

A young woman with recurrent kidney stones: questions on hypokalaemic tubular acidosis

Jill Vanmassenhove, Norbert Lameire

Renal Division, Department of Medicine, University Hospital, Gent - Belgium

ABSTRACT

This paper discusses the diagnostic and therapeutic approach to the problem of a young woman presenting with recurrent kidney stones. In the clinical work-up, a hypokalaemic normal anion gap metabolic acidosis was found. The diagnostic tests to solve this common clinical problem and some therapeutic recommendations are discussed. Question on hypokalaemic tubular acidosis:

1. What is the significance of the plasma anion gap (PAG)?
2. How does one appreciate the respiratory component of the acid base status?
3. How does one perform tests for tubular acidification disturbances?
4. What is the pathogenesis of distal tubular acidification disturbances?
5. What is the explanation of the hypokalaemia in distal tubular acidosis?
6. What is the pathogenesis of nephrolithiasis in distal tubular acidosis?
7. How does one treat a patient with distal tubular acidosis and recurrent nephrolithiasis?

Keywords: Kidney stones, Metabolic hypokalaemic tubular acidosis, Recurrent nephrolithiasis

Case report

A 33-year-old woman is referred from the urology department where she has been known for a period of 4 years for recurrent kidney stones. During this time, on two occasions, she has passed small kidney stones, which have been analysed in the stone clinic. She is currently undergoing lithotripsy.

She also complains about mild general weakness, which she has had for the last 5 years. One of her brothers was recently diagnosed with kidney stones. There is no other relevant medical history.

Examination of the cardiovascular, respiratory, and abdominal systems is unremarkable. She has mild muscle weakness and tenderness, but her neurologic examination is normal. In particular, there are no signs or symptoms suggesting Sjögren's disease.

The most relevant results of laboratory tests are as follows:

Na⁺ 140 mmol/L, K⁺ 3.1 mmol/L, Cl⁻ 109 mmol/L, HCO₃⁻ 21 mmol/L, BUN 16 mg/dL, and serum creatinine (SCr) 1.0 mg/dL. Alkaline phosphatase was 380 IU/L (normal

range, 40-140 IU/L) and aspartate aminotransferase and bilirubin levels were normal. An arterial blood gas shows pH 7.36, PaCO₂ 40 mmHg, PO₂ 106 mmHg, and HCO₃⁻ 21 mmol/L. The calculated plasma anion gap is: (140 - (109 + 21)) = 10 mmol/L.

The intact parathyroid hormone and 25-hydroxyvitamin-D3 levels are within normal ranges. Serum calcium and serum phosphorus are 8.1 and 2.4 mg/dL, respectively.

Urine volume is 1.5 L per 24 hours, pH 6.7 and 24-hour urine chemistries are calcium 2.9 mmol/day (d), phosphate 24 mmol/d, uric acid 5.5 mmol/d, oxalate 270 mmol/d, and citrate 0.6 mmol/d. Rheumatoid factor and antinuclear antibody are negative.

Urine chemistry shows the following results: K⁺ 31 mmol/L, Na⁺ 100 mmol/L, Cl⁻ 105 mmol/L.

The stones have been analysed biochemically and are composed of calcium phosphate (CaP) and calcium oxalate.

On plain abdominal x-ray, calculi are seen in both kidneys (Fig. 1).

In summary, this young woman suffers from recurrent nephrolithiasis and presents with a mild, hypokalaemic metabolic acidosis with normal anion gap, an inappropriately high urine pH and hypocitraturia.

The aim of this contribution is to discuss the clinical approach to metabolic hypokalaemic tubular acidosis in a patient with recurrent nephrolithiasis.

What is the significance of the plasma anion gap (PAG)?

Addition of hydrogen ion (H⁺) can be detected by the appearance of new anions. These new anions may remain in the body, and/or be excreted in the urine or diarrhoea fluid. Acid-

Accepted: March 2, 2017

Published online: April 14, 2017

Corresponding author:

Norbert Lameire
Renal Division
Department of Medicine
University Hospital
185, De Pintelaan
9000 Gent, Belgium
Norbert.lameire@ugent.be



Fig. 1 - Plain abdominal x-ray revealing the presence of calculi in both kidneys.

base balance is maintained if the new anions are metabolised to neutral end products, or if they are excreted in the urine along with H^+ or NH_4^+ . On the other hand, there is a net gain of H^+ in the body if these anions are retained in the body or are excreted as their Na^+ or K^+ salts. The major cation in plasma is Na^+ (PNa^+), and the major anions are Cl^- (PCl^-) and HCO_3^- ($PHCO_3^-$). The term “plasma anion gap” (PAG) is used for the difference between the plasma concentration of Na^+ and the concentrations of Cl^- and HCO_3^- , which reflect the usual excess of the other unmeasured anions over that of the other unmeasured cations in plasma. The normal plasma anion gap is positive and is on average 12 mmol/L. The major textbooks utilise this definition as do the majority of published papers. As originally conceived, serum K^+ was also included in the calculation, but serum K^+ is included in none of the major classical textbooks of nephrology and only in a minority of published papers on this subject. The rationale for omission is that the absolute change in the serum K^+ concentration observed clinically is small.

The anion gap is largely due to the net anionic valence on plasma proteins, principally plasma albumin (PAIb). If the difference is larger than the ‘normal’ value of the PAG, then other anions are present in the plasma. Because of differences in laboratory methods (particularly in the measurement of plasma chloride), there is a large difference in the mean value for the PAG gap reported by clinical laboratories. Regardless of the laboratory method used, there is a wide range within the normal values of the PAG. The clinician should know what the normal values of PAG are for his/her clinical laboratory. It should be remembered that intoxications with other halides, including bromide give falsely elevated PCl^- concentrations with low or even negative anion gap calculations.

