A Novel Vitamin K₁ 2,3-Epoxy Reductase (VKOR) Inhibitor, 3-Acetyl-5-Methyltetronic Acid, Reduces Experimental Glomerulonephritis

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ABSTRACT. Excessive proliferation of mesangial cells is observed in various types of glomerular disease including glomerulonephritis (GN), which is progressive in nature and eventually results in end-stage renal disease (ESRD). Vitamin K₁ 2,3-epoxide reductase (VKOR) and the vitamin K-dependent growth arrest-specific gene 6/Axl pathway play a key role in mesangial cell proliferation in GN. In the present study, we indicate the potential of a VKOR inhibitor, 3-acetyl-5-methyltetronic acid (AMT), to prevent the proliferation of glomerular mesangial cells and suppress the progression of GN. AMT was administered intravenously to rats once daily for 12 days and a mouse anti-Thy1 monoclonal antibody was injected intravenously after the AMT treatment on Day 6. Creatinine clearance (CCr) significantly increased and the albumin-to-creatinine ratio (ACR) significantly decreased in the AMT-treated group of the Thy-1 GN rats. In addition, glomerular and tubular damage was improved histopathologically in the AMT-treated group. AMT did not affect blood coagulation due to its unique pharmacokinetic properties. The concentration of AMT reached the IC₅₀ for VKOR in kidney, but not in liver. A novel VKOR inhibitor, AMT, reduced renal mesangial cell proliferation and could be a supportive treatment for GN.

KEY WORDS: 3-acetyl-5-methyltetronic acid (AMT), glomerulonephritis (GN), mesangial cell, Thy-1, vitamin K₁ 2,3-epoxide reductase (VKOR).

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Excessive proliferation of mesangial cells is observed in various types of glomerular disease, a progressive disorder that can eventually result in end-stage renal disease (ESRD). ESRD requires dialysis or transplantation and the number of patients with ESRD is growing worldwide year by year [7]. Glomerular mesangial cells play a major role in determining the structure of capillary loops, and their proliferation leads to renal glomerular inflammation and subsequent fibrosis [10, 33]. The proliferation of mesangial cells is one of the most important pathological events for glomerular disease.

In recent studies [41, 43], in Thy-1 glomerulonephritis (GN) rats, the expression of growth arrest-specific gene 6 (Gas6) and Axl was markedly increased in glomeruli and paralleled the proliferation of mesangial cells. The administration of warfarin, a strong inhibitor of vitamin K₁ 2,3-epoxide reductase (VKOR), inhibited the proliferation. VKOR is a major enzyme of the vitamin K cycle controlling blood coagulation, and also involved in mesangial cell proliferation via the vitamin K-dependent activation of Gas6 [43]. Gas6 acts as an autocrine growth factor for mesangial cells through its cell surface receptor Axl. These findings indicate that the vitamin K-dependent Gas6/Axl pathway plays a key role in mesangial cell proliferation in GN [42].

A VKOR inhibitor such as warfarin is effective in blocking mesangial cell proliferation and restoring renal function [42], however, it inhibits the biosynthesis of blood coagulation factors in liver and has the potential to cause bleeding [16]. Therefore, low-dose warfarin treatment is used for renal failure patients, and the monitoring of its blood concentration or a blood coagulation test is required [39].

We have been researching new low molecule weights compounds which inhibit renal mesangial cell proliferation, but not the hepatic biosynthesis of blood coagulation factors in Thy-1 GN rats. Thy-1 GN rats have been used as an acute model of mesangial proliferative GN, which is characterized by mesangial cell proliferation and matrix expansion, and a moderate decline in renal function [14]. In the present study, we indicate the potential of a new VKOR inhibitor, 3-acetyl-5-methyltetronic acid (AMT), as an inhibitor of mesangial cell proliferation and suppressor of renal disease.

