Acute decrease of urine calcium by amiloride in healthy volunteers under high sodium diet

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ABSTRACT

Background. Amiloride is a competitive blocker of the epithelial sodium channel (ENaC) in the renal collecting duct. It is a less potent diuretic than thiazides or loop diuretics, but is often used in association for its potassium-sparing profile. Whether amiloride has hypocalciuric effect similar to thiazides remains unclear. Animal studies and experiments on cell lines suggested that amiloride increases calcium reabsorption in the distal nephron, but human studies are scarce.

Methods. We performed a post hoc analysis of a study with 48 healthy males (age, 23.2 ± 3.9 years) who were assigned to a high sodium (Na)/low potassium (K) diet for 7 days before receiving 20 mg of amiloride p.o. Urinary excretions of electrolytes were measured at 3 and 6 hours afterward; we calculated the relative changes in urinary excretion rates after amiloride administration.

Results. The high Na/low K diet led to an expected suppression of plasma renin and aldosterone. Amiloride showed a mild natriuretic effect associated with a decreased kaliuresis. Urinary calcium excretion dropped substantially (by 80%) 3 hours after amiloride administration and remained low at the 6th hour. At the same time, fractional excretion of lithium decreased by a third, reflecting an increased proximal tubular reabsorption.

Conclusion. During a high Na/low K diet, amiloride had a strong acute hypocalciuric effect, most probably mediated by increased proximal calcium reabsorption, even though distal effect cannot be excluded. Further studies should establish if chronic amiloride or combined amiloride/thiazide treatment may decrease calciuria more efficiently and be useful in preventing kidney stones.

KEY LEARNING POINTS

What is already known about this subject?

- Calciuria is a major risk factor for kidney stone formation
- Thiazides are decreasing calciuria and prevent stone formation mainly through increased proximal tubular calcium reabsorption
- Amiloride, acting more distally than thiazides, may have similar effect but human data are limited

What this study adds?

- Amiloride decreases calciuria by 80% during 6 hours after a single oral dose in volunteers under high salt/low potassium diet
- The effect might be mediated by increased proximal tubular reabsorption of calcium
- A concurrent distal effect cannot be excluded

What impact this may have on practice or policy?

Amiloride might be useful in decreasing calciuria and stone risk on the long term, but needs to be studied specifically in the chronic setting and in stone formers

INTRODUCTION

Distal diuretics share hypocalciuric properties. Whereas the effect of thiazide-like diuretics on calcium excretion has been extensively investigated in animal studies [1–3] as well as in randomized placebo-controlled clinical trials on kidney stone formers [4, 5], much less is known about the effect on calcium tubular handling of amiloride, a specific epithelial sodium channel (ENaC) blocker. Furthermore, the few human studies reporting on the hypocalciuric properties of amiloride usually used combined regimes with thiazides [6–10].

Considering the animal studies performed with thiazides, it appears that an increase in proximal tubule sodium reabsorption following a thiazide-induced volume contraction is the main driving force behind the decreased calcium excretion [3, 11, 12]. Therefore, despite its more moderate diuretic effect, a similar mechanism may be considered for amiloride as well. However, some animal studies [1, 2] suggest an increase in distal calcium reabsorption under thiazides. This distal mechanism of calcium reabsorption may be important under certain conditions, especially when salt is supplemented to compensate for the diuretic-induced volume depletion. Noteworthy, most of the animal studies with amiloride [13–15] were performed before the molecular mechanisms of sodium and calcium handling in the distal nephron were elucidated. These studies suggested an increased calcium reabsorption in the distal convoluted tubule (DCT) upon amiloride exposure, although hypocalciuric effect was much less than that of thiazides [13], or it was apparent only under specific circumstances [16]. Experimental observations on distal convoluted cells [17, 18] and a mathematical model of calcium handling [19]

provide another supportive evidence for increased calcium reabsorption in the distal tubule.

We took advantage of a previous physiological study [20] to ascertain the mechanism by which amiloride administration may lead to decreased urinary calcium excretion in humans. The participants in this study were subjected to a high sodium (Na)/low potassium (K) diet to suppress endogenous aldosterone secretion. Under these conditions, the putative ENaC-dependent calcium reabsorption is minimized and other mechanisms of amiloride-induced hypocalciuria may become apparent.

