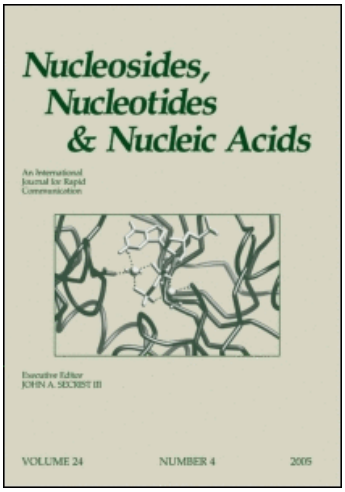

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Hitoshi Endou[ab]; Naohiko Anzai[b]
[a] Department of Pharmacology and Toxicology, Kyorin University School of Medicine, Tokyo, Japan [b] J-Pharma Co. Ltd., Tokyo, Japan

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URATE TRANSPORT ACROSS THE APICAL MEMBRANE OF RENAL PROXIMAL TUBULES

Hitoshi Endou[1,2] and Naohiko Anzai[1]

[1]*Department of Pharmacology and Toxicology, Kyorin University School of Medicine, Tokyo, Japan*
[2]*J-Pharma Co. Ltd., Tokyo, Japan*

□ *Since the molecular cloning of the renal apical urate/anion exchanger URAT1 (SLC22A12), several membrane proteins relevant to urate transport have been identified. In addition, the identification of PDZ (PSD-95, DglA, and ZO-1) domain protein PDZK1 as a binding partner of URAT1, and the emerging role of PDZ scaffold for renal apical transporters have led to a new concept of renal urate transport: urate-transporting multimolecular complex, or "urate transportsome," that may form an ultimate functional unit at the apical membrane of renal proximal tubules. Elucidation of urate transportsome will lead to the new drug development for hyperuricemia.*

Keywords Keywords Uric acid (urate); urate transport; URAT1; SLC22A12; PDZ; transportsome

Urate (uric acid) is the major inert end product of purine degradation in humans and higher primates in contrast to most other mammals because of the genetic silencing of the hepatic enzyme uricase, which oxidizes urate to water-soluble allantoin. Serum urate concentration is determined by the balance between production and elimination. The kidney plays a dominant role in urate elimination; it excretes ∼70% of the daily urate production. The transport mechanisms for urate are localized in the proximal tubule. In humans, urate is almost completely reabsorbed, resulting in the excretion of ∼10% of its filtered load. The absence of uricase and the presence of an effective renal urate reabsorption system contribute to higher blood

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Address correspondence to Hitoshi Endou, Department of Pharmacology and Toxicology, Kyorin University School of Medicine, 6-20-2, Shinkawa, Mitaka-shi, Tokyo 181-8611, Japan. E-mail: endouh@kyorin-u.ac.jp

urate levels in humans. The plasma urate concentration is much higher in humans (about 300 μM) than in other mammals. Therefore, it has been postulated that defects in tubular urate transport cause hypouricemia and that decreased renal urate clearance leads to hyperuricemia that induces gout, hypertension, and cardiovascular diseases. In both cases, urate transport proteins were proposed to be the key molecules, but the exact mechanism of renal urate transport has not been understood because of its complexity: the bidirectional transport of urate in the nephron and the species differences.

MOLECULAR IDENTIFICATION OF A URATE–ANION EXCHANGER URAT1 IN HUMAN KIDNEY

In 2002, we identified the long-hypothesized urate–anion exchanger in the human kidney (URAT1, encoded by *SLC22A12*).[1] Because urate exists primarily as a weak acid at physiological pH, we speculated that the urate transporter belongs to OAT (organic anion transporter) family. We started by scouring the human genome sequence for genes related to OAT genes and then found URAT1 gene (in silico cloning). URAT1 is expressed only in the kidney and located at the apical (luminal) membrane of proximal tubules, but not of distal tubules. URAT1 has transport activity of urate but not of various typical substrates of OATsand OCTs (organic cation transporters), indicating that URAT1 constitutes a specific pathway for urate reabsorption from the tubular lumen (extracellular) to the cytosol (intracellular) at the proximal tubules. Inhibition experiments revealed that the transport pathway is shared selectively by monovalent anions such as lactate, nicotinate, acetoacetate, and β-hydroxybutyrate. Pyrazinecarboxylic acid (PZA), nicotinate, and orotate, which are monocarboxylates with aromatic structures similar to that of urate having pyrimidine and imidazole structures, inhibited URAT1 more effectively. Various uricosuric substances known to reduce hyperuricemia such as probenecid, fenylbutazone, sulfinpyrazone, NSAIDs, diuretic drugs, and losartan effectively*cis*-inhibited URAT1 (Figure 1). Benzbromarone (a uricosuric agent clinically used in Japan) was the most potent, completely inhibiting urate uptake

