Decreased Renal Expression of H⁺-ATPase and Pendrin in a Patient with Distal Renal Tubular Acidosis Associated with Sjögren's Syndrome

Ha Yeon Kim¹, Sung Sun Kim², Eun Hui Bae¹, Seong Kwon Ma¹ and Soo Wan Kim¹

Abstract

A 31-year-old woman with no significant past medical or family history was admitted with complaints of general weakness. Laboratory tests revealed: serum potassium 3.0 mEq/L, arterial blood pH 7.28, serum bicarbonate 17.8 mEq/L and urinary pH 7.0. Double-labeling confocal fluorescence microscopy using H⁺-ATPase and pendrin antibodies demonstrated a decreased expression of these proteins in the patient's renal collecting duct compared to normal controls. Anti-Sjögren's-syndrome-related antigen A (Anti-Ro/SS-A) and anti-Sjögren's syndrome type B (anti-La/SS-B) antibodies were strongly positive with very high titers, consistent with Sjögren's syndrome. We present a case of distal renal tubular acidosis-associated Sjögren's syndrome with a defect in H⁺-ATPase and pendrin in the renal collecting duct.

Key words: hypokalemia, renal tubular acidosis, Sjögren's syndrome, H⁺-ATPase, pendrin

(Intern Med 54: 2899-2904, 2015)

(DOI: 10.2169/internalmedicine.54.4821)

Introduction

Hypokalemia often accompanies certain types of renal tubular acidosis (RTA) as a result of potassium wasting. Distal RTA, also known as type 1 RTA, is typically associated with hypokalemia. The major causes of distal RTA in adults are autoimmune diseases and hypercalciuria (1-3). Distal RTA may be the presenting manifestation of autoimmune diseases such as Sjögren's syndrome.

Sjögren's syndrome is characterized by the presence of keratoconjunctivitis, xerostomia and chronic inflammatory sialadenitis. This immune process can also affect non-exocrine organs, including the kidneys, producing an interstitial nephritis and defects in tubular function. The mechanism by which Sjögren's syndrome leads to distal RTA remains to be completely elucidated.

During the evaluation of a patient with hypokalemia, we diagnosed distal RTA associated with Sjögren's syndrome. We herein present the case of a patient with Sjögren's syndrome and RTA who was found to have a defect in H⁺-

ATPase and pendrin in the renal collecting duct.

Case Report

A 31-year-old woman presented with general weakness. Otherwise, she had no significant past medical or family history. She was afebrile, with a heart rate of 72 beats per minute in sinus rhythm and a blood pressure of 120/70 mmHg. Her blood test results were: blood urea nitrogen 13.6 mg/dL, creatinine 0.8 mg/dL, sodium 140 mEq/L, potassium 3.0 mEq/L, chloride 114 mEq/L, inorganic phosphorus 3.8 mg/dL, uric acid 4.1 mg/dL and serum osmolality 290 mOsmol/kg (Table). A urine analysis revealed urine osmolality of 312 mOsmol/kg, random urine sodium of 61 mEq/L, random urine potassium of 30.4 mEq/L, random urine chloride of 61 mEq/L and a urinary pH of 7.0. The 24-hour urinary calcium level was 140.6 mg/day. Arterial blood revealed a pH of 7.28, pCO₂ of 36.9 mmHg and serum bicarbonate of 17.8 mEq/L. Plasma aldosterone was 9.9 pg/mL, and plasma renin activity was 0.9 ng/mL/h. The serum anion gap was normal at 8.2 mEq/L, and the urine an-

¹Department of Internal Medicine, Chonnam National University Medical School, Korea and ²Department of Pathology, Chonnam National University Medical School, Korea

Received for publication December 25, 2014; Accepted for publication March 18, 2015

Correspondence to Dr. Soo Wan Kim, skimw@chonnam.ac.kr

Table. Patient's Biochemical Profile.