MATERIALS AND METHODS

Study Protocol

This is a post hoc analysis of a clinical study published previously [20]. To summarize, forty-eight healthy normotensive non-smoking white males (age, 23.2 ± 3.9 years; body mass index, 23.0 ± 2.3 kg/m^2) were enrolled in the study after providing written and informed consent. Participants were assigned to a high Na/low K (250/40 mmol/day) diet with constant protein, caloric, and water intake. After 7 days on the diet, patients received a small meal at 07:00. After completion of a 24-h urine collection, the urine was collected over 1 hour to obtain baseline values before amiloride administration (20 mg p.o.). Thereafter, urine samples were collected over 1 hour at two time points (hour 3 and hour 6 after amiloride administration) together with blood samples. Water was provided ad libitum throughout the study.

The protocol (ClinicalTrials.gov: NCT2006-005056-32) was approved by the Comité de Protection des Personnes Paris, Ile de France III (France), and all procedures were in accordance with the Declaration of Helsinki.

Laboratory Methods

Urine calcium was measured by the central laboratory of the Lausanne University Hospital using the NM-BAPTA method. Plasma calcium was not available. Na, K, lithium and creatinine have been analyzed during the course of the initial study.

Calculations

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Creatinine clearance (Cl-Crea) was calculated as Cl-Crea = (U-Crea x V) / P-Crea where U-Crea is urinary creatinine concentration, P-Crea creatinine plasmatic concentration, and V urinary flow. The clearance of sodium, potassium, and lithium was calculated in the same way. Fractional excretion of sodium, potassium, and lithium (FE-Na, FE-K, FE-Li, respectively) are ratios of their corresponding clearances to Cl-Crea. Fractional distal sodium reabsorption (FDR-Na) was estimated as FDR = (FE-Li − FE-Na) / FE- Li [21].

Statistical Analysis

Data are presented as mean ± SD or median (IQR) for normally and non-normally distributed data, respectively. Non-normally distributed data were log-transformed for statistical analysis and then back-transformed to express relative changes (95% CI) between different time points. We graphed the original non-transformed data to show the original scale. We analyzed the data by fitting a mixed-effects model for repeated-measurements over time (as implemented in GraphPad Prism 8.0). We performed post hoc multiple comparisons between different time points using Bonferroni correction. FDR-Na was analyzed by a non-parametric Friedman's test followed by a Dunn's post-hoc test. A 2-tailed $p < 0.05$ was considered statistically significant; the reported p-values are multiplicity adjusted.

RESULTS

As reflected by 24h Na and K excretion rates (252 ± 61 and 43 ± 18 mmol/24 h, respectively), participants adhered to the high sodium and low potassium diets. Plasma renin and aldosterone were suppressed accordingly. Plasma Na, K, and Creatinine were within the physiological range (Supplemental Table 1).

For clarity of presentation, changes in urine after commencing amiloride are presented in Table 1 and Figure 1; relative changes of urinary parameters (%) and results of statistical analysis are presented in Table 2.

Amiloride showed a short-term natriuretic effect with a 30% increased Na excretion at the 3rd hour after administration, which largely dissipated in the 6th hour. Accordingly, FE-Na increased by almost a half at the 3rd hour. As expected, the potassium excretion decreased by 30% in the 3rd hour and remained low over the next 3 hours (FE-K was continuously decreasing over the 6 hours). Plasma Na decreased by 2.0 mmol/l (95% CI: -2.8 to -1.3, p<0.001 versus baseline) 3 hours after amiloride administration and remained at this level at the 6th hour. The increase in plasma potassium was mild (at baseline 3.5 ± 0.2, at 3rd hour 3.6 ± 0.2, p=0.04), and plasma potassium returned to baseline values after 6 hours of amiloride administration. Amiloride did not significantly affect creatinine clearance (supplemental Table 1).

In addition to its effects on sodium and potassium urinary excretions, amiloride led to an increase in urinary flow rate by 40% (95 CI: 7.9% to 81%, p=0.007 versus baseline) at the 3rd hour. By contrast to the increased natriuresis and urine flow, urinary lithium excretion decreased. The decreased FE-Li became apparent 3 hours after amiloride administration

and it further decreased by 31 % (95% CI: -44% to -15%, p<0.001 versus baseline) at the 6th hour. The calcium excretion fell by 81% (95% CI: -85% to -74%, p<0.001 versus baseline) at the 3rd hour, and remained at the decreased level 6 hours after amiloride administration. The percent changes in urinary calcium to creatinine ratio were of similar magnitude. The fractional sodium reabsorption in the post-proximal tubule (estimated by FDR-Na) significantly decreased from 93% at baseline to 89% at 3rd and 6th hour after amiloride administration (p<0.001 and p<0.01 versus baseline for the 3rd and 6th hour, respectively).

