Toxicological and Pharmacological Effects of VKOR Inhibitors

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Abstract

Vitamin K_1 2,3-epoxide reductase (VKOR) inhibition is the main pharmacological effect of warfarin, an anticoagulant that is typically used in the prevention of thrombosis and thromboembolism. The repeated oral administration of sodium dehydroacetate (DHA-S), which has been used as a food additive, preservative, and antimicrobial agent, induced severe hemorrhage in multiple organs and prolonged blood coagulation factors with VKOR inhibition in rats. On the other hand, VKOR and the vitamin K–dependent growth arrest–specific gene 6 (Gas6)/Axl pathway play a key role in mesangial cell proliferation in glomerulonephritis (GN). We herein indicated the potential of the VKOR inhibitor, 3-acetyl-5-methyltetronic acid (AMT), to prevent the proliferation of glomerular mesangial cells and suppress the progression of GN. DHA-S-induced hemorrhage was caused by the depletion of blood VK, associated with any factors including VKOR inhibition. The novel VKOR inhibitor, AMT, reduced renal mesangial cell proliferation and may be a supportive treatment for GN.

Keywords: 3-acetyl-5-methyltetronic acid (AMT), hemorrhage, glomerulonephritis, sodium dehydroacetate (DHA-S), vitamin K_1 2,3-epoxide reductase (VKOR)

1. Introduction

The anticoagulant warfarin, which has a coumarin structure, causes hemorrhage by inhibiting the vitamin K (VK)–dependent synthesis of blood coagulation factors [1]. Blood coagulation factors undergo γ-carboxylation and have the ability to bind to calcium ions on the platelet surface, under the presence of VK and molecular oxygen. Vitamin K_1 2,3-epoxide (VKO), which is produced while this γ-carboxylation, is converted to vitamin K_1 (VK_1) by vitamin K_1 2,3-

epoxide reductase (VKOR) and is then recycled. Warfarin arrests the regeneration of VK by inhibiting VKOR and induces hemorrhage due to a VK deficiency.

Sodium dehydroacetate (DHA-S) has a wide spectrum of antimicrobial activity that accounts for its use in cosmetic products, as a preservative, and as an antimicrobial agent [2]. In Japan, it has also been used as a food preservative for cheese, butter, and margarine at a concentration of 0.5 g/kg or less as dehydroacetic acid [3, 4]. This concentration is the highest dose allowed under the Food Sanitation Law; however, the risk assessment of DHA-S has not been sufficiently performed for a food additive, and its acceptable daily intake has not yet been established. On the other hand, the toxicity of DHA-S has been widely studied. In an acute toxicity study of DHA-S using dogs, clinical signs such as salivation, vomiting, convulsions, and ataxia were reported [5]. In a subchronic toxicity study of DHA-S using dogs, body weight loss, gastric hemorrhage, and an increase in blood urea nitrogen were observed [6]. These findings were considered to be primarily due to a lack of appetite and subsequent weight loss [6]. However, the main structure of DHA-S, 2H-pyran-2-on, is included in a coumarin structure. It is reported that some derivatives of 4-hydroxy-2-pyrone, which includes similar structure of DHA-S, exhibit anti-blood coagulant activities in rats [7]. These findings suggest that DHA-S also shows the anticoagulant activity. Therefore, we investigated the effects of repeated administration of DHA-S in rats, and the amelioration by VK against DHA-S-induced hemorrhage [8].

In Thy-1 glomerulonephritis (GN) rats, the expression of growth arrest-specific gene 6 (Gas6) and Axl was found to be markedly increased in glomeruli and paralleled the proliferation of mesangial cells [9, 10]. VKOR is also related with mesangial cell proliferation through the vitamin K–dependent activation of Gas6 [10]. Gas6 is as an autocrine growth factor for mesangial cells through binding to its cell surface receptor Axl. These findings suggest that the Gas6/Axl pathway plays an important role in mesangial cell proliferation in GN [11]. VKOR inhibitors including warfarin is effective at blocking mesangial cell proliferation and improving renal function [11]; however, it inhibits the production of blood coagulation factors in the liver and has been able to cause bleeding [12]. Therefore, low-dose treatments of warfarin are used in renal failure patients, and the control of its blood concentration or the conformation of blood coagulation activities have to been required [13]. We are discovering new compounds that inhibit mesangial cell proliferation, but not hepatic production of blood coagulation factors using Thy-1 GN rats **Table 2**. Thy-1 GN rats have been used as an acute model of GN accompanied with mesangial cell proliferation, matrix expansion, and moderate decline of renal function [14]. We indicated 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 [15].

