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Protective effect of ginsenoside metabolite compound K against diabetic nephropathy by inhibiting NLRP3 inflammasome activation and NF-κB/p38 signaling pathway in high-fat diet/streptozotocin-induced diabetic mice

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ABSTRACT

Though the antidiabetic effect of ginsenoside compound K (CK) has been well studied, the effect of CK on diabetic nephropathy (DN) is not clear. Whether CK would have a protective effect against DN and it could exert the protective effect by inhibiting the oxidative stress, NLRP3 inflammasome and NF-κB/p38 signaling pathway were investigated in this study. Here, the HFD (high fat diet)/STZ (streptozotocin)-induced DN mice model was established to assess the CK effect *in vivo*. Parallel experiments uncovering the molecular mechanism by which CK prevents from DN was performed in rat glomerular mesangial cell line HBZY-1 exposed to high glucose. CK (10, 20, 40 mg/kg/day) were intragastrically administered for 8 weeks, the general status, biochemical parameters, renal pathological changes and oxidative stress-parameters were observed, and the NLRP3 inflammasome and NF-κB/p38 signaling pathway were evaluated. The results showed that the elevated fasting blood glucose, serum creatinine, blood urea nitrogen and 24-hour urine protein of the DN mice were significantly decreased, and the proliferation of glomerular mesangial matrix was alleviated by CK. In addition, the generation of ROS in the kidney was significantly decreased, and the expression of Nox1 and Nox4 proteins were down-regulated. Further, the expression of NLRP3 inflammasome components (NLRP3, ASC and Caspase-1) and the inflammatory cytokines IL-1β and IL-18 were also significantly down-regulated *in vivo* and *in vitro*. The phosphorylation of renal p38 MAPK was also inhibited by CK. MCC950 (an inhibitor of NLRP3 inflammasome) and VX-765 (a Caspase-1 Inhibitor) showed significant interaction with CK on the decrease of IL-1β concentration in HBZY-1 cells. In conclusion, our study provided evidence that the protective effect of CK on diabetes-induced renal injury is associated with down-regulating the expression of NADHP oxidase, and inhibition of ROS-mediated activation of NLRP3 inflammasome and NF-κB/p38 signaling pathway, suggesting its therapeutic implication for renal inflammation.

1. Introduction

Diabetic nephropathy (DN) is one of the most common and severe chronic complications of diabetes mellitus [1]. The pathological characteristics of DN are the glomerular mesangial hypertrophy caused by the proliferation of glomerular mesangial cells and the excessive accumulation of extracellular matrix, eventually developing into the renal fibrosis and glomerulosclerosis [2]. Although the pathogenesis of DN is not fully understood, several factors are involved in the development of DN, such as hyperglycemia, activation of polyol pathway, protein kinase C pathway and renin-angiotensin system, and production and inflammatory reaction of reactive oxygen species (ROS) [3]. Some studies have revealed that on the basis of metabolic disorder and hemodynamic abnormality, inflammation is the key factor for the occurrence and development of DN, likely playing an important role in the pathogenesis of DN as one of the downstream links in the above mechanism [4,5].

There is an imbalance between the pro-oxidation and anti-oxidation, accompanied by an increase in the production of ROS both in the early and late stages of DN [6]. The excessive ROS could regulate protein kinase C, mitogen-activated protein kinases (MAPK), and the activation of various cytokines and transcription factors, ultimately leading to an increased expression of ECM gene, and the progression to fibrosis and end-stage nephropathy [6,7]. Reducing the production of ROS and blocking the apoptotic pathway activated by the production of ROS may be a new target for the treatment of DN [8].

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NLRP3 inflammasome is composed of NLRP3 protein, Caspase-1 and ASC, and its activation is considered an important factor to aggravate the kidney inflammation and fibrosis by the processing and secretion of pro-inflammatory cytokines IL-1β and IL-18 in DN [9]. It has recently been reported that renal NLRP3 is activated in streptozotocin (STZ)-induced diabetic rats, while the inhibition of its activities could significantly reduce the inflammation of renal tissues and improve the renal functions in the rats [10]. Further, the inhibition of NLRP3 downstream pathway or silencing NLRP3/ASC or TXNIP genes can delay the development of DN [11]. It is noteworthy that recent studies consider that the targeted inhibition of NLRP3 activation may be a viable therapy for DN [12–14]. Renal NLRP3 inflammasome can be activated by uncontrolled ROS, and without doubt, the MAPK signaling pathway can be also activated by ROS [15]. A large number of evidences indicate that the signal transduction pathway activation of three important members of the MAPK family, p38 MAPK, JNK and ERK, is closely related to the development of DN, especially p38 MAPK signal transduction pathway that has attracted extensive attention. Some studies have found that these MAPKs pathways are activated in DN, and may promote the occurrence and development of DN by affecting the formation of ECM, apoptosis and cytokines [16].

