Antioxidant and Antithrombotic Therapies for Diabetic Kidney Disease

Wenjun Yan,[1] Baoqin Zhou,[1] Yunfeng Shen,[2] Gaosi Xu[3]

[1]Medical Center of the Graduate School, Nanchang University, Nanchang, China
[2]Department of Endocrinology, Second Affiliated Hospital, Nanchang University, Nanchang, China
[3]Department of Nephrology, Second Affiliated Hospital, Nanchang University, Nanchang, China

With an increasing incidence, diabetic kidney disease (DKD) has been the leading cause of chronic kidney disease and end-stage renal disease, and conventional therapies did not change this situation. This study intended to review and analyze the antioxidant and antithrombotic treatments of DKD for seeking novel therapeutic strategies. Relevant articles involved with antioxidant and antithrombotic treatments in DKD were retrieved and analyzed via systematic assessment. Meta-analysis showed that pancreatic kallikrein definitely reduced glycated hemoglobin in DKD patients (mean difference, 0.36%; 95% confidence interval, 0.08% to 0.63%; P = .01). Apart from the classic agents such as aspirin, novel drugs such as pancreatic kallikrein, sulodexide, and especially the traditional Chinese medicine including *Tripterygium wilfordii* and lumbrukinase, exert beneficial effects in DKD patients. Antioxidant and antithrombotic treatments are beneficial for DKD patients and represent promising therapeutic strategies in the future.

INTRODUCTION

Diabetes mellitus (DM) is one of the most common metabolic diseases in adults and a serious global health burden.[1] It is estimated that more than 382 million people suffer from DM worldwide and the number is expected to reach 592 million by 2035.[2] The development of DM is closely associated with systemic damages to microvessels,[3] resulting in a series of complications, which significantly affect the mortality and prognosis.[4]

Among diabetic complications, diabetic kidney disease (DKD) accounts for a significant proportion and has been the leading cause of chronic kidney disease and end-stage renal disease as well.[5] With the increasing morbidity and extension of lifespan as a results of advancement in therapies in DM, the number of patients with DKD is rising rapidly.[6] Approximately 20% to 30% of patients with type 1 DM and 40% of patients with type 2 DM will eventually develop DKD.[7] In the United States, 44% of newly diagnosed end-stage renal disease patients are closely related to DKD.[8]

End-stage renal disease is not inevitable for DKD patients.[9] With effective control of blood pressure and glycemia, mortality due to diabetic complications has decreased significantly among DKD patients and a better prognosis has been achieved. However, DKD still plays a major role in the causes of chronic kidney disease and end-stage renal disease. Thus, it is urgent to seek novel therapeutic strategies to maximally delay progression for DKD patients. The present study is intended to review and assess the efficacy of antioxidant and antithrombotic therapies in DKD treatment.

ANTIOXIDANT THERAPIES
Mechanism of Action

Oxidative stress caused by long-term hyperglycemia is closely related to the progression of DKD and risks of cardiovascular diseases.[10-12] Advanced glycation end products, including

glycosylated lipids, proteins, and nucleic acids, can be peroxided and accumulate in glomerulus, contributing to mesangial proliferation and glomerular sclerosis.[13,14] Expression of antioxidant enzymes, including superoxide dismutase and glutathione peroxidase, can be inhibited by aldose reductase in hyperglycemia.[15] Subsequent imbalance in oxidation-antioxidant system is responsible for the tissue damages.[13] Simultaneously, activated cytokines, such as platelet-derived growth factor, intercellular adhesion molecule-1, and transforming growth factor-β1, promote the proliferation in basement membrane thickness and mesangial matrix.[16] Systematic reviewing the literature, we concluded that *Tripterygium wilfordii* and pancreatic kallikrein are the most promising agents in antioxidant therapies for DKD.

Tripterygium Wilfordii

Tripterygium wilfordii is a traditional Chinese herbal medicine and is widely used in therapies of tumor and autoimmune diseases. Similar to glucocorticoids, *Tripterygium wilfordii* inhibits cellular and humoral immunity.[17,18] However, *Tripterygium wilfordii* has much lower influence on blood pressure and glycemia compared with glucocorticoids, which makes it its unique feature in treatment of DKD. Choi and colleagues[19] showed that extracts from *Tripterygium wilfordii* significantly reduced cytoplasmic oxidation and inhibited damages in cellular morphology and DNA induced by hydrogen peroxide in human dopaminergic cells, suggesting that *Tripterygium wilfordii* is able to resist oxidative stress due to hydrogen peroxide. In addition, *Tripterygium wilfordii* therapies contribute to the upregulation of expression of superoxide dismutase and glutathione peroxidase,[20] both being important factors in antioxidation. A clinical study from China showed that *Tripterygium wilfordii* inhibited the lesions in podocytes and significantly decreased albuminuria in DKD patients.[21] Hepatotoxicity and sexual inhibition may occur among the patients with long-term use of *Tripterygium wilfordii*, which needs to be monitored periodically, and if necessary, its dose should be reduced or it should be stopped.

