Review

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A Possible Therapeutic Application of The Selective Inhibitor of Urate Transporter 1, Dotinurad, for Metabolic Syndrome, Chronic Kidney Disease, and Cardiovascular Disease: A Significance of Inhibition of Urate Transporter 1 and Non-inhibition of Other Urate Transporters for Such Diseases

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A Possible Therapeutic Application of the Selective Inhibitor of Urate Transporter 1, Dotinurad, for Metabolic Syndrome, Chronic Kidney Disease and Cardiovascular Disease: A Significance of Inhibition of Urate Transporter 1 and Non-inhibition of Other Urate Transporters for Such Diseases

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Abstract: Renal uric acid (UA) reabsorption is mainly mediated by urate transporter 1 (URAT1) and glucose transporter 9 (GLUT9). Dotinurad selectively inhibits URAT1 and do not inhibit other UA transporters such as GLUT9, ATP-binding cassette transporter G2 (ABCG2) and organic anion transporter1/3 (OAT1/3). We discovered that dotinurad improved metabolic parameters and renal function in hyperuricemic patients. We consider a significance of high selectivity in inhibiting URAT1 by dotinurad for metabolic syndrome, chronic kidney disease (CKD), and cardiovascular disease (CVD). Dotinurad inhibits selectively URAT1 and increases UA in the proximal tubules, and UA may compete with glucose for GLUT9, reducing glucose reabsorption. The inhibition of UA entry via URAT1 to liver and adipose tissues by dotinurad increased energy expenditure and decrease lipid synthesis and inflammation in rats. The inhibition of URAT1 in kidney, liver and adipose tissues improve metabolic parameters. CKD patients accumulate uremic toxins such as indoxyl sulfate (IS) in the body. ABCG2 regulates renal and intestinal excretion of IS which strongly affects CKD. OAT1/3 inhibitors suppress IS uptake into kidney, increasing plasma IS, which produces oxidative stress, inducing vascular endothelial dysfunction in CKD patients. The high-selective inhibition of URAT1 by dotinurad may be beneficial for metabolic syndrome, CKD and CVD.

Keywords: ATP-binding cassette transporter G2; chronic kidney disease; dotinurad; hyperuricemia; organic anion transporter1/3; urate transporter 1

1. Introduction

Urate transporter 1 (URAT1) in the human kidney which is a urate anion exchanger regulating serum uric acid (UA) levels was identified in 2002 [1], and has been targeted by uricosuric agents. In humans, the reabsorption of UA into the blood plays a crucial role in regulating serum UA levels. The UA exchange is mediated by various molecules expressed in renal proximal tubule [2, 3] (Figure 1). UA enters the proximal tubule epithelial cells in exchange for monocarboxylate via apical URAT1 and for dicarboxylate via apical organic anion transporter (OAT) 4 [4]. OAT1 and OAT3 on the basolateral membrane of epithelial cells, transport UA from the renal interstitial into renal proximal tubule epithelial cells [4, 5]. Renal UA reabsorption is mainly mediated by URAT1 and glucose transporter 9 (GLUT9) [1, 6-8]. Apical GLUT9b plays a significant role in UA reabsorption, the reabsorbed UA exiting proximal tubule epithelial cells to blood through basolateral GLUT9a [4]. The

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ATP-binding cassette transporter G2 (ABCG2) has been identified as a high-capacity UA exporter that mediates renal and/or extra-renal (intestinal) UA excretion [9, 10].

Uricosuric agents have been developed targeting such UA transporters and have been used as therapeutic agents for hyperuricemia. Probenecid decreases serum UA by inhibiting URAT1 and GLUT9 [11]. Benzbromarone is a potent uricosuric drug that acts by inhibition of URAT 1 and GLUT 9 [12]. Lesinurad and arhalofenate are the inhibitors of URAT1 and OAT4 [11]. It has been difficult to accurately evaluate the function of URAT1 because previous uricosuric agents inhibit not only URAT1 but also GLUT9 and OAT4.

A highly selective inhibitor of URAT1, dotinurad, was developed [13] and is available in Japan. Unexpectedly, we found that dotinurad improved serum lipids, blood pressure, body weight, albuminuria and estimated glomerular filtration rate (eGFR), in addition to reduction in serum UA, in hyperuricemic patients complicated with CKD and diabetic kidney disease (DKD) [14]. Furthermore, the 24 weeks of dotinurad therapy favorably affected arterial stiffness and oxidative stress markers, suggesting off-target vascular protection of dotinurad [15].

Dotinurad is characterized by its high selectivity in inhibiting URAT1 and not inhibiting other UA transporters such as ABCG2 and OATs. Here, we will discuss on the influences of inhibition of URAT1 and non-inhibition of other UA transporters on metabolic syndrome, CKD and cardiovascular disease (CVD). Therefore, the beyond UA-lowering effects of dotinurad are also the subject of discussion.

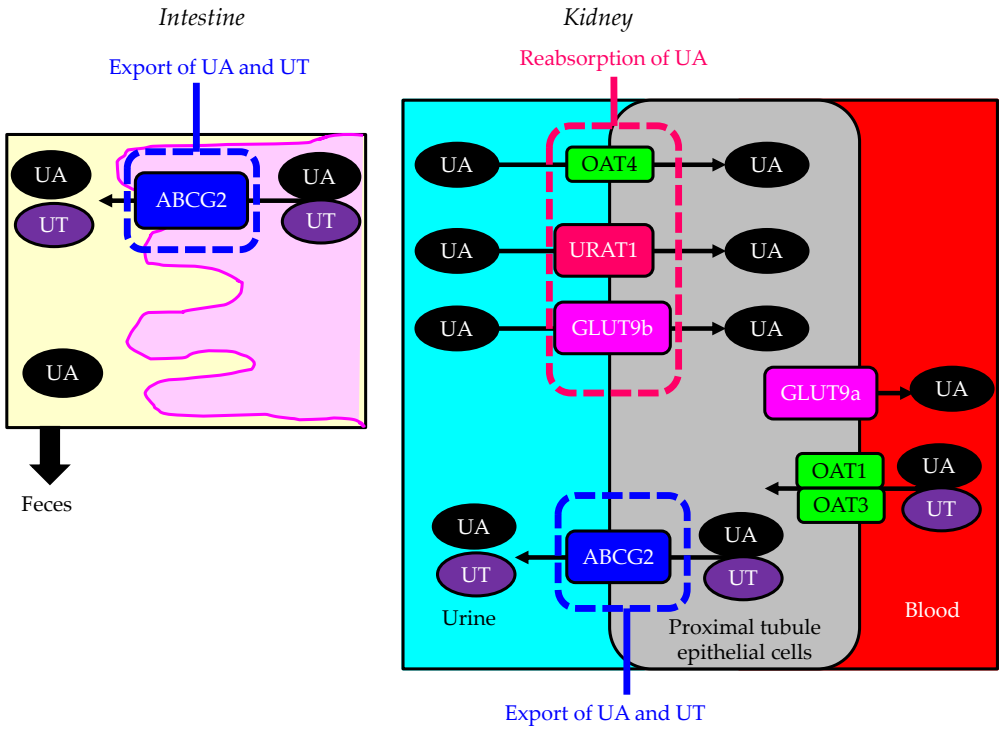

Figure 1. Urate transporters in kidney and intestine. Black arrows indicate the flow of uric acid and uremic toxins. ABCG2, ATP-binding cassette transporter G2; GLUT9, glucose transporter 9; OAT, organic anion transporter; UA, uric acid; URAT1, urate transporter 1; UT, uremic toxins.

2. The association of URAT1 and other UA transporters with metabolic syndrome

2.1. Metabolic syndrome and hyperuricemia

Hyperuricemia is significantly associated with the development and severity of metabolic syndrome. The meta-analysis showed that higher serum UA levels led to an increased risk of metabolic syndrome with a linear dose-response relationship [16]. Serum UA concentrations increased with the number of components of the metabolic syndrome adjusted for age, sex, creatinine

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clearance, and alcohol and diuretic use [17]. Multivariate analyses showed that the visceral fat area (VFA) was the strongest contributor to an elevated serum UA and a decrease in UA clearance [18]. Magnitude of insulin resistance and serum UA levels were significantly related, and insulin resistance was also significantly and inversely related to urinary UA clearance, and urinary UA clearance was significantly and inversely associated with serum UA levels [19]. Insulin resistance due to visceral fat accumulation may increase serum UA by decreasing renal UA clearance in patients with the metabolic syndrome.