Amiloride affected plasma renin and aldosterone concentrations in an opposing manner (Table 3). Plasma renin concentration increased by 17% (95% CI: 6.8% to 29%, p<0.001 versus baseline) at the 6th hour, whereas we observed a significant progressive decrease in plasma aldosterone concentrations by 26% (95% CI: -38% to -11%, p<0.001 versus baseline) and 38% (95% CI: -49% to -23%, p<0.001 versus baseline) at the 3rd and 6th hour, respectively.

DISCUSSION

In this post hoc analysis, we showed that amiloride administration caused an abrupt decrease in urinary calcium excretion. Participants in the study [20] received a high Na/low K diet to suppress endogenous aldosterone secretion, the main stimulus of ENaC activity and expression. Providing that endogenous lithium clearance serves as a surrogate marker of proximal tubule sodium reabsorption [22], the decreased fractional excretion of lithium in our study indicates proximal tubule reabsorption of calcium as a significant mechanism for the hypocalciuric effect of amiloride.

In this way, the hypocalciuric mechanism of amiloride would be similar to that of thiazides [3]. Indeed, in short-term human studies, thiazide administration caused a modest volume depletion [23, 24], which presumably enhanced the proximal tubule calcium reabsorption. Brickman *et al.* [23] could show that the replacement of thiazide-induced sodium losses with supplemental salt abolished the effect of thiazides on urinary calcium. A similar long-term effect of thiazides was observed by Bergsland *et al.* [12], who showed a reduction of distal calcium delivery and calcium excretion in patients with idiopathic hypercalciuria upon 6-months of chlorthalidone treatment (25 mg/d). In this study, the endogenous lithium clearance was also used as a surrogate marker of proximal sodium reabsorption, under the assumption that no relevant ENaC-mediated distal lithium reabsorption occurs [22, 25]. However, as the authors mentioned, a decreased urinary Na/K ratio under thiazides indicated a probable ENaC activation, which could overestimate proximal sodium reabsorption based on Li clearance.

Christensen *et al.* [26], employing a transgenic model of mice lacking αENaC in collecting ducts, demonstrated ENaC-mediated reabsorption of Li by mice on a normal salt diet. The majority of animal studies indicate that increased distal Li reabsorption occurs mainly during Na depleted states [25, 27]. Furthermore, the distal reabsorption of Li could be minimal or absent in humans even during dietary salt restriction [28, 29].

Altogether, the available evidence indicates that during high-salt states, there is probably no relevant distal Li reabsorption, wherefore Li clearance can be regarded as a non-quantitative, directional marker of reabsorption changes in the proximal nephron [22]. Even if we hypothesized a relevant Li reabsorption by ENaC in our case, amiloride administration could underestimate the proximal calcium reabsorption assessed by the reduction of Li clearance. The abrupt drop in urine calcium excretion 3 hours after amiloride ingestion compared to the rather gradual decline in FE-Li makes this hypothesis plausible. Nevertheless, the reduced FE-Li in our study is highly suggestive of increased proximal tubule calcium reabsorption.

We observed an 80% decrease in urinary calcium excretion as early as at the 3rd hour after amiloride. Considering the rather modest hypocalciuric effect of amiloride in human clinical trials [7–10] as well as animal studies [14–16], and the shared mechanism of increased proximal tubule calcium reabsorption with thiazides, the question arises whether this effect could be due to the high amiloride dose. Most of the human trials used 5 mg of amiloride, whereas the dose in our study was 20 mg, which may explain the strong hypocalciuric effect observed. However, Constanzo and Weiner [14] showed, in a series of experiments performed on dogs, a hypocalciuric effect of amiloride, which was both dose-independent and smaller than the effect of hydrochlorothiazide. Similarly, Devane

and Ryan [15] showed in a study on rats that the reduction of calcium clearance was independent of amiloride dose, while magnesium clearance was reduced in a dose-dependent manner. It is thus possible that, in the chronic setting, compensatory mechanisms engage and the hypocalciuric effect of amiloride decreases. Only a long-term study with different doses of amiloride may answer this question. Interestingly, Puig *et al.* [30] demonstrated a comparable hypocalciuric effect of spironolactone, a mineralocorticoid receptor antagonist, and hydrochlorothiazide in a long-term (52 weeks) randomized trial in hypertensive patients. There were no differences in blood pressure and body weight reduction in either group. The mean doses of both antihypertensives were rather high (spironolactone 144 ± 53 mg/d; hydrochlorothiazide 72 ± 26 mg/d); however, considering dose equivalence of potassium-sparing diuretics [31], the spironolactone dose corresponded to that of amiloride in our study.