Pancreatic Kallikrein

Kallikrein is one of the vital factors in kallikrein-kinin system (KKS) which plays a critical role in renal hemodynamics.[22] It is a serine endopeptidase consisting of 238 amino acids and pervasively distributed in tissues.[23] Activated kallikrein facilitates the hydrolysis of kininogens into kinins which exert biological effects in KKS.[24] It is promptly inactivated by several peptidases including angiotensin I-converting enzyme, endothelin converting enzyme, and neprilysin.[25] Among these kinins, bradykinin exerts the major physiological effects via combination with bradykinin receptors.[26,27] Though the renoprotective effects of KKS for DKD have been confirmed in series of animal models, clinic trials of multicenter and large sizes are needed for evaluating its efficacy in the human body.

Effects on urinary albumin excretion. Pancreatic kallikrein may significantly reduce urinary albumin excretion. Bodin and coworkers[28] showed that in kallikrein-deficient mice, the urinary albumin excretion was more than 2-fold higher than in wild-type littermates 1 month after DM induction. In fact, stimulation of bradykinin receptors activates nitric oxide synthesis in endothelial cells, reducing DNA damages and senescence induced by reactive oxygen species.[29] In addition, synthesis of prostaglandins, especially prostacyclin, induced by KKS, may decrease the production of reactive oxygen species and prevent apoptosis of tubular cells.[30]

Effects on glycosylated hemoglobin. Kallikrein improves insulin sensitivity and promotes glucose intake, partially due to KKS-induced improvement in hemodynamics and substrates exchange across the cytomembrane.[31] On the other hand, the effects of insulin on promoting glucose utilization can be partially reduced by kallikrein inhibitors.[32,33] We performed a meta-analysis to assess the effects of pancreatic kallikrein on glycosylated hemoglobin in patients with DKD. To the best of our knowledge, this is the first meta-analysis of effects of pancreatic kallikrein on glycosylated hemoglobin. The screening process of articles is shown in Figure 1 and the characteristics of included articles are listed in Table 1. All of the participants included in the control groups were treated with valsartan at a dose of 80 mg/d to 160 mg/d on the basis of conventional therapies. While participants in experimental groups were additionally treated with pancreatic kallikrein, at an intramuscular dose of 40 U/d or an oral dose of 120 U/d to 240 U/d. The meta-analysis
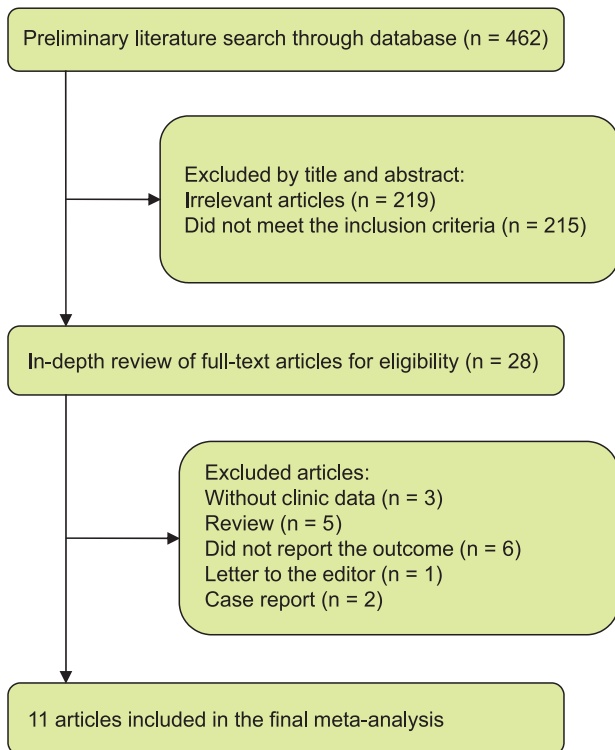
Preliminary literature search through database (n = 462)

Excluded by title and abstract:
Irrelevant articles (n = 219)
Did not meet the inclusion criteria (n = 215)

In-depth review of full-text articles for eligibility (n = 28)

Excluded articles:
Without clinic data (n = 3)
Review (n = 5)
Did not report the outcome (n = 6)
Letter to the editor (n = 1)
Case report (n = 2)

11 articles included in the final meta-analysis

Figure 1. Selection of articles included in the meta-analysis.

showed a definite role of pancreatic kallikrein in the decreases of glycosylated hemoglobin (mean difference, 0.36%; 95% confidence interval, 0.08% to 0.63%; P = .01), as shown in Figure 2.

Synergistic effects with renin-angiotensin system antagonists. It has been proved that angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers exert synergistic effects with KKS. The therapeutic efficacy of angiotensin-converting enzyme inhibitors is

attributed partially to its ability in inhibiting kinin degradation.[45] The plasma bradykinin levels can be increased by angiotensin II receptor blockers with an extent of 2-folds in DKD patients associated with hypertension,[46] which is advantageous for KKS to exert renoprotective effects.

