

Adjusting serum urate level by affecting membrane transporters involved in the disposition of urate

メタデータ	言語: eng 出版者: 公開日: 2017-10-05 キーワード (Ja): キーワード (En): 作成者: メールアドレス: 所属:
URL	http://hdl.handle.net/2297/37261

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**Adjusting serum urate level
by affecting membrane transporters
involved in the disposition of urate**

輸送体を介した血清尿酸値調節

Graduate School of
Natural Science and Technology
Kanazawa University

Major subject: Life Sciences
Course: Molecular Effects

School registration number: 1023032534

Name: Lu Yang

Chief advisor: Ikumi Tamai

Abstract:

This thesis investigates roles of urate-related transporters (e.g. URAT1/*SLC22A12*, URATv1/*SLC2A9*, and BCRP/*ABCG2*) in adjusting serum urate (SUA) level under physiological and disease conditions. In Chapter 2, functional cooperation of sodium-dependent monocarboxylate transporters (SMCTs) and urate transporter 1 (URAT1) was demonstrated indicating that SMCTs indirectly regulates urate reabsorption mediated by URAT1 function; therefore SMCT may serve as a potential target for the alteration of renal urate handling. In Chapter 3, interaction of whisky congeners with urate transporters was studied as a new rationale for their SUA-lowering effect. Since URAT1-mediated urate uptake was significantly inhibited by whisky congeners, it is conceivable that SUA-lowering effect of drinking whisky may be caused by blocking URAT1 by congeners abundantly contained in whisky. In Chapter 4, association of increased uremic toxins with extra-renal elimination of urate mediated by urate efflux transporter, breast cancer resistance protein (BCRP). In patients with chronic kidney disease, although renal function is greatly reduced, SUA level is relatively maintained. Indoxyl sulfate, as a model uremic toxin, induced significantly transport activity of BCRP in Caco-2 cells, such compensatory increase of extra-renal urate excretion may contribute to balance SUA. In conclusion, SUA can be adjusted directly or indirectly by urate transporters, providing their physiological significance in urate homeostasis.

Chapter 1: Introduction

Urate is a weak organic acid with a pKa value of 5.8. It is the end product of purine degradation in humans and some higher primates. Due to the mutation of urate oxidase gene occurred in Miocene epoch, urate in humans cannot undergo further oxidation catalyzed by urate oxidase (UOX1 or uricase) to form allantoin, a more water-soluble substance with being easily excreted. Loss of urate oxidase results in higher serum urate level (SUA) in humans, compared with those in other mammals. Epidemiologic studies show that abnormal SUA is associated with several diseases. For example, hyperuricemia (SUA over 420 μM for men, over 360 μM for women) may increase risk of gout attack as reported by many studies. It might also play a role in the development of coronary heart disease, stroke, hypertension, diabetes mellitus, renal diseases and other cardiovascular diseases. Because urate has anti-oxidant property, reduced SUA may cause harmful effect in humans. Indeed, hypouricemia (SUA lower than 120 μM) has already been linked to Hodgkin's disease and Alzheimer's disease. Thus, maintenance of normal SUA is crucial to human health. Due to the close relationship of urate with a number of diseases, it has been used as a biomarker for many diseases and is now involved in regular clinical blood test. Clinical and animal studies have also suggested the participation of urate in the generation and development of diseases, and the importance of controlling urate level as a preventing and/or treating method of such diseases.

Generation of urate primarily occurs in liver, muscles and intestine, while excretion of urate mainly occurs from the kidney and intestine. Of the urate daily produced, about two thirds is excreted from kidney and the rest is mainly eliminated directly across intestinal epithelial cells from blood. In kidney, there is a urate transport system located on renal proximal tubule which plays an important role in the regulation of SUA. Most of urate is filtrated at the glomerulus and reabsorbed by this transport system. Urate transporter 1 (URAT1, encoded by *SLC22A12*) was identified as a transporter responsible for reabsorption of urate at the apical membranes of proximal tubule cells, where it plays a predominant role in urate uptake from urine. Loss-of-function mutations of human URAT1 have been reported to result in hypouricemia. Previous studies found that many drugs (such as benzbromarone and losartan) could decrease SUA by exhibiting inhibitory effects on URAT1. These studies suggest an essential role of URAT1 in the transport of urate.