2. Main body

In a 14-day repeated toxicity study using rats, animals that received 200 or 400 mg/kg/day of DHA-S died from severe hemorrhage in various organs, such as the stomach, intestines, testes, epididymides, subcutaneous tissue, and subdural area. The profile of hemorrhage caused by

DHA-S, which occurred in multiple organs and showed differences in sensitivity among individuals, was similar to that caused by warfarin. The prothrombin time (PT) and activated partial thromboplastin time (APTT) were significantly prolonged in each surviving rat in the 100 and 200 mg/kg/day groups (**Figure 1**). These results indicated that the repeated dosing of DHA-S-induced hemorrhage in rats [8].
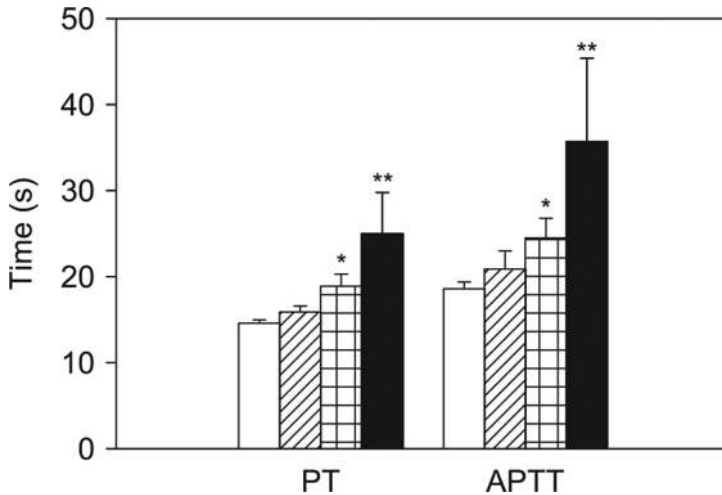

Figure 1. Effects on PT and APTT in male rats received the repeated oral administration of DHA-S at doses of 50, 100, and 200 mg/kg/day. (▢) Vehicle; (▨) 50 mg/kg/day; (▦) 100 mg/kg/day; (■) 200 mg/kg/day. Each value is presented as the mean ± SD. * $P < 0.05$, ** $P < 0.01$: Significantly different from controls at 5% and 1%.

On the other hand, DHA-S-treated rats showed no significant decrease in PLT. In the histopathological examination of the liver, marked changes, such as fibrin thrombi in the vascular system and single cell necrosis, were not observed. Therefore, hemorrhage was assumed to be caused by a deficiency in VK. In order to demonstrate this hypothesis, the effects of VK on hemorrhage were investigated using rats that received a single subcutaneous injection of VK_2 following the repeated oral administration of DHA-S at 200 mg/kg/day for 5 days. In rats, dietary VK_1 was converted to menadione in the gut and menadione was absorbed and converted to VK_2 [16]. Consequently, VK_2 (menaquinone-4) reached a maximum concentration in the liver in rats faster than after the intravenous or intraperitoneal administration of VK_1 [17]. VK_2 was also reported to exert stronger preventive effects against hemorrhage than other VK products, such as VK_1 and VK_3. As a result, DHA-S prolonged blood coagulation parameters (PT, APTT, the thrombo test (TTO), and hepaplastin test (HPT), in rats that received 200 mg/kg/day for 5 days (**Figure 2**). However, the prolongation of these parameters was suppressed in animals injected subcutaneously with VK_2 after DHA-S dosing. Since TTO and HPT are sensitive indexes of a deficiency in VK-dependent blood coagulation factors [18, 19], it was concluded that DHA-S induced hemorrhage via a deficiency in VK.
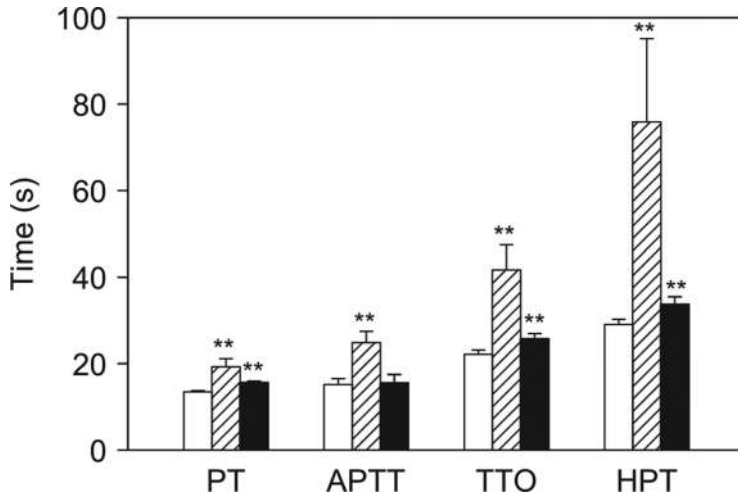
Figure 2. Effects on blood coagulation parameters (PT, APTT, TTO, and HPT) in male rats received DHA-S for 5 days following a single subcutaneous injection of vitamin K_2 (VK_2). (▢) Vehicle; (▨) DHA-S at 200 mg/kg/day for 5 days; (■) DHA-S at 200 mg/kg/day for 5 days and VK_2 at 1 mg/kg after the final DHA-S dosing on day 5. Each value is presented as the mean ± SD. ** $P < 0.01$: Significantly different from controls at 1%.