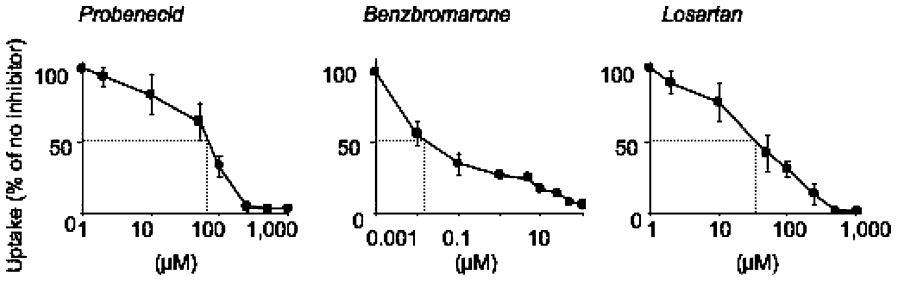

FIGURE 1 Inhibition of urate transport via URAT1 by representative uricosuric agents.

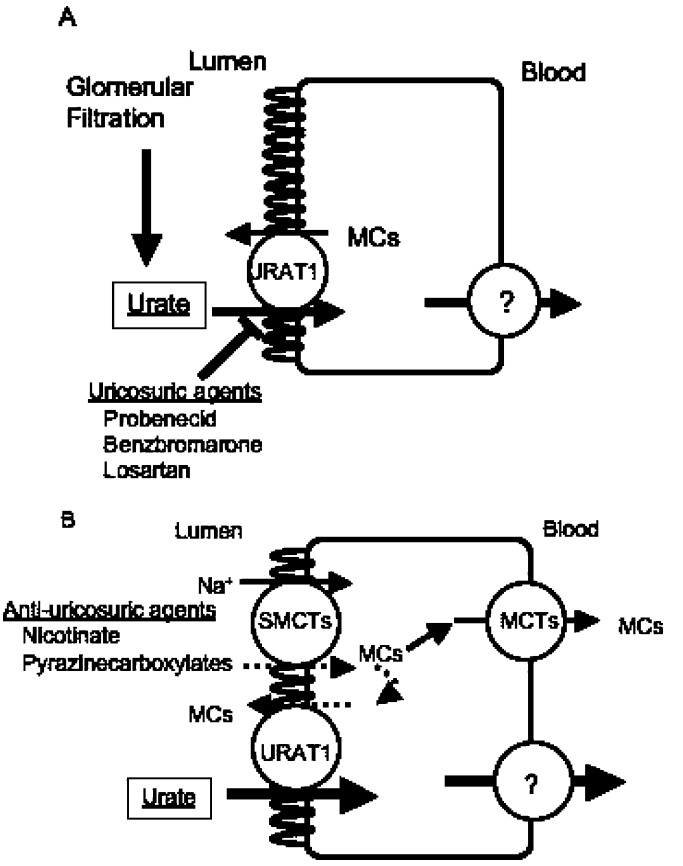

FIGURE 2 Proposed model of urate transport in renal proximal tubules. A) drugs with affinity for URAT1 will be uricosuric when acting from the lumen; B) drugs with affinity for URAT1 will be antiuricosuric by driving the influx of urate when acting from the intracellular space. MCs: monocarboxylates, MCTs: monocarboxylate transporters.

via URAT1 (Figure 1). The driving force for urate transport via URAT1 was determined, which is the exchange of luminal urate and intracellular organic anions. Intracellular accumulation of the organic anions for which URAT1 has affinity will favor the uphill reabsorption of urate in exchange for these anions, which move down their electrochemical gradients into the tubular lumen. Intracellular anions are made available mainly by their uptake from the glomerular filtrate across the luminal membrane via SMCTs (described below). Thus, drugs with affinity for URAT1 will be uricosuric when acting from the lumen, whereas they will be antiuricosuric by driving the influx of urate when acting from the intracellular space, consequently, regulating blood urate levels (Figure 2). And paradoxical effects by several agents, such as PZA, probenecid, and salicylates, will be explained as follows: at low doses, these agents cause urate retention

as a result of enhancement of urate reabsorption from the intracellular side, and at high doses, they inhibit urate rebsorption from the luminal side.

