Unravelling Renal Tubular Acidosis (RTA)

Robert Unwin
(Centre for Nephrology - London Epithelial Group - University College London)
An outline of my talk

• A definition of RTA

• Clinical aspects of RTA

• Underlying (molecular) mechanisms of RTA

• An unanswered question in (distal) RTA - the puzzle of potassium
A definition of **RTA**

Impaired renal acid excretion (as suggested by a raised urine pH) in the context of a:

- **Hyperchloraemic**, **normal** anion gap
- **metabolic acidosis** and **normal** GFR*

(*To distinguish it from 'uraemic acidosis' - CRF*)
The en(n)cumbered 'clinical' classification of RTA

| Type 1 | distal RTA (most common) | 'complete' and 'incomplete' (Oliver Wrong) | hypokalaemic |

| Type 2 | proximal RTA (often part of a renal Fanconi syndrome) |

| Type 3 | a poorly characterised mixture of 1 and 2 in infants and which may reflect an 'immature' renal tubule or, more generally accepted, CA(II) deficiency |

| Type 4 | hypoaldosteronism (real or apparent) | distal-like | hyperkalaemic (impared NH₄⁺ generation/excretion) |
To maintain systemic acid-base balance, the kidneys:

- **Reclaim** almost all the filtered $\text{HCO}_3^-$ (by $\text{H}^+$ secretion) – and also generate 'new' $\text{HCO}_3^-$ (from $\text{NH}_3/\text{NH}_4^+$ synthesis)

- **Excrete** ca. 40-70 mmol $\text{H}^+$ per day (~1 mmol/kg – by $\text{H}^+$ secretion) – depending on diet – to produce an acid (relative to plasma) urine
Main sites of $H^+$ secretion:

- **$HCO_3^-$ reabsorption** - **proximal** nephron (PT and LOH) - $>90\%$ of filtered load

- **Net acid excretion** and residual $HCO_3^-$ reabsorption (ammonium plus titratable acid minus bicarbonate) - **distal** nephron (DT & CD)
What can determine urine pH?

Urinary free $[H^+]$ depends on
- tubular $H^+$ secretion
- tubular fluid buffer content ($NH_4^+$ and $P_i$)

Urine pH can be $\uparrow$ because of
- infection (urea-splitting)
- left standing ($CO_2$ loss)
- high $NH_4^+$ content*$

(*A failure to lower urine pH following an acute acid load might indicate $\downarrow$ $H^+$ secretion OR $\uparrow$ $NH_3$ and $NH_4^+$ excretion)
Urine pH vs. Plasma bicarbonate in RTA

Plasma [HCO₃⁻] mM

Urine pH

Normal

Proximal RTA

Distal RTA

(Oxford Textbook of Nephrology - Soriano et al, 1967)
Diagnosis of **RTA**

- In the presence of systemic **acidosis** (plasma \( [\text{HCO}_3^-] \) < 20 mM), urine > 5.3

- Casual EMU (second void preferable) pH > 5.5* (and low citrate/creatinine ratio in alkaline urine)

**Tests of urinary acidification:**
- ‘short’ \( \text{NH}_4\text{Cl} \) (Wrong & Davies) (\( d\text{RTA} \))
- frusemide and fludrocortisone (modified Batlle) (\( d\text{RTA} \))
- bicarbonate loading and fractional \( \text{HCO}_3^- \) excretion (\( p\text{RTA} \))
- (urine - blood) \( p\text{CO}_2 \) (but is it valid in the presence of nephrocalcinosis?)
- urine anion (net charge) and osmolar gaps as surrogates for \( \text{NH}_4^+ \) excretion
Mechanisms of RTA

- A **failure** of tubular cell ion transport (H$^+$ or HCO$_3^-$) - including driving force (voltage defect - aldosterone, amiloride)

- A **failure** of H$^+$ generation (e.g. lack of carbonic anhydrase - CA(II))