2.2. The effect of insulin resistance on URAT1 expression

To elucidate the mechanism of obesity and metabolic syndrome-related hyperuricemia, the expression of URAT1 was investigated [20]. The protein level of URAT1 increased in the kidney of leptin-deficient mice (ob/ob mice) [20]. Further, the Quick fat diet (crude fat content: 13.6%) enhanced the protein level of URAT1 in the kidney of C57BL/6 mice [20]. Insulin resistant Otsuka-Long-Evans-Tokushima Fatty (OLETF) and its control Long-Evans Tokushima Ohtsuka (LETO) rats were used to generate a model for acute hyperuricemia [21]. OLETF rats showed a significantly higher incidence of hyperuricemia as compared to the control LETO rats, indicating that insulin resistance exacerbates the development of hyperuricemia following high-purine load [21]. Upon high-purine load, insulin resistance enhances UA reabsorption by up-regulation of URAT1 expression [21].

High-fructose diet (HFD)-feeding increased the renal expression of GLUT9 and URAT1, and serum UA concentration in rats [22]. Another study also revealed that long-term HFD-feeding significantly increased the protein expression levels of GLUT9 and URAT1 in the kidneys of mice [23]. Resveratrol is a polyphenol, non-flavonoid antitoxin that is abundant in plant materials, and has been reported to exert anti-inflammatory, anti-oxidative, to inhibit lipid peroxidation and to extend life in mice [24]. Furthermore, effects of resveratrol on amelioration of insulin resistance, and liver and kidney pathologies have been shown in several animal models [25, 26]. Compared with those in the HFD group, the protein expression levels of GLUT9 and URAT1 were significantly lower in the HFD group treated by resveratrol. Insulin resistance enhances the expression of URAT1 and GLUT9.

2.3. The effect of insulin on UA transport by other urate transporters

Insulin and hyperinsulinemia reduce renal fractional excretion of UA and play a key role in the genesis of hyperuricemia and gout. Physiological euglycemic hyperinsulinemia induced by insulin infusion in healthy volunteers acutely reduces urinary UA, suggesting a key role for insulin in the pathogenesis of hyperuricemia [27-29]. In rats, the administration of insulin decreased urinary UA excretion, with concurrent increased expression of URAT1, and decreased expression of ABCG2 [30]. There is increased expression of GLUT9 in the kidneys of streptozotocin-induced diabetic mice [31]. Heterologous expression of individual UA transporters in Xenopus oocytes revealed that insulin increased UA transport by GLUT9, OAT1 and OAT3, and decreased UA transport by ABCG2 [32].

The effects of insulin resistance and hyperinsulinemia on UA transport by each UA transporters were shown in Figure 2. Insulin resistance and hyperinsulinemia increase UA transport by URAT1 and GLUT9 and decrease UA transport by ABCG2, which may induce a decrease in renal UA clearance. Therefore, URAT1, GLUT9 and ABCG2 can be therapeutic targets for uricosuric drugs in patients with insulin resistance and hyperinsulinemia.

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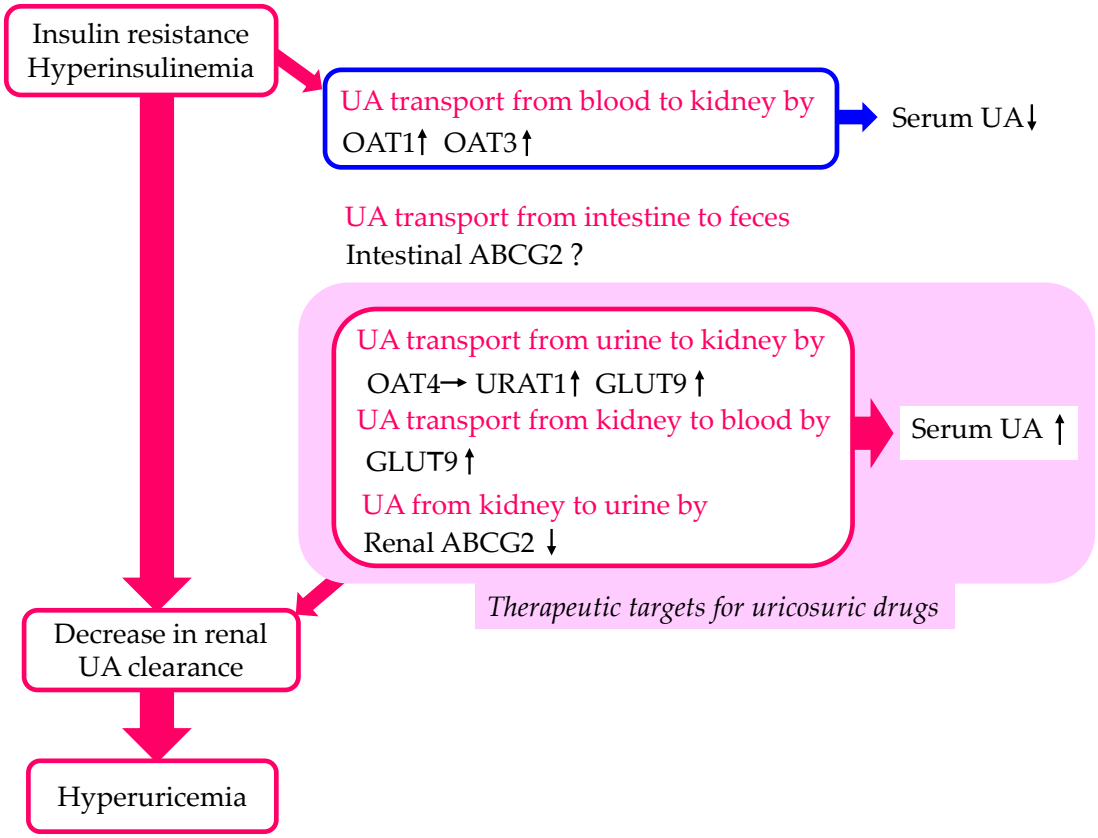

Figure 2. Changes in UA transports by UA transporters in kidney and intestine by insulin resistance and hyperinsulinemia. Up and down arrows indicate increase or decrease in substances or expression of molecules, respectively. ABCG2, ATP-binding cassette transporter G2; GLUT9, glucose transporter 9; OAT, organic anion transporter; UA, uric acid; URAT1, urate transporter 1.

2.4. The effect of inhibition of URAT1 on metabolic parameters in humans

We found that dotinurad reduced body weight, blood pressure, HbA1c, serum low-density lipoprotein-cholesterol (LDL-C), triglyceride (TG) and non-high-density lipoprotein-cholesterol (non-HDL-C), in addition to reducing serum UA, in patients with CKD and DKD [14]. To our knowledge, our study is the first to report such metabolic effects of dotinurad. We speculated that dotinurad selectively inhibits URAT1 and increases the concentration of UA in the proximal tubules, and UA may compete with glucose for apical GLUT9b, reducing glucose reabsorption, which may induce an improvement of HbA1c, serum lipids, blood pressure and body weight.

2.5. The effect of inhibition of URAT1 on metabolic parameters in mice

Tanaka, Y.; et al. found that URAT1 was also expressed in the liver and white adipose tissue (WAT) and brown adipose tissue (BAT) other than the kidneys [33]. Dotinurad administration significantly ameliorated high-fat diet-induced obesity and insulin resistance [33]. Serum TG level in high-fat diet-fed mice was increased in comparison with that in normal-fat diet-fed mice, and the treatment with dotinurad significantly decreased serum TG in both mice [33]. High-fat diet markedly induced nonalcoholic fatty liver disease (NAFLD), which were attenuated by dotinurad [33]. The development of NAFLD in high-fat diet-induced obese mice is mediated by multiple factors, such as elevated levels of pro-inflammatory cytokines released from adipose tissues, hypercholesterolemia, and hyperuricemia [34]. Hyperuricemia directly induces fat accumulation and inflammation in hepatocytes through URAT1 in vitro [35]. Dotinurad may improve NAFLD through the inhibition of extracellular UA uptake in hepatocytes via URAT1, leading to a reduction in lipid deposition and inflammation. The re-browning of brown adipose tissue (BAT) and browning of epididymal white adipose tissue (WAT) may indirectly ameliorate NAFLD via adipokines [36].

