

Study of urinary acidification in patients with idiopathic hypocitraturia

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Abstract

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Hypocitraturia (HCit) is one of the most remarkable features of renal tubular acidosis, but an acidification defect is not seen in the majority of hypocitraturic patients, whose disease is denoted idiopathic hypocitraturia. In order to assess the integrity of urinary acidification mechanisms in hypocitraturic idiopathic calcium stone formers, we studied two groups of patients, hypocitraturic (HCit, N = 21, 39.5 ± 11.5 years, 11 females and 10 males) and normocitraturic (NCit, N = 23, 40.2 ± 11.7 years, 16 females and 7 males) subjects, during a short ammonium chloride loading test lasting 8 h. During the baseline period HCit patients showed significantly higher levels of titratable acid (TA). After the administration of ammonium chloride, mean urinary pH (3rd to 8th hour) and TA and ammonium excretion did not differ significantly between groups. Conversely, during the first hour mean urinary pH was lower and TA and ammonium excretion was higher in HCit. The enhanced TA excretion by HCit during the baseline period and during the first hour suggests that the phosphate buffer mechanism is activated. The earlier response in ammonium excretion by HCit further supports other evidence that acidification mechanisms react promptly. The present results suggest that in the course of lithiasic disease, hypocitraturia coexists with subtle changes in the excretion of hydrogen ions in basal situations.

Key words

- Urinary acidification
- Hypocitraturia
- Titratable acidity
- Ammonium
- Ammonium chloride load test
- Lithiasis

Introduction

Urinary acidification defects are not rare among patients with urinary lithiasis. Initially, only cases of distal renal tubular acidosis were reported but later it was established that the proximal form was also associated with lithiasis (1-4). The incidence of this kind of disturbance varies considerably (3 to 25%) according to the series studied (1-3,5-8). Studies in which both distal and proximal tubules were evaluated have yielded the highest percentages (1,8). Hypocitraturia

(HCit) is one of the most marked characteristics of distal tubular acidosis both in its complete (9) and incomplete (10) forms. However, most cases of hypocitraturia are not accompanied by an acidification defect, corresponding to what is called idiopathic hypocitraturia (11). According to Pak (11), a diet rich in sodium or animal protein or a reduction in the gastrointestinal absorption of alkalis can be determining factors of hypocitraturia. Available studies about urinary acidification in lithiasic patients are more concerned with correlating acidifica-

tion defects with renal hypercalciuria (4,10, 12) and with associating the presence of the acidification defect with higher morbidity (1), without mentioning the urinary excretion of citrate. Therefore, a well-conducted study concerning urinary acidification in patients with idiopathic hypocitraturia is lacking. The objective of the present study was to evaluate the integrity of the urinary acidification mechanism in patients with hypocitraturia who are idiopathic calcium stone formers.

Subjects and Methods

Among the patients evaluated at the clinic of Hospital Universitário Pedro Ernesto (HUPE), we selected those for whom the secondary causes for lithiasis (hyperparathyroidism, cystinuria, renal tubular acidosis, hyperoxaluria, gout, sarcoidosis, chronic diarrhea, etc.) as well as the complications of lithiasic illness (infection, hydronephrosis) could be excluded, and who had completed a routine investigation of metabolism following a protocol described elsewhere (13). We then tested these selected patients for urinary acidification with ammonium chloride at the dose of 0.1 g/kg body weight. Two groups were studied, one with HCit (urinary citrate <320 mg/24 h; N = 21, 39.5 ± 11.5 years, 11 females and 10 males) and the other with normocitraturia (NCit, urinary citrate ≥ 320 mg/24 h; N = 23, 40.2 ± 11.7 years, 16 females and 7 males). Initially, a basal urine sample was taken and arterial blood gases were analyzed. The corresponding dose of ammonium chloride was then administered and urine samples were taken eight times per hour. In order to keep urinary flow adequate, each patient was given 150 ml of water every hour, as recommended by Wrong and Davies (14). Those patients whose urinary pH was not reduced to 5.3 or less on average in the samples taken from the third to the eighth hour after drug administration were excluded from the study. At the end of

the fourth hour a new arterial blood gas analysis was performed to ensure that the drug had been effective in producing a state of acidosis. pH, titratable acidity (TA) and ammonium (Am) (15) were measured in the basal sample and in every sample after the NH_4Cl administration. The results are reported as mean \pm SD. The difference between groups were determined by the unpaired Student *t*-test and the response of each group to ammonium chloride was evaluated by the paired Student *t*-test. The level of significance was set at $P < 0.05$.

