[3,5] In subjects with low GFR, 6 weeks of daily oral NaHCO$_3$ improved urine indices of kidney tubule damage but did not improve GFR.[8] Tubulointerstitial injury is an important component of hypertensive nephropathy,[9] it might predict its progression,[10] and many subjects with hypertensive nephropathy experience progressive kidney injury despite blood pressure (BP) reduction with regimens that include angiotensin-converting enzyme (ACE) inhibition.[11] Consequently, metabolic acidosis-induced tubulointerstitial injury that is not ameliorated by conventional kidney-protective strategies might contribute to kidney injury in subjects with low GFR due to hypertensive nephropathy. Furthermore, metabolic acidosis-induced tubulointerstitial injury in the five-sixths nephrectomy model is mediated through endothelin receptors[5] and endothelins also mediate progressive tubulointerstitial injury induced by unilateral ureteral obstruction.[12] Kidney endothelin-1 (ET-1) production is increased in the five-sixths nephrectomy model[13,14] and oral alkali decreases this production.[5] If metabolic acidosis induces tubulointerstitial injury in human hypertensive nephropathy, these animal studies support exploring endothelin as a mediator. We tested the hypothesis that oral alkali amelioration of metabolic acidosis reduces kidney endothelin production, reduces urine parameters of tubulointerstitial injury, and slows GFR decline in subjects with low GFR due to hypertensive nephropathy.

RESULTS

Table 1 shows the characteristics of each group at study entry. There were no statistically significant differences in demographic data or systolic blood pressure (SBP), venous TCO_2 ($VTCO_2$), plasma creatinine (Pcr), plasma cystatin C (Pcys), or estimated GFR (eGFR) using either Pcr (eGFRcr) or Pcys (eGFRcys) at study entry between subjects who subsequently received no Na^+ citrate (No-NaCit) or Na^+ citrate (NaCit) for 24 months. The NaCit group had a higher proportion of white subjects than the No-NaCit group (23 vs 14%) but this difference was not statistically significant ($P = 0.59$).

Table 2 shows SBP, Pcr, Pcys, and eGFR at 6 and 30 months after study entry. At 6 months, all subjects had undergone 6 months of pharmacologic SBP reduction but none had received Na^+ citrate. At 30 months, NaCit but not the No-NaCit subjects had been prescribed 24 months of

Na^+ citrate, 1 meq/kg HCO_3 equivalent daily in three divided doses and SBP reduction was maintained. Entry SBP between the NaCit and No-NaCit groups (Table 1) was not statistically different ($P = 0.611$). All subjects received ACE inhibition (Materials and Methods) and most received it as enalapril because this was the recommended ACE inhibitor on our formulary. There was no difference in the distribution of non-ACE drugs or diuretics among subjects in the two groups. Pharmacologic antihypertensive treatment from 0 to 6 months (Materials and Methods) decreased SBP significantly ($P < 0.0001$ for each group) to the 6-month values shown in Table 2. The SBP levels were similar between 6 and 30 months for both groups. The increase in Pcr and decrease in eGFRcr from months 6 to 30 within each group were statistically significant but there was no statistically significant difference in Pcr or eGFRcr at 6 or 30 months between groups. Similarly, there was a statistically significant increase in Pcys and a statistically significant decrease in eGFRcys from 6 to 30 months within each group. In contrast to the creatinine data, Pcys was statistically significantly lower and eGFRcys was statistically significant higher at 30 months in NaCit than No-NaCit.

