TUBULAR HANDLING OF URIC ACID AND FACTORS INFLUENCING ITS RENAL EXCRETION: A SHORT REVIEW

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ABSTRACT

In this review, the authors briefly examine the most recent evidence concerning the role of several proteins involved in tubular urate transport. They also analyse the influence of extracellular volume, electrolyte disorders, acid-base imbalance, and insulin-resistance on renal clearance of uric acid.

Keywords: Uric acid, urate transport, tubular handling, urate clearance.

INTRODUCTION

Uric acid is produced from purine nucleotide metabolism. The formation of uric acid involves purine degradation to inosinic acid and hypoxanthine. The latter is converted by the xanthine oxidase enzyme to xanthine and uric acid. In most mammals, uric acid is then converted to a more soluble allantoin by hepatic uricase.[1] In humans it is not further degraded and constitutes a metabolic end-product that is mainly excreted by the kidneys and, to a lesser extent, by the gastrointestinal tract.[2] Uric acid is a weak acid with a dissociation costant of pK 5.75. Thus, at a physiological plasma pH of 7.4, uric acid is in its more soluble deprotonated form. Its serum level in normal conditions is 3.5–6.8 mg/dL and is determined by the balance of synthesis and excretion rates. Higher values are associated with gout, nephrolithiasis, progression of chronic kidney disease, diabetes mellitus, hypertension, and cardiovascular damage.[3-5] Since the majority of uric acid is excreted by the kidney following the filtration, reabsorption, and secretion processes, the knowledge of uric acid tubular handling helps us to understand the main pathophysiological mechanisms underlying the alterations of its metabolism and to identify new therapeutic targets.

TUBULAR HANDLING OF URIC ACID

The kidney plays a pivotal role in uric acid homeostasis and excretion. Traditionally, uric acid was considered to be freely filtered by the glomerulus and subsequently reabsorbed and secreted along the renal tubule with a fractional excretion of ~10%. Nevertheless, the molecules responsible for bidirectional transcellular urate transport remain partially unknown. In recent years, many studies have demonstrated the role of several proteins belonging to the organic anion transporter (OAT) family, which is involved in urate transport.

Initially, urate transporter (URAT) 1, encoded by the *SLC22A12* gene, was identified as a reabsorptive URAT on the apical membrane of the renal proximal tubule.[6] In humans, a mutation of this encoding gene has been associated to Type 1 renal idiopathic hypouricaemia, characterised by low serum level and high renal excretion of uric acid.[7] Several drugs, such as probenecid and losartan, could decrease the serum urate levels by exercising

inhibitory effects on URAT1, therefore confirming the important role of this molecule in the urate transport.

Besides URAT1, other molecules belonging to the OAT family are expressed on the tubular cell membrane. They form a renal tubular secretory pathway for organic anions including drugs and toxins, however, recent evidence suggests that these proteins also play a role in urate transport.[8-10] OAT1 and OAT3, encoded by the *SLC22A6* and *SLC22A8* genes, respectively, are localised to the basolateral membrane of the renal proximal tubules. In experimental murine models, the absence of OAT1 and OAT3 determines a defective renal excretion of urate;[10] OAT4, encoded by the *SLC22A11* gene, is instead expressed in the apical cell membrane.

Glucose transporter (GLUT) 9, encoded by the *SLC2A9* gene, was initially reported as a fructose or GLUT but recent evidence suggests that it is involved in voltage-dependent urate transport.[11-13] Two isoforms have been identified in experimental studies, GLUT9a and GLUT9b, localised on the basal and apical side of the cellular membrane, respectively. Thus, it has been hypothesised that GLUT9b regulates the luminal uptake and GLUT9a regulates the interstitial exit of urate.[14-16] In humans however, exogenous expression of GLUT9 has been confirmed only at the basolateral side of renal proximal tubules.