Results

There was no difference between groups in terms of the dose of ammonium chloride administered (HCit = 6.42 ± 1.18 g vs NCit = 6.33 ± 0.08 g; $P > 0.05$). The two groups absorbed and metabolized the drug well as can be observed by the drop in blood pH and plasma bicarbonate in the blood gas analysis performed 4 h after the intake of ammonium chloride (Table 1).

Mean pH, ammonium and titratable acidity excretion in samples obtained from the 3rd to the 8th hour did not differ between groups (Table 1).

The hourly analysis of the urinary pH curve (Figure 1A) shows that both groups started off from a comparable mean value that increased during the first hour, followed by a drop. There was a significant rise in pH in the NCit group during the first hour. This rise also occurred in the HCit group, but was not statistically significant. Thus, mean pH was lower in the HCit group during the first hour ($P < 0.05$). From the second hour to the end of the test, there was a significant and similar fall in pH in both groups ($P > 0.05$).

Titratable acidity fell significantly during the first hour in both groups ($P < 0.05$). From the second hour on, however, there was a rise which continued until the end of the test (Figure 1B). Comparison of the hourly means revealed that the HCit group excreted

more TA during the control hour and during the first hour after the ingestion of NH_4Cl ($P < 0.05$) but not during the subsequent hours ($P > 0.05$). The increase (mean minus basal) in the excretion of titratable acidity was greater in the NCit group ($P < 0.05$) (Table 1).

The same analysis for ammonium chloride revealed that after NH_4Cl intake there was a rise in excretion which was already significant for the HCit group during the first hour ($P < 0.05$) and in the NCit group from the second hour on ($P < 0.05$) (Figure 1C). Comparison of the hourly means showed greater excretion in the HCit group only during the first hour ($P < 0.05$).

In Table 2, the values of urinary TA excretion for the HCit group in the basal state observed in the present study are among the highest reported in the literature, both for normal individuals and for stone-forming patients. Heilberg et al. (16) reported lower basal TA values than ours in patients with hypocitraturia. However, they included cases of urinary pH higher than 5.3 after an overload of NH_4Cl . In the work by Wrong and Davies (14), TA excretion after ammonium chloride stimulus was higher than in our study, although their group consisted of only 3 lithiasic patients (Table 2). In comparison to the values reported for normal individuals, the TA excretion of our patients after the ammonium chloride stimulus was situated in an intermediate position (Table 2).

Twenty-four-hour phosphate excretion was similar in both groups (Table 1).

Discussion

The basic mechanism that determines hypocitraturia is an increase in the tubular absorption of citrate, a situation mainly occurring in the presence of acidosis. However, in most cases of hypocitraturia there is no systemic acidosis and the mechanism of acidification, based on urinary pH, is supposed to be intact. Experimental studies re-

veal that in the case of chronic acidosis the mechanisms of urinary acidification are more active (17). It is known that in the incomplete form of distal renal tubular acidosis the exaggerate or normal excretion of ammonium prevents the appearance of acidemia, although hypocitraturia is already present. There is evidence that the incomplete form of renal tubular acidosis is an early stage of the complete form. We do not know whether there is an earlier stage. An acidification defect has not been found in most patients with hypocitraturic lithiasis (18), indicating that individuals can excrete urine with a pH equal to or lower than 5.3, which constitutes what is known as idiopathic hypocitraturia (11). According to Battle et al. (19), the capacity to excrete urine with a pH of 5.3 does not necessarily mean an intact process of hydrogen secretion through the distal tubule. In the present study, we assessed the excretion of hydrogen ions by determining