When using the calculation of the PAG for detecting the presence of new anions in plasma, one must adjust the baseline value of the PAG for the PAIb. As a rough estimate, the baseline value for the PAG rises or falls by approximately 2.5 mmol/L for every 10 g/L rise or fall in the PAIb (1, 2).

An increase in the anion gap is mostly related to an increase in unmeasured anions such as ketones or lactate. However,

when interpreting the serum anion gap the following caveats should be taken into account: (i) When acid rests are excreted in the urine, e.g., keto acids during treatment of diabetes or hippurate after glue sniffing (hippurate is rapidly removed from the circulation by the kidney through both filtration and secretion), a non-anion gap metabolic acidosis can also be found despite the presence of abnormal anions. This demonstrates that a non-anion gap metabolic acidosis is not exclusively diagnostic of renal tubular acidosis (RTA) or gastro-intestinal base losses. (ii) As stated above, the normal anion gap is positive and around 12 mmol/L. However, when the levels of the unmeasured cations are unusually high, such as in hypercalcaemia or hypermagnesaemia, the ‘normal anion gap’ will be lower.

In the patient described above, the calculated normal PAG reflects the presence of a normal PAG acidosis.

How does one appreciate the respiratory component of the acid base status?

The body responds to metabolic acidosis by trying to restore the $PCO_2/[HCO_3^-]$ ratio. This is realised by lowering the PCO_2 .

The reduction in PCO_2 is accomplished by increasing alveolar ventilation. The drop in arterial pH stimulates both the central and peripheral chemoreceptors controlling respiration, resulting in an increase in alveolar ventilation. The increase in ventilation is characterised more by an increase in tidal volume than by an increase in respiratory rate, and if the acidemia is severe, may reach a maximum of 30 L/min ($nl = 5-6$ L/min).

In its most pronounced clinical manifestation, the increase in ventilation is referred to as Küssmaul respiration.

In general, respiratory compensation results in a 1.2 mmHg reduction in PCO_2 for every 1.0 mmol/L reduction in the plasma HCO_3^- concentration down to a minimum PCO_2 of 10 to 15 mmHg.

Winter’s formula

To estimate the expected PCO_2 range based on respiratory compensation, one can also use the Winter’s formula which predicts: $PCO_2 = (1.5 \times [HCO_3^-]) + 8 \pm 2$ (3).

Another useful tool in estimating the PCO_2 in metabolic acidosis is the recognition that the pCO_2 is approximately equal to the last 2 digits of the pH.

In the above-described patient, the calculated pCO_2 according to the Winter’s formula is: $1.5 \times 21 + 8 = 39.5$, which is very similar to what was measured. Our patient has thus a completely compensated mild but normal anion gap acidosis.

How does one perform tests for tubular acidification disturbances?

Ammonium chloride or calcium chloride loading tests

When RTA is suspected but there is no severe systemic acidosis (e.g., in case of incomplete RTA), an acid loading test can be considered to demonstrate the acidification defect. The renal response to an increased acid load can be tested e.g., by administering ammonium chloride (which is chemi-

cally equivalent to adding HCl to the body). Upon administration of ammonium chloride, the urinary pH should become more acid (<5.3) and failure to do so indicates an acidification defect. Ammonium chloride 0.1 g/kg/day can be given for 3 to 5 days or alternatively a single dose may be given and the urine should be collected (under mineral oil) over the next 2 to 8 hours at 2-hour intervals. Blood samples should be taken at the end of each 2-hour urine collection to ensure that plasma bicarbonate is <20 mmol/L. The three days' test allows for a maximal increase in ammonium excretion. The presence of a pH above 5.5 in the presence of systemic acidemia and in the absence of urinary tract infection strongly suggests the diagnosis of RTA, although patients with salt-retaining diseases may respond abnormally to acidemia due to inadequate distal sodium delivery even though distal acidification is intact (4). In case of intolerance or contraindication to the use of ammonium chloride (e.g., in case of liver insufficiency), oral calcium chloride can be given at a dose of 2 mEq/kg (4). The ammonium chloride test is only informative in cases of incomplete dRTA and in mild hyperchloremic metabolic acidosis where no diagnosis can be made (5).

Urinary anion gap and urinary osmolal gap

The urinary anion gap ($\text{UNA}^+ + \text{UK}^+ - \text{UCl}^-$) and the urinary osmolal gap (Uosm) ($0.5 \times (\text{measured Uosm} - \text{calculated Uosm})$) can be applied in non-anion gap metabolic acidosis to distinguish between renal and gastrointestinal direct loss of sodium bicarbonate. Typically, in RTA, contrary to what happens in case of gastrointestinal loss such as diarrhoea, there is an impairment in NH_4^+ excretion. Unfortunately, direct ammonium measurement is rarely available in daily clinical practice (6) and one has to rely on surrogate tools such as the urinary anion gap and the urinary osmolal gap, to estimate the urinary ammonium excretion.