Similarly, organic anion transporter 4 (OAT4, encoded by *SLC22A11*) and OAT10, encoded by *SLC22A13*, are highly expressed at the apical side of proximal tubular cells and also involved in the reabsorptive transport of urate from luminal side into tubular cells. On the basolateral membranes of proximal tubular cell, a voltage-driven urate transporter, URATv1 (GLUT9) encoded by *SLC2A9* gene is recently reported as a solute carrier responsible for the urate transport from the tubular cells into blood. Hypouricemia was also found in patients with the loss-of-function mutations in URATv1, independent of genetics of URAT1. URAT1, OAT4, and OAT10 at the apical side of renal proximal tubule and URATv1 at the basolateral side of proximal tubule cells together consist of the vectorial transport from the urine to blood (as is shown in **Fig. 1**).

Since urate simultaneously undergoes secretion from blood to urine, other transporter system may also important to consider renal handling of urate in kidney. The system is possibly consisted of organic anion transporter 1 (OAT1, encoded by *SLC22A6*), and organic anion transporter 3 (OAT3, encoded by *SLC22A8*) on the basolateral side of proximal tubule cells, and sodium phosphate transporter 1 (NPT1, encoded by *SLC17A1*), NPT4 (encoded by *SLC17A3*), multidrug resistance protein 4 (MRP4, encoded by *ABCC4*), and breast cancer resistance protein (BCRP, encoded by *ABCG2*). SUA is also influenced by non-renal urate transport pathway. In humans, expression of BCRP in kidney is relatively low compared with other organs. On the contrary, expression of BCRP in intestine is high where one third of urate daily generated is excreted, compared with the expression in kidney. BCRP is expressed at the apical side of intestinal epithelial cells and mediates the urate efflux transport across the intestinal epithelial wall. Reduction of BCRP function is closely related to gout and hyperuricemia as is demonstrated by recent genome-wide association studies. Thus, BCRP can be regarded as an important efflux transporter mediating the non-renal excretion of urate.

With the development of economy, and westernization of lifestyle, the past several decades have witnessed an obvious increase in the prevalence of diseases such as cardiovascular disease, obesity, and diabetes mellitus, and the prevalence of these disease will continue to increase in the following several decades. SUA level seems to be closely associated with these diseases. Because of the important role of urate transporters in regulation of SUA level, adjusting of these urate transporters to control SUA is of critical significance. The present thesis will focus on this topic and investigate the modulation of urate transporters to affect SUA level.

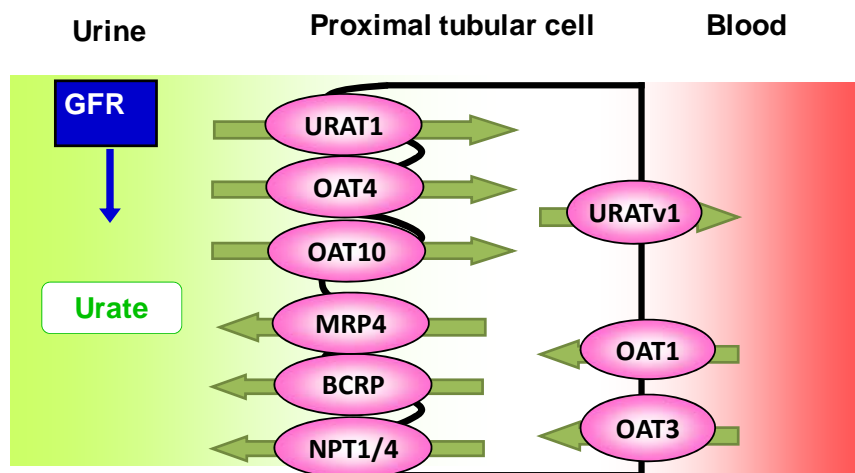


Fig. 1 Transporter mediated urate transport in renal proximal tubule. On the apical side of renal proximal tubule, URAT1, OAT4, and OAT10 are responsible for the reabsorptive transport of urate from luminal side into renal proximal tubular cell. On the basolateral side, URATv1 is responsible for the transport of urate from proximal tubular cell into blood. OAT1 and OAT3 on the basolateral side mediate the urate excretory transport from blood into proximal tubular cell. MRP4, BCRP, NPT1 and NPT4 located on the apical side functions as excretory transporter and transport urate from proximal tubular cell into urine.

Chapter 2: Functional cooperation of SMCTs and URAT1 for renal reabsorption transport of urate

SUA can be regulated by a urate transport system located on renal proximal tubule. Urate transporter 1 (URAT1) is located on the apical side of proximal tubules and is responsible for the reabsorption of urate from luminal side into tubular cells. At the same site, it has been hypothesized that sodium-coupled monocarboxylate transporters (SMCTs) are responsible for the transportation of monocarboxylates such as lactate and nicotinate, which are driving force for urate transport via URAT1 by exchange transport. Accordingly, SMCTs can enhance URAT1-mediated urate reabsorption by providing monocarboxylates for the exchange.

