Full Review

Proximal renal tubular acidosis: a not so rare disorder of multiple etiologies

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Abstract

Proximal renal tubular acidosis (RTA) (Type II RTA) is characterized by a defect in the ability to reabsorb HCO₃⁻ in the proximal tubule. This is usually manifested as bicarbonate wasting in the urine reflecting that the defect in proximal tubular transport is severe enough that the capacity for bicarbonate reabsorption in the thick ascending limb of Henle’s loop and more distal nephron segments is overwhelmed. More subtle defects in proximal bicarbonate transport likely go clinically unrecognized owing to compensatory reabsorption of bicarbonate distally. Inherited proximal RTA is more commonly autosomal recessive and has been associated with mutations in the basolateral sodium-bicarbonate cotransporter (NBCe1). Mutations in this transporter lead to reduced activity and/or trafficking, thus disrupting the normal bicarbonate reabsorption process of the proximal tubules. As an isolated defect for bicarbonate transport, proximal RTA is rare and is more often associated with the Fanconi syndrome characterized by urinary wastage of solutes like phosphate, uric acid, glucose, amino acids, low-molecular-weight proteins as well as bicarbonate. A vast array of rare tubular disorders may cause proximal RTA but most commonly it is induced by drugs. With the exception of carbonic anhydrase inhibitors which cause isolated proximal RTA, drug-induced proximal RTA is associated with Fanconi syndrome. Drugs that have been recently recognized to cause severe proximal RTA with Fanconi syndrome include ifosfamide, valproic acid and various antiretrovirals such as Tenofovir particularly when given to human immunodeficiency virus patients receiving concomitantly protease inhibitors such as ritonavir or reverse transcriptase inhibitors such as didanosine.

Keywords: drug-induced pRTA; hereditary pRTA; proximal RTA; renal tubular acidosis

Introduction

Bicarbonate is freely filtered, and its concentration in the glomerular filtrate is equal to that in plasma (~25 mEq/L). The majority of the filtered HCO₃⁻ (~80%) is reabsorbed in the proximal tubule and the remaining 20% is reclaimed by the loop of Henle, distal tubules and collecting tubules. In an individual with a glomerular filtration rate (GFR) of 100 mL/min, ~2500 mEq of HCO₃⁻ is filtered daily, and virtually all of this HCO₃⁻ is reabsorbed so that essentially none appears in the urine [1]. Because the vast majority of HCO₃⁻ is normally reclaimed in the proximal tubule, the finding of urinary HCO₃⁻ wastage is usually taken as evidence of a defect in proximal tubular HCO₃⁻ reabsorption [1]. When there is a major defect in HCO₃⁻ reabsorption in the proximal tubule, a larger quantity of filtered HCO₃⁻ is delivered to the distal segments, including the thick ascending limb, overwhelming the distal system and thereby causing urinary HCO₃⁻ wastage, which is the hallmark of the derangement underlying proximal (or Type II) RTA [1].

In recent years, there has been significant progress in the understanding of the mechanisms causing genetic proximal renal tubular acidosis (RTA) [2–8]. Moreover, new causes of drug-induced RTA have been recognized [9, 10]. In this review, we will discuss the mechanisms involved in the causation of hereditary proximal RTA as well as acquired causes with an emphasis on drug-induced proximal RTA.

Isolated proximal RTA versus Fanconi syndrome

Proximal RTA as an isolated defect in HCO₃⁻ transport is rare. It is characterized by a decreased rate of HCO₃⁻ reabsorption in the proximal tubule in the absence of alterations in the transport of other solutes. The impairment in HCO₃⁻ reabsorption was initially characterized as a decrease in the renal threshold for HCO₃⁻ reabsorption [11, 12].

More commonly, proximal RTA is associated with generalized dysfunction of the proximal tubule as part of the Fanconi syndrome [1, 9]. Isolated proximal RTA can be autosomal dominant, autosomal recessive and sporadic (Table 1). The autosomal recessive type is associated with severe growth retardation, ocular abnormalities such as glaucoma, cataracts and band keratopathy, and mental retardation [13]. Autosomal dominant proximal RTA, to our knowledge, has been reported in a single Costa Rican...
family [14]. The clinical features include mild growth retardation and reduced bone density. Sporadic isolated proximal RTA is a non-familial transient disorder that has been reported during infancy. Patients with this disorder have defective renal and intestinal bicarbonate reabsorption [13].

Autosomal dominant and autosomal recessive proximal RTA is usually permanent and requires lifelong alkali therapy. In contrast, sporadic isolated proximal RTA is transient and alkali therapy can be discontinued after several years. After discontinuation of the alkali therapy, the condition does not return. This defect has been ascribed to proximal tubular immaturity in some infants [12].

An isolated defect of proximal tubular HCO₃ reabsorption is also caused by the use of carbonic anhydrase inhibitors (see drug-induced proximal RTA). The kidney has at least two forms of carbonic anhydrase, CA II and CA IV [15]. CA II is found in the cell cytoplasm of both proximal and distal tubules and is also present in red blood cells, whereas CA IV is mainly found in the brush border of the proximal tubule. The latter is the isoform of CA involved in facilitating apical HCO₃ reabsorption by preventing the luminal accumulation of H₂CO₃ and thus creating a more favorable pH gradient for H⁺ secretion [16] (Figure 1). A derangement in CA IV expression or function could specifically lead to impairment of proximal HCO₃ reabsorption, but to our knowledge, CA IV deficiency has not been demonstrated in patients with hereditary proximal RTA. By contrast, familial RTA has been long recognized in patients with inherited deficiency of CA II in red blood cells [17–20]. These patients had features of both proximal and distal RTA (Type III RTA) as well as osteoporosis, cerebral calcification, and mental retardation [17, 19–22]. Hereditary distal RTA with associated features of proximal tubular dysfunction (low molecular weight proteinuria, generalized hyperaminoaciduria, hypophosphatemia with hyperphosphaturia, and hypouricemia with hyperuricosuria) was described in two siblings with ATP6V1B1 mutation [23]. This rare association has never been fully understood although may go away after correction of the hypokalemia.