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WAT could be converted to beige adipose tissue (browning), which possesses the brown-like features of energy dissipation through the activation of the BAT-specific protein, uncoupling protein 1 (UCP1), which improves systemic insulin resistance [36, 37]. The uptake of UA in WAT by URAT1 leads to WAT dysfunction and deterioration of systemic insulin resistance [38]. In epididymal WAT, dotinurad increased the UCP1 expression considerably under high-fat diet condition, indicating that URAT1-selective inhibitor led to the browning of epididymal WAT in high-fat diet-fed mice [33]. A previous study showed that the enhanced UA uptake into WAT via URAT1 and the subsequent increase in the intracellular UA levels led to the inhibition of the leptin- AMP-activated protein kinase (AMPK) pathway, which resulted in a reduction in the UCP1 expression in WAT [37].

The increased UCP1 expression and activity in BAT play an important role in the improvement of glucose metabolism and insulin sensitivity [39]. The UCP1 levels in BAT were significantly increased by treatment with dotinurad [33]. The uptake of UA can increase the oxidative stress in adipocytes, which induces insulin resistance [40]. The reactive oxygen species (ROS) levels in BAT were significantly reduced by treatment with dotinurad [33].

2.6. The effects of other UA-lowering drugs on metabolic parameters

Allopurinol and febuxostat are xanthine oxidase (XO) inhibitors which reduce hepatic production of UA. Allopurinol and febuxostat treatment induced significant reduction in body weight, systolic blood pressure, blood glucose, insulin, lipids in the model rats of insulin resistance and metabolic syndrome as compared with no treatment [41].

Allopurinol treatment significantly reduced hepatic steatosis, epididymal fat, serum UA, homeostatic model assessment for insulin resistance (HOMA-IR), hepatic enzyme levels, and cholesterol in the HFD-fed OLETF rats [42]. Hepatic expression of lipogenic genes, including sterol regulatory element-binding protein 1c (SREBP-1c) and stearoyl-CoA desaturase 1 (SCD-1) was significantly upregulated in the OLETF and the HFD-fed OLETF rats compared to the LETO rats. However, allopurinol significantly downregulated expressions of SREBP-1c and SCD-1 genes in the HFD-fed OLETF rats. Peroxisome proliferator-activated receptor alpha (PPARα) and carnitine palmitoyl-transferase 1 (CPT-1) were significantly downregulated in the OLETF and the HFD-fed OLETF rats compared to the LETO rats [42]. However, allopurinol treatment ameliorated the downregulation of lipid oxidation genes observed in the HFD-fed OLETF rats. Hepatic mRNA expression of tumor necrosis factor-alpha (TNF-α) was significantly increased in the OLETF and the HFD-fed OLETF rats, and this increase was abolished by allopurinol. In addition, allopurinol significantly decreased endoplasmic reticulum (ER)-stress induced protein expression, in comparison with the no-treatment group.

Insulin resistance causes an increased expression of SREBP-1c, which increases fatty acids (FA) synthesis [43]. Hepatic FA metabolism is regulated by a combination of FA uptake, FA export by very-low-density lipoprotein (VLDL) secretion, de novo FA synthesis by SREBP-1c, and FA utilization by β-oxidation. The entry of FA into mitochondria depends on CPT-1. One of the major regulators of CPT-1 is PPARα [44-47]. Activation of PPARα induces transcription of genes related to FA oxidation [44, 48, 49]. SCD1 plays a crucial role in in FA oxidation, FA synthesis and storage [50]. Recently, SCD1 was proposed to play a vital role in the explanation of obesity in Mediterranean countries [51]. Experimental animal studies have revealed the association between SCD1 and obesity and insulin resistance [52, 53]. Therefore, allopurinol-mediated downregulation of SREBP-1c and SCD-1 genes and upregulation of PPARα and CPT-1 in the HFD-fed OLETF rats suggest a beneficial effect of allopurinol on hepatic steatosis in insulin resistance.

The relationship between decrease in serum UA and visceral fat area (VFA) reduction in patients with gout was investigated [54]. The UA-lowering therapy (ULT) (febuxostat 20-80 mg/day or benzbromarone 25-50 mg/day) resulted in a decrease in serum UA level accompanied by a decrease in VFA. By multiple regression model, Δ serum UA was identified to be a significant determinant variable of decrease in VFA (beta, 0.302; $p = 0.001$). The decrease in serum UA level is positively associated with reduced VFA, providing a rationale for clinical trials to affirm whether ULT promotes loss of visceral fat in patients with gout. The ULT significantly reduced body weight, blood pressure,

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serum TG and total cholesterol levels, aspartate aminotransferase (ALT) and aspartate aminotransferase (AST).

XO inhibitor, topiroxostat treatment suppressed weight gain relative to the vehicle without any impact on food intake in diabetic obese mice [55]. However, the weight of fat pads and hepatic and muscle TG content did not change. Soletsky, B.; et al. reported a randomized, double-blinded, placebo-controlled trial (RCT) comparing 2 mechanisms of urate reduction with placebo in prehypertensive, obese, adolescents, aged 11 to 17 years [56]. Subjects were randomized to the XO inhibitor, allopurinol, uricosuric, probenecid, or placebo. Subjects treated with ULT experienced a significantly high reduction in blood pressure.

The effects of UA-lowering drugs on body weight, visceral fat, blood pressure, glucose metabolism and hepatic steatosis were shown in Table 1. These results suggest that serum UA-lowering improves metabolic parameters, regardless of whether XO inhibitors or uricosuric drugs are used.

Table 1. The effects of UA-lowering drugs on body weight, visceral fat, blood pressure, glucose and lipid metabolism and hepatic steatosis.

UA-lowering drugs	**XO inhibitors**			**Uricosuric drugs**		
	allopurinol	**febuxostat**	**topiroxostat**	**benzbromarone**	**probenecid**	**dotinurad**
Inhibition of UA transporters		**ABCG2**	**ABCG2**	**ABCG2 URAT1 GLUT9 OAT1 OAT3**	**ABCG2 URAT1 GLUT9 OAT1 OAT3**	**URAT1**
Body weight	Reduced (animal)	Reduced (animal and human)	Suppressed weight gain (animal)	Reduced (human)	No data	Reduced (animal and human)
Visceral fat	Reduced (animal)	Reduced (human)	No change (animal)	Reduced (human)	No data	Reduced (animal)
Blood pressure	Reduced (animal and human)	Reduced (animal and human)	No data	Reduced (human)	Reduced (human)	Reduced (animal and human)
Glucose metabolism	Improved (animal)	Improved (animal)	No data	No change (human)	No data	Improved (animal and human)
Serum lipids	Improved (animal)	Improved (animal and human)	No data	Improved (human)	No data	Improved (animal and human)
Hepatic steatosis	Improved (animal)	Improved (human)	No change (animal)	Improved (human)	No data	Improved (animal)

ABCG2, ATP-binding cassette transporter G2; GLUT9, glucose transporter 9; OAT, organic anion transporter; UA, uric acid; URAT1, urate transporter 1; XO, xanthin oxidase.

2.7. The possible mechanisms of an improvement in metabolic parameters by dotinurad

The possible mechanisms of an improvement in metabolic parameters by dotinurad were shown in Figure 3. In the kidney, dotinurad selectively inhibits URAT1 and increases the concentration of UA in the proximal tubules, and UA may compete with glucose for apical GLUT9b, reducing glucose reabsorption, which may induce an improvement of HbA1c, serum lipids, blood pressure and body weight. In the liver, the inhibition of UA entry to liver via URAT1 by dotinurad may upregulate genes associated with FA oxidation and may downregulate genes associated with FA synthesis and inflammation, which improve hepatic steatosis, systemic insulin resistance and serum lipids. The inhibition of URAT1 in WAT by dotinurad induces browning of WAT, and the inhibition of URAT1 in BAT increases expression of UCP-1 and decreases production of ROS, which may reduce body weight, visceral fat, and may improve insulin resistance, glucose and lipid metabolism.