Table 3 shows the months 6 and 30 values for additional parameters in No-NaCit and NaCit groups. $VTCO_2$ increased significantly in NaCit and decreased significantly in No-NaCit. By contrast, urine 8 h net acid excretion decreased significantly in NaCit but remained fairly constant in No-NaCit, reflecting ingestion of the prescribed alkali in NaCit. Urine Na^+ excretion ($U_{Na}V$) remained similar in No-NaCit but there was a statistically significant increase in NaCit, reflecting the obligate Na^+ intake with Na^+ citrate. Similarly, there was almost no change urine K^+ excretion

Table 1 | General demographic characteristics, SBP, Pcr, and eGFR at study entry in subjects before they were not treated (No-NaCit) or treated (NaCit) with Na^+ citrate

	No-NaCit (n=29)	NaCit (n=30)	P-value
Males (%)	48	47	0.891
Black/white/Hispanic (%)	55/14/31	53/23/23	0.591
	Mean ± s.d.	Mean ± s.d.	
Age (years)	53.9 ± 5.0	54.1 ± 6.4	0.928
SBP (mm Hg)	160.5 ± 8.9	161.8 ± 10.8	0.611
$VTCO_2$ (mM)	20.6 ± 0.8	20.8 ± 1.2	0.375
Pcr (mg/dl)	3.20 ± 0.89	3.27 ± 0.70	0.733
eGFRcr (ml/min)	33.4 ± 8.4	33.0 ± 8.5	0.871
Pcys (mg/l)	3.86 ± 1.09	3.88 ± 0.79	0.936
eGFRcys (ml/min)	32.3 ± 8.1	31.7 ± 8.3	0.767

Abbreviations: eGFR, estimated glomerular filtration rate; N, number of subjects per group; Pcr, plasma creatinine; Pcys, plasma cystatin C; SBP, systolic blood pressure; $VTCO_2$, venous serum total CO_2.

Table 2 | SBP, Pcr, and eGFR before (0 months) and after 24 months of No-NaCit vs NaCit

	No-NaCit (n=29)			NaCit (n=30)			P-value, NaCit vs No-NaCit	
	Month 6	Month 30	P-value, 30 vs 6 months	Month 6	Month 30	P-value, 30 vs 6 months	Month 6	Month 30
SBP	132.1 ± 6.3	131.9 ± 3.8	0.870	132.4 ± 6.2	132.7 ± 5.7	0.761	0.839	0.490
Pcr (mg/dl)	3.30 ± 0.91	4.24 ± 1.55	<0.0001	3.31 ± 0.69	3.61 ± 0.78	<0.0001	0.954	0.057
eGFRcr (ml/min)	32.5 ± 8.3	24.9 ± 9.7	<0.0001	32.7 ± 8.2	29.5 ± 8.8	<0.0001	0.945	0.066
Pcys (mg/l)	3.94 ± 1.10	5.24 ± 1.41	<0.0001	3.93 ± 0.80	4.33 ± 0.89	<0.0001	0.952	0.005
eGFRcys (ml/min)	31.7 ± 7.9	23.0 ± 6.05	<0.0001	31.4 ± 8.2	27.8 ± 7.4	<0.0001	0.885	0.008

Abbreviations: eGFR, estimated glomerular filtration rate; Pcr, plasma creatinine; Pcys, plasma cystatin; SBP, systolic blood pressure.

Table 3 | Changes in parameters after 24 months of No-NaCit vs NaCit (means ± s.e.)