ANTITHROMBOTIC THERAPIES

With significant changes in hemodynamics, which is commonly called *prethrombotic state*, the thrombotic risk is much greater among DKD patients.[47] In diabetic state, the increased production of cytokines, such as thromboxane A2, platelet activating factors, and thrombin, contributes to the activation of platelets and meanwhile the excessive expression of platelet surface adhesion molecules and receptors is directly associated with high platelet aggregation.[48] In addition, hypercoagulation, endothelial lesions and depressed fibrinolysis are also involved with the thrombotic risks.[49,50] The activated platelets and its procoagulant activity play important roles in the progression of DKD and incidence of complications.[51] Clinical trials have shown the beneficial effects of antithrombotic therapies for DKD patients as it inhibits the formation of thrombus, reduces albuminuria, stabilizes kidney function, and finally, improves outcomes for DKD patients.[52,53]

Lumbrukinase

Lumbrukinase is a group of fibrinolytic and thrombolytic enzymes extracted by Mihara and coworkers from the earthworm species, *Lumbricus rubellus*, in Japan in 1991.[54] With a molecular weight

Table 1. Characteristics of Included Articles*

| Study | Publication Year | DKD Stage | Number of Participants | | Follow-up, mo | Mean Change in Hemoglobin A1c, % | |
			Experimental†	Control		Experimental†	Control
Cai[34]	2010	Early	36	35	3	0.80 ± 0.11	0.80 ± 0.11
Chen and Chen[35]	2007	Early	26	25	3	3.00 ± 0.27	2.40 ± 0.28
Du et al[36]	2012	Early	36	32	1	0.07 ± 0.16	0.02 ± 0.19
Fu and Wang[37]	2013	Early	45	45	6	-2.80 ± 0.4	-2.90 ± 0.22
Lu and Zhang[38]	2009	Early	35	35	3	1.60 ± 0.28	1.00 ± 0.28
Ren et al[39]	2011	Advanced	44	44	3	2.02 ± 0.38	1.86 ± 0.33
Shi et al[40]	2003	Advanced	19	17	3	0.95 ± 0.12	0.35 ± 0.07
Sun et al[41]	2010	Early	32	30	6	0.70 ± 0.44	0.30 ± 0.32
Wang et al[42]	2011	Early	30	30	6	0.20 ± 0.19	0.10 ± 0.13
Wu[43]	2007	Advanced	29	29	3	1.30 ± 0.23	1.30 ± 0.23
Yang[44]	2005	Early	26	24	4	2.18 ± 0.19	1.87 ± 0.05

*DKD indicates diabetic kidney disease.
†Interventions were pancreatic kallikrein and valsartan in the experimental groups and valsartan in the control groups.
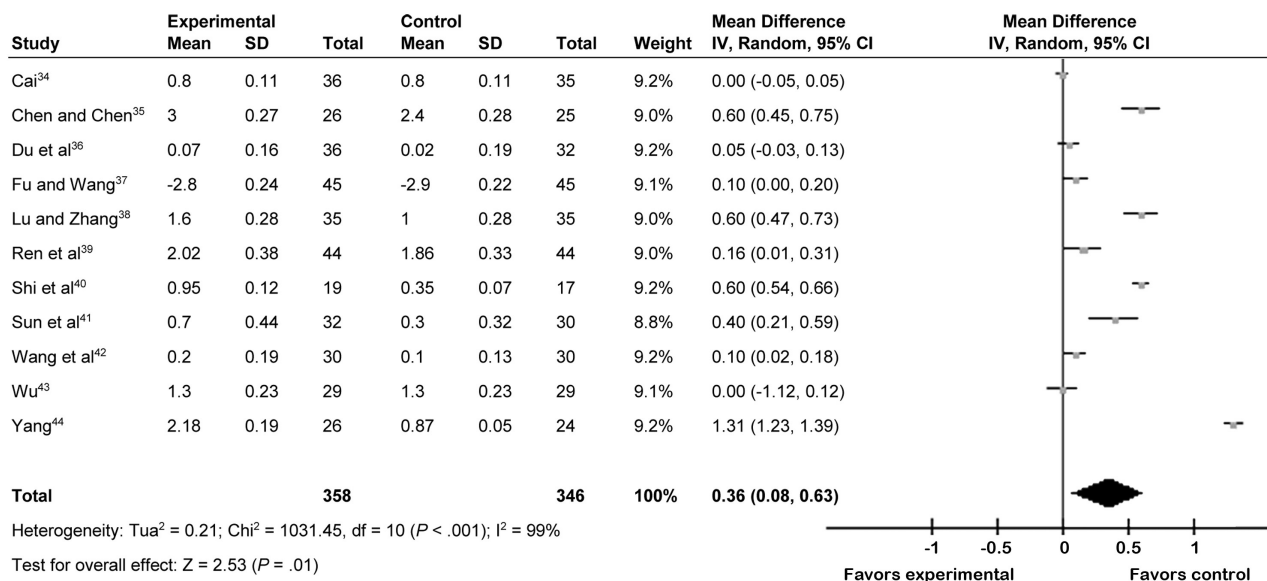
Study	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Cai[34]	0.8	0.11	36	0.8	0.11	35	9.2%	0.00 (-0.05, 0.05)
Chen and Chen[35]	3	0.27	26	2.4	0.28	25	9.0%	0.60 (0.45, 0.75)
Du et al[36]	0.07	0.16	36	0.02	0.19	32	9.2%	0.05 (-0.03, 0.13)
Fu and Wang[37]	-2.8	0.24	45	-2.9	0.22	45	9.1%	0.10 (0.00, 0.20)
Lu and Zhang[38]	1.6	0.28	35	1	0.28	35	9.0%	0.60 (0.47, 0.73)
Ren et al[39]	2.02	0.38	44	1.86	0.33	44	9.0%	0.16 (0.01, 0.31)
Shi et al[40]	0.95	0.12	19	0.35	0.07	17	9.2%	0.60 (0.54, 0.66)
Sun et al[41]	0.7	0.44	32	0.3	0.32	30	8.8%	0.40 (0.21, 0.59)
Wang et al[42]	0.2	0.19	30	0.1	0.13	30	9.2%	0.10 (0.02, 0.18)
Wu[43]	1.3	0.23	29	1.3	0.23	29	9.1%	0.00 (-1.12, 0.12)
Yang[44]	2.18	0.19	26	0.87	0.05	24	9.2%	1.31 (1.23, 1.39)
Total			**358**			**346**	**100%**	**0.36 (0.08, 0.63)**