Chapter 2 aimed to clarify the hypothesized functional cooperative relationship between URAT1 and SMCTs in the reabsorptive transport of urate. By preloading nicotinate in SMCT1-URAT1 co-expressing *Xenopus* oocytes, URAT1-mediated urate transport was stimulated. Nicotinate was taken up by SMCT1 but not by URAT1. When removing sodium ion from the uptake medium, the stimulation effect of nicotinate was decreased. When adding SMCT1 inhibitors, the stimulation effect was also reduced. The results from this study indicate the cooperative relationship of URAT1 and SMCT1, and that SMCT1 is a potential target for the alteration of renal handling of urate indirectly (**Fig. 2**).

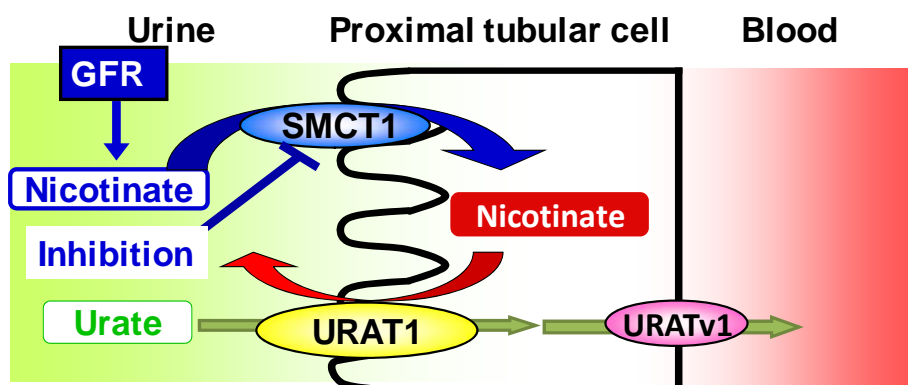


Fig. 2 Indirect regulation of SUA by affecting SMCT1.

Chapter 3: A putative mechanism of lowered SUA by whisky

Lifestyle and dietary factors are closely related to human health. Daily exposure of food is inevitable for all human beings. Influence of different kinds of food and beverage on human health has been realized since ancient times. In Chapter 3, a putative mechanism for the lowered SUA after whisky consumption was proposed and examined.

Consumption of alcoholic beverages has been a feature of many cultures throughout the world since ancient times, and especially in some Asian countries, is often associated with social gatherings. Many studies have shown that regular alcohol consumption is a risk factor for hyperuricemia and gout attack. However, recent clinical studies show that effect of moderate whisky consumption is quite different from the effect of consumption of other alcoholic beverages. Instead of increasing SUA as many other beverages do, it results in increased renal excretion of urate into urine and decreased SUA. Considering the prevalence of alcohol and whisky consumption worldwide, it is of critical significance to understand why this different effect happens. Since renal reabsorption influences SUA, we examined the effects of whisky congeners on urate transporters, URAT1 and URATv1, involved in reabsorptive transport of urate. In transporter-expressing *Xenopus* oocytes, 12- and 18-year old whisky congeners inhibited urate uptake by URAT1 with IC_{50} values of 0.084 ± 0.011 and 0.042 ± 0.006 mg/mL, respectively, while urate uptake by URATv1 was inhibited at 1 mg/mL. Results of this study suggested that decreased SUA after whisky consumption may be mainly due to inhibition of URAT1 by congeners (**Fig. 3**). Daily intake of food and beverage might influence SUA by affecting urate transporters.

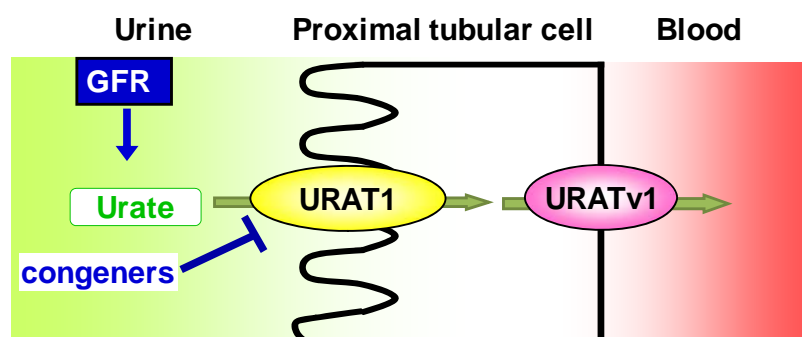


Fig. 3 Proposed mechanism for lowered SUA after whisky consumption. Whisky congeners may reduce SUA by inhibition of URAT1-mediated urate reabsorptive transport.