Breast cancer resistance protein, ATP-binding cassette sub-family G member 2 (ABCG2), is a protein expressed on the epithelial cells of several organs, especially the placenta, liver, and intestine, and mediates the transport of various chemical compounds including anti-cancer drugs. In the kidney, it is expressed at the apical side of proximal tubules. It has recently been reported to excrete urate, however given its higher expression in the liver and intestine, it likely contributes to the regulation of intestinal urate rather than renal excretion.[17,18] Paradoxically, in *ABCG2* knockout mice an increased renal excretion of urate has been reported, likely due to a compensatory effect or decreased intestinal excretion.[19] NPT (sodium-phosphate cotransporter) 1 and NPT4 proteins belong to the SLC17 family and they are expressed in the apical cellular membrane of proximal tubules. They were initially identified as sodium-dependent phosphate transporters[20] but subsequent studies revealed that they can contribute to *in vivo* excretion of several organic anions, including urate.[21]

In summary, URAT1, GLUT9, and OAT4 ensure the reabsorption of uric acid on the apical and basolateral sides of the tubular cell membrane, respectively. ABCG2, NPT1, and NPT4, provide exit from the cells in the tubular lumen (Figure 1). Other molecular mechanisms contributing to the renal uptake of uric acid at proximal tubular cells have been identified but their specific role and importance in human pathophysiology is still under investigation. Table 1 reports the various URATs, their localisation, and their pathogenetic role in human and animal disease states.

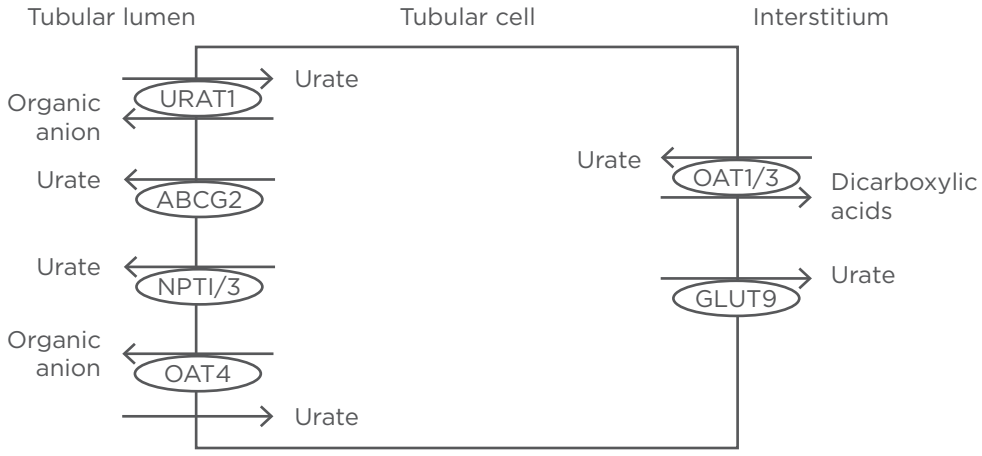

Figure 1: Schematic representation of the location and function of urate transporters on the cell membrane.

URAT1: Uric acid transporter 1; OAT: organic anion transporter; GLUT9: glucose transporter 9; NPT: sodium-phosphate cotransporter; ABCG2: ATP-binding cassette sub-family G member 2.

Table 1: Uric transporters, localisations, and pathogenetic role in human and animal disease states.

Urate transporters	Localisation	Pathogenetic role/related disease in human and animal
URAT1	Apical membrane of proximal tubule	Reabsorption of uric acid from tubular lumen/Type 1 renal idiopathic hypouricaemia in humans
OAT1/3	Basolateral membrane of proximal tubule	Luminal excretion of uric acid/ impaired renal excretion of uric acid in murine models
OAT4	Apical membrane of proximal tubule	Reabsorption of uric acid/ hyperuricaemia and gout in humans
GLUT9	Basolateral membrane of proximal tubule	Reabsorption of uric acid and interstitial exit/hyperuricaemia if upregulated, increased renal excretion if downregulated in humans. Increased renal excretion of uric acid in knockout mice
ABCG2	Apical membrane of proximal tubule, liver, intestine	Excretion of uric acid/ hyperuricaemia and gout with increased renal excretion of urate as compensatory function in humans

URAT1: Uric acid transporter 1; OAT: organic anion transporter; GLUT9: glucose transporter 9; ABCG2: ATP-binding cassette sub-family G member 2.