A VK deficiency is caused by anticoagulants, hepatobiliary disease, the inhibition of VK production by the intestinal flora, and the poor absorption of VK from the gut [20]. Prolongation of PT and APTT were also observed in the 100 mg/kg group, which did not significantly decrease in body weight. In the macroscopic observation of surviving rats, no remarkable changes were detected in the gastro-intestinal tracts. While DHA-S exhibited weak antimicrobial activity against obligatory anaerobes, the major intestinal flora [2], the effects of a high dose of DHA-S on the intestinal flora currently remain unknown. VK is a cofactor for enzymatic conversion (γ-carboxylation) of glutamic acid residues in the inactive precursors of VK-dependent proteins (blood coagulation factors) in order to convert them to their active forms. In VK cycle, accompanying with this reaction, VK_1H_2 (hydroquinone form), is oxidized to VKO, and subsequently reduced the VK_1 (quinone form). The anticoagulant warfarin is known to be a VK antagonist and is used as a rodenticide to induce hemorrhage in rats [21]. A warfarin-induced VK deficiency occurs through the inhibition of VKOR that then converts VKO to the quinone form of VK_1 in the VK cycle [1, 22, 23]. The coumarin structure of warfarin involves the main structure of DHA-S, and some derivatives of 4-hydroxy-2-pyrone have been reported to exhibit anticoagulant activities in rats [7]. The hemorrhage induced by DHA-S, which occurs in multiple organs and shows individual differences in sensitivity, is similar to that by warfarin.

The effects of DHA-S on VKOR activity were investigated using male rat liver microsomes. VKOR activity, expressed as the quantitative amount of VK_1 converted from VKO, was induced *in vitro* by a VKOR reaction and the inhibition by each compound was estimated from the percentage of VKOR activity measured in an identical incubation containing no compound. DHA-S and warfarin both showed dose-dependent suppression of VKOR activities, and DHA-

S inhibited clearly VKOR activity as well as warfarin [8]. The IC_{50} values of DHA-S and warfarin were 3149 and 2.15 µmol/L, respectively. The inhibitory effect of DHA-S on VKOR activity was expected to be approximately 1400-fold as compared with warfarin. The treatment with DHA-S at dose of 100 mg/kg (480 µmol/kg) for 5 days prolonged PT and APTT in rats by approximately 1.3-fold against that of the control. On the other hand, the repeated oral administration of warfarin at 0.2 mg/kg (0.6 µmol/kg) for 5 days elongated PT in rats [24]. Furthermore, TTO activity was reduced by approximately 50% in rats under a controlled rate (0.15 µmol/kg/day) of warfarin administration by osmotic pumps implanted subcutaneously [25]. The dose which DHA-S inhibits blood coagulation factors in rats is 800–3200-fold higher than that of warfarin. This fact explains the difference between the VKOR inhibitory activities of DHA-S and warfarin. In humans, the daily intake level of DHA is estimated to be 0.0303 mg [26] which is approximately 16,500-fold less than that inducing hemorrhage in rats. Therefore, the potential of DHA-S to induce hemorrhage in humans is low [8]. In conclusion, DHA-S induces severe hemorrhage in rats through the depletion of blood VK associated with any factors including the inhibition of VKOR [8].

On the other hand, we demonstrated that the novel VKOR inhibitor, AMT prevented renal mesangial cell proliferation, and then suppressed glomerular injury in Thy-1 GN rats [15]. The low molecular weight compound, AMT exhibits inhibitory activity against rat liver and kidney VKOR *in vitro* at IC_{50} values of 3.18 and 3.20 nmol/mL, respectively [15]. VKOR is a protein that converts the epoxide of VK back to VK, a co-factor that is essential for the post-translational γ-carboxylation of several blood coagulation factors, and is also involved in mesangial cell proliferation via the VK-dependent activation of the Gas6/Axl pathway [11, 27]. The gene of VKOR was identified and VKOR was expressed ubiquitously in the liver, kidney, lung, heart, and skeletal muscle [27, 28]. However, no information exists on the specific isoforms of VKOR in any tissue. Therefore, AMT showed no selectivity in its inhibitory activity toward rat kidney and liver VKOR. It is known that there are no selective compounds including warfarin. These findings suggest that kidney and liver VKOR have similar substrate recognition or that there was no specific isoform of VKOR, at least in the rat liver and kidney.

