Brief Report

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Therapeutic effect of Chinese prescription Kangen-karyu in patients with diabetic nephropathy

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SUMMARY Diabetic nephropathy is the kidney disease that occurs as a result of diabetes. A number of new therapies have been developed based on the pathogenic factors of diabetic nephropathy such as intensive glycemic control, precise hypertension control, lifestyle modifications including exercise and an energy-restricted diet, and numerous novel agents. The utilization of traditional Chinese medicine for patients with diabetic nephropathy has also received increasing attention due to its wide availability, weak side-effects, and proven therapeutic mechanisms and benefits. In this paper, we report the case of patients with diabetic nephropathy, stage 2 or 3. Kangen-karyu extract (7.5 g/day) was administered three times per day for 6 months. The estimated glomerular filtration rate was increased at the 6-month follow-up. The serum creatinine level decreased following administration. At that time, somatic and subjective symptoms had partially disappeared. Here, we present evidence that Kangen-karyu exerts a renoprotective effect against the development of diabetic nephropathy.

Keywords diabetic nephropathy, traditional Chinese medicine, Kangen-karyu, case report

1. Introduction

Diabetes is the leading cause of end-stage renal disease (ESRD) in most developed countries, and has driven an increase in ESRD globally over recent decades (*1,2*). There is a strong economic and health imperative to improve outcomes for people with diabetes and kidney disease. A number of promising treatments have been found to be ineffective or harmful, many of which have now been abandoned in this population (*3-5*). One feature of these failures has been the emergence of unexpected adverse effects, highlighting the importance of safety monitoring in future trials and review of what is known about the safety of existing treatments in this patient population.

Traditional Chinese medicine has received much attention as a source of novel therapeutic agents due to their multiple beneficial effects and absence of toxic and/or side effects (*6*). Kangen-karyu (Guan-Yuan-Ke-Li in Chinese), one of our major interests among traditional Chinese medicine agents, has been developed in Japan by the modification of herbal constituents of Kan-shin No. 2 (Guan-xin No. 2 in Chinese) (*7*), and is composed of six herbal formulas (Salviae Miltiorrhizae Radix, Cnidii Rhizoma, Paeoniae Radix, Carthami Flos, Aucklandiae Radix, and Cyperi Rhizoma, as shown in Table 1). Kangen-karyu has been clinically used as a treatment for cardiovascular disease (CVD), known as a risk factor for the progression of chronic kidney disease (CKD) (*8,9*). Many studies demonstrated that Kangen-karyu exhibits favorable biological activity such as anti-aging effects, platelet aggregation inhibition, hypertension suppression, anti-dyslipidemia, aiding the recovery of learning and memory impairment induced by senescence, neuroprotection, and an anti-dementia effect in animal experiments (*10-17*). Although studies have proposed the pharmacological functions of Kangen-karyu to treat various diseases, we previously reported evidence supporting its preventive and/or therapeutic potential against diabetes-induced renal damage using *db/db* mice, a type 2 diabetic animal model (*18-20*). The results of our previous study provide important evidence that Kangen-karyu exerts a renoprotective effect against the development of diabetic nephropathy. We also provide evidence supporting the use of Kangen-karyu as a therapeutic agent in a patient with diabetic nephropathy in the early stage (*21*).

On the basis of these findings, we administered Kangen-karyu to diabetic nephropathy patients, stage 2 or 3, and report its therapeutic usefulness.