Fanconi syndrome is caused by a generalized dysfunction of proximal tubules causing loss of solutes like phosphate, uric acid, glucose, amino acids, low-molecular-weight proteins and bicarbonate [8, 24]. The genetic causes of Fanconi syndrome can be primary or secondary to systemic diseases. Primary Fanconi syndrome is caused by a missense mutation in Nα phosphate cotransporter (NaPi-II) of the proximal tubular apical membrane [25]. The secondary causes of Fanconi syndrome include inherited cystinosis, galactosemia, hereditary fructose intolerance, tyrosinemia, Lowe syndrome, Alport syndrome, Wilson disease and mitochondrial disorders [8, 24, 25]. The most common inherited cause is cystinosis [26].

The acquired causes of Fanconi syndromes with proximal RTA include amyloidosis, multiple myeloma, paroxysmal nocturnal hemoglobinuria, renal transplantation, antiretroviral drugs, ifosfamide, cadmium and lead [27–30]. Of these, light-chain associated Fanconi syndrome is the most common [28]. Other causes of Fanconi syndrome are discussed under drug-induced proximal RTA (Table 2).

### Pathophysiology and clinical diagnosis

HCO₃ is transported across into the peritubular space by a basolateral sodium-bicarbonate cotransporter (NBCe1), while H⁺ re-enters the tubular lumen mainly via a sodium-hydrogen exchanger (NHE) and to a lesser extent via H⁺-ATPase-driven H⁺ secretion (Figure 1). The secreted H⁺ then reacts with filtered HCO₃ to form H₂CO₃ [31, 32]. In the proximal tubule lumen, H₂CO₃ is catalyzed by membrane-anchored CA IV and CA XIV to H₂O and CO₂ [31–33]. Conversion to CO₂ facilitates reabsorption since HCO₃ is relatively impermeable to the apical membrane of proximal tubule. In contrast, CO₂ freely diffuses across the apical membrane into the cytosol. In the cytosol, CA II catalyzes the hydration of CO₂ to form H⁺ and HCO₃ which are then transported out of the cell via specific transporters (Figure 1).

Theoretically, proximal RTA could result from a defect in the basolateral NBCe1, the apical NHE, the apical H⁺-ATPase or carbonic anhydrase deficiency of either carbonic anhydrase II (cytosolic) or IV (brush border bound) (see Figure 1). So far, mutations have only been identified in NBCe1 and carbonic anhydrase genes [5]. The diagnosis of proximal RTA is established by the finding of urinary HCO₃ wasting usually manifested by very alkaline urine. Patients with proximal RTA, however, have an intact ability to lower urine pH < 5.5 when the plasma HCO₃ is less than the renal threshold for tubular HCO₃ reabsorption (see Figure 2).

The most elegant and comprehensive way of confirming the diagnosis of proximal RTA is to assess HCO₃ reabsorption over a wide range of plasma HCO₃. This can be done when the plasma HCO₃ is increased from low levels slowly by the administration of NaHCO₃ (HCO₃ titration test). A marked increase in urinary HCO₃

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**Table 1. Causes of isolated proximal RTA**

<table>
<thead>
<tr>
<th>Genetic causes</th>
<th>Acquired causes</th>
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<tr>
<td>NBCe1 mutation</td>
<td>Carbonic anhydrase inhibitors:</td>
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<tr>
<td>CA II mutation (mixed RTA)</td>
<td>Acetazolamide, Methazolamide, Dorzolamide</td>
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<td></td>
<td>(topical only), Topiramate, Dichlorphenamide,</td>
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<td>Brinzolamide</td>
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**Fig. 1.** Proximal tubule bicarbonate reabsorption. CAII/IV: carbonic anhydrase II/IV; NHE3, Na⁺/H⁺ exchanger 3; NBC1, Na⁺/HCO₃ cotransporter.
Excretion occurs normally and this is reflected by an increase in urine pH as plasma HCO₃ rises above the renal threshold for bicarbonate reabsorption. Below a certain threshold, however, bicarbonate excretion in patients with proximal RTA ceases and the urine pH can fall almost normally (Figure 2) [34]. This is a distinctive feature from patients with distal RTA (or Type 1 RTA) in whom the urine pH cannot fall normally regardless of the degree of acidosis (Figure 2). In normal infancy, the renal threshold is ∼22 mEq/L, whereas in older children and adults it is around 26 mEq/L [35]. In the steady state, the level of plasma HCO₃ in patients with proximal RTA is close to the renal threshold. For instance, a patient with renal threshold of 18 mEq/L has a metabolic acidosis with a level of plasma bicarbonate 18 mEq/L. In this steady state, there is no bicarbonate wasting and the urine pH can be reduced close to normal levels [12].

A fractional HCO₃ excretion of ≥15% clearly establishes the diagnosis of proximal RTA [36, 37]. Even lesser degrees of HCO₃ wastage (i.e. fractional HCO₃ excretion above 5%) are, in our opinion, sufficient for the diagnosis of less severe types of proximal RTA [36–38]. When fractional bicarbonate excretion is >15%, this reflects massive bicarbonate wastage as a result of a severe defect in proximal tubular transport which is severe enough that the capacity for bicarbonate reabsorption in the thick ascending limb of Henle’s loop and more distal nephron segment is overwhelmed. More subtle defects in proximal bicarbonate transport likely go clinically unrecognized owing to compensatory reabsorption of bicarbonate in the distal nephron segment.

Glucosuria in the face of normal blood glucose, aminoaciduria, hyperphosphaturia and hyperuricosuria characterize the presence of Fanconi syndrome [39, 40]. Hypokalemia and renal potassium wasting can also be present in patients with proximal RTA associated with Fanconi syndrome [12]. Unlike patients with distal RTA, urine PCO₂, measured after HCO₃ infusion, should be normal in patients with proximal RTA (i.e. >70 mmHg), indicating that their distal H⁺ secretion is intact [41].