Therapeutics that are designed to modify URAT1 transport activities might be useful in treating pathologies that are associated with hyperuricemia such as gout and kidney stones. As described, URAT1 displays selective substrate specificity compared with other multispecific organic anion transporters, making it an attractive target of drugs that prevent urate reabsorption. Continuing studies into the pathways via URAT1 hold promise for the development of new, more effective therapeutics for hyperuricemia.

Kikuchi et al., Igarashi et al. and others have previously described patients with idiopathic renal hypouricemia (Mendelian Inheritance in Man 220150) with exercise-induced acute renal failure and chronic renal dysfunction.[2–5] Renal hypouricemia is a hereditary disease characterized by increased renal urate clearance caused by an isolated inborn error of membrane transport for urate in the renal proximal tubule (6). Patients with renal hypouricemia manifest low levels of serum urate levels (less than 2.0 mg/dl) without any underlying renal or systemic diseases such as Fanconi syndrome, Wilson disease, or drug-induced tubulopathy. They are usually asymptomatic except for the development of nephrolithiasis or exercise-induced acute renal failure. We and coworkersanalyzed *SLC22A12* (the URAT1 gene) in selected patients and found that some patients with renal hypouricemia have defects in *SLC22A12*.[1–3] Mutational analysis in a more systemic and larger population has been reported by Komoda et al.[7] and Tanaka et al.[8] Recently, Ichida et al. elucidated correlation between clinical and genetic features of patients with renal hypouricemia and described the significance of URAT1 in regulation of serum urate levels in vivo using 32 unrelated patients.[9] They detected *SLC22A12* mutations in 54 of 64 alleles, and ten mutations were identified, which included two nonsense mutations, six missense mutations, one splice-site mutation, and a 5-bp deletion. The most frequent mutation (74.1 %) was G→A transition at nucleotide 774 within exon 4 of *SLC22A12*, changing tryptophan-258 (TGG) to a stop codon (TGA) and producing premature truncated proteins that lack half of the protein (W258X).[9] Expression of the mutant cDNA in *Xenopus* oocytes revealed the truncated protein could not be targeted on the cell membrane, suggesting the mutant lost transport function completely. Interestingly, patients harboring the W258X mutation have been found in wide areas of Japan and also in Korea. We speculate how the allele was distributed throughout such wide areas. These findings obtained from the genetic studies revealed a pivotal role of URAT1 in the accumulation of higher urate levels in humans than in other mammals and bolster the case put forward that URAT1 is a key regulator of blood urate levels.

IDENTIFICATION OF URAT1 INTERACTING PROTEIN PDZK1

As described above, genetic studies of patients with renal hypouricemia indicate that URAT1 regulates blood urate levels and *vice versa*, that is, to control blood urate levels, the URAT1 transport function should be tightly regulated. Ichida et al. reported a novel genetic alteration of *SLC22A12* in a patient with renal hypouricemia, which is a 5-bp deletion (1639-1643del) that causes frameshift and amino acid sequence modification near the URAT1 extreme intracellular C-terminal region,[9] suggesting that the region is important for the function of URAT1. Interestingly, the region at the C-terminal end of URAT1 contains a binding motif for PDZ (PSD-95, DglA, and ZO-1) domain-containing proteins, which are known to participate in protein-protein interactions,[10] and it was found that the PDZ-binding motif was missing in the deletion mutation. Thus, we performed the yeast two-hybrid assay to investigate the putative URAT1 C-terminus-associated proteins that modulate its transport function.[11] We identified the multivalent PDZ domain-containing protein PDZK1[12] as an apparent partner of URAT1 in the kidney in vivo. In vitro binding assay revealed that this interaction requires the PDZ motif of URAT1 in its C-terminal region, and co-expression experiments demonstrated that URAT1 transport activities are increased by PDZK1/URAT1 interactions.[11] This results suggested function of PDZK1 as a scaffolding protein that may be a physiological regulator of URAT1 function. The identification of a URAT1 binding partner that enhances urate transport activity should lead to the development of novel type uricosuric agents by blocking the protein-protein interaction, as it is proposed in other type of PDZ interaction.[13]