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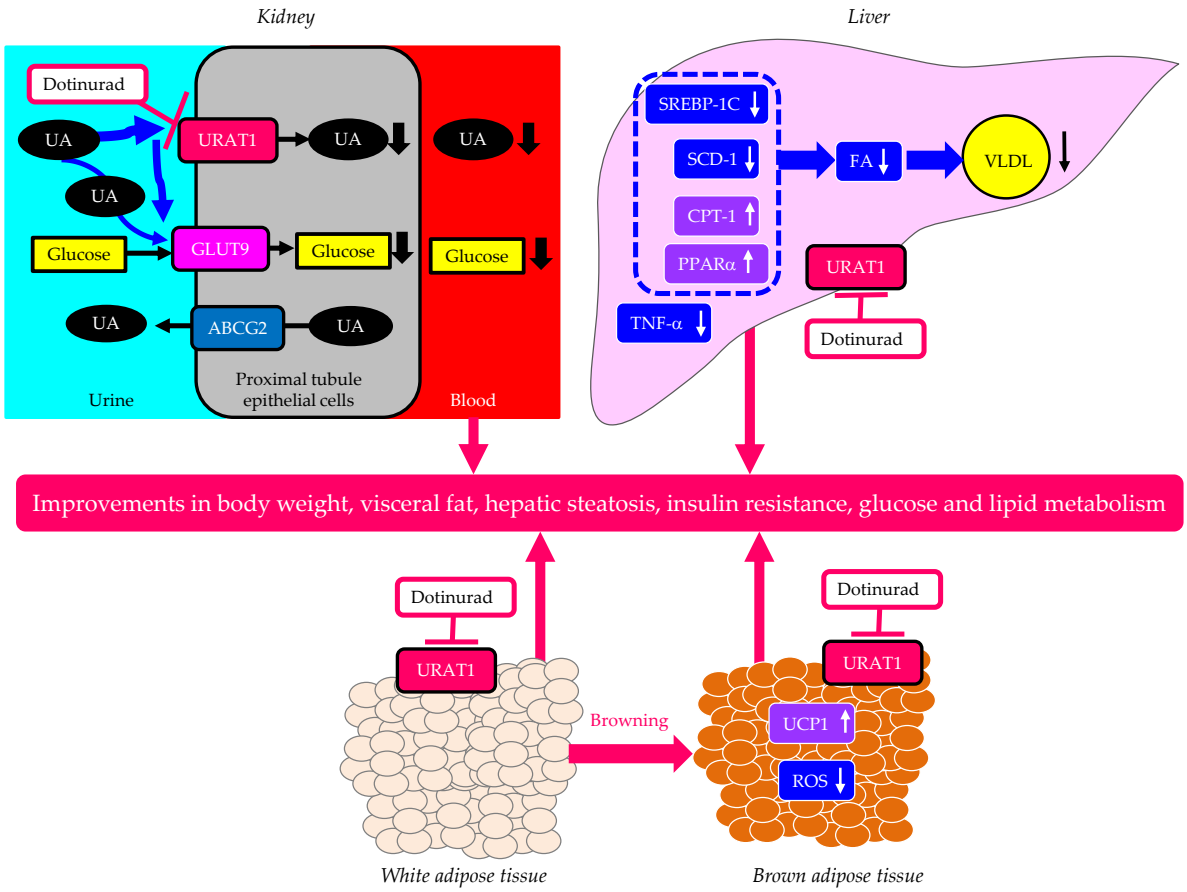

Figure 3. The possible mechanisms of an improvement in metabolic parameters by dotinurad. Up and down arrows indicate increase or decrease in substances or expression of molecules, respectively. ABCG2, ATP-binding cassette transporter G2; CPT-1, carnitine palmitoyl-transferase 1; FA, fatty acid; GLUT9, glucose transporter 9; OAT, organic anion transporter; PPARα, proliferator-activated receptor alpha; ROS, reactive oxygen species; SCD-1, stearoyl-CoA desaturase 1; SREBP-1c, sterol regulatory element-binding protein 1c; TNF-α, tumor necrosis factor-alpha; UA, uric acid; UCP1, uncoupling protein 1; URAT1, urate transporter 1; VLDL, very-low-density lipoprotein.

3. The association of URAT1 and other UA transporters with CKD

3.1. CKD and hyperuricemia

UA is known to induce hypertension through its effects on endothelial function and impaired production of nitric oxide (NO) [57]. Hypertension can be the initial trigger leading to subclinical renal damages [58]. Hyperuricemia causes the activation of both vasoactive and inflammatory processes [59], which may induce CKD. Histologic analyses showed the presence of arteriolosclerosis and tubule-interstitial injury in hyperuricemia-induced renal damage [60]. Serum UA levels were significantly correlated with vascular resistance at the afferent, but also efferent, arteriole, suggesting that hyperuricemia may be harmfully associated to glomerular perfusion [61]. Furthermore, the activation of the renin-angiotensin system (RAS) by hyperuricemia contributes to the development of CKD [62]. Renal vasoconstriction and reduced renal plasma flow can be induced by activation of RAS. UA may also increase oxidative stress, pro-inflammatory cytokines, and induce proliferation of vascular smooth muscle cells (SMC) [2]. UA crystals can cause tubular damage through inflammation mediated by direct physical mechanisms [2].

Growing evidence suggests that high serum UA levels are causally related to increased risk of CKD. A total of 2,059 community-dwelling Japanese subjects aged ≥40 years without CKD were followed for 5 years [63]. The multivariable-adjusted risk of developing CKD increased with higher serum UA levels (odds ratio [OR] 1.00 [reference] for ≤4.0, 1.21 [95% confidence interval (95%CI),

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0.84-1.74] for 4.1-4.9, 1.47 [1.01-2.17] for 5.0-5.8, and 2.10 [1.37-3.23] for serum UA ≥5.9 mg/dl, respectively). Similarly, there were positive associations between serum UA level and the adjusted risk of developing a decline of eGFR < 60 ml/min/1.73 m^2 (OR 1.00 [reference], 2.30 [1.10-4.82], 2.81 [1.34-5.88], and 3.73 [1.65-8.44]) and albuminuria (1.00 [reference], 1.12 [0.76-1.65], 1.35 [0.90-2.03], and 1.81 [1.14-2.87], respectively) [63]. This study showed that hyperuricemia is a significant risk factor for a decline of eGFR and albuminuria. A screened cohort study including 48,177 individuals showed that calculated incidences of end-stage renal disease (ESRD) per 1,000 people were 1.22 for men without hyperuricemia and 4.64 for men with hyperuricemia and 0.87 for women without hyperuricemia and 9.03 for women with hyperuricemia [64]. Hyperuricemia is significantly associated with the development and progression of CKD.

3.2. The effect of CKD on renal URAT1 expression

It has been previously considered that hyperuricemia observed in renal dysfunction was due to decreased UA clearance from the kidneys due to decreased renal function. Since various UA transporters exist in the proximal tubule of the kidney, the influence of CKD progression on these transporters must be considered. Both the mRNA expression and the immunohistochemistry of the URAT1 were decreased in the CKD model rat [65].

3.3. The effect of CKD on other urate transporters expression

Both the mRNA expression and the immunohistochemistry of GLUT9 and ABCG2 in the kidney were decreased in the CKD model rat [65]. CKD patients accumulate uremic toxins (UT) in the body, potentially require dialysis. ABCG2 is a major transporter of the UT such as indoxyl sulfate (IS) [66]. ABCG2 regulates the renal and intestinal excretion of IS and strongly affects CKD survival rates [67]. The intestinal ABCG2 may play a compensatory role in light of decreased renal clearance of UA and UT in CKD model rats [67].