In Thy-1 GN rats, kidney weights decreased significantly in a dose-dependent manner after the AMT treatment (**Table 1**). No significant changes were observed in light microscopy, while the slight enlargement and rough surface of kidneys were noted in the control [15]. Renal enlargement followed by mesangial cell proliferation is frequently observed in Thy-1 GN rats and is mostly due to edema associated with inflammation [29, 30]. Thickening of the glomerular basement membrane and fibrocellular crescent of the glomerulus were histopathologically suppressed by the AMT treatment (**Figure 3**). These pathological findings associated with the progression of renal injury [14, 29] may be caused by the inhibitory effects of AMT on mesangial cell proliferation and glomerular inflammation [15]. Additionally, tubular degeneration such as hyaline casts, hyaline droplets, tubular dilatation, and basophilic tubules followed by glomerular damage were found to be dose-dependently suppressed by the AMT treatment in Thy-1 GN rats [15].

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	$0.417 \pm 0.016^{*}$	(6)	$0.409 \pm 0.017^{*}$	(6)	$0.826 \pm 0.033^{*}$	(6)
	30 mg/kg	$0.395 \pm 0.009^{**}$	(6)	$0.383 \pm 0.010^{**}$	(6)	$0.779 \pm 0.018^{**}$	(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.

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.
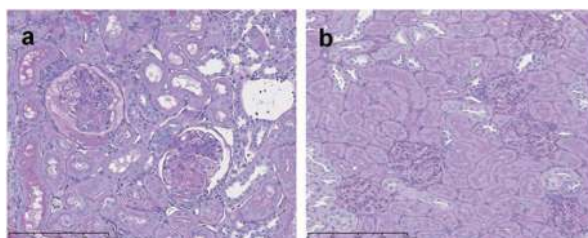

Figure 3. Light microscope of kidneys after the 12-day repeated intravenous administration of AMT to Thy-1 GN rats. Periodic acid-Schiff (PAS)-stained sections show thickening of the glomerular basement membrane and fibrocellular crescent of the glomerulus in the 0 mg/kg group (a), and no significant change in the glomerulus or tubules in the 30 mg/kg group (b).

The Thy-1 GN rat is one of the reversible models of kidney injury. An anti-Thy-1 injection induces the destruction of glomerular mesangial cells and leads to the thickening of the glomerular basement membrane in rats [14, 31]. A decline in renal function has been observed 1 week after its injection; however, renal function recovers completely 5–6 weeks later [14, 31]. In addition, marked mesangial cell proliferation has been reported in patients with chronic GN [32, 33]. Therefore, the Thy-1 GN rat is considered to be an adequate animal model of GN and is suitable for assessing the effectiveness of chemical compounds as suppressors toward the disease [34–36].

The typical VKOR inhibitor, warfarin, inhibits mesangial cell proliferation and prevents the progression of kidney diseases by inhibition of the activation of the Gas6/Axl pathway [11]. Both levels of Gas6 and its receptor Axl increased in various kidney diseases [11, 37]. Gas6 is activated by vitamin K–dependent γ-carboxylase, which converts the glutamic acid of the N-terminal domain of Gas6 to the γ-carboxyl glutamate. VKOR regulates the concentration of VK in the VK cycle, and then controls γ-carboxylase activity [9, 10]. Therefore, VKOR controls the activation of the Gas6/Axl pathway, and its inhibition has potential as a therapeutic treatment for kidney diseases [11]. Warfarin has been used as a supportive medication for

human GN in clinical. However, it inhibits not only renal VKOR, but also hepatic VKOR, which is involved in the production of blood coagulation factors, thereby increasing the bleeding risk [12].

In our experiment, following the 14-day repeated oral administration of AMT up to 1500 mg/kg to rats, no significant toxicities including hemorrhage and blood coagulation were noted in our study (data not shown). AMT, which exerts potent inhibitory effects on hepatic VKOR, did not affect blood coagulation in Thy-1 GN rats. Nevertheless, anti-inflammatory effects in kidney were observed in the AMT-treatment group, which may be caused by the inhibition of renal VKOR. We measured AMT concentrations in the kidney and liver after the intravenous administration of AMT to rats. Kidney and liver concentrations of AMT 5 min after the single intravenous administration of 30 mg/kg were 4.26 nmol/g tissue and 0.26 nmol/g tissue, respectively [15], and revealed that the concentration in the kidney reached the IC_{50} value (3.2 nmol/L) against VKOR, whereas that in liver did not (3.18 nmol/L). Renal anti-inflammatory effects and the lack of an effect on anticoagulation were both explained by VKOR inhibitory activity and tissue concentrations of AMT in Thy-1 GN rats. AMT had no selectivity against kidney and liver VKOR; however, the tissue distribution of AMT was unique, and its concentration was higher in the kidney than in the liver. At the maximum concentration after its oral administration, AMT concentrations were higher in the kidney than in the liver (data not shown). AMT is very small and highly polar, and, as such, is likely to be distributed to the kidneys and excreted. Furthermore, it has no radical scavenging potential itself (data not shown). Therefore, the suppression of mesangial cell proliferation and glomerular inflammation in Thy-1 GN rats was considered to be based on the VKOR inhibitory effects of AMT. In Thy-1 GN rat following the AMT treatment, creatinine clearance (CCr) significantly increased, and the urinary albumin-to-creatinine ratio (ACR) significantly decreased [15].