MATERIALS AND METHODS

Materials: AMT and Vitamin K₁ 2,3-epoxide were synthesized at Pharmaceutical Research Laboratories, Toray Industries, Inc. (Tokyo, Japan). Vitamin K₁ was purchased from Nacalai Tesque (Kyoto, Japan). The anti-rat Thy1.1 monoclonal antibody (ascites fluid) was purchased from CEDARLANE Laboratories (Hornby, Canada).

Animals: Male Crlj:Wistar rats (7 weeks old) from Charles River Japan (Yokohama, Japan) were maintained at 19–25°C under a 12-hr dark/light cycle. The rats were allowed unlimited access to chow (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water. The animal ex-

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periments were conducted according to the Guidelines for Animal Experiments, Research and Development Division, Toray Industries, Inc.

In vitro VKOR activity assay: The VKOR inhibitory activity was measured as described by estimating the AMT concentrations of half maximal (50%) inhibition (IC_{50}) of conversion from vitamin K_1 2,3-epoxide to vitamin K_1 using normal rat kidney and liver microsomes. The rate of biosynthesis of vitamin K_1 (VKOR activity) in the control (no AMT) was regarded as 100%. VKOR activity (%) was plotted along the Y axis, the common logarithm of the concentration of AMT (10, 100 pmol/ml, and 1, 10 nmol/ml) was plotted along the X axis, and a linear equation ($Y = A \times \log X + B$) was determined from the two points crossing 50% VKOR activity. The IC_{50} of AMT was calculated from this linear equation. The levels of vitamin K_1 and epoxide were measured by high performance liquid chromatography (HPLC) with an Acquity UPLC system (Waters, Milford, MA, U.S.A.) using a gradient of mobile phase A, acetonitrile/2-propanol/distilled water (100:8:100, v/v/v), and mobile phase B, acetonitrile/2-propanol/distilled water (100:8:2, v/v/v). The gradient was as follows; initially 1% B for 1 min then a linear increase to 99% B over the 0.5 min. The column was washed with 99% B for 1.5 min and the system was equilibrated with 1% B for 0.5 min. The flow rate was 0.80 ml/min and the column temperature was 40°C. The analytical column was Acquity UPLC BEH C18 2.1 mm $\times$ 50 mm, 1.7 μ m (Waters). Analytes were detected by UV detector at 250 nm.

Induction of experimental mesangial proliferative glomerulonephritis (Thy1 GN) and treatment of AMT: Thy1 glomerulonephritis (GN) was induced by a single intravenous injection of the mouse anti-Thy1.1 monoclonal antibody OX-7 (1 mg/10 ml/kg) in rats as described [11, 32]. AMT was administered intravenously at a dose of 0 (control group, vehicle only), 10 or 30 mg/kg once daily for 12 days (Day 1 to 12), and the mouse anti-Thy1.1 monoclonal antibody was injected intravenously after the administration of AMT on Day 6. Animals were pretreated with AMT before anti-Thy1.1 injection as same methodology as previous study [41, 43]. Each group contained 6 rats and the vehicle was saline.

Observations and examinations: Body weights were measured before the administration on Days 1 and 6, and before necropsy on Day 13. Urine was collected for 24 hr after the final administration and urinalysis was conducted on 24 hr-pooled urine. Total protein, creatinine and albumin were measured with an Auto Analyzer 7070 (Hitachi, Tokyo, Japan). At 24 hr after the final administration, Day 13, all rats were placed under inhaled anesthesia with isoflurane, and blood was withdrawn from the abdominal aorta. Blood were placed in collection tubes containing an anticoagulant, 3.2w/v% sodium citrate, and then centrifuged (approx. 1,600 $\times$ g, 10 min, 4°C) to obtain plasma for coagulation tests. Prothrombin time, activated partial thromboplastin time, thrombotest, and hepaplastin test were measured with Sysmex CA-1000 (Sysmex, Kobe, Japan). Heparinized blood was centrifuged (approx. 1,600 $\times$ g, 15 min, 4°C) to obtain plasma. Blood chemistry was conducted after the