Parameters	Patient's values
Serum values	
BUN (mg/dL)	13.6
Creatinine (mg/dL)	0.8
Sodium (mEq/L)	140
Potassium (mEq/L)	3.0
Chloride (mEq/L)	114
Inorganic phosphorus (mg/dL)	3.8
Uric acid (mg/dL)	4.1
Osmolality (mOsm/kg)	290
Anion Gap (mEq/L)	8.2
TTKG	9.42
FE_K (%)	40.53
Arterial blood gas values	
pH	7.28
PCO ₂ (mmHg)	36.9
Bicarbonate (mEq/L)	17.8
Urine values	
Minimum pH	7.0
Creatinine (mg/dL)	20
Sodium (mEq/L)	61
Potassium (mEq/L)	30.4
Chloride (mEq/L)	61
Osmolality (mOsm/kg)	312
24-h urine uric acid (mg/day)	510
24-h urine calcium (mg/day)	140.6
Uric acid /creatinine	< 1
Anion gap	30.4
No glucosuria or aminoaciduria were present	
Serum anion Gap = $S_{Na} - (S_{Cl} + S_{HCO_3})$	
Transtubular potassium gradient (TTKG) = $(U_K / Plasma_K) / (U_{Osmolality} / P_{Osmolality})$	
FE _K (%) = $U_K \times S_{creatinine} / S_K \times U_{creatinine} \times 100$	
Urine anion gap = $U_{Na} + U_K - U_{Cl}$	

ion gap was positive at 30.4 mEq/L. The transtubular potassium gradient was 9.42, indicating RTA. The urinary pH was greater than 5.5 despite metabolic acidosis, suggesting a defect in renal tubular acidification. These findings were consistent with distal RTA, a diagnosis that was further supported by the lack of any apparent proximal tubular dysfunction such as hypouricemia, hypophosphatemia or glucosuria. Ultrasonographic and radiological examination of the kidneys showed no abnormality, excluding nephrocalcinosis, medullary sponge kidney and obstructive uropathy as potential causes of distal RTA.

A kidney biopsy showed no evidence of global or segmental sclerotic lesions in the glomeruli. The glomerular basement membrane appeared normal, and there was no glomerular proliferation, inflammation or other abnormality. The interstitium was infiltrated by mononuclear leukocytes and mild inflammatory cells (Fig. 1).

Double-labeling immunofluorescence histochemical staining was performed. Formalin-fixed and paraffin-embedded renal specimens with a thickness of three μ m were prepared. The slides were deparaffinized with xylene, 100% ethanol, 95% ethanol and 70% ethanol for five minutes each, incubated in citrate buffer at 125°C for one hour and rinsed three times in phosphate buffered saline (PBS, 0.01 M, pH 7.4). The slides were then incubated with anti-aquaporin 2 antibody (goat anti-human, 1:4,000; Santa Cruz Biotechnol-

Figure 1. Renal biopsy (×200, Hematoxylin and Eosin staining). The interstitium is infiltrated by mononuclear leukocytes and mild inflammatory cells.

ogy, Santa Cruz, USA) at 4°C overnight, rinsed again in PBS and incubated at 37°C for two hours with a fluorescent-labeled secondary antibody (anti-goat; green, 1:100, Abcam, Cambridge, UK). After washing in PBS, the slides were incubated with anti-vacuolar-type H⁺-ATPase antibody (rabbit anti-human, 1:2,000; Santa Cruz Biotechnology) or anti-pendrin antibody (mouse anti-human, 1:500; Acris antibodies, San Diego, USA) at 4°C overnight. The slides were rinsed again in PBS and incubated at 37°C for two hours with the addition of fluorescent-labeled secondary antibody (H⁺-ATPase: red, anti-rabbit, 1:100, Abcam; Pendrin: red, anti-mouse, 1:100, Vector Laboratories, Burlingame, USA). The slides were rinsed three times in PBS, mounted and examined with an inverted fluorescence phase-contrast microscope.