Heterogeneity: Tua^2 = 0.21; Chi^2 = 1031.45, df = 10 (P < .001); I^2 = 99%

Test for overall effect: Z = 2.53 (P = .01)

Mean Difference IV, Random, 95% CI — scale: -1, -0.5, 0, 0.5, 1. Favors experimental / Favors control

Figure 2. The meta-analysis of mean changes in hemoglobin A1c of patients with diabetic kidney disease (random effects model).

of 20 000 D, the enzyme is thermostable and displays in a broad optimal pH range.[54] In recent years, lumbrukinase has been widely used in ischemic encephalopathy, coronary heart disease, DM, and deep vein thrombosis for its antithrombotic activity. The enzyme is able to directly dissolve fibrinogen and fibrin[55]; moreover, it facilitates the conversion of plasminogen into plasmin by activation of endogenous tissue plasminogen activator and subsequently dissolving fibrin clots.[56-58] The enzyme mechanism of action is shown in Figure 3.

Sun and colleagues[59] showed that the downregulation of matrix metalloproteinase-2 and matrix metalloproteinase-9 and induction of collagen IV were significantly attenuated by lumbrukinase in streptozotocin-induced diabetic rats, suggesting the effect of lumbrukinase on alleviating glomerulosclerosis and tubulointerstitial fibrosis. Clinic trials showed that lumbrukinase significantly decreased urinary albumin excretion
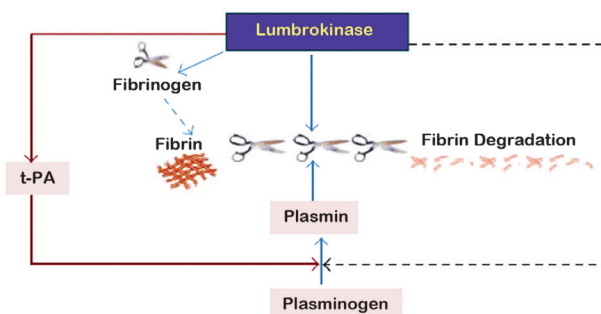

Figure 3. The lumbrokinase mechanism of action (adapted from the article by Li et al[58]).

among DKD patients,[60,61] as shown in Table 2. Compared with traditional anticlotting drugs (eg, urokinase, streptokinase, and tissue plasminogen activator), lumbrukinase is very specific to fibrin and it does not result in severe bleeding.[62,63] As an agent that can be absorbed from intestinal mucosa and keep its fibrinolytic and proteolytic activities, it avoids administration via the intravenous route,

Table 2. Studies of the Effect of Lumbrukinase on Urinary Albumin Excretion (UAE) in Patients With Diabetic Kidney Disease

Study	Publication Year	Follow-up, wk	Number of Participants Experimental	Control	Intervention Experimental	Control	Mean Change in UAE, mg/ 24 h Experimental	Control	P*
Ge[60]	2010	8	18	25	Lumbrukinase	Placebo	38.00 ± 22.92	23.00 ± 13.06	< .001
Sun and Han[61]	2003	2	11	11	Lumbrukinase	Alprostadil	97.95 ± 10.76	61.97 ± 17.14	< .001

*P values were calculated by the Welch 2-sample t test, for assessment of efficacy differences between the experimental and control groups.

which is used in traditional antithrombotic drugs.[64]

No adverse effects on the functions of cardiovascular vessels or the liver and kidney have been reported yet.[65] More large-scale rigorous clinical trials are needed for more convincing evidence of its application in DKD patients.