FACTORS INFLUENCING RENAL EXCRETION OF URIC ACID

The renal clearance of uric acid can be affected by several factors. Genome-wide association studies have demonstrated that single nucleotide polymorphisms of genes encoding for URATs can determine impaired or increased excretion of urate with consequent alteration of serum uric acid values.[22] Electrolyte alterations, especially sodium and chloride, may influence serum urate levels. It has been reported that a higher long-term sodium intake is associated with an increase of serum uric acid.[23] It has been established that each 1 g increase of sodium intake is associated with a 1.2 µmol/L increase in serum uric acid. Elevated serum uric acid could reflect the endothelial dysfunction of these subjects in response to high sodium intake, but it could also be consequent to altered function of NPTs in response to elevated sodium load into the proximal tubule. NPT activity can also be influenced by tubular chloride concentration as a result of its chloride dependence and inhibitor sensitivity.[24] In addition, electrolyte concentration can affect peritubular oncotic pressure and peritubular hydrostatic pressure or transtubular electrochemical gradient, thereby facilitating or slowing uric acid reabsorption. On the other hand, the state of hydration of the extracellular fluid compartment exerts an important influence on the tubular reabsorption of sodium and other filtered ions, including urate.[25] Contraction of extracellular fluid volume determines an activation of the renin-angiotensin-aldosterone system, leading to increased sodium ion (Na^+) reabsorption. This enhanced tubular uptake of sodium is associated with an up to 47% decrease in the clearance of uric acid and hyperuricaemia. Conversely, volume expansion results in a decrease in the net tubular reabsorption of uric acid.

As renal sodium reabsorption is the main method of regulating the extracellular volume, these findings suggest a close relation between reabsorption of sodium and urate in the proximal tubule. Other studies have suggested a similar relation between phosphate and uric acid reabsorption.[26] However, it has been reported that the enhanced urate clearance observed in patients with syndrome of inappropriate antidiuretic hormone secretion is not only due to an increase of effective vascular volume but also by an undetermined mechanism of vasopressin-1 receptor stimulation.[27] The well-known diuretic-induced hyperuricaemia may be attributed to reduced vascular volume but it has also been proposed that URAT1 could mediate urate-furosemide exchanges because furosemide is secreted into the lumen while enhancing urate uptake.[6]

Acid-base status can also influence uric acid clearance. We must first consider that OATs are not urate-specific; organic anions such as sulfate, phosphate, lactate, and ketones; as well as drugs,

metabolites, or other fixed acids excreted by the kidney can therefore interfere with the binding of uric acid to the receptors. Several studies[28,29] have demonstrated that alkalisation of urine increases uric acid excretion and that there is a direct relationship between the amount of excreted uric acid and luminal pH. Urinary pH largely changes in response to an acid or alkali load such as protein intake. Dairy consumption and an alkaline diet can reduce uric acid levels, increasing urate excretion.

In response to an acid load, the kidney increases reabsorption of filtered bicarbonate ions and excretion of hydrogen ions (H^+). H^+ is secreted in the lumen by Na^+/H^+ exchanger 3 (NHE3) and to a lesser extent by a proton pump. The Na^+/H^+ exchange by NHE3 plays a pivotal role for reabsorption of filtered bicarbonate ions and ammoniagenesis.[30] Within the tubular lumen, the secreted H^+ combines with filtered bicarbonate ions leading to carbonic acid formation. The latter is then split into H_2O and CO_2, which diffuses into the cell where it is rehydrated to carbonic acid. Ammonium (NH_3) is formed in the kidney by deamination of glutamine to glutamic acid. Once NH_3 has been protonated to ammonia (NH_4^+), this is secreted into the tubular lumen and subsequently excreted into the urine as ammonium chloride (NH_4^-Cl). Ammoniagenesis is the main renal system for the buffering and removal of H^+ from the body.