- A **failure** to maintain an H$^+$ gradient - ‘leaky’ apical (luminal) cell membrane (e.g. amphotericin)

- A **failure** of NH$_4^+$ generation (e.g. in hyperkalaemia) or its ‘circulation’ within the kidney
**Proximal RTA**

- Fanconi syndrome - drug-induced, heavy metals, inherited metabolic disorders

- CA inhibitors - acetazolamide

- CA(II) mutation(s) - autosomal recessive (Sly et al)

- Na\(^+\)-HCO\(_3^–\) (NBC1 - SLC4A4) mutation(s) - autosomal recessive (Igarashi et al)

  Hypokalaemia variable
  Nephrocalcinosis/renal stones less common
**Distal (‘classical’) RTA**

**Clinical features:**
- Plasma $\text{HCO}_3^-$ can be <10 mmol/l (in pRTA usually higher), urine pH > 6.5
- **nephrocalcinosis** in 70–80% of adults
- $K^+$-losing (can also be $Na^+$-losing) – hypokalaemia is common
- **rickets/osteomalacia** in ~25% (growth retardation)

**Acquired** – autoimmune/MSK

**Inherited** – $\text{Cl}^-/\text{HCO}_3^-$ exchanger
- ($\text{SCLC}4\text{A}1$) (Bruce et al)
- $H^+$-ATPase (deafness)
  - ($\text{ATP6V1B}1, \text{ATP6V0A}4$) (Karet et al)
Causes of nephrocalcinosis (in 194 patients)

(Wrong, Hosp Update, 1985)
The 'short' NH$_4$Cl test vs. The 'shorter' F+F test

(Walsh et al, Kidney Int, 2007)
Empirical Treatment

- **Alkali therapy** in small doses (already used in ancient India for ‘calculi’) - 1–2 mmol/kg; target plasma [HCO$_3^-$] >20 mmol/l
  - to heal bones
  - prevention of stones/(limit progression of nephrocalcinosis?)

- K$^+$ supplements may be necessary
- (Vitamin D may be necessary)
- (Thiazide diuretics may help in pRTA)
- Long-term follow-up for renal stone complications
In essence...
$P_{SF}$ and stone risk

Effect of Treatment with Alkali on Risk of Stones on Average Patient with dRTA

(WG Robertson, personal communication)
Predicted pH vs. Measured pH in 24-h urine samples from stone-formers

Predicted Urinary pH vs. Measured Urinary pH

(Uncalculated data)
Acidosis and Potassium Excretion
Orloff, Kennedy and Berliner, 1953 (J Clin Invest):

...modifications of acid-base balance suggests that a relative preponderance of one ion will depress the secretion of the other by the renal tubule.

...a relative excess or deficit of hydrogen ion is sufficient to effect an inverse change in potassium secretion without necessarily a reciprocal change in the concentration of potassium within the cell.

(From Malnic et al, Am J Physiol, 1971)
Control vs. chronic (10d) acidosis in rats

(From Stanton & Giebisch, Am J Physiol, 1982)

(Unpublished data)
Along the collecting duct - potassium and sodium

K⁺ secretion

Distal Na⁺ delivery

Aldo. ↑ / HiK diet

Aldo. ⇔ / NK diet

Aldo. ↓ / LK diet

(From Ornt et al, Clin Res, 1987)
pH and renal K$^+$ secretion

Acidosis decreases K$^+$ secretion

Distal tubule K$^+$ secretion vs Plasma pH

(From Stanton & Giebisch, Am J Physiol, 1982)

Acidosis decreases K$^+$ channel opening

PC apical K$^+$ channel P$^o$ vs pH$_i$

(From Wang et al, Am J Physiol, 1990)
Acidosis: CD K⁺ secretion

**Chronic acidosis** (K⁺ excr. ↑)

Na⁺ and fluid delivery ↑

**Acute acidosis** (K⁺ excr. ↓)

pH ↓

\[
\begin{align*}
V_{bl} & \downarrow \\
Na^+/K^+\text{-ATPase} & \downarrow \\
H^+ & \uparrow \text{secretion}
\end{align*}
\]

V_{te} ↓

-70mV

Principal cell

Na⁺ excr. ↑

(2° hyperaldosteronism)

Lumen (-ve p.d.)

