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REVIEW

Identification of Active Component of Hachimi-jio-gan Ameliorating Diabetic Nephropathy

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ABSTRACT

Conventional medicine-based Chinese herbal prescriptions, have fascinated much attention due to their extensive and unique diversity of biological effects without toxicity and/or adverse effects. Treatment with Hachimi-jio-gan (Ba-Wei-Di-Huang-Wan in Chinese) improved the dysregulated levels of hyperglycemic condition-related oxidative stress generation, advanced glycation endproduct generation, and renal function parameters. These results indicate that Hachimi-jio-gan is a prospective therapeutic agent against the pathogenesis of diabetic nephropathy. Cornel iridoid glycosides and polyphenol are the active compounds of Corni Fructus, the active component of Hachimi-jio-gan, against kidney damage caused by diabetes. Additionally, major components of the Corni Fructus, morroniside and 7-*O*-Galloyl-D-sedoheptulose (GS) are considered to be important contributors to prevent and/or delay the onset of kidney damage caused by diabetes. Chief of all, GS is expected to be developed as a novel therapeutic drug for the diabetes-accelerated kidney damage.

1. Introduction

Diabetic nephropathy is the main cause of microvascular complications in diabetic patients, and one of the leading causes of end-stage renal disease (ESRD) worldwide [1]. Multiple factors have been implicated in the pathogenesis of diabetic nephropathy, including hyperglycemia-elicited generation of advanced glycation end-products (AGE) and reactive oxygen species (ROS) [2]. A number of new therapies have been developed from experimental studies based on the pathogenic factors of diabetic nephropathy such as blood glucose control, blood pressure control, renin-angiotensin-aldosterone system

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blockade, sodium-glucose cotransporter 2 inhibitors, lifestyle modification, including exercise and an energy-restricted diet, and numerous novel agents [3-7], but the rate of ESRD due to diabetic nephropathy has still lasts high in spite of the extensive usage of various therapies, focusing on the management of factors mentioned above. Thus, interventions to effectively delay the progression of diabetic nephropathy are urgently needed.

To date, there have been various researches focusing on the treatment to prevent diabetes and secondary complications with herbal medicines including Chinese prescriptions because of their absence of toxic and/or side effects. In Japan, the Chinese prescription Hachimi-jio-gan (Ba-Wei-Di-Huang-Wan in Chinese, description in Chinese medical book "Jin Gui Yao Lue") is a Chinese prescription that contains eight medicinal herbs: root of *Rehmannia glutinosa* Libosch. var. *purpurea* Makino (Rehmanniae Radix) 27.27%, fruit of *Cornus officinalis* Sieb. et Zucc. (Corni Fructus) 13.64%, rhizome of *Dioscorea japonica* Thunb. (Dioscoreae Rhizoma) 13.64%, rhizome of *Alisma orientale* Juzep. (Alismatis Rhizoma) 13.64 %, sclerotium of *Poria cocos* Wolf (Hoelen) 13.64%, bark of *Paeonia suffruticosa* Andrews (Moutan Cortex) 11.36%, bark of *Cinnamomum cassia* Blume (Cinnamomi Cortex) 4.54%, and tuber of *Aconitium carmichaeli* Debx (Aconiti Tuber) 2.27%. Hachimi-jio-gan has long been used widely to treat several diseases, including chronic nephritis, sterility, and vegetative ataxia [8-11]. Hachimi-jio-gan has also been used widely in the treatment of several disorders related with diabetes, and it has been used to the management of renal dysfunction in human subjects, although pharmacological evidence to prove the its therapeutic effects, and the corresponding mechanisms of Hachimi-jio-gan and its active components has not been reported yet. Therefore, we evaluated the effect of Hachimi-jio-gan on diabetic kidney damage using a type 1 diabetic nephropathy rat model that underwent subtotal nephrectomy (remnant kidney model) and streptozotocin (STZ) injection (type 1 diabetic model), and Otsuka Long-Evans Tokushima Fatty (OLETF) rats as a model of type 2 diabetic model, as shown in Figure 1. The data suggest that Hachimi-jio-gan may be a novel therapeutic approach to improving diabetic nephropathy [12,13]. Furthermore, to carry out research on the phytochemical constituents of Hachimi-jio-gan, the antidiabetic effects of Corni Fructus (fruit flesh of *Cornus officinalis* Sieb. et Zucc.), one of the Hachimi-jio-gan ingredients, and its phytochemical constituents (iridoid glycosides and polyphenol compound) were investigated using STZ-induced diabetic rats (Figure 1), and we sought to elucidate the major phytochemical constituents of Hachimi-jio-gan. In the present paper, we want to introduce a series of research contents reported so far.