Table 1. Composition of Kangen-karyu

Common name	Botanical name	Family name
Salviae Miltiorrhizae Radix	*Salvia miltiorrhiza* Bunge	Labiatae
Cnidii Rhizoma	*Cnidium officinale* Makino	Umbelliferae
Paeoniae Radix	*Paeonia lactiflora* Pallas	Paeoniaceae
Carthami Flos	*Carthamus tinctorius* L.	Compositae
Aucklandiae Radix	*Aucklandia lappa* Dcne.	Compositae
Cyperi Rhizoma	*Cyperus rotundus* L.	Cyperaceae

2. Materials and Methods

2.1. Study population

This study was conducted according to the ethical guidelines for epidemiological research designated by the Japanese Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labour, and Welfare. Ethical approval was obtained from the Clinical Research Ethics Committees of Shinseikai Toyama Hospital. Written informed consent was obtained from all subjects at the time of enrollment for collection of clinical information and biosamples for archival and research purposes. The study cohort was previously diagnosed with diabetic nephropathy at Shinseikai Toyama Hospital. Both sexes (3 men and 2 women; 54-73 years, 64.0 ± 3.8 years) and stages of diabetic nephropathy (3, stage 2; and 2, stage 3) were represented. The patients continued to receive existing treatments: hypoglycemic agents (metformin: 750 mg/day, ipragliflozin: 50 mg/day), an antihypertensive agent (termisartan: 20 mg/day), antilipidemic agent (atrovastatin: 5 mg/day), and antacid-laxative (magnesium oxide: 990 mg/day). In addition, Kangen-karyu extract (7.5 g/day) was administered three times a day for 6 months. During the administration of Kangen-karyu extract, regular tests were performed to assess its effect on diabetic nephropathy. At that time, a medical interview including questions on the somatic and subjective symptoms was conducted during the study.

2.2. Measurements of study variables

All measurements were performed by the Department of Laboratory Medicine of Shinseikai Toyama Hospital using routine automated laboratory methods. Estimated GFR (eGFR) was based on the equation proposed by the Japanese Society of Nephrology (*22*). Body components were analyzed using an InBody 770 (InBody Japan Inc., Tokyo, Japan).

2.3. Assessment of somatic and subjective symptoms

The symptom checklist included the following symptoms: dizziness and palpitation, stiff shoulder and headache, coldness of the limbs and fatigability, mental stress, sleeping disorder, tension of the stomach and abdomen, pain, numbness of the waist and body, dark circles around eyes and lip symptoms, stains on face, aza skin, and tongue symptoms. The change in each symptom was assessed with a 3-point rating scale: "marked improvement" was 5 points, "improvement" was 4 points, and "slight improvement" was 2 points. The assessment of global improvement rating of subjective symptoms simply involved the addition of points.

Table 2. Laboratory data and physical characteristics on administration of Kangen-karyu for 6 months

Parameter	0 M	6 M
HbA1c (%)	7.52 ± 0.47	7.78 ± 0.39
Serum Cr (mg/dL)	0.71 ± 0.07	0.64 ± 0.09
eGFR (mL/min/1.73 m^2)	79.2 ± 8.8	88.2 ± 9.0
Urinary albumin (mg/g Cr)	56.1 ± 10.4	71.0 ± 45.4
Urinary protein (mg/g Cr)	425 ± 157	506 ± 193
BMI (kg/m^2)	28.2 ± 1.4	28.8 ± 1.3
SLM (kg)	45.8 ± 4.4	45.9 ± 4.2
BFM (kg)	25.0 ± 3.5	26.1 ± 3.3
VFA (cm^2)	119 ± 18	124 ± 19
PBF (%)	34.1 ± 3.6	35.1 ± 3.5
Score using the questionnaire	38.6 ± 2.7	27.2 ± 1.1*

M, months. Values are expressed as the mean ± SEM. of 5 patients. *$p < 0.01$ *vs.* 0 M values.

2.4. Statistical analysis

The data are expressed as the mean ± SEM. Significance was assessed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test (SPSS 11.5.1 for Windows, 2002, SPSS Inc., USA), with values of $p < 0.05$ considered to indicate significance.