### Hereditary proximal renal tubular acidosis

Inherited proximal RTA has been described as autosomal dominant, autosomal recessive or sporadic. Autosomal recessive inheritance in isolated proximal RTA has been...
linked to mutations in NBCe1 [2–7, 42]. Although the Na+/H+ exchanger 3 (NHE3) has been suggested as a candidate gene [43], to date there has been no reports of NHE3 mutations in proximal RTA patients [7]. It is of note, however, that isolated proximal RTA is very rare [2].

Autosomal dominant inheritance has been reported in a single Costa Rican family, but the gene involved has not yet been identified [14]. Lemann et al. studied some features of pRTA in two brothers from this family [14]. One brother was 20 years old, with short stature, bilateral coloboma and idiopathic subaortic stenosis. The other was 25 years old and asymptomatic. When untreated with bicitibrate, both brothers were acidic with a urine pH of ≤5.0 consistent with proximal RTA. The asymptomatic brother had serum bicarbonate ranging from 17 to 19 mEq/L, while the other brother had bicarbonate in the range of 11.5–14 mEq/L. Radiological investigation revealed reduced bone density in both brothers.

Katzir et al. described the only other family with autosomal dominant isolated proximal RTA wherein the father and all four children had a very high fractional excretion of bicarbonate (40–60%) following bicarbonate loading [2]. They did not find mutations in any of the nine candidate genes they studied including carbonic anhydrases, NBC1 and NHE3.

Most of the proximal RTA associated with inherited causes of Fanconi syndrome result from metabolites accumulating in the proximal tubules overtime, and preventing its proper functioning [10]. In the case of inherited cystinosis, there is evidence of ATP depletion in proximal tubular cells [26]. It has been suggested that ATP depletion leads to an increase in intracellular sodium concentration which would impair all transporters that depend on it for energy [10].

Na-HCO₃ cotransporter (NBCe1) mutations

The gene that encodes NBCe1 is SLC4A4 [13]. Three isoforms of NBCe1 have been described. NBCe1-A is expressed in the kidney and eye, NBCe1-B is expressed in pancreas, duodenum, colon and several other tissues, and NBCe1-C is predominantly expressed in the brain [44]. Mutations in the kidney isoform (NBCe1-A) cause proximal RTA and associated ocular abnormalities such as band keratopathy (Figure 3). Almost all cases described till now have shown autosomal recessive inheritance [3–6, 45–48].

Igarashi et al. were the first to report NBCe1 mutations in two unrelated patients having isolated proximal RTA with ocular abnormalities [5]. Both the patients were female, one presented at age 16 while the other presented at age 2. Their parents were normal. The common features were proximal RTA, short stature, bilateral glaucoma, cataracts and band keratopathy. Intellectual impairment was also reported in the 16-year-old patient. Serum amylase was interestingly elevated in both of them without signs of pancreatitis. This interesting association was attributed to the overlap between the kidney and pancreatic isoform of NBCe1 [5]. Plasma bicarbonate was low and urine was acidic. Genomic analysis revealed homozygous mutations in each patient, and heterozygous mutation in their asymptomatic parents. Missense mutations, R289S and R510, were identified [5].

Mutations leading to reduced activity and/or trafficking of the NBCe1 disrupt the normal bicarbonate reabsorption process of the proximal tubules leading to proximal RTA. Functional analysis has revealed reduced activity of R289S and R510 mutant NBCe1 [4]. Later studies have identified several other types of mutations in the NBCe1 gene [48]. Most of these mutations show abnormalities in trafficking as the likely mechanism of proximal RTA caused by NBCe1 mutations (see Figure 4).

Trafficking of mutant NBCe1 has been studied in Xenopus oocytes, ECV304 cells and polarized MDCK cells. Studies showed intracellular sequestration of mutants R410H, R510H, E91R and R342S [8]. Mutant S427L, while showing increased intracellular retention, also expressed on both apical and basolateral membranes [49]. Endoplasmic sequestration without cell surface expression was seen with mutant R881C [48]. Normal basolateral localization was observed with mutants T485S and G486R. Further investigation, however, revealed 50% reduction in transport activity [46, 47].

Using the substituted cysteine accessibility method, Zhu et al. showed that cysteine-substituted constructs R298C, S427C, T485C, G486C, L522C and A799C were processed to the plasma membrane, whereas R510C and R881C failed to process to the plasma membrane [44] (see Figure 4). In their experiment, a functional assay showed that constructs R298C, S427C, T485C, L522C and V799C have >50%, and R486C has 30–40% the transport function of NBCe1-A-5C (human NBCe1-A construct with five endogenous cysteines substituted with serines).

Carbonic anhydrase gene mutations

Deficiency of CA II is also the primary defect underlying the autosomal recessive syndrome of osteopetrosis, RTA and cerebral calcification [17, 22]. In this disorder, it causes a mixed pattern of proximal and distal RTA (Type III RTA). Early onset of hypokalemia, paroxysmal muscle weakness, moderate-to-severe mental retardation and growth retardation are the other associated manifestations.
The CA II gene is located at q22 on chromosome 8. At least 23 different mutations have been identified so far in different kindreds with mixed RTA [8, 19, 50–52]. The syndrome of CA II deficiency, caused by these mutations, has a varied phenotypic presentation and has been diagnosed in a variety of ethnic backgrounds. It is particularly common in Arab populations of the Middle East. More than 70% of the reported cases of CA II deficiency syndrome are from these populations, probably the result of both a high rate of consanguineous marriages and an increased frequency of the CA II deficiency allele [19]. Patients of Arabic origin have a unique splice junction mutation at the junction of exon 2–intron 2 of the CA II gene (c.232+1 G>A) [53].

A study done in patients from Tunisia and Algeria traced the ancestry of all affected patients studied under an old Arab tribe of Helal who had settled there in the 10th century. Clinically, Arabic patients have a very severe phenotype. Unlike American and Belgian patients (H107Y mutations), severe cognitive impairment is a consistent feature [54]. This was suggested to be because missense mutations are phenotypically less severe than splice site mutations and frameshift mutations [19, 20]. However, later studies found frameshift mutation in an American patient with only mild learning disabilities [20].