In a normal Western diet the level of UNa^+ and UK^+ is higher than UCl^- and in normal subjects, the urine anion gap is usually near zero or positive. As is the case with the serum anion gap, the interpretation of the value of the anion gap is based on the fact that there should be electro-neutrality, which means that the sum of the anions should equal the sum of the cations. The main unmeasured cation in the urine is ammonium. Urinary bicarbonate is usually quite low, thus, can be omitted from the formula (when the urine pH is below 6.5, the urinary bicarbonate is negligible), and the main unmeasured anions in the formula of the urinary anion gap are phosphate and sulfate anions. Thus, the value of the anion gap can be interpreted as being a reflection of the urinary ammonium excretion. When there is gastrointestinal loss of sodium bicarbonate (e.g., in case of diarrhoea), a high urinary ammonium excretion indicating an intact acid excretion can be expected, since there is no defect in tubular acidification. In acidosis, the ammonium excretion should be around 200–300 mmol/24-h versus 70 mmol/24-h in absence of acidosis. This evidently means that there should be a negative or normal (but certainly not a positive) urinary anion gap because a high ammonium excretion will lead to a higher urinary chloride excretion (excretion of ammonium chloride). A negative urinary anion gap is thus either due to a decrease in unmeasured anions or an increase in ammonium. A posi-

tive urinary anion gap indicates a defective ammonium excretion in response to systemic acidosis and suggests thus RTA. However, the urinary anion gap becomes unreliable if ammonium is excreted with another anion besides chloride or when there is a high excretion of other unmeasured anions such as keto-acids or hippurate (7) or in situations with avid sodium retention leading to low distal urine flow. When the urinary pH is higher than 6.5, bicarbonate becomes a significant urinary anion and should be included in the calculation. It should be emphasised that the urinary anion gap should only be used in the presence of systemic acidosis. In the patient discussed above, the urine pH of 6.7 despite the mild chronic metabolic acidosis suggests the presence of (relatively small) amounts of bicarbonate in the urine.

Unlike the urinary anion gap, the urinary osmolal gap is not influenced by the fact that ammonium can be excreted with another anion, and it detects thus all NH_4^+ salts in the urine (8). The urinary osmolal gap is therefore the best indirect test to assess whether the urinary NH_4^+ and thereby the rate of excretion of NH_4^+ is high enough in patients with chronic metabolic acidosis. It is calculated as follows: $0.5 (\text{measured Uosm} - \text{calculated Uosm})$ (divided by 2 to account for the negatively charged ions), with the calculated osmolality given by $2 \times (\text{Urinary Na}^+ + \text{K}^+) + \text{glucose}(\text{mg/dL})/18 + \text{BUN}(\text{mg/dL})/2.8$. Under non-acidotic conditions the Uosm gap will be around 70 mmHg. If the Uosm gap is >100 mOsm, one can assume an appropriate response to acidosis by increased ammonium excretion. In chronic acidosis, these values can go up to 200–300 mmol of NH_4^+ excretion per day. This adaptation by increasing ammonia excretion as a response to systemic acidosis, takes a few days to reach its maximum.

The decrease in ammonia excretion can be either due to a NH_4^+ generation defect (low GFR, starvation, defective proximal ammoniogenesis, $\text{NH}_3/\text{NH}_4^+$ recycling defect) or be due to a low NH_4^+ excretion related to an impaired distal proton secretion.

However, one should bear in mind that both methods are semi-quantitative measurements and cannot replace a direct ammonium measurement (6), which unfortunately is often unavailable in daily clinical practice.

During the short ammonium chloride test, the urinary pH in the discussed patient was persistently above 6.0 despite the PHCO_3^- dropped to 18 mmol/L. The calculation of the urinary anion gap (UAG) revealed:

$$\text{UAG} = 100 (\text{Na}^+) + 31 (\text{K}^+) - 105 (\text{Cl}^-) = 26$$

The inappropriately high urine pH during the ammonium chloride test and the positive UAG value suggest that the kidneys are unable to adequately excrete ammonium, leading to a reduction in net acid excretion and thus (mild) metabolic acidosis.

The urine osmolality gap was unfortunately not determined.

Final diagnosis in the discussed patient

In view of the inappropriately high urine pH in the presence of a chronic low-grade metabolic acidosis, the hypokalaemia, the positive UAG, and the hypocitraturia, a mild form

of renal distal tubular acidosis or type 1 tubular acidosis with recurrent nephrolithiasis, is present.

Additional diagnostic tests in RTA

Enhancement of trans-epithelial potential in the collecting duct

The distal renal response to acidosis can also be evaluated by administering sodium with a non-absorbable ion (e.g., sodium sulfate or sodium phosphate), which will affect the lumen electro-negativity of the collecting duct. The sodium reabsorption in the cortical collecting duct will be increased, especially when a sodium avid condition is present (in the presence of a low-salt diet or after administration of a mineralocorticoid such as fludrocortisone). There will also be enhanced potassium and hydrogen excretion when distal acidification is normal (9). These sodium-dependent tests will allow for additional mechanistic information and testing of the kaliuresis, compared to tests based on providing an acidemic stimulus only.

When administering sodium sulfate (500 mL of a 4% solution infused over 45–60 min) there will not be an adequate response if distal RTA is present, due to either a secretory or a voltage defect. A properly performed sodium sulfate test yields a fall in urine pH below 5.5, whether or not systemic acidosis is present. However, patients with an *incomplete* distal tubular acidosis often have a normal pH response to sodium sulfate administration. Fludrocortisone can be administered orally or IV (1 mg/kg), 12 h before the sodium sulfate test is performed, to enhance the sodium avid state. Urine collections should be continued for 2–3 hours after the end of sodium sulfate infusion, because some subjects have a delayed response.