Chapter 4: Indoxyl sulfate upregulates BCRP expression in intestinal cell line

In Chapter 4, role of indoxyl sulfate on an important efflux urate transporter, BCRP, was investigated. At chronic kidney disease (CKD), renal function is greatly reduced. This leads to the tremendous increase of the concentrations of a great number of solutes. However, the rise of SUA is not much, compared with other solutes. This phenomenon can be explained by the compensatory increase of extra-renal urate excretion. Previous study shows that intestinal Bcrp is important in controlling SUA and its expression is increased in CKD rats. But the molecular mechanism remains unknown. In this report, we examined whether indoxyl sulfate (IS) is involved in the upregulation of BCRP in intestine at CKD state. After exposure to IS, mRNA level, and protein level of BCRP in Caco-2 cells, which are used as human intestinal cultured cell model, were assayed by real-time PCR and flow cytometry, respectively. BCRP mRNA level was increased by exposure to IS for 24 h in a concentration dependent manner and reached steady state at 0.2 mM, a clinically relevant concentration. BCRP expression in the plasma membrane was also increased by 1.8 folds after treatment with 0.2 mM for three days. Basolateral to apical transcellular transport of urate in Caco-2 cells had 22% increase after IS treatment. Intracellular accumulation of pheophorbide a, a functional marker of BCRP, was also decreased by 22% after IS treatment. IS was also observed to increase BCRP mRNA expression in LS180 cells and HepG2 cells, which are human enterocytes and hepatocytes cultured cell mode, respectively. However, no protein was expressed in plasma membrane of LS180 cells. BCRP protein expression in plasma membrane of HepG2 cells was increased after IS treatment. However, no function of BCRP was observed in HepG2 cells. Results indicate that indoxyl sulfate might be involved in the up-regulation of BCRP in intestine at CKD state, thereby minimizing an increase of serum uric acid level.

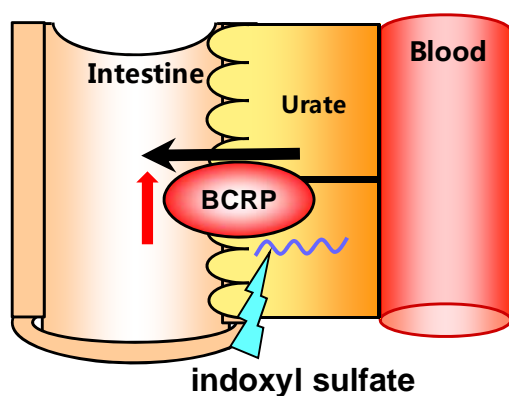


Fig. 4 Possible involvement of indoxyl sulfate in the induction of BCRP to increase intestinal urate excretion.

Chapter 5: Conclusion

This thesis shows that SUA can be regulated by directly or indirectly influencing urate transporters and humans have a self-compensatory mechanism to regulate SUA at disease state by modulation of urate transporters. The results of this thesis indicate that transporters involved in urate transport can be used as targets for developing novel compounds to control SUA.

学位論文審査結果の要旨

血清尿酸値は尿酸生合成と排泄により厳密に維持されている。高尿酸血症は痛風発作をはじめとして、高脂血症、高血圧、糖尿病など多くの生活習慣病の発症との相関が指摘されている。本研究において輸送体を介した尿酸調節機構が検討された。腎尿細管における尿酸再吸収において Na^+ 非依存性尿酸輸送体 URAT1 は支配的な役割を果たすが、従来から尿酸再吸収は Na^+ に依存することが知られていた。本研究では、腎尿細管上皮細胞に発現する Na^+ 依存性モノカルボン酸輸送体 (SMCT1) により取込まれた乳酸やニコチン酸の外向きの濃度勾配を利用した交換輸送によって URAT1 が尿酸を効率良く取込むことが *in vitro* 実験により初めて明らかにされた。また、血清尿酸値を低下させる食品中成分として古くから知られているウイスキーコンジェナーに着目し、コンジェナーによる URAT1 阻害が血清尿酸値低下の一因であることが実証された。さらに、尿酸排泄が低下する腎疾患において血中に増加する尿毒素物質であるインドキシル硫酸が小腸に発現する尿酸排泄輸送体 BCRP の発現を増加させることから、血清尿酸値維持にインドキシル硫酸を介した腎腸連関の重要性が指摘された。本研究において、複雑な尿酸排泄の分子機構が明らかにされ、食品成分あるいは病態に由来する内因性物質によっても、輸送体を介して血清尿酸値が変動することを示すことができた。以上、本研究成果は尿酸値調節を目的とした医薬品開発に有用な知見であると判断され、本論文は博士（薬学）に値すると判定された。