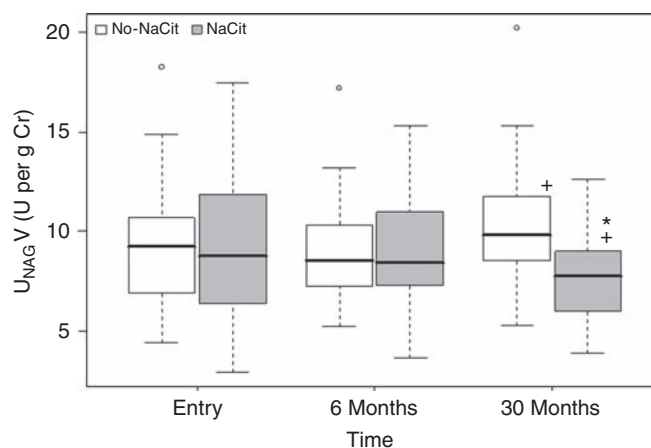|	No-NaCit (n=29)			NaCit (n=30)			P-value, NaCit vs No-NaCit	
	Month 6	Month 30	P-value, 30 vs 6 months	Month 6	Month 30	P-value, 30 vs 6 months	Month 6	Month 30
$VTCO_2$ (mM)	20.5 ± 0.8	19.6 ± 1.2	<0.0001	20.5 ± 1.1	23.8 ± 1.0	<0.0001	0.918	<0.0001
8 h Urine NAE (meq)	26.0 ± 3.1	26.1 ± 3.1	0.833	25.4 ± 3.2	11.2 ± 3.7	<0.0001	0.433	<0.0001
$U_{Na}V$ (meq/g Cr)	75.7 ± 6.7	75.3 ± 6.6	0.548	75.4 ± 7.6	88.0 ± 10.2	<0.0001	0.864	<0.0001
U_KV (meq/g Cr)	36.4 ± 5.3	36.4 ± 4.5	0.950	37.6 ± 5.7	39.7 ± 5.9	0.0001	0.421	0.019
Plasma $[Ca^{2+}]_{ionized}$ (mM)	1.12 ± 0.06	1.13 ± 0.06	0.370	1.13 ± 0.05	1.09 ± 0.05	<0.0001	0.592	0.005
Plasma PO_4 (mg/dl)	3.98 ± 0.38	4.09 ± 0.42	0.085	4.11 ± 0.31	4.15 ± 0.31	0.239	0.166	0.510

Abbreviations: N, number of subjects in each group; NAE, net acid excretion; U_KV, urine K^+ excretion; $U_{Na}V$, urine Na^+ excretion; $VTCO_2$, venous serum total CO_2.
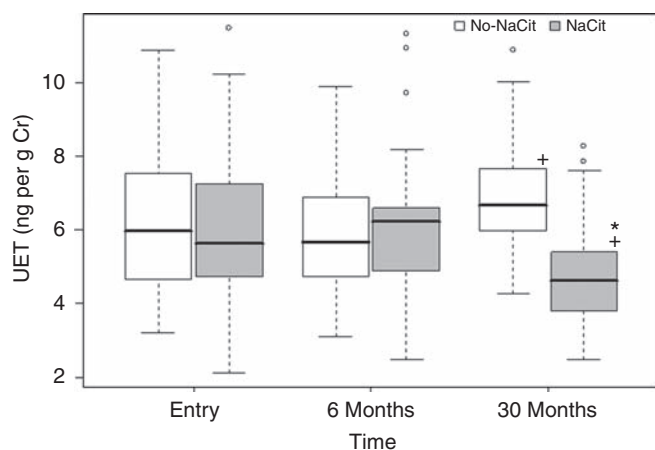
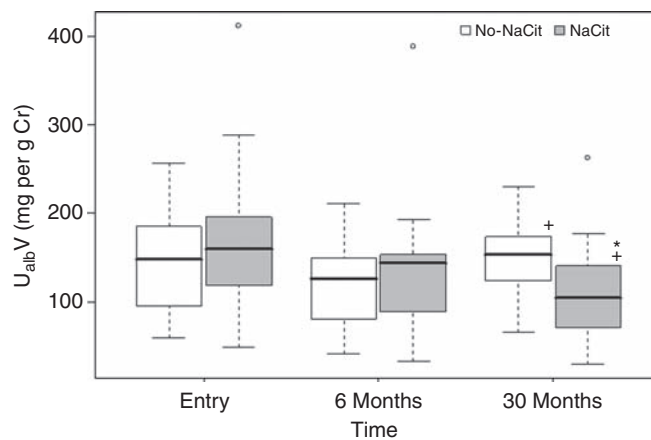**Figure 1 | Box plots showing the course of urine endothelin-1 excretion (UET) as ng/g creatinine (ng per g Cr) in spot morning specimens at study entry, after 6 months of blood pressure (BP) reduction, and after an additional 24 months of daily Na$^+$ citrate (NaCit) or no Na$^+$ citrate (No-NaCit) with continuation of BP reduction, 30 months after study entry.** The dark line through each box indicates the median UET. The box represents the inner quartile range of the differences in UET, or the 25 and 75% percentiles of the UET differences. The lines extending from the box indicate the range, or minimum and maximum, of the differences in UET. Circles indicate values that are considered outliers. *$P < 0.05$ vs No-NaCit at the indicated time point; $^+P < 0.05$ vs respective 6-month value within groups.