OAT1/3-mediated active tubular secretory clearance was estimated to decline by an additional 50% relative to the GFR decline in severe CKD, whereas change in active secretion in mild and moderate CKD was proportional to GFR [68]. The 4-pyridoxic acid (PDA) was identified as the most sensitive plasma endogenous biomarker to evaluate inhibition of OAT1 and OAT3 [69-71]. Recent clinical studies have reported an increase in plasma PDA in CKD populations [72, 73]. Changes in plasma PDA concentrations in CKD exceed those reported after probenecid inhibition and are likely a reflection of deteriorating active renal secretion. OAT1 and OAT3 play a key role in the handling of UT such as IS [74]. UT inhibits OAT1 and OAT3, which contribute to the decline in renal drug and UT clearance in patients with CKD [75].

3.4. The effect of IS on CKD

IS is accumulated in the serum of CKD patients. A part of the dietary protein-derived tryptophan is metabolized into indole by tryptophanase in intestinal bacteria. Indole is absorbed into the blood from the intestine, and is metabolized to IS in the liver [76]. IS is normally excreted into urine. In CKD, however, an inadequate renal clearance of IS leads to its elevated serum levels. IS progresses both tubulointerstitial fibrosis and glomerular sclerosis by increasing the expression of transforming growth factor-beta1, a tissue inhibitor of metalloproteinase-1 and proalpha1 (I) collagen, leading to a further loss of nephrons. Moreover, IS induces oxidative stress in tubular cells, mesangial cells, vascular SMC, endothelial cells in hypertensive rats, it is also involved in the progression of CKD.

Serum IS levels increase gradually with the decrease of renal function and reached the highest level in CKD stage 5 [77]. Serum IS concentration was measured in 604 pediatric participants (mean eGFR of 27 ± 11 ml/min/1.73m^2) at enrolment into the prospective Cardiovascular Comorbidity in Children with CKD study [78]. During a median follow up time of 2.2 years, the composite renal survival endpoint, defined as 50% loss of eGFR, or eGFR < 10ml/min/1.73m^2 or start of renal replacement therapy. Median survival time was shorter in patients with IS levels in the highest versus lowest quartile for IS (1.5 years, 95%CI [1.1,2.0] versus 6.0 years, 95%CI [5.0,8.4]). Multivariable Cox

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regression disclosed a significant association of IS with renal survival, which was independent of other risk factors including baseline eGFR, proteinuria and blood pressure.

The effects of CKD progression on UA transport by each UA transporter were shown in Figure 4. CKD progression decreases expression of OAT4, URAT1 and GLU9, which may increase serum UA, and decreases expression of OAT1 and OAT3, which may increase serum UA and UT. Furthermore, CKD progression decreases expression of renal ABCG2, which may increase renal UA and UT, and increases intestinal ABCG2, which may reduce serum UA and UT. To suppress progression of CKD, UT should be smoothly excreted from the body. For this purpose, drugs that do not inhibit ABCG2 are desired.

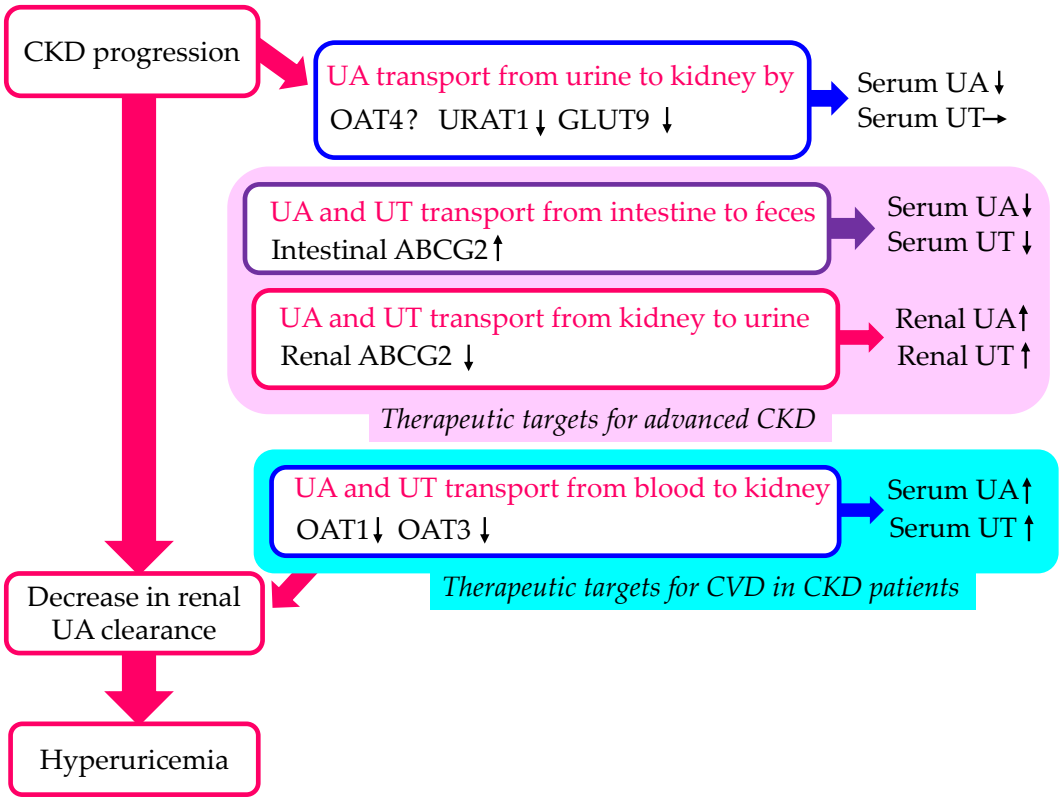

Figure 4. Changes in UA transports by urate transporters in kidney and intestine by CKD progression. Up and down arrows indicate increase or decrease in substances or expression of molecules, respectively. ABCG2, ATP-binding cassette transporter G2; CKD, chronic kidney disease; CVD, cardiovascular disease; GLUT9, glucose transporter 9; OAT, organic anion transporter; UA, uric acid; URAT1, urate transporter 1.

3.5. The effect of inhibition of URAT1 on CKD

We previously reported that the start of dotinurad decreased blood urea nitrogen (BUN) and increased eGFR, and the dose-up of dotinurad further decreased BUN and increased eGFR with reduction of UA in a diabetic patient with CKD stage G4 [79]. In this case, an improvement in albuminuria after the start of dotinurad was also observed [79]. In our study, the 6-month dotinurad treatment improved albuminuria and eGFR in hyperuricemic patients [14]. In another study, although eGFR did not significantly change in patients with $30 \leq$ eGFR < 45 and eGFR ≥ 45 ($p = 0.918$ and $p = 0.535$, respectively), dotinurad significantly improved eGFR in patients with eGFR < 30 ($p = 0.032$) [80]. The proportion of patients with improved eGFR was significantly higher in patients with eGFR < 30 ($p = 0.038$) than in patients with $30 \leq$ eGFR < 45 and eGFR ≥ 45. In the multivariate logistic regression analysis, baseline eGFR < 30 and achieving a serum UA level of ≤ 6.0 mg/dL were significantly associated with improved eGFR ($p = 0.033$ and $p = 0.015$, respectively) [80]. This study suggested that dotinurad may have a potential to improve renal function in patients with advanced CKD.

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Yanai, K.; et al. investigated the efficacy and safety of dotinurad in 34 hyperuricemic patients with advanced CKD (stage G3-5) [81]. With the 12-month dotinurad treatment, the annual change in the patients' eGFR was significantly improved from -6.0 ± 12.9 mL/min/1.73 m^2/year to -0.9 ± 4.6 mL/min/1.73 m^2/year ($p < 0.05$), but there was no change in the control group, suggesting that dotinurad can attenuate renal function decline in individuals with hyperuricemia and advanced CKD.

3.6. The effects of other UA-lowering drugs on CKD

In a RCT of 54 hyperuricemic patients with CKD, patients were randomly assigned to treatment with allopurinol or to continue the usual therapy for 12 months [82]. There was a trend toward a lower serum creatinine level in the treatment group compared with controls after 12 months of therapy, although it did not reach statistical significance ($p = 0.08$). Overall, 4 of 25 patients (16%) in the allopurinol group reached the combined end points of significant deterioration in renal function and dialysis dependence compared with 12 of 26 patients (46.1%) in the control group ($p = 0.015$).