administration of AMT on Day 13. Total protein, albumin, urea nitrogen (UN), and creatinine were measured with the Auto Analyzer 7070 (Hitachi). Creatinine clearance (CCr) was calculated from the creatinine concentration in urine, urine flow rate, and creatinine concentration in plasma. After blood collections, kidneys were removed and gross pathology was conducted. These kidneys were weighed. Relative organ weights were calculated based on body weight. The kidneys were fixed in 10vol% neutral buffered formalin at 4°C for 24 hr. They were embedded in paraffin and sections (5 μ m) were stained with hematoxylin and eosin (HE), and periodic acid-Schiff stain (PAS) for light microscopy. Histopathological observation was performed in open-labeled slides

Tissue concentrations of AMT: Kidney and liver concentrations of AMT after a single intravenous administration at a dose of 30 mg/kg to normal rats were measured by liquid chromatography tandem mass spectrometry (LC-MS) using Agilent 1,200 series RRLC system (Agilent technologies, Santa Clara, CA, U.S.A.) combined with a tandem API-5000 MS/MS system (AB SCIEX, Concord, ON, Canada). The LC system was run in the isocratic mode with a 80% acetonitrile aqueous solution containing 0.1% formic acid (v/v). The flow rate was 0.60 ml/min and the column temperature was 40°C. The analytical column was CAPCELLPAK C18 MGIII 2 mm $\times$ 50 mm, 3 μ m (Shiseido, Tokyo, Japan). The MS system was run using the Q1 multiple ion scan with the positive ion mode ($[M + H]^+ = 157.12$). Kidney and liver were homogenized using a POLYTORON PT1200C (KINEMATICA, Bohemia, NY, U.S.A.) in a two-fold volume of distilled water. Analytes were extracted by adding a 10-fold volume of methanol/acetonitrile (1:1, v/v). The extracted liquid was centrifuged (approx. 1,600 $\times$ g, 10 min, 4°C), filtrated using a 96-well GF/F filter microplate (Whatman, Clifton, NJ, U.S.A.), diluted in a 10-fold volume of distilled water, and then injected (10 μ l) into the LC-MS system. The LC-MS data was analyzed using Analyst ver. 1.4.2 (AB SCIEX). The concentrations of AMT in rat kidney and liver were quantified by absolute quantitation using a linear regression equation of the calibration curve by the least-squares method.

Statistical analysis: Values are expressed as the means $\pm$ S.E. Data on body weight, urinalysis, blood coagulation, and blood chemistry were statistically analyzed using SAS Ver. 9.1.3 (SAS institute Japan, Tokyo, Japan). The statistical significance of differences from the control group was evaluated with Dunnet's multiple comparison test for each items measured.

RESULTS

In vitro VKOR activity assay: The IC_{50} of AMT against rat kidney and liver VKOR was 3.18 μ mol/ml and 3.20 μ mol/ml, respectively.

Observations and examinations after administration of AMT in rat Thy-1 GN: There appeared to be a significant dose-dependent decrease in relative kidney weights (Table 1). A slight enlargement and rough surface of kidneys were mac-

Table 1. Relative organ weights of kidney following after 12-day repeated intravenous administrations of AMT (0, 10 and 30 mg/kg/day) in Thy-1 glomerulonephritis rats

Group	Dose	Relative kidney weight (g/100 g Body weight)					
		Right		Left		Total	
Normal ^{a)}	-	0.377 ± 0.033	(6)	0.383 ± 0.025	(6)	0.760 ± 0.057	(6)
Thy-1-administered	0 mg/kg (control)	0.478 ± 0.016	(6)	0.469 ± 0.017	(6)	0.947 ± 0.033	(6)
	10 mg/kg	0.417 ± 0.016*	(6)	0.409 ± 0.017*	(6)	0.826 ± 0.033*	(6)
	30 mg/kg	0.395 ± 0.009**	(6)	0.383 ± 0.010**	(6)	0.779 ± 0.018**	(6)

Values are means ± S.E. for the numbers of rats indicated in parentheses.