In the distal tubule, urine acidification is primarily achieved by a H^+-ATPase proton pump. In this instance, Na^+ reabsorption indirectly influences H^+ or potassium ion (K^+) excretion because the removal of cationic Na^+ from the tubular fluid makes the lumen more electronegative, thereby promoting the secretion of H^+ or K^+ into the lumen.[31] A low K^+ availability secondary to hypokalaemia as well as an acidic state determines an increased H^+ excretion in this site that contributes to urine acidification and maintenance of acid-base balance. For the same reason, anion chloride ions (Cl^-) can modify the negative charge into the tubular lumen, contributing to H^+ excretion and low urinary pH. Interestingly, urinary pH is simply a measure of free H^+ ions in urine but does not reflect renal net acid excretion because NH_4^+ and titratable acidity determine the majority of renal acid excretion. A reduced generation of NH_4^+ can determine an increase of urinary free H^+ and low urinary pH, consequently impairing renal uric acid excretion.[32,33]

Furthermore, as uric acid is a weak acid with a pK value of 5.35 in urine, a low urine pH can determine uric acid crystallisation and subsequent stone formation even if its excretion rate is normal. Observational studies as well as clinical experience suggests that the majority of pure uric acid stone formers exhibit lower urinary pH and fractional urate excretion when compared to healthy controls.[33,34] It has been postulated that the reason for a lower urinary pH in these subjects is a defective urinary ammonia excretion, likely associated to an insulin-resistant state.[33]

Diabetes mellitus and insulin resistance have been associated to low renal clearance of uric acid. The impact of insulin resistance, evaluated by a homeostasis model assessment, showed an inverse correlation between homeostasis model assessment and clearance of uric acid in patients with normal renal function.[35] Unfortunately in this study the relationship between insulin resistance and urinary pH was not investigated. Moreover, in healthy subjects, uric acid excretion was investigated after insulin infusion using the euglycaemic clamp technique. Insulin caused a statistically significant decline in fractional renal urate excretion.[36] Furthermore, uric acid stone formers showed a higher prevalence of metabolic syndrome, which is characterised by insulin resistance.[37]

Sodium-glucose cotransporter 2 (SGLT2) inhibitors are a new class of drug for the treatment of Type 2 diabetes mellitus, that act by blocking the tubular SGLT2-mediated glucose uptake. Patients treated with SGLT2 inhibitors showed that besides increased glycosuria, lower serum uric acid levels and increased urate excretion were present.[38,39] These findings support the hypothesis that hyperinsulinaemia, glucose levels, and tubular glucose uptake influence uric acid excretion. This influence can occur by regulation of GLUT9 activity which is also involved in glucose transport. Alternatively, we can hypothesise that insulin resistance determines low NH_4^+ production and that low urinary pH conditioning creates reduced uric acid excretion. Both these hypotheses could explain the epidemiological link between diabetes mellitus, hyperuricaemia, gout, and stone formation. In Table 2 the main factors influencing renal handling of uric acid in human diseases are summarised.

Table 2: Uric transporters, localisations, and pathogenetic role in human and animal disease states.

Factors influencing renal uric acid excretion	Related diseases
High sodium intake	Hypertension
Electrolyte disorders	Hypokalaemia, diarrhoea
Extracellular fluid volume	Dehydration, furosemide, SIADH
Acid-base balance	Acidosis, alkalosis
Urinary pH	Tubular acidosis, stone formation, impaired ammonia generation
Insulin resistance	Diabetes mellitus, metabolic syndrome

SIADH: syndrome of inappropriate antidiuretic hormone secretion.