3. Results and Discussion

3.1. Clinical characteristics

From samples obtained at the first timepoint, hemoglobin A1c (HbA1c) was 7.52%, showing poorly controlled blood glucose. The eGFR was 79.2 mL/min/1.73 m^2, and this corresponded to a serum creatinine (Cr) level of 0.71 mg/dL. The urinary albumin and protein levels were 56.1 and 425 mg/g Cr, respectively, indicating stage 2 to 3 diabetic nephropathy, as shown in Table 2. After the administration of Kangen-karyu extract for 6 months, eGFR was subsequently increased from 79.2 to 88.2 mL/

Table 3. Changes in eGFR and serum Cr before and after Kangen-karyu administration

Items	Kangen-karyu administration						
	-6 M	-3 M	-1 M	0 M	1 M	3 M	6 M
eGFR (mL/min/1.73 m^2)	84.6 ± 9.4	80.3 ± 7.9	79.2 ± 5.8	79.2 ± 8.8	80.7 ± 8.3	81.9 ± 7.9	88.2 ± 9.0
Serum Cr (mg/dL)	0.67 ± 0.14	0.70 ± 0.06	0.70 ± 0.05	0.71 ± 0.07	0.70 ± 0.07	0.68 ± 0.05	0.64 ± 0.09

M, months. Values are expressed as the mean ± SEM of 5 patients.

min/1.73 m^2 at the 6-month follow-up, and serum Cr was slightly decreased compared with the first timepoint. The urinary albumin level increased from 56.1 to 71.0 mg/g Cr. Urinary protein excretion also increased to 506 mg/g Cr. There was, however, no significant change in the HbA1c on the administration of Kangen-karyu extract, as shown in Table 2.

Moreover, to identify the therapeutic usefulness of Kangen-karyu extract to renal function of diabetic nephropathy patients, we investigated the eGFR and serum Cr levels in patients from the 6th month prior to Kangen-karyu extract administration. As shown in Table 3, the eGFR level of diabetic nephropathy patients was gradually decreased as time progressed until 6 months, indicating renal function decline. On the other hand, the administration of Kangen-karyu was increased from 79.2 to 80.7 mL/min/1.73 m^2 at 1 month, 81.9 mL/min/1.73 m^2 at 3 months, and 88.2 mL/min/1.73 m^2 at 6 months. When the rate of variability in eGFR was calculated using the formula shown in Table 4, its value was significantly recovered by Kangen-karyu administration. Additionally, a slight increase of serum Cr that progressed in diabetic nephropathy patients was progressively decreased by the administration of Kangen-karyu at the 6-month follow-up, as shown in Table 3. The rate of variability in serum Cr was significantly decreased by Kangen-karyu (Table 4).

Table 4. Rate of variability in eGFR and serum Cr before and after Kangen-karyu administration

Items	(At the start of administration – 6 months before administration)/6	(At the start of administration – 6 months after administration)/6
eGFR	– 0.894 ± 0.288	1.491 ± 0.406*
Serum Cr	0.007 ± 0.002	– 0.012 ± 0.003*

Significance: *$p < 0.01$ *vs.* (at the start of administration - 6 months before administration)/6 values.

3.2. Physical characteristics

There was no significant change in the physical parameters such as body mass index (BMI), soft lean mass (SLM), body fat mass (BFM), visceral fat area (VFA), or percent body fat (PBF) on the administration of Kangen-karyu extract, as shown in Table 2.

3.3. Somatic and subjective symptoms

At the 6-month follow-up of patients, the somatic and subjective symptoms such as stiff shoulder, headache, coldness of the limbs, and fatigability had disappeared. The score using the questionnaire had decreased from 38.6 to 27.2 at follow-up, being a significantly lower (30%) score, as shown in Table 2.

Diabetic nephropathy is the leading cause of ESRD, which is a threat to public health and a major financial burden for healthcare systems (*23,24*). The life expectancy of patients with ESRD has remained poor, and ESRD prevention is challenging. The prognosis of CVD patients with type 2 diabetes has markedly improved over the past 20 years, but the incidence of ESRD has decreased very little (*25*). Thus, early interventional treatment for diabetic nephropathy is important.