2. Extract of Hachimi-jio-gan

Weighed eight medicinal herbs were boiled three times for 60 times in the 10 times volume of distilled water, filtered, and the filtrate was spray-dried. The yield of the solvent free extract was 10%, by weight compared to the original preparation. To investigate the components of Hachimi-jio-gan, an aqueous extract was analyzed by three-dimensional high-performance liquid chromatography (HPLC) analysis system equipped with an LC $10AD_{vp}$ pump coupled with SPD-$M10A_{vp}$ UV-VIs detector. TSK-GEL ODS-80TS column (ϕ 4.6 x 250 mm, Tosoh, Japan) was used for separation. The mobile phase consisted of (A) 0.05 M AcOH-$AcONH_4$ and (B) CH_3CN.

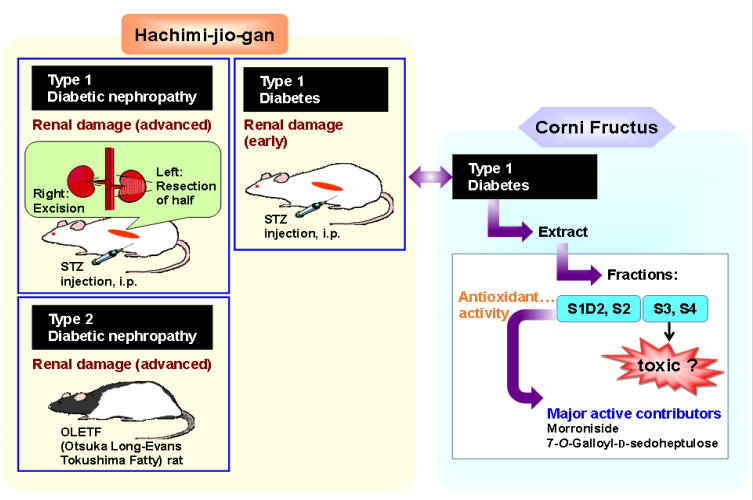

Figure 1. Schema of this research on Hachimi-jio-gan and its reactive components.

The gradient elution used was as follows: 0 min, 10% B and 60 min, 100% B. The flow rate was 1.0 mL/min. UV absorbance at 254 nm was monitored using an 3D-HPLC profiling data using an SPD-M10A$_{vp}$ UV-VIs detector. As shown in Figure 2, major components of Corni Fructus (morroniside and loganin) were the major compounds in Hachimi-jio-gan; paeoniflorin, penta-*O*-galloylglucose, benzoylmesaconine, benzoylpaeoniflorin, 16-ketoalisol A, cinnamic acid, and cinnamaldehyde were also detected. Therefore, in order to clarify the source of a particular action of Hachimi-jio-gan, we chose to evaluate the usefulness of Corni Fructus, which contains morroniside and loganin as active ingredients.

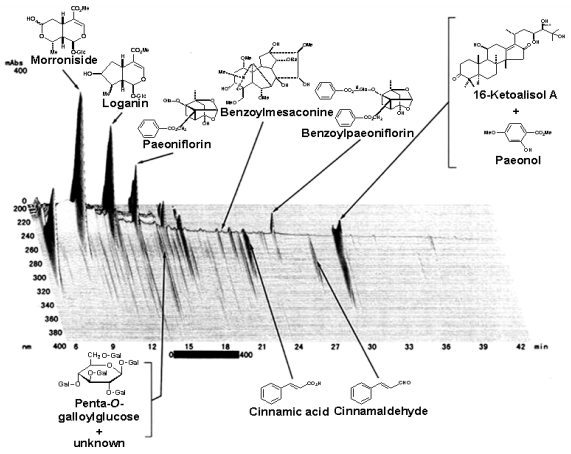

Figure 2. 3D-HPLC profile of Hachimi-jio-gan extract.