Ginsenoside compound K [20-O-beta-D-glucopyranosyl-20(s)-protopanaxadiol, CK] is the final metabolite of diol-type ginsenosides such as Rb1, Rb2 and RC under the action of intestinal bacteria (the chemical structure of CK is shown in Fig. 1A). In recent years, researchers have carried out a series of related studies on CK, and it has been found that it has high activities in anti-tumor, anti-inflammation, anti-diabetes, anti-aging and liver protection [17], especially its anti-diabetic effect that has become an attracting topic [18]. Our previous study also found that CK improved the sensitivity of rats with diabetes induced by HFD/STZ to insulin by inhibiting PI3K/Akt signaling pathway [19]. Encouragingly, Yoon et al. found that CK (10 mg/kg) had the similar antidiabetic activity to that of metformin (150 mg/kg) [20]. In addition to the anti-diabetic effect, CK also showed strong anti-inflammatory and anti-oxidant effects, such as inhibiting the NF-κB pathway [21] and MAPKs pathway in various inflammatory models [22], as well as down-regulating the expression of COX-2 and iNOS [23,24]. Furthermore, CK could promote the IRS-1/PI3K/Akt pathway by inhibiting the activation of NLRP3 inflammasome associated with oxidative stress, and in turn improve the insulin resistance in adipose tissue [25]. It is worth noting that the traditional ginsenosides Rg1 and Rb1 have a relatively low bioavailability, but CK has a higher bioavailability [26]. Although the anti-inflammatory, anti-oxidative and anti-diabetic effects of CK are well documented, its effect on DN is unclear. Therefore, based on available experimental evidences, we speculated that in addition to its antidiabetic effect, CK might have a protective effect in DN, so that whether CK would have a protective effect on the renal injury induced by HFD/STZ in mice was observed in this study, and whether its reducing oxidative stress, inhibiting NLRP3 inflammasomes and MAPK signaling pathway involved in the underlying mechanisms of this protective effect was investigated.

2. Materials and methods

2.1. Drugs and reagents

Ginsenoside compound K (CAS: 39262-14-1) was provided by Sichuan Wickqi Biotechnology Co., LTD., (HPLC ≥ 98%). Streptozotocin (STZ) was from Sigma-Aldrich (St. Louis, MO, USA). BCA Protein Assay Kit and DAB substrate kit were from ZSGB-BIO (Beijing, China). Glutathione peroxidase (GSH-Px) assay kit, malondialdehyde (MDA) assay kit and superoxide dismutase assay kit were from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Blood glucose and urine protein test kits were purchased from BioSino Bio-technology and Science Inc. (Beijing, China). Interleukin-1β assay kit and IL-18 assay kit were from eBioscience (San Diego, California, USA). Reactive oxygen species (ROS) assay kit (DHE) was purchased from Beyotime Institute of Biotechnology (Shanghai, China). Polyvinylidene difluoride (PVDF) membrane was purchased from Millipore (Billerica, MA, USA). Nox1 and Nox4 antibody, anti-NADPH oxidase1antibody, anti-TXNIP antibody, anti-ASC antibody were from Abcam (Cambridge, UK); anti-NLRP3 antibody, anti-IL-1β antibody, anti-IL-18 antibody were from Santa Cruz Biotechnology (Santa Cruz, CA, USA); anti-p38 MAPK antibody, anti-phosphorylated p38 MAPK antibody, anti-ERK antibody, anti-phosphorylated ERK antibody, anti-JNK antibody, anti-phosphorylated JNK antibody, anti-Caspase1 antibody and anti-NF-κB p65 antibody were from Cell Signaling Technology (Beverly, MA, USA). Horseradish peroxidase-conjugated IgG was from Zsbio (Beijing, China).

2.2. Animals

C57BL/6 mice, weighing 18–22 g, were purchased from Jilin University Laboratory Animal Center [the animal license No.: SCXK (2011-0004)] and raised under SPF conditions. The animal experiments were carried out in consistent with the provisions of China Animal Welfare Act and the Guide of NIH Experimental Animal Management and Use after being approved by the Ethical Committee of Experimental Animals of Changchun University of Traditional Chinese Medicine. The mice were kept at 21 ± 1 °C and in a 12-hour light (8: 00 a.m.–8: 00 p.m.)/12-hour dark cycle, and all the experiments were conducted at 8: 00–17: 00.

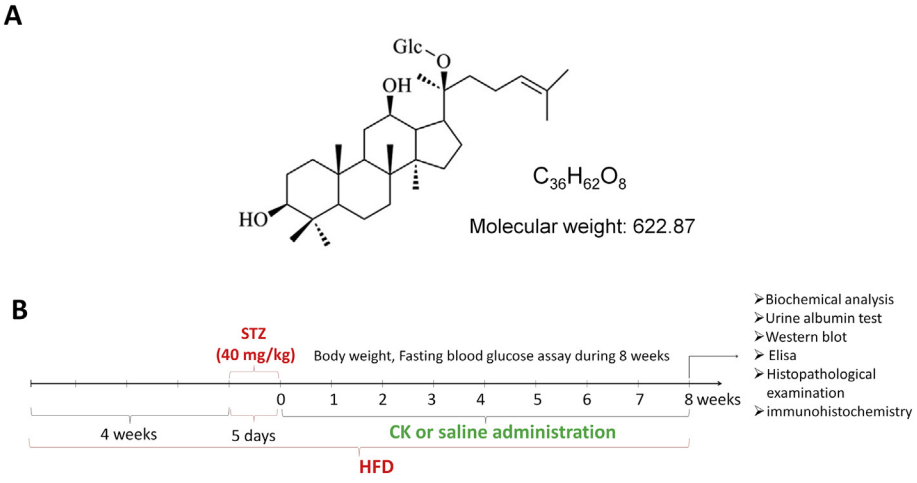

Fig. 1. Chemical structure of CK (A) and schematic representation of the experimental procedure (B).