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Effects of Zishentongluo in Patients with Early-Stage Diabetic Nephropathy

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Abstract: The purpose of this study was to assess the efficacy of zishentongluo (ZSTL) for the treatment of diabetic nephropathy (DN) and its related mechanisms. Forty-five patients with DN were randomized to receive either ZSTL ($n = 25$) or benazepril ($n = 20$), an angiotensin converting enzyme inhibitor, for 12 weeks. Conventional biochemical tests were performed to determine fasting blood glucose (FBG), glycated hemoglobin (HbA1c), serum creatinine (SCr), endogenous creatinine clearance rate (Ccr), total cholesterol (TC), and triglyceride (TG) levels. The urinary albumin excretion rate (UAER), and endothelin 1 (ET-1), and atrial natriuretic peptide (ANP) levels were determined with a radioimmunoassay, and vascular endothelial growth factor (VEGF) was detected using an enzyme-linked immunosorbent assay. The primary endpoint was change from the baseline to post-treatment in HbA1c. Secondary endpoints were change from baseline to post-treatment in FBG, TC, TG, UAER, SCr, Ccr, VI-C, ANP, ET-1, and VEGF. ZSTL was significantly more effective at improving the primary (i.e., HbA1c) and secondary (i.e., FBG, TC, TG, UAER, SCr, ANP, ET-1, and VEGF) outcomes than benazepril ($p < 0.05$). These findings suggest that ZSTL is superior to benazepril at improving the metabolic and renal functioning in patients with early-stage DN, in part, by modifying ANP, ET-1, and VEGF.

Keywords: Diabetic Nephropathy; Hyperglycemia; Diabetes Mellitus; Traditional Chinese Medicine; Atrial Natriuretic Peptide; Endothelin; Vascular Endothelial Growth Factor.

Introduction

Diabetic nephropathy (DN), which is characterized by increasing albuminuria and declining renal function, is a common microvascular complication of diabetes mellitus (DM), and a leading cause of end-stage renal disease (ESRD) worldwide (Gupta *et al.*, 2011). In addition, DN is associated with an increased risk of cardiovascular morbidity and mortality (Cao and Cooper, 2011). The increasing incidence of DM worldwide is resulting

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in an increase in the prevalence of DN and its associated complications, which are becoming major healthcare burdens (Cao and Cooper, 2011).

The pathogenesis of DN is very complex and involves numerous metabolic and hemodynamic abnormalities (Cooper, 2001). Current treatment modalities for DN are directed at delaying the progression of DN towards ESRD via glycemic and blood pressure control, reducing albuminuria and proteinuria, and interfering with the renin-angiotensin system (RAS) with the use of angiotensin converting enzyme (ACE) inhibitors and angiotensin type-1 receptor blockers (ARB), along with dietary modification and cholesterol lowering agents (Cao and Cooper, 2011; Gupta *et al.*, 2011; Abdel-Rahman *et al.*, 2012). However, despite the available multifactorial therapeutic approaches, clinical evidence suggests that the effectiveness of these interventions, even in combination, only partially reduce the progression of DN in patients with DM (Cao and Cooper, 2011; Gupta *et al.*, 2011; Abdel-Rahman *et al.*, 2012), indicating the importance of developing novel and more effective intervention strategies.

Recently, studies have shown that traditional Chinese medicine is effective in treating DN patients (Wang *et al.*, 2012a). For example, Li *et al.* (2009) demonstrated that ginkgo leaf extract appeared to improve the structure and function of kidneys in rats with type 2 DM, as well as correct lipid metabolism, reduce urinary albumin excretion, and improve vascular endothelial function in early-stage DN patients. Before this approach will be accepted by Western medical practice and regulatory authorities, further rigorous research (both pharmacological and mechanistic) that evaluates the use of different herbs commonly used in traditional Chinese medicine for DN is needed (Liu *et al.*, 2012; Wang *et al.*, 2012b).