The response to administering sodium phosphate is comparable to sodium sulfate but sodium phosphate is considered a stronger stimulus to the generation of carbon dioxide compared to bicarbonate (4) (see paragraph on U-B PCO_2).

Another mechanism by which the lumen negative potential can be enhanced is by administration of furosemide. This will inhibit the NKCC2 channel in the ascending loop of Henle and will enhance the distal delivery of sodium and chloride which should stimulate distal Na reabsorption. This in turn will create a higher degree of luminal electro-negativity, which should stimulate H^+ and K^+ secretion under normal conditions. This response can be abolished by administration of amiloride, thus a normal response to amiloride (20 mg) implies that voltage dependent H^+ and K^+ secretion is essentially intact. Urine should be collected at 2-hourly intervals from 4–6 hours after an oral dose of furosemide (10). The furosemide test should not replace the ammonium chloride test because a failure to acidify the urine after furosemide does not mean there is a definite irreversible acidification defect, e.g., in case of dehydration when distal sodium delivery can be insufficiently enhanced by furosemide, there will be a reversible distal acidification defect. The maximum acidification effect of furosemide is delayed, taking place about 120 to 180 min after administration of the drug. As mentioned before, furosemide can be administered either orally or IV but an advantage of the IV administration is that it also stimulates the RAAS system, which

is of critical importance in the evaluation of a patient with hyperkalaemic RTA (11).

Although transport in the cortical collecting duct is essentially sodium dependent, H^+ and K^+ secretion in the medullary collecting duct is not sodium dependent, which means that tests enhancing the luminal electro-negativity can help to differentiate between cortical, voltage and medullary collecting duct defects. In case of a cortical defect or a voltage-dependent distal acidification defect (where there is a primary defect in sodium reabsorption, unrelated to aldosterone) the urinary pH will not decrease in response to these tests whereas in case of a unique medullary defect, urinary pH will decrease.

Urine PCO_2 /U-B PCO_2

Defects in acidification resulting from decreased proton secretion are associated with low urinary PCO_2 . Defects arising from other mechanisms are associated with a normal urinary PCO_2 (4). When the urine is rich in bicarbonate, it has a PCO_2 usually 40 mmHg greater than that of blood and rises above 70 mmHg after maximal alkalinisation of the urine (pH 7.8 or greater). This can be explained by the delayed hydration of carbonic acid because carbonic anhydrase (CA) is not present on the luminal membrane of the distal nephron. Delayed hydration of carbonic acid leads to formation of CO_2 in areas that are unfavourable for CO_2 diffusion, resulting in elevated urinary CO_2 tensions (12).

This mechanism explains the rationale of bicarbonate loading and measurement of pCO_2 or the U minus blood PCO_2 value as a test for intact distal hydrogen excretion.

Bicarbonate loading can be achieved by IV administration of 2.75% sodium bicarbonate at a rate of 4 mL/kg/h. Urine and blood samples are taken at 2-hourly intervals until plasma bicarbonate concentration reaches 26 mmol/L. The values of urine to blood PCO_2 and fractional excretion of bicarbonate are calculated when urine pH is raised to 7.5/7.8. The test is terminated after the pH of the last three consecutive urine collections is above 7.8. An infusion lasting 180 to 260 minutes is usually required.

There are some pitfalls/difficulties when performing this test: (i) the urine needs to be alkaline because high amounts of bicarbonate need to be present for the reaction to occur, (ii) during the test, urine should be collected under mineral oil, in order to avoid diffusion of CO_2 and (iii) the test assumes that there is no difference between systemic PCO_2 and renal cortex PCO_2 , which is not completely true. It might therefore be better to look at the PCO_2 and the PCO_2 evolution itself, instead of at the urine minus blood PCO_2 tension (13). The urine pCO_2 test can also be performed during sodium phosphate infusion. At a urine pH of about 6.8 (= pK_a of the phosphate buffer) half of the phosphate will occur in its acid form and serve as a proton donor for bicarbonate titration and PCO_2 formation, whereas in a highly alkaline urine, phosphate concentration plays no role in the generation of urine PCO_2 . By infusing neutral phosphate (1 mmol/L total body water, dissolved in 180 mL of isotonic saline at a rate of 1 mL/h for 3 hours), urine phosphate must increase to at least 20 mmol/L in 2 to 3 successive collections after the beginning of the phosphate infusion. Under these conditions, urine PCO_2 is

consistently 25 mmHg above that in blood. The use of sodium phosphate infusion versus bicarbonate loading can help to identify a distal tubular acidification defect that is caused by acid back-leak. Phosphate will stimulate H^+ secretion and form acid phosphate, which will react with bicarbonate to form carbonic acid and CO_2 , but because this reaction is delayed, the hydration of carbonic acid will take place at a site in the distal tubule where there will no longer be back diffusion. This will lead to an increase in PCO_2 tension when sodium phosphate compared to bicarbonate is administered in patients with distal tubular acidosis if the defect is caused by back diffusion, whereas this is not the case in presence of a H^+ secretory defect. The latter will lead to an abolished response in both tests (either bicarbonate loading or sodium phosphate infusion).

The following discussion focusses on type 1 tubular acidosis and does not include the approach to type 2, or type 4 tubular acidosis or uraemic acidosis. Complete discussion on all renal acidosis types can be found in many classical textbooks of nephrology (14).

What is the pathogenesis of distal tubular acidification disturbances?

