Mechanism underlying treatment of diabetic kidney disease using Traditional Chinese Medicine based on theory of Yin and Yang balance

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Abstract
The pathogenic mechanism of diabetic kidney disease (DKD) is complex. The development of DKD cannot be fully explained by a single mechanism. Traditional Chinese Medicine (TCM) has been applied extensively for the treatment of DKD in China. However, studying the mechanism of DKD using theories and methods that are appropriate for TCM characteristics and searching for theoretical bases for TCM clinical application are topics that still need to be explored and researched. Activation of the transforming growth factor (TGF)-β1/Smad and PI3K/Akt/mTOR signaling pathways functions as a self-protection mechanism against renal microinflammation in DKD. However, the persistent abnormal overactivation of reactions causes secondary cell dysfunction, cell apoptosis, increased extracellular matrix (ECM) secretion, and eventually renal fibrosis. During this process, the dysregulation of self-balance among a variety of signaling pathways and the loss of self-feedback regulatory mechanisms downstream of these signaling pathways are critical causes of the occurrence and development of DKD. TCM may both inhibit the expression or activation of "hyperactive" signaling pathways (NF-κB, Smad3, and PI3K/Akt/mTOR) and increase the expression or activation of "deficient" signaling pathways (Smad7 and PTEN) to restore balance to cells with an abnormal pathophysiological status and achieve the goal of DKD treatment.

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Keywords: Diabetic kidney disease; Traditional Chinese Medicine; Yin and Yang theory

INTRODUCTION
Diabetic kidney disease (DKD) is one of the most common chronic complications of diabetes mellitus (DM) and is the major cause of mortality in DM. In developed countries, DKD is the leading cause of end-stage renal diseases.1 According to data in the hemodialysis registration system in China, although DKD is currently the second most common primary disease in hemodialysis patients, its incidence increases rapidly every year.2,3 Because of the explosive growth of the incidence of DM in China in the past 30 years, the chronic complications associated with the progression of the DM disease course will definitely cause rapid changes in the composition of etiologies of end-stage renal disease (ESRD) in China. In the near future, DKD will definitely surpass chronic glomerulonephritis to become the main cause of ESRD in China. Moreover, patients with ESRD caused by DKD have additional DM-associated complications. Their medical expenses are much higher than those of patients with ESRD caused by other etiologies. In the USA, the medical expenses of patients with DKD-associated ESRD are more than double those of other ESRD patients.4 Therefore, pre-
venting the occurrence and development of DKD is an urgent and important issue. To quote the title of a review article published by the International Society of Nephrology for 2010 World Kidney Day, we must “act now or pay later”.

The occurrence and development of DKD is associated with the overexpression of TGF-β1, the abnormal activation of the PI3K/Akt/mTOR signaling pathway, advanced glycation end products (AGEs), oxidative stress reactions, and microinflammation status in kidney tissues. However, no single mechanism can fully explain the occurrence and development of DKD. Currently, there have been no cases in which the above single pathway has been successfully used to develop drugs for the prevention and treatment of DKD. Traditional Chinese Medicine (TCM) is applied extensively for the treatment of DKD in China, and reports from many small-scale clinical studies have suggested the utility of TCM in the prevention and treatment of DKD. Research on the mechanism underlying DKD treatment by TCM is critical for the promotion and application of TCM. However, the composition of Chinese medicines is complicated, and there might be various ingredients have effective functions on multiple diseases, and studying focusing on unique mechanism is not consistent with the clinical therapeutic characteristics of TCM. It is worthwhile to study the mechanisms underlying DKD treatment using Chinese medicines based on TCM theories.

PATHOGENIC MECHANISM OF DKD

The strict control of blood glucose and blood pressure, especially the administration of angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs), and lipid-lowering statins for the control of hypercholesterolemia as well as the restriction of dietary protein intake are currently the main clinical measures to delay the progression of DKD. However, the efficacy of these measures is very limited, and patients with clinical DKD still inevitably gradually progress to ESRD. Therefore, there is an active global search for new drugs to treat DKD. However, some drugs with significant prevention and treatment effects for DKD in animal experiments and in vitro experiments have been terminated after entering clinical trials due to ineffectiveness and severe side effects; these drugs include AGE inhibitors, aldose reductase inhibitors, and specific anti-oxidative inflammatory cytokine Nr2f2 activators. In clinical practice, many patients receive integrated TCM and Western medicine for the treatment of DKD. The results of many small-scale clinical observation studies have suggested that Chinese medicines function to prevent and treat DKD. Many scholars have studied the mechanism underlying DKD treatment of with Chinese medicines from different perspectives, including the effects of Chinese medicines on the inhibition of TGF-β1 in DKD, anti-inflammatory factors, inhibition of aldose reductases, inhibition of the expression of AGES and their receptors, and anti-oxidative functions. The current status of exploration for new therapeutic drugs for DKD shows that it is difficult to successfully identify therapeutic drugs using a single target. Although it has been suggested that Chinese medicines, especially compound Chinese medicines, function to prevent and treat DKD in clinical application, the optimal research would not follow the footsteps of Western Medicine by studying the mechanisms using a single target or search for the so-called effective components in Chinese medicine because the components of Chinese medicines are complex, and this approach is not consistent with the principles of TCM. A better research topic would be how to apply theories and methods that are consistent with the principles of TCM to study the mechanisms and identify the theoretical basis of the clinical promotion and application of Chinese medicines.