CONCLUSIONS

Hyperuricaemia is associated with gout, hypertension, diabetes mellitus, and renal and cardiovascular damage. Serum uric acid levels mainly depend on uric acid synthesis and its renal excretion. Several proteins identified in recent years are involved in tubular urate transport and influence its renal clearance. In addition, clinical and experimental studies have shown that several haemodynamic and metabolic derangements can affect uric acid renal excretion. Therefore, genetic mutations, drugs, electrolyte disorders, acid-base imbalance, variation of effective vascular volume such as renin-angiotensin system alteration, reduced ammonia generation, and insulin resistance can influence urate renal clearance. These are all contributing factors to the occurrence of hyperuricaemia, gout, renal stone formation, and renal failure. The knowledge of the intrinsic homeostatic systems regulating uric acid excretion has a great impact in clinical practice because it provides us with a better understanding of pathophysiological alterations as well as aiding the development of further therapeutic strategies and novel drugs.

REFERENCES

1. Wu XW et al. Two independent mutational events in the loss of urate oxidase during hominoid evolution. J Mol Evol. 1992;34(1):78-84.

2. Sica DA, Schoolwerth AC. "Renal handling of organic anions and cations: excretion of uric acid", Brenner BM (ed.), The Kidney (2000), Philadelphia: WB Saunders, pp.680-700.

3. Feig DI et al. Uric acid and cardiovascular risk. N Engl J Med. 2008;359(17):1811-21.

4. Mazzali M et al. Elevated uric acid increases blood pressure in the rat by a novel crystal-independent mechanism. Hypertension. 2001;38(5):1101-6.

5. Mazzali M et al. Hyperuricemia induces a primary renal arteriolopathy in rats by a blood pressure-independent mechanism. Am J Physiol Ren Physiol. 2002;282(6): F991-7.

6. Enomoto A et al. Molecular identification of a renal urate anion exchanger that regulates blood urate levels. Nature. 2002;417(6887):447-52.

7. Ichida K et al. Clinical and molecular analysis of patients with renal hypouricemia in Japan influence of URAT1 gene on urinary urate excretion. J Am Soc Nephrol. 2004;15(1):164-73.

8. Ahn SY, Nigam SK. Toward a systems level understanding of organic anion and other multispecific drug transporters: a remote sensing and signaling hypothesis. Mol pharmacol. 2009 Sep;76(3):481-90.

9. Motohashi H et al. Gene expression levels and immunolocalization of organic ion transporters in the human kidney. J Am Soc Nephrol. 2002;13(4):866-74.

10. Sekine T et al. Molecular physiology of renal organic anion transporters. Am J Physiol. Renal Physiol 2006;290(2): F251-61.

11. Doblado M, Moley KH. Facilitative glucose transporter 9, a unique hexose and urate transporter. Am J Physiol Endocrinol Metabol. 2009;297(4):E831-5.

12. Vitart V et al. SLC2A9 is a newly identified urate transporter influencing serum urate concentration, urate excretion and gout. Nature genetics 2008;40(4):437-42.

13. Anzai N et al. Plasma urate level is directly regulated by a voltage-driven urate efflux transporter URATv1 (SLC2A9) in humans. J Biol Chem. 2008; 283(40):26834-8.

14. Anzai N et al. Urate transport: Relationship with serum urate disorder. Curr Rheumatol Rev. 2011;7:123-31.

15. Augustin R et al. Identification and characterization of human glucose transporter-like protein-9 (GLUT9):alternative splicing alters trafficking. J Biol Chem. 2004;279(16): 16229-36.

16. Reginato AM et al. The genetics of hyperuricaemia and gout. Nat Rev Rheumatol. 2012;8(10):610-21.

17. Woodward OM et al. Identification of a urate transporter, ABCG2, with a common functional polymorphism causing gout. Proc Natl Acad Sci U S A. 2009;106(25): 10338-42.