*, ** Significantly different from control, $P < 0.05$ (*), $P < 0.01$ (**).

a) Normal rats without anti-Thy-1 injection.

Fig. 1. Representative light micrographs of kidneys following after 12-day repeated intravenous administrations of AMT in Thy-1 glomerulonephritis rats. Periodic acid-Schiff (PAS) stained sections showing a thickening of the glomerular basement membrane and fibro-cellular crescent of the glomerulus at 0 mg/kg (a), and no remarkable change in the glomerulus and tubule at 30 mg/kg (b).

roscopically observed in the control group, but no remarkable change was observed in the 30 mg/kg group. Moderate and slight thickening of the glomerular basement membrane were histopathologically observed in 4/6 and 2/6 animals of the control group, respectively. Moderate and slight fibro-cellular crescent of glomerulus were observed in 1/6 and 5/6 rats of the control group, respectively (Table 2). However, these findings were very slight in the AMT-treatment group, and suppressed dose-dependently by AMT (Fig. 1). In addition, hyaline casts, hyaline droplets, tubular dilatation, and basophilic tubules caused by glomerular injury were also suppressed dose-dependently by AMT (Table 2). In blood chemistry and urinalysis, CCr was increased significantly, and urinary albumin-to-creatinine ratio (ACR) was decreased significantly, by the AMT treatment (Tables 3, 4). In blood coagulation tests, no significant change was observed (Table 5).

Tissue concentrations of AMT: Kidney and liver concentrations of AMT 5 min after a single intravenous administration of 30 mg/kg were 4.26 $\mu\text{mol/g}$ tissue and 0.26 $\mu\text{mol/g}$ tissue, respectively (Table 6).

DISCUSSION

In the present study, we demonstrated that a novel VKOR inhibitor, AMT, prevented renal mesangial cell proliferation, and then suppressed glomerular injury in Thy-1 GN rats. A low molecular weight compound, AMT has inhibitory activity against rat liver and kidney VKOR *in vitro*. VKOR is a protein which converts the epoxide of vitamin K back to vitamin K, a cofactor essential for the posttranslational γ -carboxylation of several blood coagulation factors, and is also involved in mesangial cell proliferation via vitamin K-dependent activation of the Gas6/Axl pathway [38, 42]. The gene of VKOR was identified and VKOR showed to be expressed ubiquitously, in liver, kidney, lung, heart and skeletal muscle [15, 38]. However, no information exists concerning the specific isoform of VKOR in any tissue. In the present study, AMT showed no selectivity in its inhibitory activity forward rat kidney and liver VKOR. In addition, there were no selective compounds including warfarin in our chemical library (data not shown). These results suggest that kidney and liver VKOR have similar substrate recognition or there was no specific isoform of VKOR at least in rat liver and kidney.

Table 2. Histopathology after 12-day repeated intravenous administrations of AMT (0, 10 and 30 mg/kg/day) in Thy-1 glomerulonephritis rats

Findings	Grade ^{a)}	AMT		
		0 mg/kg	10 mg/kg	30 mg/kg
Basophilic tubule	—	0/6	4/6	4/6
	—/+	4/6	2/6	1/6
	+	2/6	0/6	1/6
	++	0/6	0/6	0/6
	+++	0/6	0/6	0/6
Fibro-cellular crescent of glomerulus	—	0/6	1/6	2/6
	—/+	0/6	3/6	2/6
	+	5/6	2/6	2/6
	++	1/6	0/6	0/6
	+++	0/6	0/6	0/6
Hyaline cast	—	0/6	0/6	2/6
	—/+	0/6	4/6	2/6
	+	5/6	2/6	2/6
	++	1/6	0/6	0/6
	+++	0/6	0/6	0/6
Hyaline droplet	—	0/6	4/6	4/6
	—/+	3/6	2/6	2/6
	+	3/6	0/6	0/6
	++	0/6	0/6	0/6
	+++	0/6	0/6	0/6
Thickening of glomerular basement membrane	—	0/6	1/6	2/6
	—/+	0/6	0/6	1/6
	+	2/6	5/6	3/6
	++	4/6	0/6	0/6
	+++	0/6	0/6	0/6
Tubular dilatation	—	0/6	6/6	4/6
	—/+	1/6	0/6	2/6
	+	5/6	0/6	0/6
	++	0/6	0/6	0/6
	+++	0/6	0/6	0/6

The number of abnormal/normal of rats used. a) Not remarkable, —/+ : Very slight, + : Slight, ++ : Moderate, +++ : Marked.