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

In Thy-1 rats, a marked decline in CCr and increase in ACR involved with the glomerular disorder have been reported [9, 17, 37]. In our study, serum creatinine levels were only suppressed by the 10 mg/kg AMT treatment, whereas urine creatinine levels were suppressed

and CCr was subsequently increased by the 10 and 30 mg/kg treatments in Thy-1 rats. In addition, ACR was also slightly decreased in a dose-dependent manner. Previous studies demonstrated that ACR is the most important index in GN and accurately assesses the progression of kidney injury [18, 24]. Therefore, we consider that the CCr and ACR to be more sensitive than plasma and/or serum creatinine. On the other hand, no significant change was observed in blood coagulation tests such as PT, APTT, TTO, and HPT. Based on results, AMT suppressed glomerular mesangial cell proliferation and the progression of glomerular disease in Thy-1 GN rats at a non-toxic dose. Recently, warfarin-related nephropathy (WRN) has been reported in patients with and without chronic kidney disease (CKD) [38, 39]. Clinical studies showed that mortality rates in 1 year were higher in WRN patients than in other patients. This result coincides with previous findings of increased mortality rates in warfarin-treated chronic hemodialysis patients [40, 41]. However, the increased mortality rate associated with WRN is related to the complications of diabetes, hypertension, cardiovascular disease, etc., and the mechanisms and risks of WRN currently remain unclear. Warfarin is a widely used anticoagulant for thrombotic complications and increases mortality rates in CKD patients. Further studies are necessary to assess how these complications are related to the mechanisms and risks [41].

GN is a major process of end-stage renal disease (ESRD) along with diabetes and hypertension [42]. However, there are no appropriate therapeutic treatments for GN. In therapy for GN, steroids are widely used as anti-inflammation drugs for patients, particularly those with IgA nephropathy, which is typical mesangial proliferative GN [43, 44]. As steroids reduce proteinuria, they prevent the progression of kidney failure. However, extensive and severe side effects concerning the immune, circulating, and metabolic systems have been reported in patients with steroid therapy [45, 46]. Both angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) are widely treated for kidney disease in patients with diabetes and hypertension [47–49]. ACE inhibitors and ARBs control blood pressure by inhibiting the production of angiotensin II or preventing it from binding to angiotensin II receptors in the renin-angiotensin system, and, thus, reduce hypertension. In addition, nonclinical and clinical studies have demonstrated that ACE inhibitors and ARBs decrease proteinuria and prevent kidney failure [47, 50, 51]. In Thy-1 GN rats, treatments with ACE inhibitors or ARBs were found to inhibit increases in blood pressure and proteinuria, and subsequently the decline in renal function [52]. Persistent hypertension and glomerular hypertension may stretch the glomerular capillary wall, and result in endothelial damage and glomerular sclerosis, followed by a rise in protein glomerular filtration [53]. Schmieder [54] and Mochizuki et al. [55] reported that these compounds inhibited the kidney-specific renin-angiotensin aldosterone system and prevented end-organ damage in the kidney beyond these antihypertensive effects. On the other hand, the VKOR inhibitor, AMT, prevents mesangial cell proliferation in the kidneys and suppresses the progression of GN. Unfortunately, AMT is not as effective by oral administration due to its low bioavailability. If improvements are archived in the biological stability of AMT, which has unique pharmacokinetic properties and is distributed to the kidney at a higher concentration than to the liver, VKOR inhibitors may be useful for the treatment for GN, particularly in combination with existing medications such as ACE inhibitors or ARBs.

In conclusion, the novel VKOR inhibitor, AMT, reduced renal mesangial cell proliferation and may be a supportive treatment for GN.

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References

[1] Sutcliffe FA, MacNicoll AD, Gibson GG. Aspects of anticoagulant action: a review of the pharmacology, metabolism and toxicology of warfarin and congeners. Rev Drug Metab Drug Interact. 1987;5(4):225–272. doi:10.1515/DMDI.1987.5.4.225.

[2] Cosmetic Ingredient Review. Final report on the safety assessment of sodium dehydroacetate and dehydroacetic acid. Int J Toxicol. 1985;4(3):123–159. doi:10.3109/10915818509078671.

[3] Kanai K, Asada-Kubota M, Kanamura S. Morphological studies on the liver of mice administered with dehydroacetic acid. Kankyo Kagaku Sogo Kenkyujo Nenpo. 1981;8:27–31.