The characteristic pathological changes of DKD are glomerular basement membrane thickening, glomerular mesangial widening, an increase in the extracellular matrix (ECM), and the gradual formation of the characteristic acellular Kimmelstiel-Wilson nodules. The pathological process of DKD can be divided into 3 stages: (a) glomerular hypertrophy and hyperfiltration; (b) microinflammation of glomeruli and tubulointerstitial regions; and (c) reduction of cell number by apoptosis and the accumulation of ECM. The results of recent studies on the occurrence and development of DKD have shown that DKD represents a series of closely related pathophysiological events promoted by the interactive effects of a variety of genetic and environmental factors; these pathophysiological events form a complex network. It has been shown that there is close relationship among a variety of pathophysiological pathways associated with the occurrence and development of DKD and that this relationship is very important for a comprehensive understanding of the mechanism underlying the occurrence and development of DKD and the search for safer and more effective treatment regimens; nonetheless, in-depth studies are currently lacking.

Persistent hyperglycemia and the status of renal hyperfiltration and hyperperfusion are driving factors behind the occurrence of DKD. A variety of renal intrinsic cells produce a large amount of oxygen free radicals under high glucose conditions. The production of intracellular oxygen free radicals can induce the activation of intracellular inflammation-associated signaling pathways (such as the JAK/STAT signaling pathway) and eventually cause the activation and translocation of the cytoplasmic transcription factor NF-κB into the cell nucleus to activate the expression of many inflammatory cytokines [such as interleukin-1 (IL-1) and tumor necrosis factor α (TNF-α)]. The microinflammatory status of the kidneys in the early stage of DKD has been confirmed in clinical and animal studies; however, its association with the subsequent renal fibrosis
remains unclear. TGF-β1 is a key factor for renal fibrosis in chronic kidney diseases, including DKD by stimulating the production of ECM and inhibiting its degradation; however, few researchers have noted that TGF-β1 is also an anti-inflammatory cytokine. TGF-β1 gene knockout mice exhibit a lethal inflammatory reaction and die at the age of 3 weeks. Studies have shown that TGF-β1 inhibits NF-κB-mediated renal inflammation by inducing the expression of IkBα, an inhibitor of NF-κB. Therefore, we hypothesize that the increase in TGF-β1 expression in DKD is a mechanism by which the kidney maintains self-protection or balance to fight inflammation. The treatment of DKD by blocking the TGF-β1/Smad signaling pathway alone might not succeed. Studies in animal models have shown that the PI3K/Akt/mTOR signaling pathway exhibits an abnormal activation status in DKD. Animal experiment results have suggested that the PI3K/Akt/mTOR signaling pathway inhibitor rapamycin could relieve the pathological changes in the kidney in a DKD animal model. However, the excessive application of rapamycin in renal transplantation patients would cause proteinuria with pathological findings of increased glomerular podocyte apoptosis and focal segmental glomerulosclerosis through an unknown mechanism. Our present ongoing study has shown that even a small dose of rapamycin (an intraperitoneal injection of 0.5 mg/kg every other day) can significantly reduce the urinary albumin excretion rate in rats with streptozotocin (STZ)-induced diabetes; however, these rats had a significantly lower body weight compared with that of the DM model control group and exhibited obvious wasting. The mortality rate of the large-dose rapamycin group (intraperitoneal injection of 5 mg/kg every other day) was significantly higher (70%) than that of the DM model control group. These findings suggest that treating DKD by inhibiting the PI3K/Akt/mTOR signaling pathway alone eventually might not be clinically feasible due to severe toxic side effects. The mechanism underlying the abnormal activation of the PI3K/Akt/mTOR signaling pathway in DKD is unclear, and its relationship with the simultaneous abnormal activation of the TGF-β1/Smad signaling pathway is also unclear. The activation of the PI3K/Akt/mTOR signaling pathway has functions of inhibiting cell apoptosis and maintaining cell survival. Many studies on cell functions have shown that the functions of the PI3K/Akt/mTOR and TGF-β1/Smad signaling pathways usually have opposite effects. For example, many studies of tumor cells have shown that the activation of the TGF-β1/Smad signaling pathway induces cell apoptosis, which can be inhibited by activation of the PI3K/Akt/mTOR signaling pathway. The relationship between the activation of the PI3K/Akt/mTOR signaling pathway and the inflammatory pathways in DKD remains unclear. However, studies of the mechanism of cardiac inhibition induced by endotoxin have shown that the activity of the PI3K/Akt/mTOR signaling pathway in cardiomyocytes decreased, while the activation of inflammation-associated signaling pathways eventually activated NF-κB to produce large amounts of inflammatory factors. The Chinese medicine extract thaliporphine can increase the activity of the PI3K/Akt/mTOR signaling pathway to achieve the inhibition of inflammation, suggesting that the activation of the PI3K/Akt/mTOR signaling pathway might also function to inhibit inflammation. Therefore, we speculate that the increased activity of the PI3K/Akt/mTOR signaling pathway in DKD is also a self-balance mechanism based on the inhibition of renal inflammation and renal cell apoptosis induced by TGF-β1/Smad. In addition, molecules downstream of the TGF-β1/Smad and PI3K/Akt/mTOR signaling pathways exhibit self-balancing or antagonistic mechanisms between positive and negative aspects. Studies have shown that the activation of Smad3 (downstream of the TGF-β1/Smad signaling pathway) in DKD is a critical cause of TGF-β1-mediated renal fibrosis. The expression and activation of Smad7 can antagonize Smad3 activation, which is also a major mediator of the inhibition of kidney inflammation by TGF-β1. PTEN is an inhibitor of the PI3K/Akt/mTOR signaling pathway, and the activity of PTEN in the kidney decreases in DKD.