Aspirin

Aspirin is one of antiplatelet agents with the longest histories, and it has been widely used in therapies of cardiac-cerebrovascular diseases, venous thrombosis, and inflammatory states. However, research of utilization of aspirin in DKD treatment is still relatively insufficient and its efficacy for DKD is not very clear. Yet it is certain that for patients with DKD, aspirin does not have significant benefits in reducing risks of cardiovascular death, stroke, and myocardial infarction.[66, 67]

However, there are studies supporting its use in DKD treatment for its effects on renoprotection. Treatment with aspirin may stabilize kidney function via reducing platelet hypersensitivity and the production of thromboxanes, which in turn decreases the constrictor activity in the glomerular vessels and improves the renal microcirculation.[53] For patients with DM, aspirin therapy is beneficial in reducing risk of DKD,[68] while for DKD individuals, it significantly reduces urinary protein.[69,70] Makino and colleagues showed that aspirin inhibited upregulation of connective tissue growth factor and transforming growth factor-β1 induced by hyperglycemia in streptozotocin-induced DKD rats, which affected mesangial expansion and kidney sclerosis.[71] No adverse event reports of aspirin have been recorded in the DKD treatment yet, and its application in DKD patients needs further experimental and clinical evaluation.

Sulodexide

Sulodexide is a group of highly purified glycosaminoglycans extracted from porcine intestine mucosa, which consists of about 80% low-molecular-weight heparin, 20% dermatan sulfate, and less than 1% chondroitin sulfate. It has been the most extensively studied glycosaminoglycan for DKD patients with its antithrombotic activity. Though bearing similar biochemical characteristics to heparin, it does not have anticoagulation properties when given orally,[72] and it does not cause severe bleeding.[73] The exact mechanism of sulodexide in DKD therapies remains unclear, yet many potential therapeutic effects has been observed in animal models. It has been proved effective in suppression of mesangial matrix expansion and thickening of glomerular basement membrane mediated by transforming growth factor-β1.[74,75] In addition, restorative effects of sulodexide on glomerular basement membrane anionic charge barrier were also observed.[76,77] However, its efficacy in the treatment of DKD does not seem to be so optimistic. In a series of clinical trials, it was found that sulodexide only reduced the urinary albumin in early DKD, while for advanced DKD, it did not show significant kidney protective effects.[78-80]

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract. 2010;87:4-14.

2. Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res Clin Pract. 2014;103:137-49.

3. Kern TS, Engerman RL. Microvascular metabolism in diabetes. Metabolism. 1986;35:24-7.

4. Huang ES, Brown SE, Ewigman BG, Foley EC, Meltzer DO. Patient perceptions of quality of life with diabetes-related complications and treatments. Diabetes Care. 2007;30:2478-83.

5. Tuttle KR, Bakris GL, Bilous RW, et al. Diabetic kidney disease: a report from an ADA Consensus Conference. Am J Kidney Dis. 2014;64:510-33.

6. Ayodele OE, Alebiosu CO, Salako BL. Diabetic nephropathy--a review of the natural history, burden, risk factors and treatment. J Natl Med Assoc. 2004;96:1445-54.

7. Alicic RZ, Tuttle KR. Novel therapies for diabetic kidney disease. Adv Chronic Kidney Dis. 2014;21:121-33.

8. Centers for Disease Control and Prevention (CDC). Incidence of end-stage renal disease attributed to diabetes among persons with diagnosed diabetes---United States and Puerto Rico, 1996-2007. MMWR Morb Mortal Wkly Rep. 2010;59:1361-6.

9. Mudaliar SR, Henry RR. Role of glycemic control and protein restriction in clinical management of diabetic kidney disease. Endocr Pract. 1996;2:220-6.

10. Lapolla A, Fedele D. [Oxidative stress and diabetes: role in the development of chronic complications]. Minerva Endocrinol. 1993;18:99-108. Italian.

11. Haidara MA, Yassin HZ, Rateb M, Ammar H, Zorkani MA. Role of oxidative stress in development of cardiovascular complications in diabetes mellitus. Curr Vasc Pharmacol. 2006;4:215-27.

12. Arora MK, Singh UK. Oxidative stress: meeting multiple targets in pathogenesis of diabetic nephropathy. Curr Drug Targets. 2014;15:531-8.

13. Ceriello A, dello RP, Amstad P, Cerutti P. High glucose induces antioxidant enzymes in human endothelial cells in culture. Evidence linking hyperglycemia and oxidative stress. Diabetes. 1996;45:471-7.

14. Yamagishi S, Matsui T. Advanced glycation end products, oxidative stress and diabetic nephropathy. Oxid Med Cell Longev. 2010;3:101-8.