[4] Uchida O, Naito K, Yasuhara K, Ohba S, Sato C, Shimonuri S, Kobayashi K, Tobe M. Studies on the acute oral toxicity of dehydroacetic acid, sorbic acid and their combination compound in rats. Eisei Shikenjo Hokoku. 1985;103:166–171.

[5] Seevers MH, Shideman FE, Woods LA, Weeks JR, Kruse WT. Dehydroacetic acid (DHA). II. General pharmacology and mechanism of action. J Pharmacol Exp Ther. 1950;99(1):69–83.

[6] Spencer HC, Rowe VK, McCollister DD. Dehydroacetic acid (DHA). I. Acute and chronic toxicity. J Pharmacol Exp Ther.1950;99(1):57–68.

[7]   Rehse K, Schinkel W, Siemann U. Anticoagulant 4-hydroxy-2-pyrones. Arch Pharm (Weinheim). 1980;313(4):344–351. doi:10.1002/ardp.19803130411.

[8]   Sakaguchi Y, Suga S, Oshida K, Miyamoto-Kuramitsu K, Ueda K, Miyamoto Y. Anticoagulant effect of sodium dehydroacetate (DHA-S) in rats. J Appl Toxicol. 2008;28(4):524–529. doi:10.1002/jat.1304.

[9]   Yanagita M, Arai H, Ishii K, Nakano T, Ohashi K, Mizuno K, Varnum B, Fukatsu A, Doi T, Kita T. Gas6 regulates mesangial cell proliferation through Axl in experimental glomerulonephritis. Am J Pathol. 2001;158(4):1423–1432. doi:10.1016/S0002-9440(10)64093-X.

[10] Yanagita M. The role of the vitamin K-dependent growth factor Gas6 in glomerular pathophysiology. Curr Opin Nephrol Hypertens. 2004;13(4):465–470.

[11] Yanagita M. Gas6, warfarin, and kidney diseases. Clin Exp Nephrol. 2004;8(4):304–309. doi:10.1007/s10157-004-0305-z.

[12] Limdi NA, Beasley TM, Baird MF, Goldstein JA, McGwin G, Arnett DK, Acton RT, Allon M. Kidney function influences warfarin responsiveness and hemorrhagic complications. J Am Soc Nephrol. 2009;20(4):912–921. doi:10.1681/ASN.2008070802.

[13] Woo KT. Current therapeutic strategies in glomerulonephritis. Ann Acad Med Singapore. 1999;28(2):272–278.

[14] Jefferson JA, Johonson RJ. Experimental mesangial proliferative glomerulonephritis (the anti-Thy-1.1 model). J Nephrol. 1999;12(5):297–307.

[15] Uchida M, Sakaguchi Y, Miyamoto Y. A novel vitamin K_1 2,3-epoxide reductase (VKOR) inhibitor, 3-acetyl-5-methyltetronic acid, reduces experimental glomerulonephritis. J Vet Med Sci. 2012l;74(7):863–869. doi:10.1292/jvms.11-0530.

[16] Billeter M, Bollinger W, Martius C. Untersuchungen uber die umwandlung von verfutterten K-vitaminen durch austausch der seitenkette und die rolle der darmbakterien hierbei. Biochem Z. 1964;340:290–303.

[17] Sakamoto N, Kimura M, Hiraike H, Itokawa Y. Changes of phylloquinone and menaquinone-4 concentrations in rat liver after oral, intravenous and intraperitoneal administration. Int J Vitam Nutr Res. 1996;66(4):322–328.

[18] Motohara K, Endo F, Matsuda I. Effect of vitamin K administration on carboxy prothrombin (PIVKA-II) levels in newborns. Lancet. 1985; 3(8449):242–244. doi:10.1016/S0140-6736(85)90291-0.

[19] Roncaglioni MC, Reyers I, Cerletti C, Donati MB, De Gaetano G. Moderate anticoagulation by salicylate prevents thrombosis without bleeding complications. An experimental study in rats. Biochem Pharmacol. 1988;37(24):4743–4745. doi:10.1016/0006-2952(88)90346-2.

[20] Shearer MJ. Vitamin K and vitamin K-dependent proteins. Br J Haematol. 1990;75(2): 156–162. doi:10.1111/j.1365-2141.1990.00156.x

[21] Bentley EW. A review of anticoagulant rodenticides in current use. Bull World Health Organ. 1972;47(3):275–280.

[22] Park BK. Warfarin: metabolism and mode of action: a review. Biochem Pharmacol. 1988;37(1):19–27. doi:10.1016/0006-2952(88)90750-2.

[23] Cain D, Hutson SM, Wallin R. Warfarin resistance is associated with a protein component of the vitamin K 2,3-epoxide reductase enzyme complex in rat liver. Thromb Haemost. 1998;80(1):128–133.

[24] Chan K, Lo AC, Yeung JH, Woo KS. The effects of Danshen (Salvia miltiorrhiza) on warfarin pharmacodynamics and pharmacokinetics of warfarin enantiomers in rats. J Pharm Pharmacol. 1995;47(5):402–406. doi:10.1111/j.2042-7158.1995.tb05819.x.