Blood

Acute acidosis

Na⁺ and fluid delivery ↑

3Na⁺ → 3Na⁺

2K⁺ → 2K⁺

K⁺ → K⁺

Na⁺ excr. ↑

(2° hyperaldosteronism)
Hypokalaemia in clinical RTA

Generally thought to be corrected by alkali therapy (though not always true in clinical practice)

Patients with ‘incomplete’ dRTA are also often hypokalaemic and require K supplements

Evidence for persistent K wasting in corrected RTA

(From Sebastian et al, J Clin Invest, 1971a, 1971b)

Possible explanations include:

- $\text{HCO}_3^-$ therapy itself (pRTA)
- secondary hyperaldosteronism
- nephrocalcinosis/TID in autoimmune dRTA
The anion exchanger AE1

**Erythrocyte:**
- structurally and functionally important
- 25% of erythrocyte surface membrane

**α-Intercalated cell:**
- acid-secreting cell of distal nephron
- different isoform (kAE1)
The anion exchanger AE1

**Erythrocyte:**
- structurally and functionally important
- 25% of erythrocyte surface membrane
- glycophorin A (GPA) acts as a chaperone protein transporting AE1 to cell membrane

**α-Intercalated cell:**
- acid-secreting cell of distal nephron
- different isoform (kAE1)
- no GPA - AE1 is transported to cell membrane as a dimer/tetramer
Distal RTA (dRTA) and AE1

\[ \text{H}^+ \text{ATPase} \]

\[ \text{CO}_2 + \text{H}_2\text{O} \Rightarrow (\text{CO}_2 + \text{H}_2\text{O}) \]

\[ \text{CA(II)} \]

\[ \text{HCO}_3^- \]

\[ \text{Cl}^- \]

\[ \text{?mutant AE1} \]
Three dRTA mutants are ‘trapped’ or mistargeted in MDCK cells

- **R589H** and **S613F** do not reach the plasma membrane
- **R901Stop** is mistargeted to the apical membrane

(G609R, a novel mutation is, in part, also mistargeted to the apical membrane, Rungroj et al, J Biol Chem, 2004)

(Toye et al, J Cell Sci, 2004)
### Band 3 (AE1) Mutations and Familial dRTA

#### European/US

- **ARG<sub>589</sub>⇒HIS/CYS/SER** - red cell SO<sub>4</sub><sup>2-</sup> transport ↓
- **SER<sub>613</sub>⇒PHE** - red cell SO<sub>4</sub><sup>2-</sup> transport ↑

#### SE Asian

- **GLY<sub>701</sub>⇒ASP** - red cell SO<sub>4</sub><sup>2-</sup> transport →
- **ALA<sub>858</sub>⇒ASP** - red cell SO<sub>4</sub><sup>2-</sup> transport ↓ ↓
- **ΔVAL<sub>850</sub>** - red cell SO<sub>4</sub><sup>2-</sup> transport ↓ ↓
- **SAO*/compound** - red cell SO<sub>4</sub><sup>2-</sup> transport ↓ ↓ ↓

Δ400-408* (AD - loss of anion transport)  
*Hypokalaemic paralysis  
(Bruce *et al*, J Clin Invest, 1997; Wrong *et al*, Kidney Int, 2002)
AE1 mutation

N-  
  R589H  
  R589C  
  R589S  
  R602H  
  G609R  
  S613F  
  G701D (SAO/S773P)  
  ΔV850 (SAO)  
  A858D (SAO)  
  Q795H (SAO)  

C-  
  A888L  
  R901X  
  V488M (red cell HS)