3. Corni Fructus, One of the Major Ingredient of Hachimi-jio-gan

Corni Fructus, the dried ripe fruit of *Cornus officinalis* Sieb. et Zucc. (Cornaceae), is an important traditional herbal medicine used in Chinese medicine due to exhibiting various pharmacological effects, including blood glucose-lowering, tumor growth-inhibiting, and microbial growth-inhibiting effects, and to improve liver and kidney functions [14-17]. To determine whether Corni Fructus exhibits the principal role in Hachimi-jio-gan, we investigated the effects of Corni Fructus using the same dose as for Hachimi-jio-gan, in order to compare the effect in STZ-induced diabetic rats. Administration of Corni Fructus inhibited hyperglycemic condition, proteinuria, renal AGE accumulation, and involved protein expressions, *i.e.*, receptor for AGEs, N^{ε}-(carboxymethyl) lysine (CML), nuclear factor-kB, and transforming growth factor-β_1 with these effects being alike to those of Hachimi-jio-gan. In addition, treatment with Corni Fructus attenuated renal dysfuntion, shown through serum creatinine (Cr) and Cr clearance, in diabetic rats [18]. Therefore, we hypothesized that Corni Fructus plays a central role in the pathogenesis of diabetes, *i.e.*, mitigating hyperglycemia-associated toxicities, ameliorating glycation-associated kidney damage, and improving diabetic condition-associated renal dyfunction and damage. As a result, this study may promote a renewed perception of traditional herbal medicine to elucidate the mechanisms of Chinese herbal formula, and Corni Fructus would be a main contributor to the renoprotective effect of Hachimi-jio-gan.

4. Fractionation and Chemical Characterization of Bioactive Compounds from Corni Fructus

Under the supposition that Corni Fructus would be a main contributor to the anti-diabetic and reno-protective effects of Hachimi-jio-gan, we attempted to identify the active fraction and components of Corni Fructus [19]. As shown in Figure 3, Corni Fructus extract (100 g) was applied to a column (32 x 5 cm) of Sephadex LH-20 column. Column was eluted with 10% stepwise increasing amounts of MeOH in water and ultimately 60% acetone to yield fractions: S1 (94.5 g), S2 (1.2 g), S3 (2.2 g), and S4 (1.6 g). Fraction S1 was further divided into two fractions (S1D1 and S1D2) by column (28 x 5 cm) of Diaion HP-20SS eluted with water-MeOH (0-100%, 10% stepwise mobile phase gradient elution) gave S1D1 (85.6 g) and S1D2 (7.9 g). TLC and HPLC analyses indicated that fraction S1D1 and S1D2 mainly composed sugars and iridoid glycosides, and fraction S2, S3 and S4 were contained phenolic substances. Fraction S2 was subjected to MCI-gel CHP20P column chromatography (28 x 2 cm) using water-MeOH (0-10%) to give the chromatographically pure 7-*O*-galloyl-D-sedoheptulose. Further separation of S1D2 (1 g) by MCI-gel CHP20P (30 x 3.4 cm) using water-MeOH (0-100%, 10% stepwise mobile phase gradient elution) gave five subfractions: S1D2-1 (127.0 mg), S1D2-2 (68.8 mg), S1D2-3 (157.0 mg), S1D2-4 (405.2 mg), and S1D2-5 (136.6 mg). Subsequent chromatography of the fraction S1D2-1, S1D2-2, and S1D2-3 over Chromatorex ODS column chromatography (26 x 2.8 cm) using water-MeOH (0-20%, 0-30%, and 0-45%) yielded mevaloside (37.0 mg), loganic acid (34.8 mg), and 5-hydroxymethyl-2-furfural (9.6 mg), respectively. A similar separation of S1D2-4 afforded morroniside (167.0 mg) and loganin (138.3 mg). Their chemical structures were identified by spectral analysis (COSY, HSQC, and HMBC) and NMR comparison (^{1}H- and ^{13}C-NMR). The chemical characterization isolated from S1D2 or S2 fraction is shown in Figure 3.