[25] Mosterd JJ, Thijssen HH. The relationship between the vitamin K cycle inhibition and the plasma anticoagulant response at steady-state S-warfarin conditions in the rat. J Pharmacol Exp Ther. 1991;260(3):1081–1085.

[26] Ishiwata H, Nishijima M, Fukasawa Y. Estimation of preservative concentration in foods and their daily intake based on official inspection results in Japan in fiscal year 1998. Shokuhin Eiseigaku Zasshi 2001;42(6):404–412.

[27] Wang Y, Zhen Y, Shi Y, Chen J, Zhang C, Wang X, Yang X, Zheng Y, Liu Y, Hui R. Vitamin K epoxide reductase: a protein involved in angiogenesis. Mol. Cancer Res. 2005;3(6): 317–323. doi:10.1158/1541-7786.MCR-04-0221.

[28] Li T, Chang CY, Jin DY, Lin PJ, Khvorova A, Stafford DW. Identification of the gene for vitamin K epoxide reductase. Nature. 2004;427(6974):541–544. doi:10.1038/nature02254.

[29] Yamamoto T, Mundy CA, Wilson CB, Blantz, RC. Effect of mesangial cell lysis and proliferation on glomerular hemodynamics in the rat. Kidney Int. 1991;40(4):705–713. doi:10.1038/ki.1991.264.

[30] Zoran LB. Principles of genitourinary radiology. 2nd ed. Thieme Medical Publishers; 1994.

[31] Shimizu F, Kawachi H, Orikasa M. Role of mesangial cell damage in progressive renal disease. Kidney Blood Press Res. 1999;22(2):5–12. doi:10.1159/000025903.

[32] Ito S, Kuriyama H, Iino N, Iguchi S, Shimada H, Ueno M, Narita I, Nakano M, Nishi S, Karasawa R, Gejyo F. Patient with diffuse mesangial and endocapillary proliferative glomerulonephritis with hypocomplementemia and elevated anti-streptolysin O treated with prednisolone, angiotensin-converting enzyme inhibitor, and angiotensin II receptor antagonist. Clin Exp Nephrol. 2003;7(4):290–295.

[33] Noël LH, Gubler M. C. Histological classification of chronic glomerular diseases. Rev. Prat. 2003;53(18):2005–2012.

[34] Gohda T, Ra C, Hamada C, Tsuge T, Kawachi H, Tomino Y. Suppressive activity of pemirolast potassium, an antiallergic drug, on glomerulonephritis. Studies in glomerulonephritis model rats and in patients with chronic glomerulonephritis concurrently affected by allergic rhinitis. Arzneimittelforschung. 2008;58(1):18–23. doi:10.1055/s-0031-1296461.

[35] Mii A, Shimizu A, Masuda Y, Ishizaki M, Kawachi H, Iino Y, Katayama Y, Fukuda Y. Angiotensin II receptor blockade inhibits acute glomerular injuries with the alteration of receptor expression. Lab. Invest. 2009;89(2):164–177. doi:10.1038/labinvest.2008.128.

[36] Sadakane C, Kase Y, Koseki J, Hasegawa Y, Shindo S, Maruyama H, Takeda S, Takeda H, Hattori T. Effects of TJN-598, a new selective phosphodiesterase type IV inhibitor on anti-Thy1 nephritis in rats. Clin Exp Nephrol. 2011;15(1):14–24.

[37] Eng PC, Chua WC, Suk Peng Chew V, Wong PT, Yin JL, Hambly B, McLachlan CS. Chronic angiotensin-converting enzyme inhibition up-regulates mouse kidney growth arrest specific-6 protein and the AXL subfamily of receptor tyrosine kinases. J Renin Angiotensin Aldosterone Syst. 2008;9(4):238–241. doi:10.1177/1470320308098342.

[38] Makibayashi K, Tatematsu M, Hirata M, Fukushima N, Kusano K, Ohashi S, Abe H, Kuze K, Fukatsu A, Kita T, Doi T. A vitamin D analog ameliorates glomerular injury on rat glomerulonephritis. Am J Pathol. 2001;158(5):1733–1741. doi:10.1016/S0002-9440(10)64129-6.

[39] Pavkov ME, Knowler WC, Hanson RL, Bennett PH, Nelson RG. Predictive power of sequential measures of albuminuria for progression to ESRD or death in Pima Indians with type 2 diabetes. Am J Kidney Dis. 2008;51(5):759–766. doi:10.1053/j.ajkd.2008.01.011.

[40] Brodsky SV, Satoskar A, Chen J, Nadasdy G, Eagen JW, Hamirani M, Hebert L, Calomeni E, Nadasdy T. Acute kidney injury during warfarin therapy associated with obstructive tubular red blood cell casts: a report of 9 cases. Am J Kidney Dis. 2009;54(6):1121–1126. doi:10.1053/j.ajkd.2009.04.024.