Table 3. Blood chemistry after 12-day repeated intravenous administrations of AMT in Thy-1 glomerulonephritis rats

	0 mg/kg		10 mg/kg		30 mg/kg	
Total protein (g/100 ml)	5.06 ± 0.15	(6)	5.25 ± 0.06	(6)	5.06 ± 0.06	(6)
Albumin (g/100 ml)	1.85 ± 0.02	(6)	1.97 ± 0.05	(6)	1.71 ± 0.06	(6)
UN (mg/100 ml)	22.4 ± 1.2	(6)	19.0 ± 1.1	(6)	19.9 ± 2.2	(6)
Creatinine (mg/100 ml)	0.30 ± 0.01	(6)	0.26 ± 0.01**	(6)	0.32 ± 0.02	(6)

Values are means ± S.E. for the numbers of rats indicated in parentheses. * Significantly different from control, $P < 0.01$ (**).

After AMT treatment, kidney weights were significantly decreased dose-dependently, and no remarkable changes were observed, while a slight enlargement and rough surface of kidneys were macroscopically observed in the control group. Renal enlargement accompanied by mesangial cell proliferation is often observed in Thy-1 GN rats and is mostly due to an inflammatory edema [40, 45]. Thickening of the glomerular basement membrane and fibro-cellular crescent of glomerulus were histopathologically suppressed

in the AMT-treatment group. These findings were involved with the progression of renal injury in Thy-1 GN rats [14, 40]. These pathological findings may be ascribable to an inhibitory effect of AMT on mesangial cell proliferation and glomerular inflammation. In addition, hyaline casts, hyaline droplets, tubular dilatation and basophilic tubules followed by glomerular injury were dose-dependently suppressed by the AMT treatment in Thy-1 GN rats.

Thy-1 GN rat is one of the reversible models of kidney

Table 4. Urinalysis after 12-day repeated intravenous administrations of AMT (0, 10 and 30 mg/kg/day) in Thy-1 glomerulonephritis rats

	0 mg/kg		10 mg/kg		30 mg/kg	
Total protein (mg/day)	106 ± 24	(6)	129 ± 51	(6)	91 ± 119	(6)
Albumin (mg/day)	74.8 ± 9.1	(6)	75.9 ± 22.9	(6)	59.8 ± 19.1	(6)
Creatinine (mg/day)	8.10 ± 0.19	(6)	10.5 ± 0.9*	(6)	12.8 ± 0.4**	(6)
ACR ^{a)} (mg/mg)	9.26 ± 1.18	(6)	6.98 ± 1.69	(6)	4.77 ± 1.61	(6)
CCr ^{b)} (ml/min/kg)	1.91 ± 0.08	(6)	2.76 ± 0.22**	(6)	2.87 ± 0.20**	(6)

Values are means ± S.E. for the numbers of rats indicated in parentheses. a) ACR is albumin-to-creatinine ratio. b) CCr is calculated from the urine creatinine concentration, urine volume and the plasma creatinine concentration. *, ** Significantly different from control, $P < 0.05$ (*), $P < 0.01$ (**).