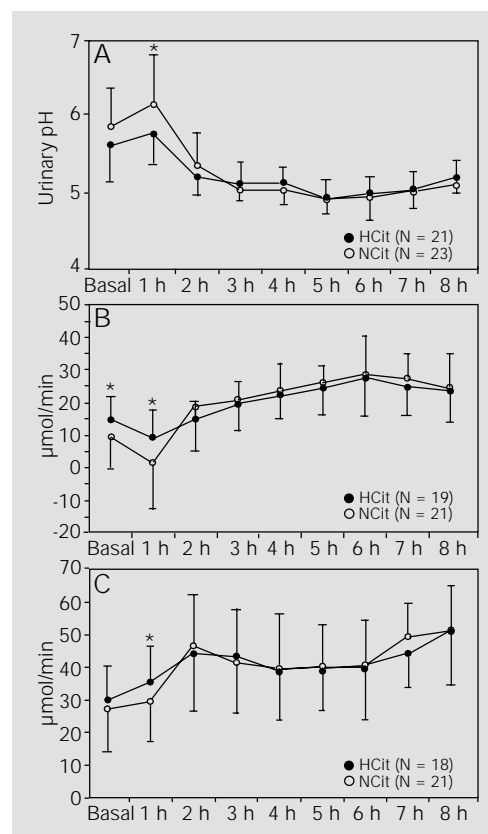


Figure 1 - Effect of ammonium chloride administration on urinary pH (A), titratable acidity (B), and ammonium excretion (C) by patients with hypocitraturia (filled circles) and normocitraturia (blank circles). Data are reported as means \pm SD for 18-23 measurements for each group. * $P < 0.05$ (Student t-test).

the pH and TA and ammonium levels.

The present results show that hypocitraturic patients excrete more titratable acidity in the basal sample and during the first hour. It is known that titratable acidity basically depends on the pK of the buffer, on the

availability of the buffer and on the urine pH (20). Since the HCit group displayed a lower urinary pH during the first hour, it was expected to excrete more TA exactly during the same hour, as was indeed the case. The inverse correlation between urinary pH and

Table 1 - Effect of NH₄Cl in blood and urinary chemistry.

Values are reported as mean \pm SD. *P<0.05 4 h or mean vs basal (paired Student t-test); **P<0.05 HCit vs NCit (unpaired Student t-test). HCit, Hypocitraturia; NCit, normocitraturia; TA, titratable acidity; Am, ammonium.

Parameter	Citrate	Basal	4 h	Mean (3-8 h)	Mean minus basal	Urine (24 h)
Blood pH	HCit	7.41 \pm 0.04	7.34 \pm 0.04*	-	-	-
	NCit	7.42 \pm 0.04	7.35 \pm 0.05*	-	-	-
Blood HCO ₃ (mEq/l)	HCit	24.4 \pm 3.11	19.4 \pm 2.29*	-	-	-
	NCit	24.9 \pm 2.23	18.7 \pm 3.04*	-	-	-
Urinary pH	HCit	5.63 \pm 0.48	-	5.07 \pm 0.18*	-	-
	NCit	5.87 \pm 0.49	-	5.01 \pm 0.23*	-	-
TA (μ Mol/min)	HCit	17.6 \pm 7.6	-	26.6 \pm 7.1*	9.01 \pm 6.24	-
	NCit	9.4 \pm 8.8**	-	26.1 \pm 7.8*	16.5 \pm 9.11**	-
Am (μ Mol/min)	HCit	29.8 \pm 10.5	-	45.5 \pm 9.2*	-	-
	NCit	27.1 \pm 12.9	-	45.4 \pm 12.7*	-	-
Urine phosphate (mg/24 h)	HCit	-	-	-	-	443 \pm 171
	NCit	-	-	-	-	510 \pm 219

Table 2 - Titratable acidity (TA) and ammonium (Am) excretion in the basal state and after NH₄Cl administration.

*Backman et al. used a dose of 0.2 g/kg of NH₄Cl. In the other studies a dose of 0.1 g/kg was used. HUPE, Hospital Universitário Pedro Ernesto; HCit, hypocitraturia; NCit, normocitraturia.