We performed confocal laser immunofluorescence microscopy to investigate whether there were changes in the expression of H⁺-ATPase and pendrin along the connecting tubule and collecting duct. In the cortex, aquaporin 2 was present exclusively in the principal cells of the connecting tubule and collecting duct in the kidneys from a control patient and the Sjögren's syndrome patient of this case. Immunolabeling of aquaporin 2 was comparable between the control patient and the patient with Sjögren's syndrome. Double labeling revealed that H⁺-ATPase expression was absent from the connecting tubule and collecting duct of the kidney from the Sjögren's syndrome patient, whereas H⁺-ATPase was present in the intercalated cells of the connecting tubule and collecting duct of the control kidney (Fig. 2). Double labeling of aquaporin 2 and pendrin exhibited similar labeling patterns for pendrin in the control and the Sjögren's syndrome patients. In contrast, there was a distinct red fluorescence in the normal kidney, whereas the Sjögren's syndrome patient's specimen revealed a weak red fluorescence of H⁺-ATPase and pendrin (Fig. 3).

To investigate the secondary cause of this patient's distal RTA, an evaluation of autoimmune diseases was performed. Tests for antinuclear antibodies, rheumatoid factor [87.3 IU/mL (normal 0-14)], anti-dsDNA antibody, anti-nucleosome

Figure 2. Double-labeling immunofluorescence histochemical staining revealed that H^+ -ATPase expression was absent from the connecting tubule and collecting duct of the kidney from the Sjögren's syndrome patient, while H^+ -ATPase was present in the intercalated cells of the connecting tubule and collecting duct of the control kidney. A: Control, AQP2+DAPI, B: Control, H^+ -ATPase+DAPI, C: Control, merge, D: Patient, AQP2+DAPI, E: Patient, H^+ -ATPase+DAPI, F: Patient, merge. AQP2: Aquaporin 2, DAPI: 4',6-diamidino-2-phenylindole nuclear stain

antibody and anti-smooth muscle antibody all yielded positive results. Both anti-Ro [also known as anti-Sjögren's-syndrome-related antigen A (SS-A)] and anti-La [also known as Sjögren's syndrome type B (SS-B)] antibodies were strongly positive with very high titers of >200 U/mL (>20 is positive). Results of autoimmunity studies showed findings consistent with Sjögren's syndrome. A repeated, thorough physical examination revealed the patient to have dry eyes and a dry mouth. However, her symptoms were not serious, and she refused further evaluation by means of a salivary gland biopsy, Schirmer tear test or sialogram. Hepatitis viral marker and complement were within normal limits, and thyroid function test results were also normal. These findings, together with the presence of distal RTA, raised a high degree of suspicion of Sjögren's syndrome.

The administration of potassium citrate corrected the patient's hypokalemia and metabolic acidosis, leading to a recovery of her general weakness. Her dry eyes improved with the administration of pilocarpine. To date, the patient has been maintained on potassium citrate and has been followed as an outpatient on a regular basis.

Discussion

The patient in our case had metabolic acidosis with a normal anion gap, a positive urinary anion gap, a urine pH of

7.0 and an absence of hypouricemia, hypophosphatemia and glucosuria, consistent with a diagnosis of distal RTA. Distal RTA is generally regarded as a condition characterized by a failure of renal intercalated cells to acidify the urine normally, resulting in hyperchloremic, hypokalemic metabolic acidosis. This dysfunction of the renal intercalated cells results in a failure to excrete hydrogen ions, and the urine pH cannot reach maximal acidity despite systemic acidemia. Our patient had hypokalemia associated with normal anion gap acidosis. The pathogenesis of hypokalemia in this patient with an H^+ -ATPase pump defect might be attributed to a relative increase in K^+ secretion because cortical tubule function is stimulated appropriately by a rise in luminal electronegativity. In addition, Gueutin et al. suggested that the up-regulation of the renal outer medullary potassium channel (ROMK) and the large conductance calcium-activated potassium channel (BKCa) in the collecting duct in type 1 distal RTA, *atp6v1b1*^{-/-} mice (which have inactivation of the B1 proton pump subunit), would induce excessive K^+ secretion through the renal potassium channel in the collecting duct (4).