In the present study, we chose Kangen-karyu extract for the following reasons. Kangen-karyu was developed by the modification of herbal constituents of Kan-shin No. 2 in Japan (*7*). It has been clinically used as a treatment for CVD (*8*). Kangen-karyu has received much attention as a source of new therapeutic agents based on pre-clinical animal experiments related to various human diseases (*10-17*). To add to these findings, we report evidence supporting its preventive and/or therapeutic potential against diabetes-induced renal damage (*18-20*). The administration of Kangen-karyu reduced the increased serum glucose level in type 2 diabetic mice, and decreased the elevated oxidative and inflammatory biomarkers in the serum and kidney. The increased serum Cr and urea nitrogen levels, which reflect renal dysfunction, and renal structural changes, representing glomerular enlargement, were significantly improved by Kangen-karyu administration. The results of our previous study suggest that Kangen-karyu improves diabetes-induced renal damage through pleiotropic effects on the development of diabetic nephropathy. The utilization of traditional Chinese medicine to treat diabetic nephropathy has received increasing attention due to its wide availability, weak side-effects, and proven therapeutic mechanisms and benefits.

In the present patients, there was an improvement in diabetic nephropathy following the administration of Kangen-karyu for 6 months. Because of the short follow-up period, the effect of the long-term administration of Kangen-karyu on progressive nephropathy remains unknown. However, eGFR was subsequently increased

from 79.2 to 88.2 mL/min/1.73 m^2 at the 6-month follow-up. The serum Cr level decreased from 0.71 to 0.64 mg/dL. In addition, the score using the questionnaire was significantly decreased during the follow-up. We present the therapeutic option using Kangen-karyu to treat renal disease patients with diabetic nephropathy. Interesting findings were also obtained with regard to eGFR: the level of eGFR gradually decreased at 6, 3, 1, and 0 months prior to Kangen-karyu extract administration. The administration of Kangen-karyu for 6 months increased this level, and the rate of variability in eGFR was significantly recovered. There were, however, no improvement in the urinary albumin and protein levels on the administration of Kangen-karyu.

Albuminuria is characterized clinically as an early predictor for progression of diabetic nephropathy (*26*). Proteinuria is the universal finding in progressive renal disease, and viewed as a measure of the severity and determinant for diabetic renal disease progression (*27*), whereas eGFR is estimated using endogenous plasma or serum filtration markers, most commonly Cr (*28,29*). With regard to Cr, we have shown that Cr reacts with hydroxyl radical to quantitatively and non-enzymatically produce 5-hydroxycreatinine, which partially decomposes to methylguanidine, a stronger uremic toxin. These reactions have been reported to occur not only *in vitro* but also *in vivo* (*30*). Moreover, we suggested that the efficacy of Kangen-karyu on diabetic nephropathy in type 2 diabetic *db/db* mice was dependent on several oxidative stress-related parameters and exerted a renoprotective effect (*18-20*). Thus, Kangen-karyu may function as an ameliorator of oxidative stress and show beneficial effects to diabetic nephropathy patients.

Diabetic nephropathy is among the main causes of ESRD. Multiple factors such as metabolic and hemodynamic alterations, oxidative stress, activation of the renin-angiotensin system, and inflammation may interdepend on various levels, causing progressive nephropathy (*31,32*). In the present study, there was an improvement in diabetic nephropathy following the administration of Kangen-karyu extract for 6 months, although we cannot come to a conclusion on the pathway that was affected. In addition, the score using the questionnaire was decreased during the follow-up. Herein, we present a therapeutic option using Kangen-karyu in the early phase of diabetic nephropathy.

In conclusion, we report evidence supporting the use of Kangen-karyu as an adjunctive therapy in patients with diabetic nephropathy corresponding to stage 2 or 3. Kangen-karyu exhibits good efficacy in the treatment of patients with diabetic nephropathy.

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