In a RCT of 113 patients with eGFR < 60 ml/min, patients were randomly assigned to treatment with allopurinol 100 mg/day or to continue the usual therapy [83]. In the control group, eGFR decreased by 3.3 ± 1.2 ml/min/1.73 m^2, and in the allopurinol group, eGFR increased by 1.3 ± 1.3 ml/min/1.73 m^2 after 24 months. The post hoc analysis of a long-term follow-up after completion of the 2-year RCT showed that during the initial and long-term follow-up (median, 84 months), 9 patients in the allopurinol group had a renal event compared with 24 patients in the control group (hazard ratio [HR], 0.32; 95% confidence interval [CI], 0.15-0.69; $p = 0.004$) [84].

A greater reduction in serum UA with febuxostat was associated with an increase in eGFR and a tendency toward decreased proteinuria in patients with CKD stages 3b, 4 and 5 [85]. In a 1-year cohort study of 73 hyperuricemic patients with eGFR < 45 ml/min, treatment in 51 patients was changed from allopurinol to febuxostat, and the other 22 patients were continued on allopurinol [86]. The serum UA levels significantly decreased from 6.1 ± 1.0 to 5.7 ± 1.2 mg/dl in the febuxostat group and significantly increased from 6.2 ± 1.1 to 6.6 ± 1.1 mg/dl in the allopurinol group. The eGFR decreased from 27.3 to 25.7 ml/min in the febuxostat group and from 26.1 to 19.9 ml/min in the allopurinol group, suggesting that febuxostat slowed the progression of renal disease in the CKD cohort in comparison with allopurinol. In an RCT, febuxostat for 12 weeks reduced the urinary levels of fatty acid-binding protein 1 (FABP1), albumin, and β2-microglobulin, whereas the levels of these markers did not change in the control group [87]. Urinary FABP1 and β2-microglobulin are the markers for proximal tubular impairment [88, 89]. However, the meta-analysis showed no significant differences in the changes in serum creatinine from baseline between the febuxostat and allopurinol groups [90]. The changes in eGFR were not significantly different at 3 months. A significant difference did exist in the changes in albuminuria levels from baseline between the febuxostat and allopurinol groups (mean difference [MD], -80.47 mg/ gram creatinine [gCr]; 95% CI, -149.29 to -11.64 mg/gCr; $p = 0.02$) [90]. A Nationwide Database Analysis showed a lower risk of progression to dialysis was observed in pre-dialysis stage 5 CKD febuxostat users without compromising survival [91].

Topiroxostat treatment resulted in significant reduction in serum UA, systolic blood pressure (-8.9 mmHg), diastolic blood pressure (-5.0 mmHg), and urinary protein excretion (-795.5 mg/gCr) compared with baseline values [92]. However, serum creatinine and urinary N-acetyl-beta-D-glucosaminidase (NAG) which is the marker for renal tubular impairment [93], and eGFR did not change significantly [92]. Another study reported that topiroxostat significantly improved eGFR and reduced the urinary albumin/creatinine ratio compared to placebo [94].

A 13-year inception cohort study showed that compared with allopurinol, benzbromarone therapy was associated with a reduced risk of progression to dialysis, the adjusted HR was 0.50 (95% CI, 0.25-0.99) [95]. We could not find any RCTs and meta-analysis which investigated the effect of probenecid on CKD.

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3.7. The effects of febuxostat and dotinurad on advanced CKD

Serum IS levels increase gradually with the decrease of renal function and reached the highest level in CKD stage 5 [77]. Serum IS concentration is significantly associated with renal survival [78]. Therefore, ABCG2-mediated excretion of IS [66], may be more critical for patients with CKD stage 4 or 5. The start of dotinurad which did not inhibit ABCG2 improved eGFR in our case with CKD stage 4 [79]. In this case, an improvement in albuminuria after the start of dotinurad was also observed [79]. Although eGFR did not significantly change in patients with 30 ≤ eGFR < 45 and eGFR ≥ 45, dotinurad significantly improved eGFR in patients with eGFR < 30 [80]. However, in the cohort study of 778 gout patients, febuxostat reduced eGFR (19.1 ml/min/1.73 m^2 at baseline) by 0.7 ml/min/1.73 m^2 in patients with CKD stage 4, 5 [96]. Another study showed that the eGFR of 63 patients with stage 4-5 CKD, excluding dialysis patients, was 19.84 ± 7.08 mL/min/1.73 m^2 when they began to take febuxostat and 23.49 ± 16.67 mL/min/1.73 m^2 after 12 months (p = 0.13) [97], suggesting that febuxostat did not improve eGFR in patients with CKD stage 4, 5.

Table 2. The effects of UA-lowering drugs on renal function, and renal outcome.

UA-lowering drugs	**XO inhibitors**			**Uricosuric drugs**		
	allopurinol	**febuxostat**	**topiroxostat**	**benzbromarone**	**probenecid**	**dotinurad**
Inhibition of UA transporters		**ABCG2**	**ABCG2**	**ABCG2 URAT1 GLUT9 OAT1 OAT3**	**ABCG2 URAT1 GLUT9 OAT1 OAT3**	**URAT1**
Albuminuria	No data	Improved	Improved	No data	No data	Improved
eGFR or serum creatinine	Improved	Improved	Improved	No data	No data	Improved
eGFR in patients with CKD stage 4 and 5	No data	Not improved	No data	No data	No data	Improved
Proximal tubular impairment	No data	Improved	Not improved	No data	No data	No data
Renal outcomes	Improved	Improved	No data	Improved	No data	No data

ABCG2, ATP-binding cassette transporter G2; GLUT9, glucose transporter 9; OAT, organic anion transporter; UA, uric acid; URAT1, urate transporter 1; XO, xanthin oxidase.

To suppress progression of CKD, drugs that do not inhibit ABCG2 which excretes UT such as IS are desired. Febuxostat was reported to be a strong ABCG2 inhibitor [98], and dotinurad does not inhibit ABCG2. Taniguchi, T.; et al. evaluated whether hypouricemic agents including dotinurad affect IS clearance in rats [99]. Intact and adenine-induced acute renal failure rats were orally administered hypouricemic agents, and both endogenous IS and exogenously administered stable isotope-labeled-IS in the plasma and kidney were measured. Febuxostat caused renal IS accumulation remarkably by suppressing its excretion in intact rats. Dotinurad did not significantly affected the clearance of IS under both conditions.

4. The association of URAT1 and other UA transporters with the development of CVD

4.1. The association of URAT1 with atherogenesis

High levels of UA are associated with the development of CVD. The molecular mechanisms by which UA induces vascular damage have not been completely elucidated. The plasma membrane enzyme, named ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1), also known as plasma cell antigen-1, has been shown to inhibit insulin receptor function and having high expression levels in cells of insulin-resistant subjects [100]. Cultures of human umbilical vein endothelial cells were stimulated with insulin, UA and URAT1 inhibitor probenecid [101]. UA inhibited insulin-

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induced Akt/ endothelial nitric oxide synthase (eNOS) axis [101], suggesting that UA has a key role in reducing Akt–eNOS axis activity, which induces endothelial dysfunction [101]. UA induced ENPP1 binding to insulin receptor that resulted in an impairment of insulin signaling. Probenecid reverted such UA effects, suggesting that UA intracellular uptake by URAT1 is required for its action.

The expression of URAT1 on human aortic vascular SMC was reported [102]. Expression of URAT1 was localized to the cell membrane. Evidence that the URAT1 was functional was provided by the finding that uptake of ^{14}C-urate was significantly inhibited in the presence of probenecid, URAT1 inhibitor. URAT1 may provide a mechanism by which UA enters the human vascular SMC [103]. UA (6 to 12 mg/dl) upregulated C-reactive protein (CRP) mRNA expression in human vascular SMC (HVSMC) and human umbilical vein endothelial cells (HUVEC) with a concomitant increase in CRP release into cell culture media [104]. UA stimulated HVSMC proliferation whereas UA inhibited serum-induced proliferation of HUVEC, which was attenuated by co-incubation with probenecid, suggesting that entry of UA into cells is responsible for CRP expression. UA also increased HVSMC migration and inhibited HUVEC migration. In HUVEC, UA reduced NO release. Treatment of vascular cells with anti-CRP antibody revealed a reversal of the effect of UA on cell proliferation and migration in HVSMC and NO release in HUVEC. The entry of UA to cells via URAT1 may induce endothelial dysfunction and the proliferation of SMC, by inducing inflammation.