Carbonic anhydrase II deficiencies are recessive mixed proximal–distal (Type 3) RTA [18]. Predominance of distal type of RTA (dRTA) has been reported in some
cases [55, 56]. The characteristic biochemical findings are bicarbonate wasting, inability to lower urine pH <5.5, a low urine-to-blood pCO2 difference in an alkaline urine and decreased NH4 excretion [8].

Studies in mice

Gawanes et al. described that a null NBCe1−/− mice, prepared through disrupting the SLC4A4 gene, exhibited severe metabolic acidosis, growth retardation, reduced plasma sodium, hyperaldosteronism, splenomegaly, abnormal dentition, intestinal obstruction and death before weaning [57]. Blood pH and bicarbonate were very low in the mutants. Impaired transepithelial bicarbonate secretion in the colon was reported, but there was no histopathological evidence of any pancreatic abnormalities [57].

Nakamura et al. reported proximal RTA in NHE3 (NHe3−/−) mutant mice wherein the mutant mice had reduced bicarbonate and fluid reabsorption in the proximal convoluted tubules by 61% and 69%, respectively [58]. Impaired intestinal absorption with diarrhea, lower blood pressure and increased aldosterone was also noted. TWIK-related acid-sensitive K+ channel 2 (TASK2) in the renal proximal tubules is involved in volume regulation [59]. Warth et al. produced Task−/− mutant mice, and reported a reduction in blood pH, and bicarbonate concentration in the mutant mice, whereas urine pH and bicarbonate were increased [60]. These findings were consistent with proximal RTA, and further studies suggested coupling of TASK2 activity to bicarbonate transport through external alkalinization [60].

Bicarbonate wastage and RTA have been well documented with Fanconi syndrome secondary to cystinosis in humans. A Ctns (cystinosis) (−/−) knock-out mouse model, however, did not show any significant difference in urinary bicarbonate excretion or urinary pH when compared with control wild-type mice at 10 months of age [61]. Increased urinary excretion of other biochemical parameters such as glucose, phosphate and potassium were noted in the knock-out mice consistent with Fanconi syndrome.

There has been a report of glucosuria and mild proximal RTA in patients with maturity onset diabetes of the young (MODY) Type 3, a condition resulting from a defect in a transcription factor called hepatocytes nuclear factor 1α (HNF1α). A knock-out mouse model of HNF1α, which developed glucosuria with evidence of Fanconi syndrome, suggested its important role in renal transport [62]. In a later study, knock-out mice had reduced expression of sodium/phosphate cotransporters 1 and 4 (NaPi-I and NaPi-IV). Interestingly, no effect could be shown on the major renal phosphate transporter NaPi-II [63].

Drug-induced proximal RTA

Carbonic anhydrase inhibitors cause isolated proximal RTA, whereas several drugs have been linked to the development of proximal RTA associated with Fanconi syndrome. Those include ifosfamide, oxaplatin, aminoglycosides, tenofovir, cidofovir, adefovir, didanosine, topiramate, valproic acid and others [9, 10] (Table 2).

Carbonic anhydrase inhibitors

It has been long recognized that carbonic anhydrase inhibitors, such as acetazolamide used to manage conditions such as glaucoma or increased intracranial pressure, cause isolated proximal RTA [9]. The main isoform of carbonic anhydrase found in the kidney is the membrane-bound and cytoplasmic carbonic anhydrase isoform, namely CA IV and CA II, respectively [71, 72]. CA II is more widespread and is present in almost all cells of the nephron, whereas the membrane-bound carbonic anhydrase, CA IV, has limited expression and is found mainly in the proximal tubule and is absent or expressed weakly in most segments of the collecting duct and the final segment of the proximal tubule [72]. Both isoforms of carbonic anhydrase play an important role in acid–base transport throughout the nephron [72]. In the tubular lumen, CA IV catalyzes the formation of CO2 and H2O from H2CO3. In the cytosol of tubular cells, CA II favors the formation of bicarbonate and hydrogen ion from CO2 and H2O that enter the cell (Figure 1) [72].

Other inherited proximal tubulopathies with acidosis

Lowe syndrome (oculocerebrorenal syndrome, OCRL) is an X-linked disease characterized by eye anomalies (mostly cataracts), mental retardation and Fanconi-like proximal tubulopathy [64]. The syndrome is attributed to mutations in the gene OCRL, encoding alpha-phosphatidylinositol (4,5)-biphosphate phosphatase (PIP2P) [65]. Most patients with Lowe syndrome develop significant metabolic acidosis and the disease should be distinguished from other hereditary renal acidosis disorders.

Dent’s disease is an X-linked recessive proximal tubulopathy, characterized by low-molecular-weight proteinuria and hypercalciuria with nephrocalcinosis and nephrolithiasis, as well as progressive renal failure [66]. Mutations of two different genes produce Dent’s disease: CLCN5 gene in two-thirds of cases [67], and OCRL, the same gene that causes Lowe’s syndrome in one-third of the patients [68]. Metabolic acidosis can be found in those patients with Dent’s disease due to OCRL gene mutations, and therefore should be considered in the differential diagnosis of proximal RTA, whereas those with CLCN5 mutations commonly do not develop metabolic acidosis. Patients with genetic Fanconi syndrome of Dent’s disease, Lowe syndrome and autosomal dominant idiopathic Fanconi can be differentiated on the basis of their distinct urinary proteomes and metabolomes using mass spectrometry and H-NMR spectroscopy [69].