The kidney plays an important role in acid-base regulation by secreting excess acid and reabsorbing the filtered bicarbonate. The final regulation occurs in the connecting tubule and collecting duct, where the intercalated cells play an important role. At least two types of intercalated cells, type

Figure 3. Double-labeling immunofluorescence histochemical staining showed similar labeling patterns for pendrin in the control and the Sjögren's syndrome patient, but there was a distinct red fluorescence in the normal kidney, whereas the Sjögren's syndrome patient's specimen revealed a weak red fluorescence of H⁺-ATPase and pendrin. A: Control, AQP2+DAPI, B: Control, Pendrin+DAPI, C: Control, merge, D: Patient, AQP2+DAPI, E: Patient, Pendrin+DAPI, F: Patient, merge. AQP2: Aquaporin 2, DAPI: 4',6-diamidino-2-phenylindole nuclear stain

A and type B, are located in the distal nephron. Type A intercalated cells play a role in H⁺ secretion via vacuolar H⁺-ATPase in the apical plasma membrane and subapical vesicles of the connecting tubule and collecting duct. Type B intercalated cells secrete HCO₃⁻ via a novel Cl/HCO₃⁻ exchanger, pendrin. Pendrin mediates the secretion of HCO₃⁻ and chloride reabsorption in the distal tubules (5, 6). Genetic ablation of pendrin in mice abolishes the luminal chloride-bicarbonate exchanger activity in type B intercalated cells, suggesting that this protein is a component of the apical bicarbonate extruding pathway (7). In order to maintain normal acid-base homeostasis, the expression and activity of pendrin is regulated by the systemic acid-base status, dietary electrolyte intake and hormones, such as angiotensin II and aldosterone (8).

In the present study, we demonstrated that the protein expression of H⁺-ATPase and pendrin in the cortical collecting duct markedly decreased in the kidney of a patient with Sjögren's syndrome, which was associated with normal anion gap metabolic acidosis and a distal tubule acidification defect. The development of metabolic acidosis was accompanied by decreased immunolabeling of H⁺-ATPase in the collecting duct. These findings suggest that the renal acidification defect could be caused by impaired H⁺ secretion via H⁺-ATPase in the collecting duct. This defect could result in an inability of type A intercalated cells to decrease urine pH

and might result in the impaired secretion of H⁺ and the development of acidosis. In contrast, pendrin immunolabeling was also markedly diminished in the kidney of our patient with Sjögren's syndrome, and this reduction was associated with the development of metabolic acidosis. It can be speculated that decreased immunoreactivity of pendrin in the collecting duct plays a compensatory role, acting to decrease the secretion of HCO₃⁻ and prevent the development of acidosis, but the change is not able to fully compensate for the acidosis (thus, H⁺-ATPase might play a key role).

Patients with secondary distal RTA require a diagnostic work-up to determine the etiology, which can include such drugs as ifosfamide, amphotericin B, or lithium, hypercalcemic conditions, medullary sponge kidney, nephrocalcinosis, obstructive uropathy and autoimmune diseases, including Sjögren's syndrome (2, 9-11). Distal RTA occurs in up to 25% of patients with Sjögren's syndrome (12). A few case reports of Sjögren's syndrome associated with hypokalemia or hypokalemic periodic paralysis and distal RTA have been reported (13-17). However, the mechanism by which Sjögren's syndrome leads to distal RTA still remains to be completely elucidated. With respect to renal involvement of Sjögren's syndrome, tubulointerstitial nephritis with defects in tubular function is the most common finding, and pathological examination reveals predominantly a focal or diffuse lymphocytic cell infiltrate in the interstitial tissue (18, 19).