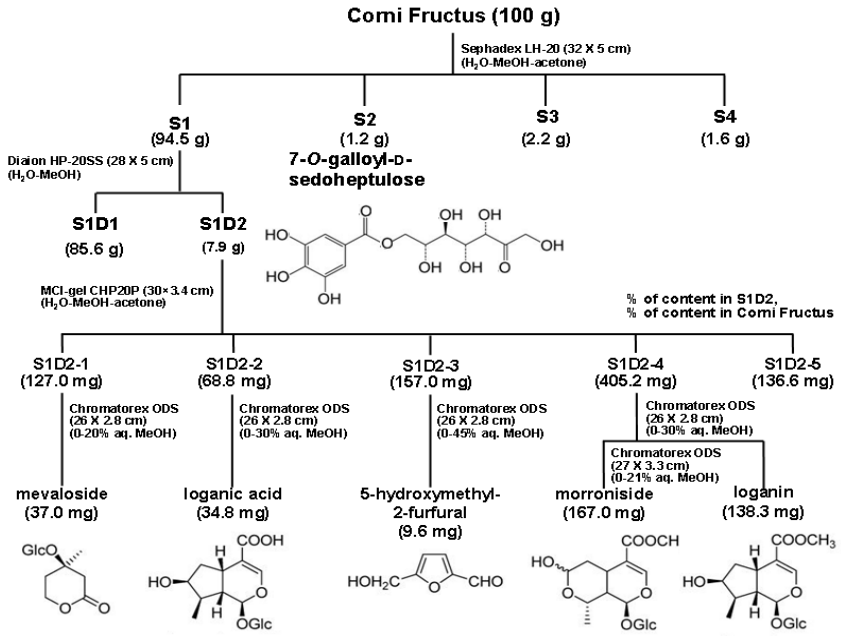

Figure 3. Fractionation and isolation of compounds from Corni Fructus.

5. Antidiabetic Potential of Fractionated Cornel Iridoid Glycosides and Polyphenol Fractions from Corni Fructus

As described above, we prepared Corni Fructus fractions and determined the fraction containing the potential constituents against diabetes, using one iridoid glycoside fraction (S1D2) and three polyphenol fractions (S2, S3, and S4), which were expected to possess potential activities than Corni Fructus, administered orally, respectively. Iridoid glycoside fraction (S1D2) and the low-molecular-weight polyphenol fraction (S2) could inhibit the pathogenesis of diabetic kidney damage, with each having different mechanisms: S1D1 (iridoid glycoside fraction) successfully reduced the hyperglycemic state and affected renal AGE accumulation, such as N^{ε}-(carboxyethyl)lysine (CEL) and CML, while the S2 (low-molecular-weight polyphenol fraction) could decrease renal lipid peroxidation, the receptor for AGE, and inducible nitric oxide synthase. In addition, S3 and S4 fractions (the other polyphenol fractions) reduced body weight gain compared with the control group, although these fractions significantly inhibited the levels of increased thiobarbituric acid-reactive substances (TBARS) in the kidney, proposing that these two fractions may have radical scavenging activities but may include some toxic ingredients. Ultimately, these results suggest that antidiabetic potential of iridoid glycoside (S1D2) and low-molecular-weight polyphenol (S2) fractions obtained from Corni Fructus improve metabolic parameters related with the development of diabetic kidney damage [19].

6. Protective Effects of Morroniside and Loganin Isolated from Corni Fructus against Kidney Damage in Diabetic Rats

Iridoid glycosides such as morroniside and loganin are well-known as the major bioactive compounds of Corni Fructus [20-22]. This study investigated whether morroniside and loganin isolated from Corni Fructus exerts a beneficial effect on kidney damage in STZ-treated diabetic rats. Oral treatment of diabetic rats with morroniside resulted in significant reductions in elevating levels of serum glucose and urinary protein. The reduced levels of serum albumin and total protein in diabetic rats were significantly improved by morroniside administration. Moreover, morroniside modulated the enhanced level of serum urea nitrogen and showed a tendency to decrease Cr clearance. Morroniside also significantly reduced the increased serum glycosylated protein, and serum and renal TBARS levels. Renal protein expressions related to the AGE such as CEL as well as oxidative stress such as heme oxygenase-1 were significantly reduced following the treatment of morroniside [23,24]. These results suggest that morroniside is effective against diabetic kidney damage, which mediates action of the hyperglycemia and oxidative stress in serum and renal tissue. However, loganin has a weaker effect than morroniside against diabetic kidney damage, but has an indirect effect of the improving metabolic diseases in other organs such as hepatic tissue [24,25]. Therefore, these

results indicate that morroniside is one component with a partial role for the renoprotective effects of Hachimi-jio-gan and Corni Fructus against kidney damage caused by diabetes.