Mode of inheritance
(D, dominant; R, recessive)

Caucasian

SE Asian

(After Shayakul & Alper, Clin Exp Nephrol. 2004)
Clustering of dRTA-associated AE1 mutations in SE Asia

(Wrong et al, Kidney Int, 2002)
Renal biopsy tissue from a male patient with inherited ‘incomplete’ dRTA and the S613F AE1 mutation

Control  Bric 155 (AE1 C-term. ab)  S613F

Red, AQ2; Green, BRIC

(Walsh et al, NDT, 2007)
Renal biopsy tissue from a male patient with inherited ‘incomplete’ dRTA and the S613F AE1 mutation

Control  Bric 155 (AE1 C-term. ab)  S613F

Red, AQ2; Green, BRIC

(Walsh et al, NDT, 2007)
Renal biopsy tissue from a male patient with inherited ‘incomplete’ dRTA and the S613F AE1 mutation

Control, E11 (vH⁺-ATPase), S613F

Red, AQ2; Green, E11

(Walsh et al, NDT, 2007)
Autoimmune dRTA

Renal biopsy tissue from a female patient with inherited ‘complete’ autoimmune dRTA due to Sjogren’s syndrome

Bric 155

E11

Red, AQ2 ab; Green, BRIC ab

(Walsh et al, NDT, 2007)
More from the red cell - a clinical oddity or physiological clue?

Hereditary *spherocytosis* (HS)  
~20% have AE1 mutations

1 report of *dRTA* - *C*-terminal truncation (Rysava *et al*, NDT, 1997)

Family P - mother and 2 daughters with diagnosis of 'HS', but all were hyperkalaemic with $P_K > 6 \text{ mM}$: 'pseudo-hyperkalaemia' - temp.-dependent 'leak'

In fact, have a form of *stomatocytosis*

(Urinary acidification abnormal in mother, but normal in daughters?)
AE1 may not function only as an exchanger

AE1, in fish (trout), can function as a cation channel, which is NPPB-sensitive:

(Guizouarn et al, J Physiol, 2001)
AE1 mutations have now been identified in several families with ‘leaky’ rbc and familial spherocytosis/stomatocytosis, including Family P (S731P), all of which can behave as a ‘cation channel’:

*(From Bruce et al, Nat Genet, 2005)*
Our initial hypothesis

Schematic membrane topography of AE1

(Cation-leaky” in HSt

SE Asian

R589  S609  S613

Ext.

Int.

SA0

G701

R901

Anion transport - $^{36}$Cl$^-$ influx and intracellular pH

Cation transport - $^{86}$Rb$^+$ influx
Cation transport - $^{36}\text{Cl}^-$ influx inhibition and intracellular cation measurements

(Unpublished observations)
Is the cation channel activity of G701D stable when heterodimerised in the absence of GPA?

**Cation transport** - $^{86}\text{Rb}^+$ influx: wtAE1 and G701D AE1 without GPA (0°C)

This is consistent with functionally cation leaky AE1 being also present at the α-IC cell membrane in G701D heterozygotes

(Unpublished observations)
Prevalence and distribution of the G701D mutation

G701D is common in NE Thailand:
• allele frequency of 0.8%
• (Yenchitsomanus et al, Hum Genet 2003)

In NE Thailand-Idiopathic hypokalemia is common:
• Hypokalemic paralysis
• Hypokalemic sudden death syndrome (SUDS/Lai Tai)
  • (Nilwarangkur et al, QJM 1990, Nammanit, Lancet 1991)

Survivors and relatives of Lai Tai have:
• Low serum potassium and high erythrocyte sodium concentrations
  • (Tosukhowong et al, Am J Nephrol 1996)

Could the cation leaky behaviour of G701D contribute to these phenomena?
"An idea, like a ghost, must be spoken to a little before it will explain itself."

(Charles Dickens, 1812-1870)

“A merry Christmas to everybody! A happy New Year to all the world!”