The underlying causes of distal RTA are multifactorial and due to a genetic defect, immune mechanism or drug-induced kidney damage. As causes of hereditary distal RTA, many mutations have been identified in the anion exchanger 1, the B1 and a4 subunits of H⁺-ATPase and cytosolic carbonic anhydrase II leading to the dysfunction of intercalated cells in the collecting tubules (20-25). The histological findings in the present study were characterized by a prominent interstitial infiltrate composed of mononuclear leukocytes and mild inflammatory cells (26, 27). It could be possible that this kind of inflammatory process disrupts the common components of cytoskeleton assembly and regulatory proteins essential for both H⁺-ATPase and pendrin. However, tubulointerstitial inflammation occurs infrequently, and this speculation remains unsubstantiated. Evidence for autoantibodies to renal tubule cells were described in a group of patients with distal RTA (28). Circulating autoantibodies against H⁺-ATPase and pendrin have been shown to be involved in the pathological mechanism of distal RTA, thus implying that the immune abnormalities underlying Sjögren's syndrome might have caused the distal RTA in this patient (29). However, the mechanisms involved in the autoantibody development and causal relationship in the pathogenesis of distal RTA remain to be elucidated. In addition, no immunocytochemical evidence for anti-intercalated cell autoantibodies was observed in a subset of patients with Sjögren's syndrome, although absent H⁺-ATPase immunostaining in the cortical collecting duct has been demonstrated (26, 30). To test the hypothesis that circulating autoantibodies in the patient's serum might be reacting with renal intercalated cells, the patient's serum was incubated with normal human and rat kidney tissue. However, the patient's serum did not react with the kidney samples (26). Thus, precisely what role the autoantibodies might have played in the development of distal RTA, and how the immune system decreases the activity of H⁺-ATPase in the collecting duct in patients with Sjögren's syndrome remain unclear.

In conclusion, we herein presented a case that demonstrates the possibility that defects in the H⁺-ATPase pump and pendrin are associated with distal RTA in Sjögren's syndrome.

The authors state that they have no Conflict of Interest (COI).

Financial Support

This study was supported by a grant (CRI13903-21) from the Chonnam National University Hospital Biomedical Research Institute.