Fanconi–Bickel syndrome is a rare but well-defined autosomal recessive entity, characterized by hepatorenal glycogen accumulation, proximal renal tubular dysfunction and impaired utilization of glucose and galactose, possibly as a result of a primary defect in monosaccharide transport across the tubular membranes. RTA is part of the clinical manifestations in the affected patients [70].
CA inhibitors have been used in clinical practice mainly to reduce elevated intraocular pressure in glaucoma or to treat mountain sickness [73]. Three of them (acetazolamide, methazolamide and dichlophenamide) can be administered systemically. The other two CA inhibitors (brinzolamide and dorzolamide) are applied topically. All CA inhibitors are sulfonamide derivatives and have the potential to cause proximal RTA [73].

The defect in bicarbonate reabsorption with CA inhibitors can be explained by inhibition of CA IV located in the apical membrane of the proximal tubule cells. It has been demonstrated that some CA inhibitors, such as acetazolamide and benzolamide, are less membrane-permeable than others and as such are not as effective inhibitors of cytosolic CA as membrane-bound CA [74]. Typically CA inhibitors, therefore, cause a pure proximal RTA as a result of inhibition of the membrane-bound CA IV isoform resulting in the isolated inhibition of bicarbonate reabsorption without any associated features of Fanconi syndrome [75].

**Ifosfamide**

Ifosfamide is an alkylating agent, which is used in the treatment of various cancers such as bone sarcomas, soft tissue sarcomas and testicular cancer [76, 77]. It is a synthetic analog of cyclophosphamide and, like cyclophosphamide, can cause hemorrhagic cystitis. The use of Mesna has reduced the occurrence of hemorrhagic cystitis but has shown no preventive effect on its tubular toxicity which leads to Fanconi syndrome [78].

The incidence of Fanconi syndrome in treated patients has been reported between 1.4 and 5% [78]. The toxicity of ifosfamide and its late onset following discontinuation have been well recognized in several studies [78–81]. Rossi et al. did a follow-up study of 75 patients who had received ifosfamide for various malignancies [78]. Over 31 months of follow-up, five patients developed renal Fanconi syndrome as demonstrated by the presence of hyperaminoaciduria, phosphaturia, glucosuria and low serum bicarbonate [78]. Seven patients developed generalized subclinical tubulopathy which was defined as an impairment of three or all four parameters of proximal tubular solute transport (amino acids, phosphate, glucose and sodium) in the absence of acidosis or metabolic bone disease [78]. They reported that generalized subclinical tubulopathy occurred before the development of Fanconi syndrome in all five cases and moderate reduction in creatinine clearance was also reported in them [78]. Most information on ifosfamide nephrotoxicity comes from children since the use of ifosfamide in pediatric oncology is common [79–81]. By contrast, studies reporting ifosfamide-related Fanconi syndrome in adult patients are rare [82, 83]. In a recent report, Farry et al. did a long-term assessment of ifosfamide-related renal toxicity in adult patients and reported a steady decline in the estimated GFR although none of the patients progressed to end-stage renal disease [84]. Given the development of renal toxicity after completion of ifosfamide therapy, it has been suggested that patients should be followed for the development of proximal RTA [85, 86].

Nissim et al., based on their studies on rats, demonstrated that the active metabolites of ifosfamide, chloroacetalddehyde (CAA), causes renal injury by inhibiting nicotinamide adenine dinucleotide (reduced) (NADH): ubiquinone oxidoreductase (C-I), one of the enzymes in the oxidative phosphorylation pathway [87] (Figure 5). These authors showed for the first time that CAA accumulates in the renal cortex following ifosfamide treatment. They further showed that the inhibition of (C-I) led to increased NADH and decreased nicotinamide adenine dinucleotide (NAD). Furthermore, administration of agmatine (AGM), a metabolite of arginine decarboxylation, with ifosfamide prevented these changes and raised cAMP level. AGM, therefore, has been suggested as one of the potential therapies for prevention against ifosfamide-induced tubular dysfunction including Fanconi syndrome [87]. Later studies by Yaseen et al. examined the adverse effect of CAA and found that it inhibited endocytosis in the rat proximal kidney tubules which was attributed to a CAA-induced decrease in ATP levels and inhibition of V-ATPase [88] (Figure 5).

**Oxaplatin and cisplatin**

Oxaplatin-induced proximal RTA has been described as both isolated proximal RTA and as part of Fanconi syndrome. It was first described in a patient who was being treated with it for adenocarcinoma of the colon and subsequently developed hypokalemic, hyperchloremic metabolic acidosis with a normal anion gap. Evidence of Fanconi syndrome was based on glycosuria and low serum phosphate level [89]. In another report, in a similar clinical setting of oxaplatin use for adenocarcinoma of colon, the patient developed bicarbonate wasting and severe hypokalemic, hyperchloremic metabolic acidosis with a normal anion gap but no other abnormalities, thus suggesting isolated proximal RTA [90].

Fanconi syndrome has also been described with cisplatin [91]. In mice, a marked increase in urinary concentrations of glucose, amino acids such as alanine, valine, leucine, methionine and trichloroacetic acid cycle metabolites such as pyruvate and lactate was found within the first 48 h of administration of cisplatin [92]. The amino acid profile present in the urine of cisplatin-treated mice preceded the elevations in serum creatinine. Electron microscopy data at Day 3 revealed a cytotoxic effect on the S3 segment of the proximal tubules. Aminoaciduria was explained by the toxic effect of cisplatin on amino acid transporters in the proximal convoluted tubule as almost 90% of amino acid transport occurs in the proximal convoluted tubule. It has been suggested that one of the possible mechanisms of cisplatin-induced proximal tubule nephrotoxicity is reduced expression and function of sodium-dependent glucose transporters (Figure 6) [92].

Portilla et al. have reported in previous studies that cisplatin inhibits peroxisome proliferator-activated receptor-alpha activity and consequently fatty acid oxidation resulting in proximal tubule cell death [92, 93]. Fibrate such as bezafibrate prevents this inhibition and thus, may be protective against cisplatin-induced proximal tubule cell death [93].
The uptake of glucose into the epithelial cells is mediated by luminal sodium-dependent transporters such as SLC5A1, SLC5A2, SLC6A18 and SLC16A7 [94]. Xu et al. reported that the entry of cisplatin into the tubular epithelial cells results in the transcriptional down-regulation of HNF1R and HNF1, which, in turn, leads to the reduction of messenger RNA (mRNA) levels of SLC5A1, SLC5A2 and collectrin [94]. Cisplatin also leads to the reduction of SLC6A18 and SLC16A7 mRNA. Collectrin, an analog of ACE2, which lacks ACE2 activity is involved in proximal tubule absorption of amino acids [95]. Inhibition of collectrin may be part of the proximal tubule defect causing proximal RTA but this, to our knowledge, has not been studied.