As in the studies in dogs [14], some [8, 10] but not all [7] of the studies in humans showed that the addition of amiloride potentiated the hypocalciuric effect of thiazides. This additive effect suggests either an increased natriuresis upon administration of both distal diuretics—hence increased volume contraction and proximal tubule calcium reabsorption as discussed above—or different sites of enhanced calcium reabsorption within the tubule. The site of enhanced calcium reabsorption could be located either in the thick ascending limb or in the distal convolution. By the design of our study, we are not able to confirm/exclude any distal reabsorption or discriminate between the two sites.

Yet some experimental data provide a valid basis for the distal calcium reabsorption. In microperfusion studies in rats, Constanzo [13] showed an increased Ca reabsorption in the late segments of DCT after amiloride administration. The degree of Ca reabsorption

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was highly correlated with the inhibition of Na reabsorption. She proposed that the decreased apical sodium reabsorption hyperpolarized the apical membrane of distal tubular cells. This would consequently facilitate the movement of calcium into the cells. Others [18, 32] validated this hypothesis in animal experiments with isolated DCT or connecting tubule cells. At the same time, the apically located TRPV5 (transient receptor potential cation channel subfamily V member 5), responsible for the distal nephron calcium reabsorption, features a hyperpolarization-dependent activation [33], which further supports this hypothesis. However, thiazide administration still induced hypocalciuria in TRPV5-deficient mice [3], underlying the significance of proximal tubule calcium reabsorption.

After amiloride intake, plasma renin activity increased, but plasma aldosterone decreased. Whereas the increase in plasma active renin further indicates a relevant amiloride-induced volume depletion, the decrease in plasma aldosterone is less obvious. It might be related to the known circadian rhythm of aldosterone levels and might suggest that a strong circadian rhythm activity of aldosterone overcame the regulation by volume and renin [34]. However, while the relative changes in the concentrations of both hormones appear to be substantial, the absolute difference and the biological significance of these changes under high Na diet are probably small.

Our study has several limitations. Given its post hoc design, the plasma calcium values are not available; therefore, we cannot calculate calcium clearance. The parathormone and vitamin D levels are not available either; owing to the rapid onset of urinary changes after amiloride administration, it appears unlikely that acute changes in vitamin D were

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involved. Only Caucasian males were included in the study, so that the conclusion cannot be extended to females or other ethnicities.

To conclude, amiloride showed a strong acute hypocalciuric effect that is mediated, at least partly, by an increased proximal tubular reabsorption of calcium in salt-replete male healthy volunteers. Further studies are needed to evaluate the effect of chronic amiloride administration on calcium metabolism. Likewise, the addition of amiloride to thiazide therapy in patients with hypercalciuria and kidney stone will request additional prospective evidence.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest regarding this study. M. Azizi has received research grants from the French Ministry of Health, the European Horizon 2020 program, ReCor Medical and Idorsia; and has received personal fees from, Astra Zeneca, Alnylam Pharmaceutical, and Poxel Pharma.

The results presented in this paper have not been published previously in whole or part.

AUTHORS' CONTRIBUTIONS

OB and GW designed this study. XJ, MA, AB and GW designed the initial study. GW and AB collected the samples. MM measured plasma aldosterone and renin activity. DH, OB

and GW analyzed the data. DH and OB wrote the manuscript. All authors approved the final version of the manuscript.

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Table 1.

Urinary parameters at baseline, three and six hours after amiloride administration

Parameters	Baseline	Hour 3	Hour 6
UCreaV (umol/min)	12 (11, 14)	11 (9.5, 13)	12 (11, 14)
UCaV (umol/min)	6.9 (4.1, 9.7)	1.2 (0.86, 1.8)	1 (0.54, 1.6)
UCa/UCrea (mmol/mmol)	0.53 (0.37, 0.65)	0.11 (0.09, 0.15)	0.08 (0.04, 0.12)
Flow rate (ml/min)	2.1 (1.3, 3.2)	2.9 (2.1, 3.8)	2.4 (1.9, 3.3)
FE-Na (%)	1.4 (1.0, 1.8)	2.1 (1.7, 2.3)	1.5 (1.2, 1.8)
FE-Li (%)	19 (14, 25)	17 (12, 23)	14 (11, 18)
FE-K (%)	7.4 (5.1, 13)	5.6 (4.8, 7.3)	4.1 (3.7, 6.2)
FDR-Na (%)	93 (91, 96)	89 (83, 91)	89 (83, 92)

UCreaV, urinary creatinine excretion; UCa/UCrea, urinary calcium/creatinine ratio; FE-Na fractional excretion of sodium; FE-Li, fractional excretion of lithium; FE-K, fractional excretion of potassium. FDR-Na, fractional distal reabsorption of sodium. Data are presented as median (IQR).