The kidneys manage to regulate acid base balance with a net acid excretion of around 1 mmol/kg/day by reabsorbing most of the filtered bicarbonate in the proximal tubule, and by generating new bicarbonate through buffer titration (mainly phosphate) and excretion of ammonia. RTA is a group of disorders presenting as high chloride metabolic acidosis (HCMA) due to either renal loss of bicarbonate or inability to generate bicarbonate by the kidneys.

Distal RTA (dRTA) reflects a failure to regenerate HCO_3^- by intercalated cells in the collecting duct, resulting in persistent alkaline urine. It is characterised by impaired acid secretion and the inability to reduce urinary pH to ≤ 5.3 when confronted with spontaneous acidaemia or during acid loading. The defect in H^+ secretion in the collecting duct leads to reduced net acid excretion (NAE) subsequent to decreased NH_4^+ , titratable acid excretion, and some degree of bicarbonaturia. This results in a decrease in serum HCO_3^- concentration and generation of hyperchloraemic metabolic acidosis.

The spectrum of renal tubular acidosis (RTA) can be classified according to the site of injury (proximal vs. distal tubule) or according to the pathophysiological mechanism involved. Tubulopathy can be caused by genetic or acquired factors or be associated with auto-immune disorders such as Sjögren's disease.

Whereas proximal tubular acidosis is due to a decreased reabsorption of bicarbonate in the proximal tubule leading to bicarbonaturia when serum bicarbonate levels are above the threshold for reabsorption, dRTA is related to an impaired distal acidification.

In the last 2 decades, the pathophysiology of hypokalaemic dRTA (type 1 RTA) has been more clearly elucidated by studying the genetic and molecular bases of the inherited forms of this disease. Most studies suggest that the inherited complete forms of dRTA are due to defects in the transport mechanisms which participate in the secretion of H^+ ions in the urine carried out by alpha (α)-intercalated cells in cortical and medullary collecting ducts.

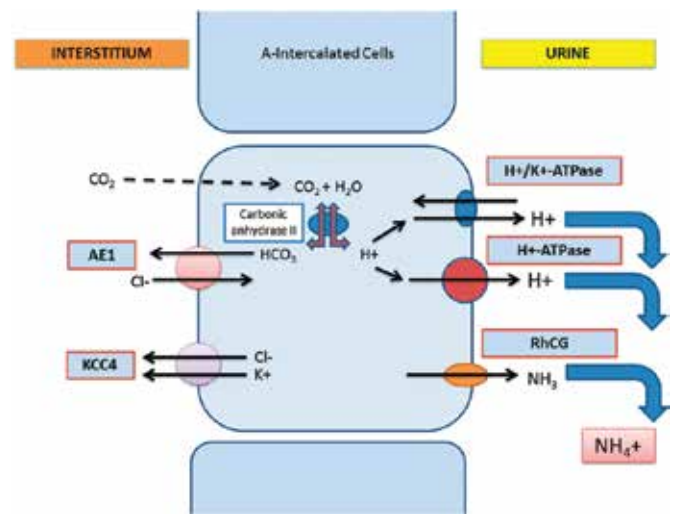


Fig. 2 - Acidification of the urine depends on the entry of CO_2 into the cell and its reaction with water to create bicarbonate and H^+ ions. This reaction is catalysed by carbonic anhydrase II (CAII). The bicarbonate is reabsorbed via cellular exit through the anion exchanger 1 (AE1). The H^+ is excreted via H^+/K^+ -ATPase or H^+ -ATPase. Lastly, ammonia is transported into the tubule via the Rh C glycoprotein (RhCG) channel. Defects in H^+ ion secretion, backflow of H^+ ions into the cell, defects in AE1 or CAII or in ammonia production or transport can all lead to forms of distal RTA (15), with permission from Clin J Am Soc Nephrol. 2015;10(3):530-539.

Figure 2 taken from Rosner et al (15) illustrates the handling of acid balance in the distal tubule by the α -intercalated cells.

Acidification of the urine depends on the entry of CO_2 into the cell and its reaction with water to create bicarbonate and H^+ ions. This reaction is catalysed by carbonic anhydrase II (CAII). The bicarbonate is reabsorbed via cellular exit through the anion exchanger 1 (AE1). The H^+ is excreted via H^+/K^+ -ATPase or H^+ -ATPase. Lastly, ammonia is transported into the tubule via the Rhesus C glycoprotein (RhCG) channel. Defects in H^+ ion secretion, backflow of H^+ ions into the cell, defects in AE1 or CAII or in ammonia production or transport can all lead to forms of distal RTA.

Distal H^+ excretion requires thus cytosolic carbonic anhydrase II (CAII) to generate an acid-base pair (H^+ and HCO_3^-) to furnish substrate for acid-base transporters. CAII produces H^+ hydrogen ions and simultaneously, bicarbonate is reabsorbed through the Cl^-/HCO_3^- exchangers, including the AE1 isoform in the basolateral cell membrane. Tubular H^+ secretion is mediated by two proton pumps located on the apical cell membrane, a V (acuolar)-type H^+ -ATPase and an H^+K^+ -ATPase. The H^+ -ATPase is regulated by aldosterone, while the H^+K^+ -ATPase responds inversely to the serum potassium level (16, 17).

The urinary ammonium excretion mechanism takes place in two stages: firstly, there is uptake from the interstitium to the cytoplasm via hyperpolarisation – activated cyclic nucleotide-gated (HCN)2 voltage-activated ammonium channels (18) and Rhesus B Glycoprotein (RhBG) and Rhesus C Glycoprotein (RhCG) ammonia channels (19). HCN2 channels are constitutive, they may uptake ammonium and/or sodium

and are not regulated by metabolic acidosis (18). By contrast, RhCG ammonia channels are located both in apical membranes and in basolateral membranes and their destination to the membranes is regulated by metabolic acidosis (19, 20).