In the present study, we assessed the effects and underlying mechanism of zishentongluo (ZSTL) in the treatment of early-stage DN. ZSTL consists of the following 11 Chinese herbs: raw astragalus, angelica, safflower, zedoary turmeric, Dodder, Radix Rehmanniae, dogwood, Poria, Epimedium, earthworm, and Schisandra.

Materials and Methods

Patients

Patients who met the inclusion criteria in Air Force Hospital, were included in the study. Eligible patients (aged 18 to 65 years) had a diagnosis of type 2 DM, according to the guidelines of the World Health Organization (1999) and early-stage DN, according to the criteria of Mogensen (1986), where the urinary albumin excretion rate (UAER) was determined via urinalysis to be within 20–200 μg/min at two consecutive assessments or 30–300 mg/24 h within a three-month period. Excluded patients included pregnant or lactating women, those that were allergic to zishentongluo or benazapril or both, and those suffering from other diseases or complications, including congestive heart failure, elevated serum transaminases, essential hypertension, and other serious primary heart, brain, lung, and liver diseases. Patients were also excluded if they had experienced diabetic ketoacidosis or urinary tract infection within the past month. The study was approved by the Institutional Review Board (IRB) of the Air Force Hospital, and written informed consent was obtained from all patients prior to trial entry.

Treatments

All patients were provided with a DN diet to ensure a protein intake of 0.8–1 g/kg/day. They were also treated with oral gliquidone (Glurenorm) and metformin or injected insulin to control their blood glucose levels. The study group received decocting-free ZSTL granules (Shenzhen Sanjiu Pharmaceutical Company, Shenzhen, China). The ZSTL prescription consisted of the following 11 Chinese herbs: raw astragalus (30 g), angelica (10 g), safflower (10 g), zedoary turmeric (10 g), Dodder (10 g), Radix Rehmanniae (10 g), dogwood (10 g), Poria (10 g), Epimedium (10 g), earthworm (20 g), and Schisandra (10 g). The amount of each herb in ZSTL was determined using information from Pharmacopoeia Commission of the People's Republic of China (1995) and Practical Dictionary for Chinese herbs and medicine (Tian, 2003). The granules were prepared as described in the Chinese Pharmacopoeia (Chinese Pharmacopoeia Commission, 2010). Briefly, each herb was first extracted in water by decocting for a certain period of time, and then concentrated into a powder, which was subsequently packaged into granules. Prior to ingestion, the granules were brewed with warm water. Patients ingested half of the ZSTL solution (150 ml) twice daily for three consecutive months. Patients in the control group received 10 mg of benazepril (Lotensin®, Novartis, Basel Switzerland) once daily for three consecutive months.

Measurements

Conventional biochemical evaluated the serum levels of fasting blood glucose (FBG), glycated hemoglobin (HbA1c), serum creatinine (SCr), endogenous creatinine clearance rate (Ccr), cholesterol (TC), and triglyceride (TG). The urinary albumin excretion rate (UAER) was determined with a radioimmunoassay (Beijing North Institute of Biological Technology, Beijing, China). Serum type IV collagen content was detected using an enzyme-linked immunosorbent assay (ELISA; Beijing Bangding Taike Biotechnology Co., Ltd., Beijing, China). Endothelin 1 (ET-1) and atrial natriuretic peptide (ANP) levels were determined by radioimmunoassay (Research Institution of Radioimmunology, People's Liberation Army General Hospital, Beijing, China) and vascular endothelial growth factor 189 (VEGF) was detected by using an ELISA (Jingmei Biotech, Beijing, China).

Outcomes

The primary endpoint was changes from baseline to post-treatment (week 36) in HbA1c serum levels. Secondary endpoints included changes from baseline to post-treatment in FBG, TC, TG, UAER, SCr, Ccr, VI-C, ANP, ET-1, and VEGF.

Statistical Analysis

Because of small sample, continuous and categorical variables were compared using Mann-Whitney U test and chi-square tests, respectively. Differences between the baseline and last visit (i.e., week 36) in both groups were determined with a Wilcoxon Signed Ranks

Test. Continuous data are presented as median (interquartile range), whereas categorical data are expressed as a number (n) and percentage (%). For all analyses, a two-sided p-value of < 0.05 was considered significant. Statistical analyses were performed using SPSS 15.0 statistics software (SPSS Inc, Chicago, IL, USA).