References

- Rodriguez Soriano J. Renal tubular acidosis: the clinical entity. *J Am Soc Nephrol* **13**: 2160-2170, 2002.
- Buckalew VM Jr. Nephrolithiasis in renal tubular acidosis. *J Urol* **141**: 731-737, 1989.
- Caruana RJ, Buckalew VM Jr. The syndrome of distal (type 1) renal tubular acidosis. Clinical and laboratory findings in 58 cases. *Medicine* **67**: 84-99, 1988.
- Gueutin V, Vallet M, Jayat M, et al. Renal β -intercalated cells maintain body fluid and electrolyte balance. *J Clin Invest* **123**: 4219-4231, 2013.
- Roy A, Al-Bataineh MM, Pastor-Soler NM, et al. Collecting duct intercalated cell function and regulation. *Clin J Am Soc Nephrol* **10**: 305-324, 2015.
- Alper SL, Natale J, Gluck S, Lodish HF, Brown D. Subtypes of intercalated cells in rat kidney collecting duct defined by antibodies against erythroid band 3 and renal vacuolar H⁺-ATPase. *Proc Natl Acad Sci USA* **86**: 5429-5433, 1989.
- Wagner CA, Finberg KE, Stehberger PA, et al. Regulation of the expression of the Cl⁻/anion exchanger pendrin in mouse kidney by acid-base status. *Kidney Int* **62**: 2109-2117, 2002.
- Wagner CA, Mohebbi N, Capasso G, Geibel JP. The anion exchanger pendrin (SLC26A4) and renal acid-base homeostasis. *Cell Physiol Biochem* **28**: 497-504, 2011.
- Battle DC, Sabatini S, Kurtzman NA. On the mechanism of toluene-induced renal tubular acidosis. *Nephron* **49**: 210-218, 1988.
- McCurdy DK, Frederic M, Elkinton JR. Renal tubular acidosis due to amphotericin B. *N Engl J Med* **278**: 124-130, 1968.
- Skinner R, Pearson AD, English MW, et al. Risk factors for ifosfamide nephrotoxicity in children. *Lancet* **348**: 578-580, 1996.
- Soy M, Pamuk ON, Gerenli M, Celik Y. A primary Sjögren's syndrome patient with distal renal tubular acidosis, who presented with symptoms of hypokalemic periodic paralysis: report of a case study and review of the literature. *Rheumatol Int* **26**: 86-89, 2005.
- Poux JM, Peyronnet P, Le Meur Y, et al. Hypokalemic quadriplegia and respiratory arrest revealing primary Sjögren's syndrome. *Clin Nephrol* **37**: 189-191, 1992.
- Bae EH, Han CW, Lee JH, et al. The case. Hypokalemia associated with nephrocalcinosis. Distal renal tubular acidosis associated with Sjögren's syndrome. *Kidney Int* **75**: 443-444, 2009.
- Aygen B, Dursun FE, Dogukan A, Ozercan IH, Celiker H. Hypokalemic quadriparesis associated with renal tubular acidosis in a patient with Sjögren's syndrome. *Clin Nephrol* **69**: 306-309, 2008.
- Duffles Amarante GB, Zotin MC, Rocha E, Delgado AG, Leite M Jr, Gomes CP. Renal tubular dysfunction in patients with primary Sjögren syndrome. *Clin Nephrol* **81**: 185-191, 2014.
- Yılmaz H, Kaya M, Özbek M, Üreten K, Safa Yıldırım İ. Hypokalemic periodic paralysis in Sjögren's syndrome secondary to distal renal tubular acidosis. *Rheumatol Int* **33**: 1879-1882, 2013.
- Tu WH, Shearn MA, Lee JC, Hopper J Jr. Interstitial nephritis in Sjögren's syndrome. *Ann Intern Med* **69**: 1163-1170, 1968.
- Shioji R, Furuyama T, Onodera S, Saito H, Ito H, Sasaki Y. Sjögren's syndrome and renal tubular acidosis. *Am J Med* **48**: 456-463, 1970.
- Battle D, Haque SK. Genetic causes and mechanisms of distal renal tubular acidosis. *Nephrol Dial Transplant* **27**: 3691-3704, 2012.
- Bruce LJ, Cope DL, Jones GK, et al. Familial distal renal tubular acidosis is associated with mutations in the red cell anion exchanger (Band 3, AE1) gene. *J Clin Invest* **100**: 1693-1707, 1997.
- Nagai R, Kooh SW, Balfe JW, Fenton T, Halperin ML. Renal tubular acidosis and osteopetrosis with carbonic anhydrase II deficiency: pathogenesis of impaired acidification. *Pediatr Nephrol* **11**: 633-636, 1997.
- Battle D, Ghanekar H, Jain S, Mitra A. Hereditary distal renal tubular acidosis: new understandings. *Annu Rev Med* **52**: 471-484, 2001.
- Karet FE, Finberg KE, Nelson RD, et al. Mutations in the gene encoding B1 subunit of H⁺-ATPase cause renal tubular acidosis with sensorineural deafness. *Nat Genet* **21**: 84-90, 1999.
- Yashima T, Noguchi Y, Kawashima Y, Rai T, Ito T, Kitamura K.

- Novel ATP6V1B1 mutations in distal renal tubular acidosis and hearing loss. *Acta Otolaryngol* **130**: 1002-1008, 2010.
26. DeFranco PE, Haragsim L, Schmitz PG, Bastani B. Absence of vacuolar H⁺-ATPase pump in the collecting duct of a patient with hypokalemic distal renal tubular acidosis and Sjögren's syndrome. *J Am Soc Nephrol* **6**: 295-301, 1995.
27. Siamopoulos KC, Elisaf M, Drosos AA, Navridis AA, Moutsopoulos HM. Renal tubular acidosis in primary Sjögren's syndrome. *Clin Rheumatol* **11**: 226-230, 1992.
28. Konishi K, Hayashi M, Saruta T. Renal tubular acidosis with autoantibody directed to renal collecting duct cells. *N Engl J Med* **331**: 1593-1594, 1994.
29. van den Wildenberg MJ, Hoorn EJ, Mohebbi N, et al. Distal renal tubular acidosis with multiorgan autoimmunity: a case report. *Am J Kidney Dis* **65**: 607-610, 2015.
30. Cohen EP, Bastani B, Cohen MR, Kolner S, Hemken P, Gluck SL. Absence of H⁺-ATPase in cortical collecting tubules of a patient with Sjögren's syndrome and distal renal tubular acidosis. *J Am Soc Nephrol* **3**: 264-271, 1992.