**Antiretrovirals**

Fanconi syndrome has been reported in human immunodeficiency virus (HIV)-positive patients on antiretroviral therapy [96]. Earle et al. described three cases of Fanconi in such patients. Each of these patients showed generalized tubular dysfunction with hypophosphatemia, metabolic acidosis, phosphaturia, glucosuria and generalized aminoaciduria. All three patients had glucosuria, two had proteinuria (1.6 g/24 h and 2.6 g/24 h), urinary phosphorus was high in all and aminoaciduria was present in two of them [96]. Serum analysis revealed hypophosphatemia in all, but serum bicarbonate was low normal. Two of them recovered on discontinuation of their respective antiretroviral drugs: tenofovir and adefovir. The third patient, who had been treated with cidofovir, an acyclic nucleoside phosphonate, for cytomegalovirus (CMV) retinitis 9 months before presentation, continued to require electrolyte replacement. Of note, all these antiretroviral drugs were nucleotide reverse transcriptase inhibitors. Proximal RTA has also been reported with two other nucleoside reverse transcriptase inhibitors lamivudine and stavudine [97].

In a review of Food and Drug Administration (FDA) Adverse Event Report System of Fanconi syndrome associated with Tenofovir use, it was found that the protease inhibitor, ritonavir (74%), and the nucleoside reverse transcriptase inhibitor, didanosine (43%), were most commonly used in combination with tenofovir, a nucleotide reverse transcriptase inhibitor [98]. In view of a previous drug interaction wherein the coadministration of lopinavir/ritonavir with tenofovir increased the systemic levels of tenofovir, it was suggested that these drug combinations are a risk for the development of Fanconi syndrome [99]. Therefore, patients receiving these combination drugs should be closely monitored for renal toxicity [98, 100]. It has also been suggested that factors other than drug combination may also potentiate tenofovir toxicity as 17% of the patients who did not take these combination drugs with tenofovir still developed Fanconi syndrome [98]. It is interesting to note that although tenofovir is used both to treat HIV and hepatitis B infection, Fanconi syndrome has been described more commonly in HIV patients [101]. This again points to the potentiation of tenofovir toxicity in HIV patients either through other concomitant drugs and/or HIV-associated renal damage.

Three mechanisms of tenofovir nephrotoxicity have been suggested including drug excretion in proximal...
Fig. 6. Schematic representation of the proximal tubule reabsorption processes perturbed in cisplatin- or gentamicin-induced renal Fanconi-like syndromes that are manifested by the urinary elevation of glucose, NAAs and monocarboxylates. The uptake of glucose, NAA and monocarboxylate by the epithelial cells is mediated by luminal sodium-dependent transporters such as SLC5A1, SLC5A2, SLC6A18 and SLC16A7. The sodium electrochemical gradient across the luminal membrane is provided by the activity of the basolateral sodium/potassium ATPase. The entry of cisplatin (denoted as ‘C’) or gentamicin (denoted as ‘G’) into the tubular epithelial cells results in the transcriptional down-regulation of HNF1R and HNF1, which in turn leads to the reduction of mRNA levels of SLC5A1, SLC5A2 and collectrin. Cisplatin or gentamicin treatment also leads to the reduction of SLC6A18 and SLC16A7 mRNA through unknown transcription factors. Both nephrotoxins also induce hypoxia in renal tubular cells, which leads to the transcriptional up-regulation and post-translational stabilization of hypoxia-inducible factor HIF1R. An increased amount of HIF1 up-regulates the transcription of basolateral GLUT transporters as one of the adaptive responses to proximal tubule injury. Up-regulated gene names are shown in red color, while down-regulated ones are shown in blue color. For schematic convenience, these regulated genes are shown next to each other, although they are located on different chromosomes inside the nucleus (source: Xu et al. [94]).

There have been few reports wherein didanosine has been implicated in the development of Fanconi’s syndrome in HIV patients [104–106]. D’Ythurbide et al. reported Fanconi syndrome and diabetes insipidus in an HIV patient being treated with didanosine, lamivudine, atazanavir and ritonavir [104]. On admission, the patient had hypophosphatemia, hypouricemia, hyperchloremic metabolic acidosis with a normal anion gap, normoglycemic glycosuria and low-molecular-weight proteinuria. High urinary phosphate excretion and high fractional excretion of phosphate indicated renal phosphate wasting. One month following discontinuation of didanosine, while continuing to treat with other drugs, the patient recovered completely from glycosuria, tubular proteinuria, metabolic acidosis and hyponatremia [104].

Anti-convulsant therapies

Topiramate is an anti-epileptic drug which is used mainly for seizure disorders and migraine prophylaxis [107]. Several studies have described the development of metabolic acidosis following treatment with topiramate [107–109]. Sacre et al. reported a 47-year-old woman treated with topiramate for 12 months for migraine prophylaxis who developed hyperchloremic metabolic acidosis with a normal GFR and positive urinary anion gap suggesting both proximal and distal RTA [110].

Studies have suggested inhibition of CA II by topiramate as the cause for the development of mixed RTA [73, 111]. Winum et al. found that topiramate is a very potent inhibitor of human CA Types II and XII and a medium potency inhibitor of Type IV CA [73]. Maryanoff et al. by contrast, found topiramate to have low activity against CA II [112]. However, X-ray crystallography studies have revealed a very tight association between bound topiramate and the active site of CA II consistent with topiramate’s very potent inhibitory activity against CA II [111]. This inhibition of cytosolic CA could explain very well the development of mixed RTA.