Figure 2 | Box plots in the previously described format showing the course of urine N-acetyl-β-D-glucosaminidase (NAG) excretion (U$_{NAG}$V) as U/g creatinine in spot morning specimens at study entry, after 6 months of blood pressure (BP) reduction, and after an additional 24 months of daily Na$^+$ citrate (NaCit) or no Na$^+$ citrate (No-NaCit) with continuation of BP reduction, 30 months after study entry. *$P < 0.05$ vs No-NaCit at the indicated time point; $^+P < 0.05$ vs respective 6-month value within groups.

Figure 3 | Box plots in the previously described format showing the course of urine albumin excretion (U$_{alb}$V) as mg/g creatinine in spot morning specimens at study entry, after 6 months of blood pressure (BP) reduction, and after an additional 24 months of daily Na$^+$ citrate (NaCit) or no Na$^+$ citrate (No-NaCit) with continuation of BP reduction, 30 months after study entry. *$P < 0.05$ vs No-NaCit at the indicated time point; $^+P < 0.05$ vs respective 6-month value within groups.

(U_KV) in No-NaCit but U_KV increased significantly in NaCit. Plasma ionized [Ca^{2+}] decreased significantly in NaCit but remained fairly constant in No-NaCit. The change in plasma PO_4 was not significant in either group. No subject met Ca^{++}/PO_4 guidelines[15] to warrant PO_4 binders or vitamin D therapy.

Figure 1 shows that despite no statistically significant differences (means ± s.d.) in kidney endothelin production as measured by urine ET-1 excretion ($U_{ET-1}V$) between NaCit and No-NaCit at study entry (5.97 ± 2.31 vs 6.17 ± 1.96 ng/g Cr, respectively, $P = 0.73$) and at 6 months after SBP reduction (6.06 ± 1.97 vs 5.95 ± 1.55 ng/g Cr, respectively, $P = 0.81$), $U_{ET-1}V$ was statistically significantly lower in NaCit than No-NaCit (4.83 ± 1.47 vs 6.92 ± 1.67 ng/g Cr, respectively, $P < 0.0001$) at month 30, that is, after 24 months of Na$^+$ citrate. Also, 30- vs 6-month values for $U_{ET-1}V$ decreased significantly in NaCit ($P < 0.0001$) but increased significantly in No-NaCit ($P < 0.0001$). Figure 2 shows that despite no statistically significant differences in tubulointerstitial injury as measured by urine N-acetyl-β-D-glucosaminidase (NAG) excretion ($U_{NAG}V$) between NaCit and No-NaCit at study entry (9.32 ± 3.53 vs 9.26 ± 3.19 U/g Cr, respectively, $P = 0.95$) and at 6 months (8.93 ± 2.92 vs 9.01 ± 2.70 U/g Cr, respectively, $P = 0.92$), $U_{NAG}V$ was statistically significantly lower in NaCit than No-NaCit (7.72 ± 2.14 vs 10.37 ± 3.15 ng/g Cr, respectively, $P = 0.0004$) at month 30. Also, 30- vs 6-month values for $U_{NAG}V$ decreased significantly in NaCit ($P < 0.004$) yet increased significantly in No-NaCit ($P < 0.0001$). These data support

that kidney tubulointerstitial injury decreased with alkali therapy but increased without it, following the same directional pattern described for kidney ET-1 production.