15. Thomas T, Rauscher F, Sanders R, Veltman J, Watkins JB, III. Effects of aldose reductase inhibitors on antioxidant defense in rat and rabbit liver. Toxicol Sci. 2000;53:145-9.

16. Navarro-Gonzalez JF, Mora-Fernandez C. The role of inflammatory cytokines in diabetic nephropathy. J Am Soc Nephrol. 2008;19:433-42.

17. Zheng J, Feng K, Gu K. [Screening of anti-inflammatory, immunosuppressive and antifertility components of Tripterygium wilfordii V. Effects of 7 diterpene lactone epoxide compounds on the proliferation of T and B lymphocytes in vitro]. Zhongguo Yi Xue Ke Xue Yuan Xue Bao. 1994;16:24-8. Chinese.

18. Zheng J. [Screening of active anti-inflammatory, immunosuppressive and antifertility components of Tripterygium wilfordii. III. A comparison of the antiinflammatory and immunosuppressive activities of 7 diterpene lactone epoxide compounds in vivo]. Zhongguo Yi Xue Ke Xue Yuan Xue Bao. 1991;13:391-7. Chinese.

19. Choi BS, Sapkota K, Kim S, Lee HJ, Choi HS, Kim SJ. Antioxidant activity and protective effects of Tripterygium regelii extract on hydrogen peroxide-induced injury in human dopaminergic cells, SH-SY5Y. Neurochem Res. 2010;35:1269-80.

20. Zhao HY, Du ZP, Li HH, et al. [Effect of qubi recipe on changes of oxygen free radical metabolism and hypoxia-inducible factor-1alpha in collagen-induced arthritis rats]. Zhongguo Zhong Xi Yi Jie He Za Zhi. 2014;34:1108-12.

21. Ge Y, Xie H, Li S, et al. Treatment of diabetic nephropathy with Tripterygium wilfordii Hook F extract: a prospective, randomized, controlled clinical trial. J Transl Med. 2013;11:134.

22. Tschope C, Reinecke A, Seidl U, et al. Functional, biochemical, and molecular investigations of renal kallikrein-kinin system in diabetic rats. Am J Physiol. 1999;277:H2333-H2340.

23. Okada Y, Tsuda Y, Tada M, et al. Development of potent and selective plasmin and plasma kallikrein inhibitors and studies on the structure-activity relationship. Chem Pharm Bull (Tokyo). 2000;48:1964-72.

24. Kaplan AP, Joseph K, Silverberg M. Pathways for bradykinin formation and inflammatory disease. J Allergy Clin Immunol. 2002;109:195-209.

25. Cyr M, Lepage Y, Blais C, Jr., et al. Bradykinin and des-Arg(9)-bradykinin metabolic pathways and kinetics of activation of human plasma. Am J Physiol Heart Circ Physiol. 2001;281:H275-H283.

26. Schmaier AH. The plasma kallikrein-kinin system counterbalances the renin-angiotensin system. J Clin Invest. 2002;109:1007-9.

27. Tang SC, Leung JC, Lai KN. The kallikrein-kinin system. Contrib Nephrol. 2011;170:145-55.

28. Bodin S, Chollet C, Goncalves-Mendes N, et al. Kallikrein protects against microalbuminuria in experimental type I diabetes. Kidney Int. 2009;76:395-403.

29. Kayashima Y, Smithies O, Kakoki M. The kallikrein-kinin system and oxidative stress. Curr Opin Nephrol Hypertens. 2012;21:92-6.

30. Villa E, Rabano A, Ruilope LM, Garcia-Robles R. Effects of cicaprost and fosinopril on the progression of rat diabetic nephropathy. Am J Hypertens. 1997;10:202-8.

31. Dietze G, Wicklmayr M, Bottger I, et al. The kallikrein-kinin system and muscle metabolism: biochemical aspects. Agents Actions. 1980;10:335-8.

32. Wicklmayr M, Dietze G, Gunther B, et al. The kallikrein-kinin system and muscle metabolism--clinical aspects. Agents Actions. 1980;10:339-43.

33. Potier L, Waeckel L, Fumeron F, et al. Tissue kallikrein deficiency, insulin resistance, and diabetes in mouse and man. J Endocrinol. 2014;221:297-308.

34. Cai J. Clinical investigation of treatment consisting of valsartan and pancreatic kininogenase for diabetic nephropathy. Strait Pharm J. 2010;22:131-2.

35. Chen R, Chen ZG. Effects of valsartan combined with pancreatic kallikrein in treatment of diabetic nephropathy. Prac Clin Med. 2006;7:40-3.

36. Du W, Zhou YY, Yang P. Effects of kallidinogenase combined with valsartan on serum cystatin C and urinary albumin excretion rate in patients with early diabetic nephropathy. J Xinxiang Med College. 2012;29:594-6.