7. 7-*O*-Galloyl-D-sedoheptulose (GS) is a New Therapeutic Drug in the Diabetic Kidney

From the S2 fraction, we isolated GS of low-molecular-weight polyphenol [19]. To our knowledge, this compound is only detected from *Cornus officinalis*, and its biological activity has not yet been found. That being so, we decided to clarify the completed protective mechanisms against diabetic kidney disease, and examined whether the oral administration of GS reduces diabetes-induced oxidative stress and glycation products in the kidney.

Polyphenols, including flavonoids and phenolic acid are the most abundant antioxidants in human diet, being common constituents of fresh vegetables, fruits, and beverages include tea. They have been reported to have various beneficial effects, such as the prevention and/or delay of cancer [26], neurodegenerative disorder [27], atherosclerotic disorder [28], and diabetes [29], as well as indicate slow down the ageing process effects [30], because an imbalance between production and accumulation of oxygen reactive species is believed to influence many disorders. Our previous study showed that (-)-epigallocatechin 3-*O*-gallate, a major green tea polyphenol, exerted a protective effect on kidney damage caused by hyperglycemic condition-related oxidative stress complicated in renal lesions index of diabetic nephropathy [31]. Based on the previous study, we tested GS, and exhibited its renoprotective effect on the diabetic condition; to date, our study is the only results on biological research. We revealed its beneficial effects on abnormal metabolism-related renal symptoms, including renal glucose uptake, AGE generation [methylglyoxal, glycoaldehyde (GA), receptor for AGE, CEL, CML, and GA-pyridine], and oxidative stress [TBARS and heme oxygenase-1], which is thought to play a key role in the pathogenesis of diabetic kidney disease. GS had strong effects on the suppression of AGE generation *via* the regulation of Maillard reaction and lipid peroxidation, as shown in Figure 4. In addition, GS effectively suppressed the rises in levels of serum Cr and urinary protein to nearly non-diabetic control values. The serum albumin level was significantly increased in the GS-administered mice [32]. These effects were consistent with those of Hachimi-jio-gan and/or Corni Fructus. The role of GS is expected to provide a novel therapeutic strategy against diabetic kidney disease.

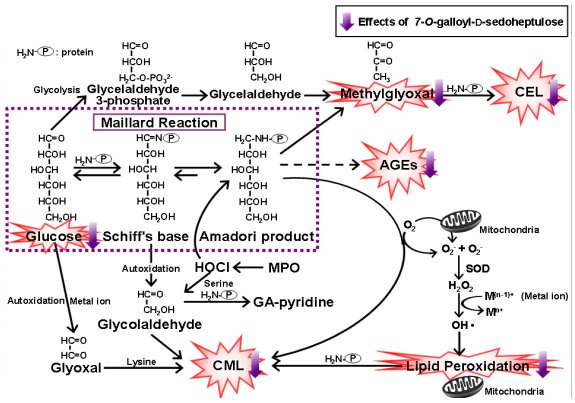

Figure 4. Effect of GS on the possible mechanisms of AGE generation from glucose through the Maillard reaction, autoxidation, glycoxal or methylglyoxal pathways, lipid peroxidation, and the myeloperoxidase (MPO) system.

8. Conclusions

The discovery of efficacious components is an important prerequisite for clarification of the precise mechanisms of herbal medicines. However, studies on the biological activities of the active components isolated from herbal medicines and Chinese prescriptions are limited. Therefore, we evaluated the effects of Hachimi-jio-gan ameliorating diabetic kidney damage using a diabetic nephropathy rat model. Hachimi-jio-gan treatment suppressed the higher levels of hyperglycemic condition-induced oxidative stress, AGE generation, renal protein dysexpression, and proteinuria. These results indicate that Hachimi-jio-gan is a prescribed herbal medication with potential against kidney damage caused by diabetes. In addition, we established that Corni Fructus, one ingredient of Hachimi-jio-gan, could ameliorate glucose-associated metabolic disorders and its mechanisms were closely related to Hachimi-jio-gan. Fraction S1D2 (containing iridoid glycosides) and S2 (containing low-molecular-weight polyphenols) were active fractions of Corni Fructus, the active ingredient of Hachimi-jio-gan, against kidney damage caused by diabetes. Furthermore, according to the identification of active ingredients of the traditional herbal medicine, Corni Fructus, morroniside and GS are expected to provide a novel therapeutic drug against kidney damage caused by diabetes. Chief of all, GS, which is a polyphenolic compound of Corni Fructus, is expected as a new therapeutic drug.

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