References	N	Baseline TA	NH ₄ Cl TA	Baseline Am	NH ₄ Cl Am	Unit	Baseline pH	NH ₄ Cl pH
Normals								
4	7	-	-	-	55.0	μ Eq/min	-	5.00
14	10	-	37.9	-	50.4	μ Eq/min	-	4.81
16	10	10.9	38.8	28.3	51.5	μ Eq/min	6.30	5.50
22	17	8.0	-	37.1	-	μ Eq/min	5.51	-
23	10	-	37.9	-	52.8	μ Eq/min	-	5.00
24	5	13.0	25.0	20.0	33.0	μ Eq/min	5.41	4.83
Kidney stone formers								
12	26	10.0	-	23.0	-	μ Eq kg ⁻¹ h ⁻¹	-	-
14	3	-	45.0	-	46.7	μ Eq/min	-	4.85
16	15	8.6	22.5	21.9	48.1	μ Eq/min	6.40	5.40
25*	39	-	29.0	-	62.0	μ Eq/min	-	4.80
HUPE, HCit	18	17.6	26.6	29.8	45.5	μ Eq/min	5.63	5.07
HUPE, NCit	21	9.4	26.1	27.1	45.4	μ Eq/min	5.87	5.01

phosphate-dependent excretion of titratable acidity only occurs at pH ranges equal to or higher than 5.5. Due to the pK of phosphate (6.8), when the pH is lower than 5.5 almost all dibasic phosphate (HPO_4^{2-}) has been transformed into monobasic phosphate (H_2PO_4^-) and the buffering ceases unless there is an increase in phosphate excretion. The higher TA excretion in the HCit group in the basal situation in which the urinary pH was equivalent in the two groups suggests a greater availability of phosphate buffer during this hour. A greater availability of phosphate early in the morning after the night abstinence from food suggests that this difference is not due to an acute effect of diet. Twenty-four-hour phosphate excretion was similar in both groups (Table 1), suggesting that the difference in TA cannot be attributed to phosphate. However, some peculiarities of the circadian rhythm of phosphate do not totally rule out the possibility of phosphate contributing to a greater excretion of TA. Serum phosphate presents a circadian rhythm that reaches a peak in the early hours and a maximum fall at around 10:00 a.m.; coincidentally, urinary phosphate excretion is lower between 7:00 and 10:00 a.m. than during the other 21 h of the day (21). Therefore, although 24-h urinary phosphate excretion did not differ between the two groups of patients, we cannot rule out a difference in segments of the daily excretion curve in our patients. The time of 10:00 h in the morning also coincided with the drop in titratable acidity excretion experienced by both groups right after the intake of ammonium chloride, which was nevertheless lower in the HCit group. It may be assumed that in the HCit group this drop was attenuated by a higher excretion of titratable acidity in the basal samples of the circadian rhythm, which could

justify a higher excretion of titratable acidity in the samples during the first hour. The origin of phosphate may correspond to a chronic effect of diet or even to bone, although this possibility is merely speculative. After the first hour of the NH_4Cl intake it is no longer detected a difference in the excretion of TA or in the urinary pH between the two groups.

The NCit group, starting from a lower basal value of TA, was more capable of increasing the excretion of titratable acidity than the HCit group, suggesting that in the latter group the process of activation was already close to the saturation point. Mean ammonium excretion was equal in both groups. However, the HCit group presented an early response in terms of ammonium excretion, supporting the idea that acidification mechanisms are hyper-reactive, as observed in the chronic acidosis situation. The test demonstrated that the HCit group excreted an important part of the acid load during the first hour while in the NCit group this phenomenon occurred later. It is most probable that this phenomenon reflects a chronic effect of diet in hypocitraturic patients. It is known that in hypocitraturia with normal urinary acidification the factors involved are a diet rich in sodium or animal protein or a reduction in the gastrointestinal absorption of alkalis (11).

The results obtained here suggest that in the course of lithiasic disease, hypocitraturia coexists with subtle changes in the excretion of hydrogen ions in basal situations, possibly representing the earlier feature of an acidification defect that precedes incomplete distal renal tubular acidosis. A further follow-up study is required to assess if these patients will show one of the forms of distal renal tubular acidosis in the future.

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