37. Fu D, Wang QY. The effect of Pancreatic Kininogenase Combined with Valsartan for the Treatment of Early Diabetic Nephropathy and its Influence on Cys-C and Urine β2-GM Level. Chin J Lab Diagn. 2013;17: 2152-5.

38. Lu J, Zhang ZF. Treatment with pancreatic kallikrein united valsartan for early diabetic nephropathy. Shan xi Med J. 2009;38:653-4.

39. Ren X, Cao HY, Chen YH, et al. Observation of the valsartan combined with enteric-coated pancreatic kallikrein in treatment of diabetic nephropathy. J Modern Clin Med. 2011;37:423-4.

40. Shi H, Qin WH, Yin T, Zhu JY. Effects of Valsartan combined with pancreatic kallikrein for diabetic nephropathy. Acta Acad Med Nantong. 2003;23:172-4.

41. Sun YM. Curative effects of pancreatic kallidinogenase and valsartan for early type 2 diabetic nephrophathy patients. Guide Chin Med. 2010;8:93-5.

42. Wang GH, Ren HQ, Wang F. The therapeutic effect of combined kallidinogenase and valsartan on early stage of type 2 diabetic nephropathy. Chin J Diabetes. 2011;19:585-7.

43. Wu YF. Investigation of pancreatic kallikrein and valsartan for diabetic nephropathy therapy. J Chin Prac Diagn Ther. 2007;21:62-3.

44. Yang YQ. Clinical observation on effect of kallikrein united valsartan in the treatment of the elder type 2 diabetic nephropathy. Chin J Gerontol. 2005;25:758-9.

45. Maric C. Vasoactive hormones and the diabetic kidney. Sci World J. 2008;8:470-85.

46. Campbell DJ, Krum H, Esler MD. Losartan increases bradykinin levels in hypertensive humans. Circulation. 2005;111:315-20.

47. Hillegass WB, Brott BC, Dobbs JC, Papapietro SE, Misra VK, Zoghbi GJ. Oral antiplatelet therapy in diabetes mellitus and the role of prasugrel: an overview. Vasc Health Risk Manag. 2011;7:445-59.

48. Natarajan A, Zaman AG, Marshall SM. Platelet hyperactivity in type 2 diabetes: role of antiplatelet agents. Diab Vasc Dis Res. 2008;5:138-44.

49. Schafer A, Bauersachs J. Endothelial dysfunction, impaired endogenous platelet inhibition and platelet activation in diabetes and atherosclerosis. Curr Vasc Pharmacol. 2008;6:52-60.

50. Newby DE, Wright RA, Labinjoh C, et al. Endothelial dysfunction, impaired endogenous fibrinolysis, and cigarette smoking: a mechanism for arterial thrombosis and myocardial infarction. Circulation. 1999;99:1411-5.

51. Omoto S, Nomura S, Shouzu A, et al. Significance of platelet-derived microparticles and activated platelets in diabetic nephropathy. Nephron. 1999;81:271-7.

52. Best PJ, Steinhubl SR, Berger PB, et al. The efficacy and safety of short- and long-term dual antiplatelet therapy in patients with mild or moderate chronic kidney disease: results from the Clopidogrel for the Reduction of Events During Observation (CREDO) trial. Am Heart J. 2008;155:687-93.

53. Donadio JV, Jr., Ilstrup DM, Holley KE, Romero JC. Platelet-inhibitor treatment of diabetic nephropathy: a 10-year prospective study. Mayo Clin Proc. 1988;63:3-15.

54. Mihara H, Sumi H, Yoneta T, et al. A novel fibrinolytic enzyme extracted from the earthworm, Lumbricus rubellus. Jpn J Physiol. 1991;41:461-72.

55. Zhao J, Li L, Wu C, He RQ. Hydrolysis of fibrinogen and plasminogen by immobilized earthworm fibrinolytic enzyme II from Eisenia fetida. Int J Biol Macromol. 2003;32:165-71.

56. Park S, Kye KC, Lee HB, Kim JS, Lee MH. Antithrombotic effect of intravenous administration of lumbrokinase, an earthworm fibrinolytic enzyme, on thrombin-induced thromboembolism in mice. Thromb Haemost. 1991;65:1195.

57. Jin L, Jin H, Zhang G, Xu G. Changes in coagulation and tissue plasminogen activator after the treatment of cerebral infarction with lumbrokinase. Clin Hemorheol Microcirc. 2000;23:213-8.

58. Li G, Wang KY, Li D, Wang N, Liu D. Cloning, expression and characterization of a gene from earthworm Eisenia fetida encoding a blood-clot dissolving protein. PLoS One. 2012;7:e53110.

59. Sun H, Ge N, Shao M, et al. Lumbrokinase attenuates diabetic nephropathy through regulating extracellular matrix degradation in Streptozotocin-induced diabetic rats. Diabetes Res Clin Pract. 2013;100:85-95.