[41] Brodsky SV, Nadasdy T, Rovin BH, Satoskar AA, Nadasdy GM, Wu HM, Bhatt UY, Hebert LA. Warfarin-related nephropathy occurs in patients with and without chronic kidney disease and is associated with an increased mortality rate. Kidney Int. 2011;80(2):181–189. doi:10.1038/ki.2011.44.

[42] Constantini E. United States Renal Data System 2006 Annual Data Report: Atlas of Chronic Kidney Disease & End-Stage Renal Disease in the United States. USRDS 2006 ADR: Precis-Background on the US ESRD program. Am J Kidney Dis. 2007;49(Suppl 1):S17–S32. doi:10.1053/j.ajkd.2006.11.004.

[43] Nolin L, Courteau M. Management of IgA nephropathy: evidence-based recommendations. Kidney Int. 1999;55(Suppl 70):S56-S62. doi:10.1046/j.1523-1755.1999.07008.x.

[44] Zhou YH, Tang LG, Guo SL, Jin ZC, Wu MJ, Zang JJ, Xu JF, Wu CF, Qin YY, Cai Q, Gao QB, Zhang SS, Yu DH, He J. Steroids in the treatment of IgA nephropathy to the improvement of renal survival: a systematic review and meta-analysis. PLoS ONE 2011;6(4):e18788. doi:10.1371/journal.pone.0018788.

[45] Hodson EM, Knight JF, Willis NS, Craig JC. Corticosteroid therapy for nephrotic syndrome in children. Cochrane Database Syst Rev. 2001;3:CD001533. doi:10.1002/14651858.CD001533.pub5.

[46] Olney RC. Mechanisms of impaired growth: effect of steroids on bone and cartilage. Horm Res. 2009;72(Suppl 1):30–35. doi:10.1159/000229761.

[47] Brewster UC, Perazella MA. The renin-angiotensin-aldosterone system and the kidney: effects on kidney disease. Am J Med. 2004;116(4):263–272. doi:10.1016/j.amjmed.2003.09.034.

[48] Ruggenenti P, Perna A, Gherardi G, Benini R, Remuzzi G. Chronic proteinuric nephropathies: outcomes and response to treatment in a prospective cohort of 352 patients with different patterns of renal injury. Am J Kidney Dis. 200;35(6):1155–1165. doi:10.1016/S0272-6386(00)70054-0.

[49] THE GISEN GROUP. Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy. Lancet. 1997;349(9069?):1857–1863. doi:10.1016/S0140-6736(96)11445-8.

[50] Pugsley MK. The angiotensin-II (AT-II) receptor blocker olmesartan reduces renal damage in animal models of hypertension and diabetes. Proc West Pharmacol Soc. 2005;48:35–38.

[51] Tomita M, Sogabe H, Nakazato S, Nakatsuji S, Noto T, Hamada K, Kawachi H, Shimizu F, Matsuo M, Mutoh S. Monoclonal antibody 1-22-3-induced glomerulonephritis in uninephrectomized rats as a model of progressive renal failure. Nephrol Dial Transplant. 2005;20(11):2358–02367. doi:10.1093/ndt/gfi062.

[52] Villa L, Boor P, Konieczny A, Kunter U, van Roeyen CR, Denecke B, Gan L, Kupper MB, Hoffmann K, Eitner F, Ostendorf T, Floege J. Effects and mechanisms of angiotensin II receptor blockade with telmisartan in a normotensive model of mesangioproliferative nephritis. Nephrol Dial Transplant. 2011;26(10):3131–3143. doi:10.1093/ndt/gfr096.

[53] Qian Y1, Feldman E, Pennathur S, Kretzler M, Brosius FC 3rd. From Fibrosis to Sclerosis; Mechanisms of glomerulosclerosis in diabetic nephropathy. Diabetes. 2008;57(6):439–1445. doi:10.2337/db08-0061.

[54] Schmieder RE. Mechanisms for the clinical benefits of angiotensin II receptor blockers. Am J Hypertens. 2005;18(5):720–730. doi:10.1016/j.amjhyper.2004.11.032.

[55] Mochizuki S, Dahlöf B, Shimizu M, Ikewaki K, Yoshikawa M, Taniguchi I, Ohta M, Yamada T, Ogawa K, Kanae K, Kawai M, Seki S, Okazaki F, Taniguchi M, Yoshida S, Tajima N; Jikei Heart Study group. Valsartan in a Japanese population with hypertension and other cardiovascular disease (Jikei Heart Study): a randomised, openlabel, blinded endpoint morbidity-mortality study. Lancet. 2007;369(9571):1431–1439. doi: 10.1016/S0140-6736(07)60669-2.