Rarely, long-term treatment with valproic acid, another anti-convulsant drug, can also cause Fanconi syndrome. Knorr et al. reported that an 8-year-old boy on valproic acid for 7 years was admitted to the hospital for status epilepticus with laboratory findings of Fanconi syndrome. On discontinuation of valproate, laboratory findings returned to normal 2 months later [113]. More recent reports have described similar findings with laboratory values returning to normal following discontinuation of valproate [114]. The mechanism is not clear but a direct effect of valproic acid on the mitochondria of the proximal tubules has been implicated [115, 116].

Aminoglycosides

There are a few case reports of aminoglycoside-induced Fanconi syndrome [117–119]. Ghiculescu et al. reported the development of Fanconi syndrome in a 53-year-old man treated for respiratory infections with gentamicin [117]. The patient was profoundly hypophosphatemic and moderately hypocalcemic, and discontinuation of gentamicin resulted in recovery.
The presence of glucosuria and aminoaciduria after exposure to gentamicin has also been reported [120]. In the case of gentamicin, recent in vitro and in vivo studies performed in LLCPK1 cells, as well as in mouse kidney tissue, have shown that aminoglycoside antibiotics reduce glucose reabsorption in kidney tissue by reducing mRNA, protein expression and function of the sodium-dependent glucose transporter, which is located in the apical membrane of the proximal tubule (Figure 6) [121].

Other drugs

Other antivirals used for opportunistic infections in HIV have also been implicated in the development of Fanconi’s syndrome [122]. Vittecoq et al. [122] reported the development of tubular dysfunction in HIV patients treated for CMV retinitis with cidofovir. On the fifth day of cidofovir treatment, a patient developed low serum bicarbonate, low serum phosphorous, nonselective proteinuria and glycosuria. Fanconi syndrome was diagnosed and a renal biopsy revealed degeneration and necrosis of proximal tubular cells [122]. Fanconi syndrome has also been reported after the administration of capicabine, irinotecan and bevacizumab [123]. L-Cationic amino acids, such as lysine and l-arginine, have a profound inhibitory effect on proximal bicarbonate reabsorption and can potentially cause proximal RTA [124].

Heavy metals

Heavy metals such as lead, cadmium and mercury have been reported to be associated with proximal RTA [125]. Chronic cadmium exposure has been reported to cause Fanconi syndrome [126]. Cadmium accumulates in the proximal tubular cells through receptor-mediated endocytosis of metallothionein-bound Cd (Cd–MT). Cd–MT complexes are degraded in endosomes and lysosomes which release free Cd$^{2+}$ into the cytosol. In the cytosol, it generates reactive oxygen species which leads to a cascade of damaging cellular events that can cause generalized proximal tubular dysfunction [126].

Miscellaneous causes

Proximal RTA as part of Fanconi’s syndrome has been reported with several conditions including vitamin D deficiency, multiple myeloma, amyloidosis, renal transplantation and paroxysmal nocturnal hemoglobinuria [127].

There have been several reports of proximal RTA, with or without Fanconi’s syndrome, in children with nutritional vitamin D deficiency or resistance to vitamin D action [128, 129]. There have also been reports of Fanconi syndrome in adult patients with vitamin D deficiency [127]. Taylor et al. reported that a 33-year-old African American woman with nutritional vitamin D deficiency, possibly as a result of various medical problems including paraparesis, developed Fanconi syndrome [127]. The patient was acidic with hypocalcemia and aminoaciduria. Using an ammonium chloride loading test and a bicarbonate infusion test, proximal RTA was diagnosed which resolved following 2 years of vitamin D and calcium therapy [127]. To our knowledge, the exact mechanism by which vitamin D deficiency leads to Fanconi syndrome is unknown.

Messiaen et al. have reported several cases of Fanconi syndrome as a result of multiple myeloma [28]. Although the exact pathophysiology of Fanconi syndrome in multiple myeloma has not been elucidated, it has been shown in several studies that kappa light chain accumulates in the lysosomes of the proximal tubular cells and is not degradable [28, 130, 131]. Possible mechanisms could be disruption of apical membrane recycling, and/or impaired ATP production [28]. In a more recent report, accumulation of kappa light chains in the absence of crystals was demonstrated in a case of Fanconi syndrome in a patient with Waldenstrom macroglobulinemia [131]. Rochman et al. reported development of Fanconi syndrome in a patient with chronic lymphocytic leukemia who presented with proximal RTA with hypokalemia, phosphaturia and glycosuria [27]. Kappa light chains in the urine, peritubular deposits and amyloid casts were found [27]. In the light of these studies, kappa light chain has been implicated as the key factor causing Fanconi syndrome in these patients [28, 130, 131]. Rikitake et al., however, reported lambda light chain proteinuria in a 57-year-old female who developed Fanconi syndrome and had renal amyloidosis with nephrotic syndrome for the last 5 years [132].

Both proximal and distal RTA have been reported after kidney transplantation, the distal type being much more frequently found [133, 134]. Friedman et al. reported the development of Fanconi syndrome alongside rejection episode 4.5 years following a renal transplant [134]. An immunologically mediated tubular dysfunction was suggested as the possible cause [134].

Hsiao et al. reported Fanconi syndrome and chronic kidney disease in a patient with paroxysmal nocturnal hemoglobinuria (PNH) and hemosiderosis [135]. The patient was a 57-year-old female who had PNH for the last 17 years and history of multiple blood transfusion. She had the following features of Fanconi syndrome: proximal RTA, hyperphosphaturia, glucosuria and aminoaciduria. A renal biopsy revealed heavy hemosiderin deposits in the proximal tubular cells and that explained the signs of the generalized proximal tubular dysfunctions in this patient [135].