Because CAII is also involved in proximal tubular acidification, defects in CAII lead to mixed proximal and distal RTA.

Distal RTA can be caused by the following: impaired proton pump function (defective H^+K^+ -ATPase, defective H^+ -ATPase), decreased potential difference in cortical collecting tubule (CCT) or voltage dependent RTA, decreased capacity to maintain steep pH gradients (or back-leak dRTA), rate-dependent RTA, or abnormal anion exchange. Patients with impaired collecting duct H^+ secretion and complete dRTA also exhibit uniformly low excretory rates of NH_4^+ when the degree of systemic acidosis is taken into account. Low NH_4^+ excretion equates with inappropriately low renal regeneration of HCO_3^- , which indicates that the kidney is responsible for causing or perpetuating the chronic metabolic acidosis. Low NH_4^+ excretion in classical hypokalaemic distal RTA occurs because of the failure to trap NH_4^+ in the medullary collecting duct as a result of higher-than-normal tubule fluid pH in this segment and loss of the disequilibrium pH (pH > 6.0). The high urine pH indicates impaired H^+ secretion (15, 16, 21).

When aldosterone deficiency/resistance is involved in tubular acidosis then the latter is classified as type 4 RTA. Both the voltage type dRTA and type 4 RTA are associated with hyperkalaemia, whereas the other forms of distal tubular acidosis are characterised by hypokalaemia (15, 16, 21). It is tempting to speculate that the ammonia channel RhCG may be another candidate gene for dRTA in humans. So far, direct sequencing of patients with recessive forms of dRTA yielded no evidence for RhCG mutations. Arguably, such patients may be difficult to detect, as testing for an incomplete distal RTA remains challenging and is not a standard procedure in most centres.

Defects in H^+ secretion can be acquired, most often in association with auto-immune diseases, such as Sjögren syndrome, systemic lupus erythematosus, cryoglobulinaemia, thyroiditis, and Graves' disease. Sjögren syndrome is the autoimmune disorder most commonly associated with distal RTA; up to 3%-5% of patients will have overt distal RTA, and a much higher percentage (25%-35%) will have incomplete distal RTA (22-24). In some of the autoimmune diseases, including Sjögren's disease, medullary interstitial disease commonly occurs and may, in addition to distal RTA, impair NH_4^+ excretion by interrupting the medullary counter-current system for NH_4^+ .

What is the explanation of the hypokalaemia in distal tubular acidosis?

Hypokalaemia is a common feature of both pRTA and dRTA. The degree of hypokalaemia varies and is often more severe in patients with dRTA. In hereditary dRTA, hypokalaemia is a cardinal clinical feature, independent of the genetic mutation, although it is more severe in autosomal recessive forms due to a mutation in subunits of the H^+ -ATPase pump. One mechanism possibly explaining the hypokalaemia is the loss of positively charged potassium ions accompanying the poorly reabsorbable bicarbonate anions. The hypokalaemia

may also be due to a recently described signalling pathway involving activation and release of prostaglandin E_2 by β -intercalated cells that directly communicate to enhance sodium absorption and potassium secretion by activation of the epithelial sodium channel (ENaC) in collecting duct principal cells (25). A defect in the H^+K^+ -ATPase pump in α -intercalated cells of the distal convoluted tubule could also lead to potassium loss and metabolic acidosis, but such an abnormality has not yet been well documented. The mechanism of hypokalaemia may also involve an increase in aldosterone levels due to the sodium wasting (16).

It may here be reminded that in the voltage-dependent tubular acidosis and the type 4 tubular acidosis, hyperkalaemia is the rule (14, 16).

What is the pathogenesis of nephrolithiasis in distal tubular acidosis?

The history of nephrolithiasis is typical of distal RTA (26) and, interestingly, a considerable fraction of the literature on incomplete dRTA actually stems from patients with kidney stones. While most patients with calcium oxalate stones can maximally acidify their urine, up to 30% of patients with CaP stones have reduced ability to lower the urine pH. Impaired maximal acidification is also more common in recurrent stone formers (26) and those with bilateral disease [for references see (27, 28)]. The alkaline pH of the urine fosters precipitation of CaP, leading to stones and/or nephrocalcinosis. Luminal alkalisation inhibits calcium reabsorption resulting in hypercalciuria. Progression of nephrocalcinosis may lead to chronic renal failure.

A recent study revealed that the prevalence of incomplete dRTA was 23% in a non-selected study population with densitometrically proven osteopenia/osteoporosis patients (28). Importantly, long-term alkali treatment in addition to conventional therapy of osteopenia/osteoporosis improved bone mass at lumbar spine and possibly at other bone sites in these patients.

It is conceivable that incomplete distal RTA is in part due to allelic variants of genes recognised to cause the overt form. Indeed, in a family carrying an autosomal-recessive V-ATPase B1 subunit mutation, some heterozygous members were also affected by recurrent calcium nephrolithiasis (27).

In the presence of metabolic acidosis, the bone serves as an important buffer and exchanges H^+ ions for sodium, calcium, and potassium that are complexed to carbonates and phosphates (26) [for review see (29)]. Over time, this leads to increased bone resorption with increased calcium and phosphate release and hypercalciuria with hyperphosphaturia. Compounding this is that metabolic acidosis leads to increased citrate reabsorption in the proximal tubule and a decrease in urine citrate, a prime inhibitor of stone formation (26).