Figures 3 and 4 show the course of change for the remaining two urine indices of kidney injury. Figure 3 shows that despite no statistically significant differences in urine albumin excretion ($U_{alb}V$) between NaCit and No-NaCit at study entry (160.6 ± 74.8 vs 145.6 ± 54.7 mg/g Cr, respectively, $P = 0.38$) and at 6 months (132.0 ± 66.3 vs 119.1 ± 46.2 mg/g Cr, respectively, $P = 0.39$), $U_{alb}V$ was
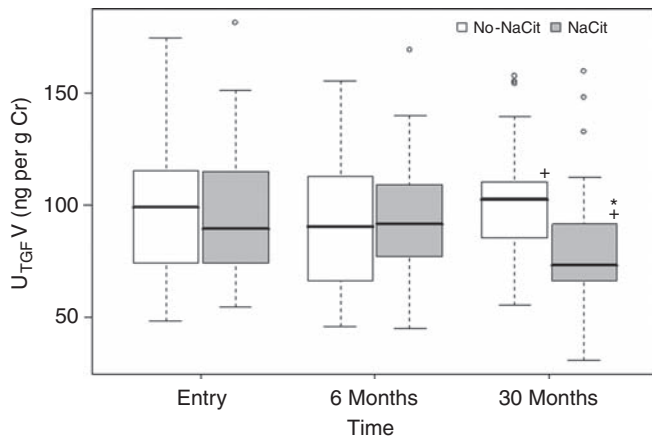
Figure 4 | Box plots in the previously described format showing the course of urine transforming growth factor-β1 excretion ($U_{TGF}V$) as ng/g creatinine (ng/g Cr) in spot morning specimens at study entry, after 6 months of blood pressure (BP) reduction, and after an additional 24 months of daily Na^+ citrate (NaCit) or no Na^+ citrate (No-NaCit) with continuation of BP reduction, 30 months after study entry. *$P < 0.05$ vs No-NaCit at the indicated time point; $^+P < 0.05$ vs respective 6-month value within groups.

statistically significantly lower in NaCit than No-NaCit (107.1 ± 48.7 vs 146.9 ± 44.6 mg/g Cr, respectively, $P = 0.002$) at month 30. Also, 30- vs 6-month values for $U_{alb}V$ decreased significantly in NaCit ($P < 0.0001$) but increased significantly in No-NaCit ($P < 0.0001$). In addition, entry compared to 6-month $U_{alb}V$ was lower in both No-NaCit ($P < 0.0001$) and NaCit ($P < 0.0001$) groups before either received alkali therapy, likely reflecting the effects of SBP reduction. Figure 4 shows that despite no statistically significant differences in urine transforming growth factor-β excretion ($U_{TGF}V$) between NaCit and No-NaCit at study entry (97.0 ± 29.1 vs 99.1 ± 32.1 ng/g Cr, respectively, $P = 0.79$) and at 6 months (91.5 ± 28.3 vs 96.6 ± 29.1 ng/g Cr, respectively, $P = 0.78$), $U_{TGF}V$ was statistically significantly lower in NaCit than No-NaCit (80.8 ± 30.1 vs 102.2 ± 26.5 ng/g Cr, respectively, $P = 0.005$) at month 30. Also, 30- vs 6-month $U_{TGF}V$ decreased significantly in NaCit ($P < 0.003$) but increased significantly in No-NaCit ($P = 0.004$). Together, these data support that kidney injury worsened with time in these hypertensive nephropathy subjects with metabolic acidosis but that daily Na^+ citrate for 24 months decreased kidney injury, possibly mediated through decreased kidney ET-1 production.

Comparisons using linear mixed models showed that the yearly rate of eGFR decline was significantly slower in NaCit than No-NaCit for eGFRcr (-1.60 ± 0.13 vs -3.79 ± 0.30 ml/min per year, $P < 0.0001$) and eGFRcys (-1.82 ± 0.08 vs -4.38 ± 0.98 ml/min per year, $P < 0.0001$).