Table 5. Blood coagulation after 12-day repeated intravenous administrations of AMT in Thy-1 glomerulonephritis rats

	0 mg/kg		10 mg/kg		30 mg/kg	
Prothrombin time (sec)	15.3 ± 0.3	(6)	15.1 ± 0.18	(6)	15.5 ± 0.3	(6)
Activated partial thromboplastin time (sec)	17.9 ± 1.0	(6)	18.3 ± 0.7	(6)	16.9 ± 0.5	(6)
Thrombotest (%)	572 ± 61	(6)	709 ± 117	(6)	815 ± 100	(6)
Hepaplastin test (%)	65.4 ± 3.9	(6)	72.1 ± 5.3	(6)	74.9 ± 4.1	(6)

Values are means ± S.E. for the numbers of rats indicated in parentheses.

Table 6. Tissue concentrations after single intravenous administration of AMT (30 mg/kg) in normal rats

Time after administration (hr)	Tissue concentration (μmol/g tissue)	
	Kidney	Liver
0.083	4.25 ± 0.29	0.26 ± 0.02
1	0.09 ± 0.00	<LLOQ ^{a)}
24	<LLOQ ^{a)}	<LLOQ ^{a)}

The data were presented as a mean ± S.E. (n=3).

a) Concentrations of AMT in all animals were under low limit of quantification (0.04 nmol/g tissue).

injury. Anti-Thy-1 injection brings on the destruction of the glomerular mesangial cells and leads to a thickening of the glomerular basement membrane in rats [14, 32]. At 1 week after the injection, a decline in renal function was observed, but then 5 to 6 weeks later, the renal function recovered completely [14, 32]. On the other hand, marked mesangial cell proliferation was observed in patients with chronic glomerulonephritis [13, 21]. Therefore, the Thy-1 GN rat is considered the adequate animal models of GN and is suited to assessing the effectiveness of chemical compounds as suppressors toward the disease [11, 19, 30].

A strong VKOR inhibitor, warfarin, inhibits mesangial cell proliferation and prevents the progression of kidney diseases by interfering with the activation of the Gas6/Axl pathway [42]. Levels of Gas6, a growth factor for glomerular cells, and its receptor Axl are both increased in various kidney diseases [8, 42]. Gas6 is activated by vitamin K-dependent γ -carboxylase which converts glutamic acid of N-terminal domain of Gas6 to γ -carboxyl glutamate. VKOR regulates the concentration of vitamin K in the vitamin K cycle, and then controls the γ -carboxylase activity [41, 43]. Therefore, VKOR controls the activation of the Gas6/Axl pathway and inhibition of VKOR would be a potential treatment for

kidney diseases [42]. Actually, warfarin has been used as a supportive medication for GN in clinical. However, warfarin inhibits not only kidney VKOR, but also liver VKOR involved in the biosynthesis of blood coagulation factors, increasing the risk of bleeding [16]. In the animal experiments, warfarin has been well known as a rodenticide [1]. On the other hand, following 14-days repeated oral administration of AMT up to 1,500 mg/kg to rats, no significant toxicity including hemorrhage and blood coagulation was shown in our study (data not shown). In the present study, AMT which had a potent inhibitory effect on liver VKOR, did not affect blood coagulation, nevertheless a renal anti-inflammatory effect was observed in the AMT-treatment group, which might be caused by the inhibition of kidney VKOR. We measured concentrations of AMT in kidney and liver after the intravenous administration of AMT in rats, and revealed that the concentration in kidney reached the IC_{50} against VKOR, but not that in liver. The renal anti-inflammatory effect and lack of an effect on anticoagulation were both explained by the VKOR inhibitory activity and tissue concentration of AMT in the Thy-1 GN rats. In the present study, AMT had no selectivity against kidney and liver VKOR, however, the tissue distribution of AMT was unique and the concentration of AMT was higher in kidney than liver. At the maximum concentration after oral administration of AMT, concentrations of AMT were higher in kidney than liver (data not shown). AMT is very small and highly polar. Therefore, it is likely to be distributed to the kidneys and excreted. Furthermore, AMT has no radical scavenging potential itself (data not shown). From these results, it was considered that the suppression of mesangial cell proliferation and glomerular inflammation in Thy-1 GN rats were based on the VKOR inhibitory effect of AMT.

In Thy-1 GN rats, following AMT treatment, CCr increased significantly and ACR decreased significantly.