**MECHANISM UNDERLYING DKD TREATMENT BY CHINESE MEDICINES BASED ON THEORY OF YIN AND YANG BALANCE**

The occurrence and development of DKD is due to microinflammation in the kidney induced by long-term hyperglycemia and renal hyperfiltration on a background of numerous susceptibility genes. The activation of the TGF-β1/Smad and PI3K/Akt/mTOR signaling pathways is a self-protection mechanism against microinflammatory reactions in the kidney. However, the sustained excessive reaction of abnormal activation results in secondary cell dysfunction, cell apoptosis, increased ECM secretion, and eventually renal fibrosis. During this process, the dysregulation of self-balance among different signaling pathways and the loss of self-feedback regulatory mechanisms downstream of these signaling pathways are critical in causing the occurrence and development of DKD. How to regain balance among these signaling pathways and restore the self-feedback regulatory mechanisms downstream of them are key questions in the search for new methods of DKD treatment. Plain questions: The manifestation of yin and yang from the macrocosm to the microcosm refers to "Yin and Yang, the way of heaven and earth, discipline of all things, parents of all changes, origin of life and death, the house of mind". The results of modern medical research into the pathology and physiology of all diseases have shown that the presence of a positive factor is definitely counterbalanced by a negative factor for checks and balances. The Yin and Yang...
theory of TCM can explain all pathological and physiological phenomena from the macrocosm to the microcosm. Currently, most studies only focus on the relationship between one overactive factor and a disease, ignoring the relationship between insufficient factors and the disease; therefore, the exploration of therapeutic methods only has "purge excess" and does not have "reinforce insufficiency". Plain Questions: Treatise on the communication of the force of life with heaven referred to "Yin and Yang in relative balance, the spirit can be cured". We believe that using the concept of "reinforce insufficiency and purge excess" to achieve "Yin and Yang in relative balance" based on TCM may be a better route to study the mechanisms underlying the treatment of DKD using Chinese medicines. There has long been an understanding in TCM that long-term DM (emaciation-thirst disease) can cause edema. For example, Complete Record of Sacred Be-nevolence by Zhao Ji of the Song Dynasty noted that "when the duration of diabetes mellitus gets longer, kidney Qi get impaired. Since kidney governs water and fluid, when kidney Qi is weak, edema occurs due to abnormal Qi transformation and impaired fluid metabolism". Therefore, through clinical observation, ancient TCM physicians already understood that DM had many chronic complications, including clinical presentations with descriptions similar to DKD. Although ancient physicians had a different understanding of the TCM mechanism in DKD treatment, most scholars consider spleen and kidney deficiency as well as blood stasis and internal resistance to be the most common mechanisms. According to these disease mechanisms, we generated the Chinese compound medicine "Huangqi-Tujian decoction" (composed of Radix astragali, Cuscutae semen, Euonymus alatus, and Stephaniae tetrandrae) for research. Our previous studies showed that the intragastric administration of Tujian decoction into rats with single kidney resection and STZ-induced diabetes could significantly decrease urinary protein secretion, decrease the pathological changes of glomerular hypertrophy and increased glomerular ECM, inhibit the phenotypic transition of glomerular mesangial cells, and inhibit the expression of TGF-β1 in the kidney. Tujian decoction was found to inhibit protein kinase C activity in the renal cortex of diabetic rats. A serum containing Tujian decoction inhibited the activation of Akt (Thr308) in glomerular mesangial cells cultured under high-glucose conditions and increased the activity of the Akt inhibitor PTEN.