4.2. The association of other UA transporters with atherogenesis

NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) is an intracellular sensor that detects a broad range of microbial motifs, endogenous danger signals and environmental irritants, resulting in the formation and activation of the NLRP3 inflammasome. Assembly of the NLRP3 inflammasome leads to caspase 1-dependent release of the pro-inflammatory cytokines interleukin (IL)-1β and IL-18 [105]. Soluble UA absorbed by cells through UA transporters accumulates intracellularly, activates the NLRP3 inflammasome and thereby increases IL-1β secretion. ABCG2 excludes intracellular UA. GLUT9 and ABCG2 were expressed in macrophage-like J774.1 cells, however, URAT1 was not expressed in these cells. The entry of soluble UA via GLU9 increased mRNA and protein levels of ABCG2 in macrophage-like J774.1 cells, and an ABCG2 inhibitor, febuxostat, but not dotinurad, enhanced IL-1β production in cells pretreated with soluble UA, suggesting that inhibition of ABCG2 enhances IL-1β production especially under hyperuricemic conditions by increasing intracellularly accumulated UA in macrophage-like cells [106].

4.3. The effect of inhibition of URAT1 on atherosclerosis

Cardio-ankle vascular index (CAVI), which is a marker of arterial stiffness measured from the origin of the aorta to the ankle, was developed in 2004 [107]. Several studies have demonstrated that CAVI is high in patients with various atherosclerotic risk factors, and treatment of cardiovascular risk factors and lifestyle modifications improve CAVI [107]. The multicenter prospective cohort study with a 5-year follow-up period, including patients (aged 40–74 years) with CVD risks was performed [108]. The CAVI predicted the primary outcome (HR, 1.38; 95% CI, 1.16–1.65; $p < 0.001$). When the CAVI was incorporated into a model with known CVD risks for predicting CV events, the global $\chi 2$ value increased from 33.8 to 45.2 ($p < 0.001$), and the net reclassification index was 0.254 (P=0.024), suggesting that the CAVI predicted CV events. The 24- week-treatment with dotinurad significantly reduced CAVI from 9.29 to 8.92 ($p = 0.044$), suggesting that dotinurad may favorably affect arterial stiffness [15]. The derivatives of reactive oxygen metabolites concentration at week 24 was significantly lower than that at baseline [15]. The beneficial vascular effects might have been partly caused by URAT1 inhibition by dotinurad at the urate-entry site on vascular walls and resultant attenuation of ROS production [104, 105].

4.4. The effects of other UA-lowering drugs on endothelial function

Endothelial dysfunction is an initial phase in the vascular damage and atherosclerotic process. Hyperuricemia and advanced CKD especially are related to endothelial dysfunction by impairing the

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NO bioavailability and markers of endothelial dysfunction are associated with stage of CKD [109]. XO inhibitors produce benefits concerning endothelial function by reducing oxidative stress [110]. A meta-analysis of RCTs showed that allopurinol therapy is associated with significantly improved endothelial function in subjects at risk of CVD, and the beneficial effects of allopurinol seemed to be more remarkable in patients with normal UA at baseline [111]. Allopurinol has an antioxidant property that might partially reverse endothelial dysfunction in patients with certain comorbidities. The importance of this property and the magnitude of the beneficial effect are likely to be related to the relative contribution of XO into the oxidative stress associated with different underlying pathologies [110].

There is growing evidence that the elevated expression of the eNOS inhibitor asymmetric dimethylarginine (ADMA) is associated with the development of endothelial dysfunction [112-115]. Further, the elevation of ADMA is associated with an increased risk of CVD. The 8-week febuxostat treatment did not show improvements in serum ADMA and high sensitivity-CRP, and vascular stiffness measured by ankle brachial index in patients with CKD patients [116]. Febuxostat treatment did not alter endothelial function assessed by flow mediated dilation during a 2-year study period in patients with asymptomatic hyperuricemia [117]. Furthermore, a RCT showed that topiroxostat nor febuxostat had any significant effects on arterial stiffness measured by CAVI over 24 weeks' treatment [118].

Nakata, T.; et al. determined and compared the effects of benzbromarone and febuxostat on endothelial function. This randomized, cross-over, open-label study initially recruited 30 patients with hyperuricemia. They were divided into two groups, treated initially with benzbromarone or febuxostat for three months and then were switched for the next three months [119]. Endothelial function was defined as reactive hyperemia indexes (RHI) determined by using Endo-PAT 2000 before and at three and six months after medication using the two agents. Adiponectin and the RHI have significantly increased after treatment with benzbromarone. The changes in RHI ($p = 0.026$) and adiponectin levels ($p = 0.001$) were found to be significantly greater in patients treated with benzbromarone than febuxostat. Benzbromarone has increased adiponectin besides reducing UA levels, and thus, this might confer more benefits on endothelial function than febuxostat.

4.5. The effects of UA-lowering drugs on CVD

The meta-analysis was done to determine the association of 2 ULT commonly used in clinical practice (febuxostat vs. allopurinol) on major adverse cardiac events (MACE), by using 10 RCTs [120]. No significant association was also noted of either ULT with all-cause mortality, myocardial infarction, and stroke. The retrospective cohort study used data from the Japanese healthcare record database including 152,166 patients showed that ULT for patients with asymptomatic hyperuricemia did not prevent the development of CVD [121]. In the subgroup analysis, subjects prescribed topiroxostats had a higher risk of developing CVD (HR, 1.89; 95% CI, 1.18 to 3.03; $p = 0.01$). The meta-analysis showed that in patients without atherosclerotic disease, febuxostat likely has a similar CV risk profile to allopurinol [122]. However, in patients with a history of CVD, allopurinol treatment is associated with less CV mortality as compared with febuxostat.

In the large population-based cohort of gout patients, allopurinol was associated with an increased risk of composite CV events and all-cause mortality compared to benzbromarone [123]. In the large cohort of 38,888 elderly gout patients, treatment with probenecid appears to be associated with a modestly decreased risk of CV events compared with allopurinol [124].

IS accumulates in the body in CKD. In the renal proximal tubules, IS excretion is mediated by OAT1/3 and ABCG2 (Figure 1 and 4). OAT1 and OAT3 are inhibited by probenecid and benzbromarone. OATs inhibitors, such as probenecid, suppress IS uptake into the kidney, leading to increased plasma IS concentration, which is harmful for CVD in CKD patients [99]. Therefore, hypouricemic agents that do not affect OATs and ABCG2 are effective therapeutic options for the treatment of hyperuricemia complicated by CKD.

As a major component of uremic syndrome, CVD is largely responsible for the high mortality observed in CKD. Preclinical studies have evidenced an association between serum IS levels and

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vascular alterations. The association between serum IS, vascular calcification, vascular stiffness, and mortality was investigated in a cohort of CKD patients [125]. In crude survival analyses, the highest IS tertile was a powerful predictor of overall and cardiovascular mortality ($p = 0.001$ and $p = 0.012$, respectively). Serum IS may have a significant role in the development of CVD and higher mortality in CKD patients.

5. The summary of unfavorable effects of the inhibition of ABCG2, OAT1 and OAT3 on the kidney and vascular endothelial cells in CKD patients

The summary of unfavorable effects of the inhibition of ABCG2 and of OAT1 and OAT3 on the kidney and vascular endothelial cells in CKD patients were shown in Figure 5.

Among CKD patients, the inhibition of renal ABCG2 may increase renal UT accumulation which produces ROS, resulting in renal damage. The inhibition of intestinal ABCG2 and renal OAT1/3 increase plasma UT which produces ROS, inducing endothelial dysfunction. Endothelial dysfunction causes renal dysfunction. The inhibition of ABCG2 induces UA accumulation in macrophage due to reduced excretion of UA by ABCG2, which induces increased secretion of IL-1β. Such inflammatory cytokine induces endothelial dysfunction.