DISCUSSION

We tested the hypothesis that the recommended alkali treatment of metabolic acidosis associated with low GFR[2] reduces kidney endothelin production, reduces urine

parameters of tubulointerstitial injury, and slows GFR decline in subjects with low GFR due to hypertensive nephropathy. Alkali prescribed as Na^+ citrate was given after pharmacologic SBP reduction because BP reduction was necessary to show the ameliorating effects of alkali therapy on kidney injury in the five-sixths model of low GFR.[5] In addition, the antihypertensive regimens of these hypertensive nephropathy subjects with higher than normal $U_{alb}V$ included ACE inhibition as recommended for hypertensive nephropathy subjects with albuminuria[16] and because of the suggestion that ACE inhibition reduces hypertensive nephropathy progression to chronic kidney disease (CKD) stage 5.[17] The data support that Na^+ citrate reduces kidney ET-1 production as measured by $U_{ET-1}V$, reduces $U_{NAG}V$, the marker of kidney tubulointerstitial injury, and slows eGFR decline. By contrast, subjects not prescribed Na^+ citrate had increases in $U_{ET-1}V$ and $U_{NAG}V$ as well as had faster eGFR decline, supporting that this therapy also prevented what otherwise would have been progressive kidney injury with faster eGFR decline. This reduction in kidney injury and better eGFR preservation was in addition to any provided by the conventional kidney protection strategies of SBP reduction and ACE inhibition. These studies suggest that treating metabolic acidosis associated with low GFR due to hypertensive nephropathy ameliorates progressive kidney injury, some of which might be induced by endothelins, and is an effective adjunct to SBP reduction and ACE inhibition as a kidney protection strategy.

Earlier studies suggest mechanisms by which amelioration of metabolic acidosis associated with low GFR reduces $U_{ET-1}V$. Kidney ET-1 production is increased by dietary acid in animals with intact nephron mass[18,19] and by metabolic acidosis in animals with reduced nephron mass.[5,13] This increase includes higher kidney cortical interstitial ET-1 in acid-ingesting animals with intact nephron mass[18] and those with reduced nephron mass and metabolic acidosis.[14] Metabolic acidosis in subjects with low GFR might increase kidney ET-1 through acid-induced ET-1 release from kidney microvascular endothelium because an acid extracellular environment within the physiologic range increases ET-1 release from human kidney microvascular endothelium *in vitro*.[20] ET-1 release from vascular endothelium is predominantly basolateral[21] and so microvascular endothelium might add ET-1 to cortical interstitium adjacent to the interstitial space.[22] Cortical epithelium secretes and releases basolateral ET-1[23] and might also be a source of kidney cortical interstitial ET-1.

The present studies specifically explored tubulointerstitial injury as a mechanism for metabolic acidosis-induced kidney injury because it is a consistent and important feature of hypertensive nephropathy.[9] The net increase in $U_{NAG}V$ in No-NaCit and its net reduction in NaCit supports that metabolic acidosis induces and its amelioration with Na^+ citrate reduces tubulointerstitial injury. Nevertheless, Na^+ citrate therapy was also associated with reduced $U_{alb}V$ and reduced $U_{TGF}V$ and its absence was associated with increases

in each. Because tubulointerstitial injury contributes to $U_{alb}V$ in some nephropathies,[24] the suggested increase in tubulointerstitial injury might contribute to the increase in $U_{alb}V$. Tubulointerstitial injury mediates GFR decline in five-sixths nephrectomy[3,5] and the present data support that Na^+ citrate reduces eGFR decline rate and leads to higher eGFRcys after 24 months of therapy. Because eGFR calculations with Pcys more accurately reflect GFR than those carried out with Pcr,[25] we consider that the statistically higher eGFRcys in NaCit than No-NaCit supports better GFR preservation with Na^+ citrate, just as 2 years of $NaHCO_3$ led to better preservation of creatinine clearance in subjects with low GFR for various causes.[7]

There are some limitations to be pointed out for this study. The choice of treatment and not treatment with Na^+ citrate was not determined in a randomized manner for the reasons stated in Materials and Methods. Nevertheless, the two groups were very similar regarding entry characteristics as detailed. In addition, the group numbers are small although the indicated differences between them were highly significant. It remains possible, however, that greater subject numbers would yield different outcomes. This illustrates the need for a larger-scale study to explore the efficacy of this potentially effective and comparatively inexpensive adjunctive kidney protection strategy.