Renal function can be evaluated by measuring the glomerular filtration rate (GFR) [29]. As it is not easy to measure the GFR directly, the serum/plasma and urine creatinine concentration is often used to assess renal function [2, 27]. CCr is better practical measure of GFR and renal function than the serum creatinine levels in animal models [6]. CCr is calculated from the urine creatinine concentration, urine flow rate and serum/plasma creatinine concentration. The urinary albumin-to-creatinine ratio (ACR) is widely used for the measurement of renal function in patients with GN, especially in diabetic renal disease [18, 24]. In Thy-1 rats, a marked decline in CCr and increase in ACR involved with the glomerular disorder are observed [9, 17, 37]. In the study, serum creatinine level was suppressed only by 10 mg/kg AMT treatment, but urine creatinine level was suppressed and subsequently CCr increased by 10 and 30 mg/kg treatments in Thy-1 rats. In addition, ACR also showed a tendency to decrease in a dose-dependent manner. Some reports demonstrate that ACR is a most important index in GN and well assesses the progression of kidney injury [18, 24]. Therefore, we think that the CCr and ACR are more sensitive than plasma (not serum) creatinine. AMT suppressed the glomerular mesangial cell proliferation and the progression of glomerular disease in Thy-1 GN rats.

In some recent clinical studies, warfarin-related nephropathy (WRN) was reported in patients with and without chronic kidney disease (CKD) [17, 24]. The cohort studies showed the 1-year mortality was higher in the patients with WRN than those without WRN. This finding is consistent with the previous reports of increased mortality rate in warfarin-treated chronic hemodialysis patients [4, 5]. However, the increased mortality rate associated with WRN is related to the comorbidities of diabetes, hypertension, and cardiovascular disease, and then the mechanisms and risks of WRN are unclear. Warfarin is widely used anticoagulant to treat or prevent thrombotic complications and increase mortality rate in CKD patients. We think that further study might be required to assess how these comorbidities are related to the mechanisms and risks [5].

Glomerulonephritis (GN) is a major cause of ESRD along with diabetic mellitus and hypertension [7]. However, there are no appropriate treatments for GN. For the anti-inflammatory therapy in GN, steroids are widely used in patients especially with IgA nephropathy which is typical mesangial proliferative GN [22, 44]. Steroids have great anti-inflammatory potency and reduce proteinuria, and then prevent the progression of kidney failure. However, extensive and severe side effects concerning immune, circulating and metabolic system are certainly observed in patients with the steroid-therapy [12, 23]. ACE inhibitors and ARBs are both widely used for kidney disease in patients with hypertension and diabetes [3, 28, 34]. ACE inhibitors and ARBs control blood pressure by inhibiting the production of Angiotensin II or preventing angiotensin II from binding to angiotensin II receptors in the renin-angiotensin system, and reduce hypertension. In addition, preclinical and clinical studies show that ACE inhibitors and ARBs decrease proteinuria and prevent kidney failure [3, 25, 35]. In Thy-1 GN rats, treatment with

ACE inhibitors or ARBs inhibited increases in both blood pressure and proteinuria, and then slowed the decline in renal function [36]. Persistent hypertension and glomerular hypertension can cause stretching the glomerular capillary wall, and result in endothelial damage and glomerular sclerosis, and then a rise in protein glomerular filtration [26]. Schmieder [31] and Mochizuki *et al.* [20] reported that these compounds inhibited kidney-specific renin-angiotensin-aldosterone system and showed the prevention of end-organ damage in kidney beyond these antihypertensive effects. On the other hand, the VKOR inhibitor AMT directly prevents renal mesangial cell proliferation and suppresses the progression of GN. Unfortunately, AMT is not as effective via oral route due to its low bioavailability. If improvements could be made to the biological stability of AMT, which has unique pharmacokinetic properties and distributes to kidney at a higher concentration than in liver, VKOR inhibitors would be useful for the treatment for GN, especially in combination with existing medications like ACE inhibitors or ARBs.

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