18. Noguchi K et al. Human ABC transporter ABCG2/BCRP expression in chemoresistance: Basic and clinical perspectives for molecular cancer therapeutics. Pharmacogenomics Pers Med. 2014;7:53-64.

19. Ichida K et al. Decreased extra-renal urate excretion is a common cause of

hyperuricemia. Nat Commun. 2012;3:764

20. Murer H et al. Proximal tubular phosphate reabsorption: molecular mechanisms. Physiol Rev. 2000;80(4): 1373-409.

21. Jutabha P et al. Human sodium phosphate transporter 4 (hNPT4/ SLC17A3) as a common renal secretory pathway for drugs and urate. J Biol Chem. 2010;285(45):35123-32.

22. Anzai N et al. Recent advances in renal urate transport: Characterization of candidate transporters indicated by genome-wide association studies. Clin Exp Nephrol. 2012;(1):89-95.

23. Forman JP et al. Association between sodium intake and change in uric acid, urine albumin excretion, and the risk of developing hypertension. Circulation. 2012; 125(25):3108-16.

24. Iharada M et al. Type 1 Sodium-dependent Phosphate Transporter (SLC17A1 Protein) is a Cl(-)-dependent Urate Exporter. J Biol Chem. 2010;285(34): 26107-13.

25.Weinman EJ et al. The influence of the extracellular fluid volume on the tubular reabsorption of uric acid. J Clin Invest. 1975; 55(2):283-91.

26. Staum BB et al. Tracer microinjection study of renal tubular phosphate reabsorption in the rat. J Clin Invest. 1972; 51(9):2271-6.

27. Decaux G et al. Evidence in hyponatremia related to inappropriate secretion of ADH that V1 receptor stimulation contributes to the increase in renal uric acid clearance. J Am Soc Nephrol. 1996;7(5):805-10.

28. Kanbara A et al. Urine alkalization facilitates uric acid excretion. Nutr J. 2010; 9:45.

29. Kanbara A et al. Effect of urine pH changed by dietary intervention on uric acid clearance mechanism of pH-dependent excretion of urinary uric acid. Nutr J. 2012;11:39.

30. Bruno CM, Valenti M. Acid-base disorders in patients with chronic obstructive pulmonary disease: A pathophysiological review. J Biomed Biotechnol. 2012;915150.

31. Eaton MP, Stone AM, "Neuromuscular Blockade", Rose BD, Post TW, (e.ds), Clinical physiology of acid-base and electrolyte disorders (2001), New York: McGraw-Hill, pp.406-26.

32. Kamel KS et al. Studies on the pathophysiology of the low urine pH in patients with uric acid stones. Kidney Int. 2002;61(3):988-94.

33. Sakhaee S et al. Pathophysiologic basis for normouricosuric uric acid nephrolithiasis. Kidney Int. 2002;62(3): 971-9.

34. Pak CYC et al. Biochemical profile of idiopathic uric acid nephrolithiasis. Kidney Int. 2001;60(2):757-61.

35. Perez-Ruiz F et al. Renal clearance of uric acid is linked to insulin resistance and lower excretion of sodium in gout patients. Rheumatol Int. 2015;35(9): 1519-24.

36. Ter Maaten JC et al. Renal handling of urate and sodium during acute physiological hyperinsulinaemia in healthy subjects. Clin Sci. 1997;92(1):51-8.

37. Fraile JM et al. Uric acid metabolism in patients with primary gout and the metabolic syndrome. Nucleosides Nucleotides Nucleic Acids. 2010;29(4-6): 330-4.

38. Ferrannini E et al. A Phase IIb, randomized, placebo-controlled study of the SGLT2 inhibitor empagliflozin in patients with Type 2 diabetes. Diabetes Obesity Metab. 2013;15(8):721-8.

39. Lytvyn Y et al. Glycosuria-mediated urinary uric acid excretion in patients with uncomplicated Type 1 diabetes mellitus. Am J Physiol Renal Physiol. 2015;308(2): F77-83.