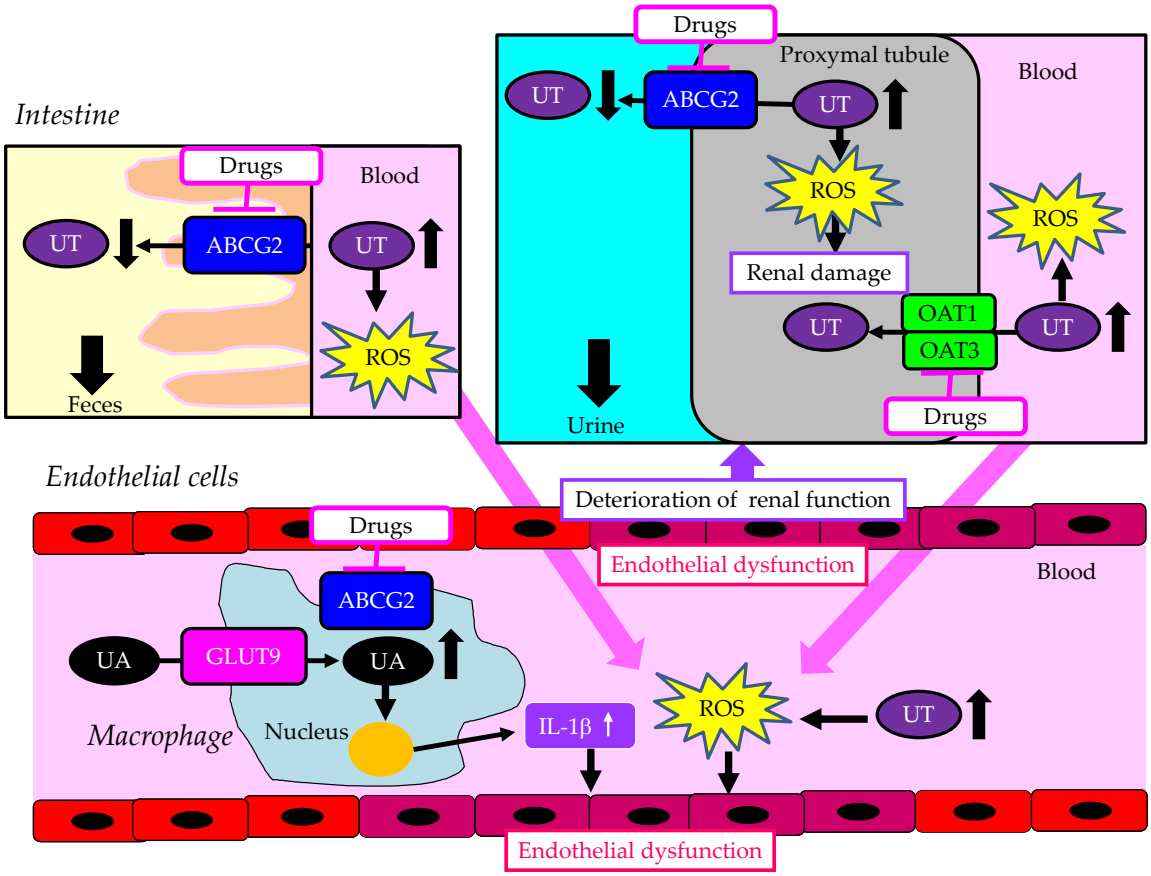

Figure 5. The summary of unfavorable effects of the inhibition of ABCG2, OAT1 and OAT3 on the kidney and vascular endothelial cells in CKD patients. Up and down arrows indicate increase or decrease in substances. ABCG2, ATP-binding cassette transporter G2; GLUT9, glucose transporter 9; IL-1β, interleukin-1β; OAT, organic anion transporter; ROS, reactive oxygen species; UA, uric acid; UT, uremic toxins.

6. The summary of beneficial effects of dotinurad on the kidney and atherosclerosis in CKD patients

The summary of beneficial effects of dotinurad on the kidney and atherosclerosis in CKD patients were shown in Figure 6. Dotinurad do not inhibit intestinal ABCG2 and renal OAT1 and

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OAT3, which do not increase plasma UA and UT. This property is beneficial for endothelial function. URAT1 inhibition in endothelial cells and vascular SMC by dotinurad may prevent the development and progression of atherosclerosis. In the kidney, dotinurad reduces renal UA accumulation by inhibiting UA reabsorption, which may increase excretion of UT into urine due to reduced competition against UA for ABCG2. This property is beneficial for renal function in CKD patients.

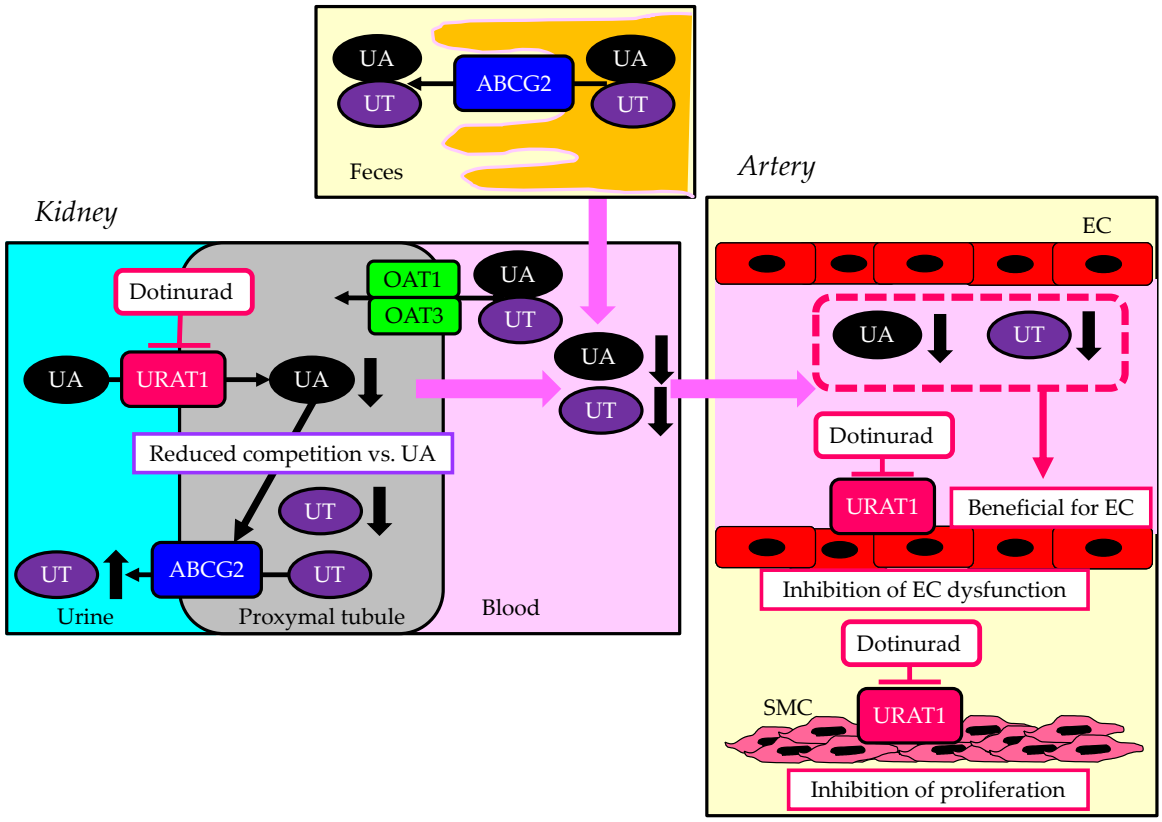

Figure 6. The summary of beneficial effects of dotinurad on the kidney and atherosclerosis in CKD patients. Up and down black arrows indicate increase or decrease in substances. ABCG2, ATP-binding cassette transporter G2; EC, endothelial cells; OAT, organic anion transporter; ROS, reactive oxygen species; SMC, smooth muscle cells; UA, uric acid; URAT1, urate transporter 1; UT, uremic toxins.

7. Conclusions

Dotinurad selectively inhibits URAT1 and do not inhibit ABCG2 and OATs. These properties of dotinurad are beneficial for an improvement in metabolic parameters in patients with metabolic syndrome/obesity, and for renal function and atherogenesis in CKD patients.

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