In summary, these studies show that Na^+ citrate, as recommended for subjects with low GFR and $VTCO_2$ <22 mM, reduces kidney ET-1 production, reduces urine parameters of kidney injury including tubulointerstitial injury, and slows eGFR decline with better GFR preservation in subjects with low GFR due to hypertensive nephropathy. These studies support that Na^+ citrate treatment of subjects with low GFR due to hypertensive nephropathy be further explored as an adjunctive kidney protection strategy to SBP reduction and ACE inhibition.

MATERIALS AND METHODS

This prospective interventional study hypothesized that oral Na^+ citrate, 1 meq of HCO_3 equivalent/kg body weight per day in three divided doses as recommended for subjects with low GFR and $VTCO_2$ <22 mM,[2] reduces kidney endothelin production and tubulointerstitial injury in subjects with low GFR due to hypertensive nephropathy. Primary outcome was urine ET-1 excretion ($U_{ET-1}V$), a surrogate of kidney endothelin production[18] that mediates progressive kidney injury in experimental CKD models.[5,12,19] Secondary outcomes included urine excretion of parameters of kidney injury and eGFR by MDRDS formula using Pcr.[26] Subsequent to protocol completion, cystatin C-derived eGFR (eGFRcys) was calculated using the CKD-EPI equation.[27] Urine NAG excretion ($U_{NAG}V$) was measured as a marker of kidney tubulointerstitial injury,[28] albumin excretion ($U_{alb}V$) was measured as a general marker of progressive kidney injury,[29] and transforming growth factor-β1 excretion ($U_{TGF}V$) because it reflected kidney injury induced by dietary acid in an experimental model of CKD[5] and because it might be a mediator of hypertensive nephropathy.[30] Urine net acid excretion was calculated by measuring urine titratable acidity (TA), ammonium (NH_4^+), and HCO_3 ($[NH_4^+] + [TA] - [HCO_3]$).

These parameters were followed in hypertensive nephropathy subjects after lowering SBP toward recommended levels[16] over 6 months and maintaining the reduced SBP thereafter. Subjects with hypertensive nephropathy, eGFR $\geqslant 20$ but <60 ml/min, and $VTCO_2$ <22 mM were recommended Na^+ citrate[2] but some either refused it because of its bad taste and/or because they could not afford it (it is not covered by the local indigent health plan). Those who refused or could not afford Na^+ citrate were offered $NaHCO_3$ tablets. Those who accepted, could afford, and tolerated $NaHCO_3$ were treated as such but were not followed in this study. Those who did not tolerate $NaHCO_3$ (most commonly due to bloating), refused it and Na^+ citrate outright, and agreed to participate in the study were enrolled and followed as controls to the NaCit subjects and followed an additional 24 months. They were instructed to take no over-the-counter medications. Follow-up data were available for all recruited subjects. Our local institutional review board approved the protocol.