Diagnosis of proximal RTA

The diagnosis of proximal RTA requires the demonstration of urinary HCO$_3^-$ wastage. This is evident whenever urine pH is high when the plasma bicarbonate is normal or slightly reduced. Patients with proximal RTA, however, have an intact ability to lower urine pH <5.5 when the plasma HCO$_3^-$ is lower than the renal threshold for bicarbonate reabsorption. This is in contrast to patients with classic or Type I RTA who cannot lower urine pH maximally regardless of the degree of the acidosis (Figure 2). Skeletal abnormalities and rickets are less common with proximal RTA than in classic, distal RTA.
Nephrocalcinosis and nephrolithiasis are usually absent in proximal RTA but are prominent features of distal RTA [31]. Growth retardation is also seen in proximal RTA as a result of metabolic acidosis [31].

Traditionally, a fractional HCO$_3^-$ excretion of ≥15% is said to be needed to establish the diagnosis of proximal RTA [36]. Given the large capacity of the distal nephron to reabsorb HCO$_3^-$, however, even minor degrees of HCO$_3^-$ wastage (i.e. fractional HCO$_3^-$ excretion ~5%) are, in our opinion, sufficient for the diagnosis. The most definitive way to diagnose proximal RTA is to assess HCO$_3^-$ excretion when plasma HCO$_3^-$ is increased by administration of NaHCO$_3$ (HCO$_3^-$ titration test). When proximal RTA is present, a marked increase in urinary HCO$_3^-$ excretion and urine pH occurs as plasma HCO$_3^-$ rises above the renal threshold, whereas HCO$_3^-$ excretion decreases and the urine pH falls when the plasma HCO$_3^-$ is reduced. Hypokalemia and renal potassium wasting are characteristic of distal RTA [137]. In proximal RTA, hypokalemia can also occur particularly during bicarbonate therapy as discussed under treatment. Glucosuria in the face of normal blood glucose, aminoaciduria, hyperphosphaturia and hyperuricosuria characterize the presence of Fanconi’s syndrome [39]. Additionally, the urinary proteines and metabolonemeses can be examined using mass spectrometry and $^1$H-NMR spectroscopy as it has been done in genetic forms of the renal Fanconi syndrome [69]. Ifosfamide nephrotoxicity results in tubular proteinuria, whereas the urine of a Fanconi syndrome patient contains mainly albumin. Under both the conditions, excretion of amino acids is greatly augmented [69].

Patients with a distal acidification defect (e.g. distal RTA) typically have a positive urine anion gap because of low NH$_4^+$ excretion, whereas in diarrheal states associated with metabolic acidosis, the urine anion gap is negative, reflecting the fact that NH$_4^+$ excretion is appropriately increased [138]. Information regarding NH$_4^+$ excretion from subjects with proximal RTA is limited. The excretion rates of NH$_4^+$ may not be reduced when compared with those of control subjects [11, 139, 140]. For instance, in a study by Brenes and Sanchez, the response to a 3-day acid loading test with NH$_4$Cl was evaluated in 8 patients with isolated proximal RTA and in 10 healthy control subjects [141]. In the basal state, all subjects with proximal RTA had rates of NH$_4^+$ excretion similar to those of the control subjects, suggesting a normal pattern of ammonium renal handling. On the third day of acid loading, however, their NH$_4^+$ excretion rates were significantly lower than those of controls, demonstrating impairment in maximal urinary NH$_4^+$ excretion [14, 141]. Given this latter finding, it is likely that the urinary anion gap in proximal RTA may not be as negative as in normal people with metabolic acidois. Although reduced, the amount of urinary ammonium may still be sufficient to result in a negative urine anion gap. Since these previous studies did not provide information on the urinary anion gap (UAG) in proximal RTA, additional information on this parameter is needed for the proper interpretation of UAG in proximal RTA. In distal RTA, the urine anion gap is consistently positive without exceptions [138].

**Therapy of proximal RTA**

Drug-induced proximal RTA caused by CA inhibitors is usually mild and readily reversible. One has to be very careful in patients with compromised kidney function because in this setting, metabolic acidosis can be severe with the use of CA inhibitors. Drug-induced proximal RTA associated with Fanconi syndrome usually reflects severe proximal tubular nephrotoxicity. In some cases, there are profound defects in bicarbonate and phosphate transport, such that the degree of metabolic acidosis and hypophosphatemia is severe requiring drug discontinuation. Discontinuation of the offending drugs such as thiazidrivorials usually leads to recovery, but the defect can be persistent after discontinuation with some agents such as ifosfamide.

Patients with inherited and acquired proximal RTA should be given HCO$_3^-$ supplements [140, 142]. In children, in particular, HCO$_3^-$ replacement therapy is critical for the prevention of growth retardation due to acidosis [142]. The magnitude of the bicarbonaturia that occurs when serum HCO$_3^-$ is normalized requires that large amounts of HCO$_3^-$ be administered (5–15 mEq/kg body weight). By adding a thiazide diuretic (e.g. hydrochlorothiazide 25–50 mg daily), the amount of bicarbonate can be reduced. Thiazides enhance proximal tubule and loop HCO$_3^-$ reabsorption by reducing the extracellular volume. A reduction in extracellular volume is sensed by the kidneys as a stimulus for tubular reabsorption of Na$^+$ and HCO$_3^-$, This way the use of thiazide diuretics by producing mild volume depletion allows for a reduction in the amount of bicarbonate to be given daily [143].

The combination of sodium bicarbonate and thiazide administration can unfortunately aggravate hypokalemia by promoting K$^+$ secretion in the cortical collecting tubule (CCT) as a result of enhanced distal HCO$_3^-$ delivery. By being relatively poorly reabsorbed in the CCT, the presence of bicarbonate in large amounts generates a negative transepithelial voltage that promotes K$^+$ secretion and urinary potassium wastage. Therefore, plasma K$^+$ must be carefully monitored when these therapies are used. A mixture of sodium and potassium salts is usually needed. We recommend Polycitra (K-Shohl solution, a mixture of sodium and potassium citrate). This oral solution contains 1 mEq of K$^+$ and 1 mEq of Na$^+$ per mL, and the citrate, when metabolized, is equivalent to 2 mEq of HCO$_3^-$ per mL.

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