One of our ongoing studies has revealed that both the PI3K/Akt/mTOR singling pathway inhibitor rapamycin and Huangqi-Tujian decoction can significantly reduce the urinary albumin excretion rate of rats with STZ-induced diabetes, and the treatment effects of the two groups did not differ significantly. However, compared with the Chinese medicine treatment group, the rats in the small-dose rapamycin group had significantly lower body weight and a significantly higher mortality rate; moreover, the mortality of the large-dose rapamycin treatment group reached 70% after 16 weeks of treatment. These results suggest that the attempt to generate drugs for DKD treatment by inhibiting the PI3K/Akt/mTOR signaling pathway alone may not easily lead to success. Recently, some studies have suggested that renal protective functions of Chinese medicines on DKD are related to signaling pathways. Huang et al have reported that Quefengtonglu decoction ameliorates the renal fibrosis and reduces 24 h urinary protein in DKD model rats, which might be the results from Quefengtonglu decoction’s inhibition of PI3K/Akt signaling pathway via activating PTEN and inhibiting TGF-β expression. Chen et al proved that herbal mixture of Radix Puerariae and Fructus Crataegi inhibits glomerular mesangial matrix expansion, renal capsule constriction and renal tubular epithelial cell edema, and reduces protein levels of PI3K, Akt, a-SMA and collagen IV in the kidneys of type 2 diabetic rats, suggesting the herbal mixture may prevent renal injury via inhibiting the PI3K/AKT pathway in the diabetic rats. Mao et al observed that Huangkui capsule attenuates renal fibrosis in diabetic nephropathy rats through regulating oxidative stress and p38MAPK/Akt pathway in kidneys of DKD rats. Zhang et al reported that Mo-mordica Saponin increases the expression of PTEN, inhibits the activation of PI3K/Akt pathway, and protects the kidneys in DKD mice. Huang demonstrated that tripterygium glycosides improves glomerular hypertrophy via inhibiting the activity of PI3K/Akt/mTOR signaling pathway in renal tissue of DKD rats. In vitro, Jia et al reported that Panax notoginseng Saponins increase the expression of BMP-7 and connexin 43, down-regulate the percentage of alpha-SMA positive cells, and decrease the expression of P-Akt and P-Akt/Akt in NRK-52E cell line, suggesting Panax notoginseng Saponins might ameliorate renal interstitial fibrosis via its inhibition of PI3K/Akt signaling pathway. Overall, these results suggest that Chinese medicines may achieve the goal of treating DKD by means of restoring balance of "hyperactive" and "insufficient" signaling pathways in cells with abnormal pathological physiological state, which includes the inhibition of "hyperactivity" signaling pathway factors (NF kappa B, Smad3, PI3K/Akt/mTOR) expression or activation in renal cells in DKD, and improving "insufficient" signaling pathway factors (Smad7 and PTEN) expression or activation (Figure 1).

CONCLUSIONS

The pathogenic mechanism of DKD is complex. The search for therapies for DKD based on the inhibition of a single overactive factor is unlikely to succeed. Only by applying the concept of "reinforce insufficiency and purge excess" to achieve "Yin and Yang in relative balance" based on the Yin and Yang balance theory of TCM can studies elucidate the mechanism underlying the DKD treatment using Chinese medicines and pro-
Figure 1 Mechanism underlying diabetic kidney disease treatment by Chinese medicines based on theory of yin and yang balance.

AGEs: advanced glycation end products; mTORC: mammalian target of rapamycin complex; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; PTEN: phosphatase and tensin homolog; TGF: transforming growth factor; TβRI: transforming growth factor β1 receptor I; TβRII: transforming growth factor β1 receptor II.

Provide evidence for the promotion and application of Chinese medicines for DM treatment.

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