Table 2. Relative changes in urinary parameters at baseline, three and six hours after amiloride administration

Parameters	Hour 3 vs. Baseline	Hour 6 vs. Baseline	Hour 6 vs. Hour 3
UCaV	-81% (-85% to -74%)§	-85% (-89% to -79%)§	-23% (-43% to 6%)
UNaV	33% (9% to 62%)‡	16% (-7% to 44%)	-13% (-27% to 3%)
UKV	-30% (-45% to -12%)‡	-39% (-51% to -24%)§	-13% (-26% to 3%)
ULiV	-25% (-40% to -6%)‡	-34% (-49% to -15%)§	-12% (-30% to 10%)
UCreaV	-13% (-23% to -2%)†	1.7% (-10.8% to 16.0%)	17% (2% to 35%)†
UCa/UCrea	-78% (-83% to -71%)§	-85% (-89% to -79%)§	-32% (-47% to -12%)‡
Flow rate	40% (7.9% to 81%)‡	21% (-7% to 57%)	-13% (-28% to 3%)
FE-Na	46% (25% to 70%)§	13% (-7% to 36%)	-23% (-29% to -16%)§
FE-Li	-18% (-34% to 1.3%)	-31% (-44% to -15%)§	-16% (-30% to 1.2%)
FE-K	-27% (-39% to -12%)§	-43% (-54% to -30%)§	-23% (-32% to -12%)§

UCaV, urinary calcium excretion; UNaV, urinary sodium excretion; UKV, urinary potassium excretion; ULiV, urinary lithium excretion; UCreaV, urinary creatinine excretion; UCa/UCrea, urinary calcium/creatinine ratio; FE-Na, fractional excretion of sodium; FE-Li, fractional excretion of lithium; FE-K, fractional excretion of potassium. Data are presented as ratio of geometric means (95% CI). P-values adjusted for multiplicity with Bonferroni correction.

† p≤0.05; ‡ p≤0.01; § p≤0.001

Table 3.

Plasma renin and aldosterone absolute values and relative changes at baseline, three and six hours after amiloride administration

Parameters	Absolute values, median (IQR)		
	Baseline	Hour 3	Hour 6
Active renin (pg/ml)	12 (10, 18)	14 (10, 18)	16 (11, 19)
Aldosterone (pg/ml)	18 (12, 25)	12 (8.0, 21)	12 (6.3, 17)
	Relative changes (95% CI)		
	Hour 3 vs. Baseline	Hour 6 vs. Baseline	Hour 6 vs. Hour 3
Active renin	11% (-0.6% to 25%)	17% (6.8% to 29%)§	5.3% (-2.8% to 14%)
Aldosterone	-26% (-38% to -11%)§	-38% (-49% to -23%)§	-16% (-29% to 0.1%)

Absolute values are presented as median (IQR); relative changes are presented as ratio of geometric means (95% CI). P-values adjusted for multiplicity with Bonferroni correction.
§ p≤0.001

Figure legend:

FIGURE 1: Urinary excretion of electrolytes at baseline, three and six hours after amiloride administration. Median

and IQR. ** $p \leq 0.01$; *** $p \leq 0.001$; ns, not significant.
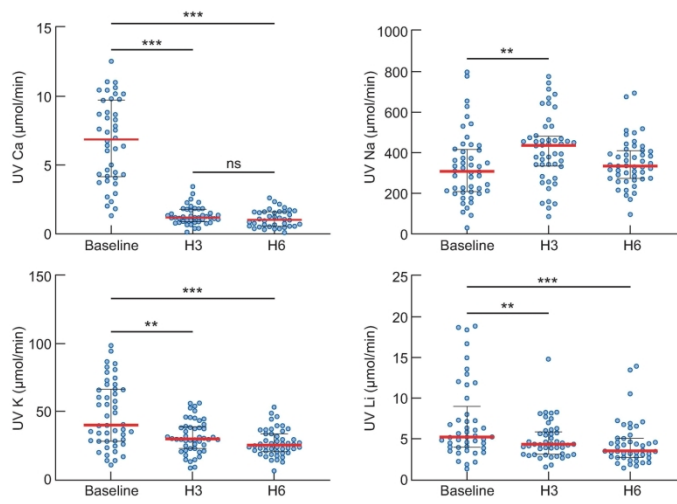
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