60. Ge N. Protection and Mechanism research of earthworm and lumbrokinase on diabetic kidney disease [dissertation]. Guangzhou Univ Chin Med. 2010. Available from: http://www.dissertationtopic.net/doc/1540133

61. Sun SF, Han WH. Clinical observation on effect of lumbrukinase and benazepril for diabetic nephropathy. Capital Med. 2003;2:39-40.

62. Cao YJ, Zhang X, Wang WH, et al. Oral fibrinogen-depleting agent lumbrokinase for secondary ischemic stroke prevention: results from a multicenter, randomized, parallel-group and controlled clinical trial. Chin Med J (Engl). 2013;126:4060-5.

63. Hrzenjak T, Popovic M, Bozic T, Grdisa M, Kobrehel D, Tiska-Rudman L. Fibrinolytic and anticoagulative activities from the earthworm Eisenia foetida. Comp Biochem Physiol B Biochem Mol Biol. 1998;119:825-32.

64. Yan XM, Kim CH, Lee CK, Shin JS, Cho IH, Sohn UD. Intestinal Absorption of Fibrinolytic and Proteolytic Lumbrokinase Extracted from Earthworm, Eisenia andrei. Korean J Physiol Pharmacol. 2010;14:71-5.

65. Cooper EL, Ru B, Weng N. Earthworms: sources of antimicrobial and anticancer molecules. Adv Exp Med Biol. 2004;546:359-89.

66. Sasso FC, Marfella R, Pagano A, et al. Lack of effect of aspirin in primary CV prevention in type 2 diabetic patients with nephropathy: results from 8 years follow-up of NID-2 study. Acta Diabetol. 2015;52:239-47.

67. Ikeda Y, Shimada K, Teramoto T, et al. Low-dose aspirin for primary prevention of cardiovascular events in Japanese patients 60 years or older with atherosclerotic risk factors: a randomized clinical trial. JAMA. 2014;312:2510-20.

68. Romero R. [Diabetic nephropathy: strategies for prevention]. Nefrologia. 2002;22 Suppl 1:46-50.

69. Khajehdehi P, Roozbeh J, Mostafavi H. A comparative randomized and placebo-controlled short-term trial of aspirin and dipyridamole for overt type-2 diabetic nephropathy. Scand J Urol Nephrol. 2002;36:145-8.

70. Hopper AH, Tindall H, Davies JA. Administration of aspirin-dipyridamole reduces proteinuria in diabetic nephropathy. Nephrol Dial Transplant. 1989;4:140-3.

71. Makino H, Mukoyama M, Sugawara A, et al. Roles of connective tissue growth factor and prostanoids in early streptozotocin-induced diabetic rat kidney: the effect of aspirin treatment. Clin Exp Nephrol. 2003;7:33-40.

72. Suzuki N, Kitazato K, Takamatsu J, Saito H. Antithrombotic and anticoagulant activity of depolymerized fragment of the glycosaminoglycan extracted from Stichopus japonicus Selenka. Thromb Haemost. 1991;65:369-73.

73. Andriuoli G, Mastacchi R, Barbanti M. Antithrombotic activity of a glycosaminoglycan (sulodexide) in rats. Thromb Res. 1984;34:81-6.

74. Wang ZQ, Liang KH, Pahl MV, Vaziri ND. Effect of heparin on mesangial cell growth and gene expression of matrix proteins. Nephrol Dial Transplant. 1998;13:3052-7.

75. Caenazzo C, Garbisa S, Onisto M, Zampieri M, Baggio B, Gambaro G. Effect of glucose and heparin on mesangial alpha 1(IV)COLL and MMP-2/TIMP-2 mRNA expression. Nephrol Dial Transplant. 1997;12:443-8.

76. Nader HB, Buonassisi V, Colburn P, Dietrich CP. Heparin stimulates the synthesis and modifies the sulfation pattern of heparan sulfate proteoglycan from endothelial cells. J Cell Physiol. 1989;140:305-10.

77. Ofosu FA. Pharmacological actions of sulodexide. Semin Thromb Hemost. 1998;24:127-38.

78. Packham DK, Wolfe R, Reutens AT, et al. Sulodexide fails to demonstrate renoprotection in overt type 2 diabetic nephropathy. J Am Soc Nephrol. 2012;23:123-30.

79. Rossini M, Naito T, Yang H, et al. Sulodexide ameliorates early but not late kidney disease in models of radiation nephropathy and diabetic nephropathy. Nephrol Dial Transplant. 2010;25:1803-10.

80. Lewis EJ, Lewis JB, Greene T, et al. Sulodexide for kidney protection in type 2 diabetes patients with microalbuminuria: a randomized controlled trial. Am J Kidney Dis. 2011;58:729-36.

Correspondence to:
Gaosi Xu, MD, PhD
Department of Nephrology, Second Affiliated Hospital, Nanchang University, No 1, Minde Rd, Nanchang, Zip Code: 330006, China
Tel: +86 791 862 70383
Fax: +86 791 862 70383
E-mail: gaosixu@163.com

Wenjun Yan and Baoqin Zhou contributed equally to this work as first
authors.