The study sought subjects whose exclusive cause for low GFR was hypertensive nephropathy. Candidates had been referred for control of 'resistant' hypertension. Such referred subjects at our clinic undergo duplex Doppler ultrasonography and serum aldosterone/renin ratios to help rule out renal artery stenosis[31] and hyperaldosteronism,[32] as contributors to 'resistant' hypertension. Inclusion criteria were (1) age $\geqslant 18$ years and able to give consent, (2) $\geqslant 2$ visits with their primary care providers showing compliance with clinic visits, and (3) $20 \leqslant$ eGFR <60 ml/min. Exclusion criteria were (1) known primary kidney disease or findings consistent thereof such as $\geqslant 3$ red blood cells per high-powered field of urine or urine cellular casts; (2) history of diabetes or fasting blood glucose $\geqslant 110$ mg/dl; (3) history of malignances, chronic infections, pregnancy, or clinical evidence of cardiovascular disease; (4) peripheral edema or diagnoses associated with edema such as heart/liver failure or nephrotic syndrome; (5) history of taking Al^{++}-containing products; (6) and Doppler studies and/or serum aldosterone/renin ratios consistent with renal artery stenosis and/or primary hyperaldosteronism, respectively; or (7) history of medication noncompliance.

Improved BP control reduces the rate of GFR decline in subjects with hypertensive nephropathy[33] and guidelines recommend that ACE inhibitors be included in the drug regimens of such subjects.[16] Consequently, all subjects underwent a BP reduction protocol[34] including ACE inhibition as tolerated for 6 months that was maintained through 30 months. Those unable to tolerate ACE inhibition were excluded. Subjects had visits at least every 6 months with measurements of the indicated parameters and were followed for 30 months.

Analytical methods

Serum and urine creatinine and urine albumin were measured using the Sigma Diagnostics Creatinine Kit (Procedure No. 555; Sigma Diagnostics, St Louis, MO, USA).[35] Cystatin C was measured using a particle-enhanced immunonephelometric assay (N Latex Cystatin C; Dade Behring, Somerville, NJ) with a nephelometer (BNII; Dade Bering).[25,36] Urine ET-1 was measured using a RIA kit (Peninsula Laboratories, Belmont, CA) after extraction using Bound Elut c/8 columns (Varian, Harbor City, CA, USA) preconditioned with methanol, H_2O, and acetic acid as carried out previously.[37] Urine TGF-β was measured using quantitative sandwich enzyme immunoassay.[38] Urine NAG was measured using a colorimetric assay (Boehringer Mannheim, Mannheim, Germany).[3] The IRMA SL Series 2000 blood analysis system (Edison, NJ) measured venous plasma/blood pH, pCO_2, and ionized calcium. This system calculated TCO_2.

Serum phosphate was measured with the Hitachi autoanalyzer (Norcross, GA, USA). Urine TA was measured by correction to the ambient serum pH by NaOH addition, NH_4^+ by the formalin titrimetric (to ambient serum pH) method,[39] and urine HCO_3 (as TCO_2) was measured by ultrafluorometry.[40] All urine parameters were expressed per gram creatinine of a spot a.m. specimen.

Statistical methods

Patient characteristics at the time of study enrollment were tabulated or described by mean and standard deviation (s.d.) as appropriate. At this time point, categorical variables were compared between the treatment and control group with the χ^2-test and continuous measures were compared between the groups with the two-sample t-test. Similarly, measurements of markers at baseline, 6, and 30 months for each group were plotted and described by mean and s.d. The change from 6 to 30 months within each group was considered with a one-sample t-test. The differences between the two groups at each time point were considered with a two-sample t-test. We considered whether the rate of change between 6 and 30 months was different between the two groups. We fit linear mixed models with terms for treatment (NaCit vs No-NaCit), time (6 vs 30 months), and the interaction between the two. Linear mixed models are used in situations in which data are correlated over more than two time points, as with this study. An important choice in a linear mixed model, which affects the validity of hypothesis tests, is the error/correlation structure within each subject. For this study, the measurements are not equally spaced in time and so the error/ correlation structure within each patient over time was modeled using a general symmetric structure. Because 'practical experience based on many longitudinal studies has led to the empirical observation that variances are rarely constant over time',[41] account was also taken of the possibility that the variance changed over time. We used restricted maximum likelihood estimation to fit each model. Analyses were performed using R 2.4.1 (R Core Development Team, 2006). All statistical tests were two sided and P-values less than 0.05 were considered statistically significant. No adjustments were made for multiple statistical tests.

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