Modulation of citrate excretion in the kidney is influenced by multiple factors; however, pH (systemic, tubular, and intracellular) has the strongest impact. It has long been known that acidosis decreases renal citrate excretion, whereas alkalosis increases it. There are several mechanisms through which pH exerts these effects. Citrate is reabsorbed through the sodium citrate cotransporter as citrate²⁻ but exists predominately as citrate³⁻ within renal tubules. Lowering tubular pH increas-

es the concentration of citrate² available for transport and reduces the concentration of citrate³, thereby limiting its competitive inhibition. Even small decreases in proximal tubular pH (7.4 to 7.2) significantly increase tubular reabsorption. Acute acidosis is associated with increased activity of the sodium-dependent dicarboxylate transporter -1(NADC-1) transporter, and chronic acidosis leads to increased transporter messenger ribonucleic acid and the transporter itself (for review see (30). Hypocitraturia, generally defined as urinary citrate excretion less than 320 mg (1.67 mmol) per day for adults, is a common metabolic abnormality in stone formers, occurring in 20% to 60%. It should be noted that in the patient described in the case report a very low citrate excretion was noted. Citrate is a known inhibitor of stone formation, working through a variety of mechanisms. In the renal tubule citrate complexes with calcium, increasing its solubility and reducing the concentration of free calcium in the urine. This calcium-citrate complex limits calcium supersaturation and prevents nucleation of both calcium oxalate and CaP, at least partly through interactions with Tamm-Horsfall protein. Additionally, citrate prevents crystal agglomeration and growth through its ability to bind to the crystal's surface and may also prevent adhesion of calcium oxalate to renal epithelial cells.

How does one treat a patient with distal tubular acidosis and recurrent nephrolithiasis?

Correction of chronic metabolic acidosis can usually readily be achieved in patients with complete dRTA by administration of alkali in an amount sufficient to neutralise the production of metabolic acids derived from the diet. The goal is to correct the plasma HCO₃⁻ concentration to normal (25 mmol/L), and the concentration should be monitored frequently. In adult patients with complete distal RTA, the amount of bicarbonate administered may be equal to no more than 1 to 3 mmol/kg/day (31). In growing children, endogenous acid production is usually between 2 and 3 mmol/kg/day but may on occasion exceed 5 mmol/kg/day. Larger amounts of bicarbonate must therefore be administered to fully correct the acidosis and maintain normal growth.

Citrate is also a mainstay in the treatment of patients with distal RTA, in whom it is used to treat both acidosis and hypocitraturia. The goals of citrate therapy are stabilisation of bone disease as well as stone prevention (32). Correction of metabolic acidosis may lower urine calcium, and improve CaP supersaturation. These patients have a high urine pH at baseline, which may worsen with citrate treatment, and thiazide may need to be added to control hypercalciuria and supersaturation. However, in a study of nine patients with incomplete distal RTA, Preminger and associates (33) found that despite a significant increase in urinary pH, treatment with potassium citrate (60-80 mmol per day) led to a decrease in stone formation during 34 months of follow up compared with pretreatment rates. Urine calcium and calcium oxalate supersaturation fell, while CaP supersaturation remained stable. However, therapy with alkaline citrate should be monitored carefully with follow-up 24-hour urine collections to avoid an excessive rise in urinary pH and potential worsening of CaP supersaturation.

Summary

A case with recurrent nephrolithiasis with underlying mild distal RTA is presented. The most relevant clinical tests to explore the defective tubular acidification and the management of these patients are described.

Abbreviations

PNa ⁺	Plasma sodium
PCl ⁻	Plasma chloride
PHCO ₃ ⁻	Plasma bicarbonate
Palb	Plasma albumin
PAG	Plasma anion gap
UAG	Urinary anion gap
HCMA	High chloride metabolic acidosis
RTA	Renal tubular acidosis
pTA	Proximal tubular acidosis
dTA	Distal tubular acidosis
CA II	Carbonic anhydrase II
AE 1	Anion exchanger 1
NKCC2	Sodium-potassium 2 chloride channel
NADC-1	Sodium-dependent dicarboxylate transporter -1
Rhbg	Rhesus B glycoprotein channel
Rhcg	Rhesus C glycoprotein channel

Disclosures

Financial support: No grants or funding have been received for this study.

Conflict of interest: None of the authors has financial interest related to this study to disclose.

References

1. Adrogué HJ, Madias NE. Assessing acid-base status: physiologic versus physicochemical approach. *Am J Kidney Dis.* 2016; 68(5):793-802.
2. Kraut JA, Nagami GT. The serum anion gap in the evaluation of acid-base disorders: what are its limitations and can its effectiveness be improved? *Clin J Am Soc Nephrol.* 2013;8(11): 2018-2024.
3. Winters RW. Terminology of acid-base disorders. *Ann Intern Med.* 1965;63(5):873-884.
4. Kurtzman NA. Acquired distal renal tubular acidosis. *Kidney Int.* 1983;24(6):807-819.
5. Kim S, Lee JW, Park J, et al. The urine-blood PCO gradient as a diagnostic index of H(+)-ATPase defect distal renal tubular acidosis. *Kidney Int.* 2004;66(2):761-767.
6. Kirschbaum B, Sica D, Anderson FP. Urine electrolytes and the urine anion and osmolar gaps. *J Lab Clin Med.* 1999;133(6): 597-604.
7. Carlisle EJ, Donnelly SM, Vasuvattakul S, Kamel KS, Tobe S, Halperin ML. Glue-sniffing and distal renal tubular acidosis: sticking to the facts. *J Am Soc Nephrol.* 1991;1(8):1019-1027.
8. Kamel KS, Briceno LF, Sanchez MI, et al. A new classification for renal defects in net acid excretion. *Am J Kidney Dis.* 1997;29(1):136-146.
9. Battle D, Haque SK. Genetic causes and mechanisms of distal renal tubular acidosis. *Nephrol Dial Transplant.* 2012; 27(10):3691-3704.
10. Han JS, Kim GH, Kim J, et al. Secretory-defect distal renal tubular acidosis is associated with transporter defect in H(+)-