URATE-TRANSPORTING MULTIMOLECULAR COMPLEX, URATE TRANSPORTSOME AT THE RENAL APICAL MEMBRANE

In the last few years, many studies concerning the PDZ interaction network have been performed in kidney proximal tubules.[14,15] In kidneys, the proximal tubule plays important roles for both the reabsorption of filtered substances and the secretion of water-soluble drugs, toxins, and metabolic waste products. These two transports of opposite direction are simultaneously accomplished by the coordinated action of ion channels and transporters located both in the apical and basolateral membrane. Thus, the polarized expression of these membrane proteins is essential for the function of the proximal tubular cells. Scaffolding proteins underneath the plasma membrane such as PDZ proteins are thought to contribute to the generation of cell polarity by the formation of junctional complex and by protein anchoring. In addition, proximal tubular transport processes are regulated by a number of factors that include hormones such as PTH. For efficient and specific signal transduction, for enhancement of membrane expression, and

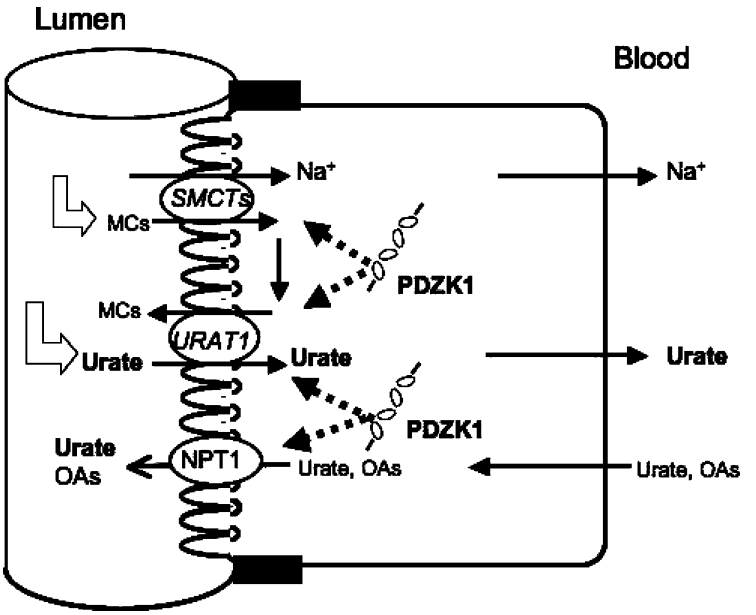

FIGURE 3 Model of urate transportsome. PDZK1 may tether URAT1 and NPT1, and/or URAT1 and SMCTs. OAs: organic anions.

for direct modulation of transport processes, the formation of functional complexes including transport proteins, hormonal receptors, and intracellular signaling elements are beneficial. This supramolecular structure supported by the scaffolds such as PDZ proteins are proposed to be an ultimate functional unit of membrane transport (Figure 3).

Although the introduction of molecular approaches led to the identification of several transport molecules and to examinations of the activity of single transport proteins, the precise mechanisms of renal apical handling of various organic anions are largely unknown. Application of the novel proteomics approaches based on the concept of a urate-transporting multimolecular complex, or "urate transportsome" may, however, contribute to the comprehensive understanding of the apical urate transport phenomenon in the proximal tubules.[16] Moe suggested in his short commentary[17] that this scaffold provides a platform for regulated apical urate uptake based on the ability of PDZK1 to cluster two urate-transporting proteins NaPi-I (mouse NPT1) and URAT1. This coupling may be one of the components of the apical urate transportsome. We have already observed that the Na^+-dependent monocarboxylate transporter 1 (SMCT1) C-terminus has a PDZ motif that binds to PDZK1.[18] Coupling between URAT1 and SMCT1 through a PDZ protein can thus form a single complex. With other unknown signaling elements, the apical urate transportsome is at least composed of several membrane transporters and PDZ

scaffolds (Figure 3). It may be responsible for the concerted regulation of urate transport at the apical membrane.

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