- ATPase and anion exchanger-1. *J Am Soc Nephrol*. 2002;13(6):1425-1432.
11. Rodríguez Soriano J. Renal tubular acidosis: the clinical entity. *J Am Soc Nephrol*. 2002;13(8):2160-2170.
 12. DuBose TD Jr. Hydrogen ion secretion by the collecting duct as a determinant of the urine to blood PCO₂ gradient in alkaline urine. *J Clin Invest*. 1982;69(1):145-156.
 13. Halperin ML, Goldstein MB, Haig A, Johnson MD, Stinebaugh BJ. Studies on the pathogenesis of type I (distal) renal tubular acidosis as revealed by the urinary PCO₂ tensions. *J Clin Invest*. 1974;53(3):669-677.
 14. DuBose TD Jr. Acid base homeostasis. In: Skorecki KL CG, Marsden PA, Yu ASL, Taal MW., eds. *Brenner & Rector's the kidney*. Vol 10. Boston: Elsevier; 2016:511.
 15. Rosner MH, Perazella MA, Choi MJ. American Society of Nephrology quiz and questionnaire 2014: acid-base and electrolyte disorders. *Clin J Am Soc Nephrol*. 2015;10(3):530-539.
 16. Soleimani M, Rastegar A. Pathophysiology of renal tubular acidosis: core curriculum 2016. *Am J Kidney Dis*. 2016;68(3):488-498.
 17. Winter C, Schulz N, Giebisch G, Geibel JP, Wagner CA. Non-genomic stimulation of vacuolar H⁺-ATPases in intercalated renal tubule cells by aldosterone. *Proc Natl Acad Sci USA*. 2004;101(8):2636-2641.
 18. Carrisoza-Gaytán R, Rangel C, Salvador C, et al. The hyperpolarization-activated cyclic nucleotide-gated HCN2 channel transports ammonium in the distal nephron. *Kidney Int*. 2011;80(8):832-840.
 19. Weiner ID, Verlander JW. Recent advances in understanding renal ammonia metabolism and transport. *Curr Opin Nephrol Hypertens*. 2016;25(5):436-443.
 20. Weiner ID, Verlander JW. Ammonia transport in the kidney by Rhesus glycoproteins. *Am J Physiol Renal Physiol*. 2014;306(10):F1107-F1120.
 21. Both T, Zietse R, Hoorn EJ, et al. Everything you need to know about distal renal tubular acidosis in autoimmune disease. *Rheumatol Int*. 2014;34(8):1037-1045.
 22. Pertovaara M, Korpela M, Kouri T, Pasternack A. The occurrence of renal involvement in primary Sjögrens syndrome: a study of 78 patients. *Rheumatology (Oxford)*. 1999;38(11):1113-1120.
 23. Ram R, Swarnalatha G, Dakshinamurthy KV. Renal tubular acidosis in Sjögrens syndrome: a case series. *Am J Nephrol*. 2014;40(2):123-130.
 24. Ren H, Wang WM, Chen XN, et al. Renal involvement and follow-up of 130 patients with primary Sjögrens syndrome. *J Rheumatol*. 2008;35(2):278-284.
 25. Gueutin V, Vallet M, Jayat M, et al. Renal β -intercalated cells maintain body fluid and electrolyte balance. *J Clin Invest*. 2013;123(10):4219-4231.
 26. Krieger NS, Frick KK, Bushinsky DA. Mechanism of acid-induced bone resorption. *Curr Opin Nephrol Hypertens*. 2004;13(4):423-436.
 27. Zhang J, Fuster DG, Cameron MA, et al. Incomplete distal renal tubular acidosis from a heterozygous mutation of the V-ATPase B1 subunit. *Am J Physiol Renal Physiol*. 2014;307(9):F1063-F1071.
 28. Sromicki JJ, Hess B. Abnormal distal renal tubular acidification in patients with low bone mass: prevalence and impact of alkali treatment. *Urolithiasis*. 2016.
 29. Alexander RT, Cordat E, Chambrey R, Dimke H, Eladari D. Acidosis and urinary calcium excretion: insights from genetic disorders. *J Am Soc Nephrol*. 2016;27(12):3511-3520.
 30. Zuckerman JM, Assimos DG. Hypocitraturia: pathophysiology and medical management. *Rev Urol*. 2009;11(3):134-144.
 31. Morris RC Jr, Sebastian A. Alkali therapy in renal tubular acidosis: who needs it? *J Am Soc Nephrol*. 2002;13(8):2186-2188.
 32. Xu H, Zisman AL, Coe FL, Worcester EM. Kidney stones: an update on current pharmacological management and future directions. *Expert Opin Pharmacother*. 2013;14(4):435-447.
 33. Preminger GM, Sakhaee K, Skurla C, Pak CY. Prevention of recurrent calcium stone formation with potassium citrate therapy in patients with distal renal tubular acidosis. *J Urol*. 1985;134(1):20-23.