Results

A total of 45 patients with early-stage DN were enrolled into the study between January 2009 and September 2011. Patients were randomized to receive either ZSTL ($n = 25$) or 10 mg of benazepril ($n = 20$). The demographic and baseline characteristics were similar between treatment groups ($p > 0.05$) (Table 1). Both groups consisted of 40% males and the median durations of diabetes were 15 years and 16 years for ZSTL and benazepril groups, respectively.

Comorbidities were present in a subset of patients. Fourteen patients (zishentongluo $n = 8$; control $n = 6$) had hypertension and were treated with calcium channel blockers to maintain values within normal ranges. Nine patients (zishentongluo $n = 6$; control $n = 3$) had coronary heart disease and were treated with nitrates and were continually monitored throughout the study. Eight patients (zishentongluo $n = 8$; control $n = 3$) had hyperuricemia and all were treated with allopurinol and monitored throughout the study.

Table 1. Demographic and Baseline Characteristics of Patients Treated with Zishentongluo or Benazepril ($n = 45$)

	Zishentongluo Recipe ($n = 25$)	Benazepril ($n = 20$)	p Value
Demographic Characteristics			
Age (years)[1]	57 (57, 58)	57 (56, 58)	0.088
Gender, n (%)[2]			1.000
Male	10 (40.0)	8 (40.0)	
Female	15 (60.0)	12 (60.0)	
Duration of diabetes (years)[1]	15 (13, 16)	16 (12, 17)	0.413
Baseline Characteristics			
HbA1c (%)[1]	10.68 (8.48, 13.96)	12.08 (10.21, 14.08)	0.197
FBG (mmol/L)[1]	12.54 (10.57, 14.80)	13.34 (10.56, 15.23)	0.775
Total cholesterol (mmol/L)[1]	6.12 (5.47, 7.33)	5.99 (5.30, 6.72)	0.123
Triglyceride (mmol/L)[1]	2.35 (1.72, 3.05)	2.75 (2.12, 3.00)	0.222
UAER (μg/min)[1]	211.52 (164.58, 243.89)	203.21 (150.32, 230.21)	0.332
SCr (μmol/L)[1]	87.17 (70.59, 110.25)	83.69 (72.55, 109.47)	0.802
Ccr (mL/min)[1]	139.86 (129.58, 149.52)	139.09 (133.06, 146.76)	0.623
Serum type IV collagen (ng/ml)[1]	161.08 (142.53, 188.54)	172.92 (152.42,192.56)	0.178
ANP (ng/L)[1]	266.53 (236.53, 343.56)	289.44 (235.51, 360.97)	0.591
ET-1 (ng/L)[1]	118.52 (84.27, 149.56)	106.96 (80.75, 134.28)	0.304
VEGF (pg/ml)[1]	200.97 (100.80, 345.10)	201.02 (155.97, 269.17)	0.698

Note: Data are expressed as median (interquartile range), unless otherwise specified. p values are based on [1]Mann-Whitney U test and [2]Chi-square test. Abbreviations: HbA1c, glycated hemoglobin; FBG, fasting blood glucose; UAER, urinary albumin excretion rate; SCr, serum creatinine; Ccr, endogenous creatinine clearance rate; ANP, atrial natriuretic peptide; ET-1, endothelin; VEGF, vascular endothelial growth factor.

Table 2. Effects of a 36-Week Treatment with Zishentongluo and Benazepril on the Primary and Secondary Endpoints ($n = 45$)

Mean Change from Baseline	Zishentongluo ($n = 25$)	Benazepril ($n = 20$)	p Value
Primary endpoint			
HbA1c (%)	−4.29 (−5.85,−2.79)†	−3.26 (−4.06,−1.96)†	0.016*
Secondary endpoints			
FBG (mmol/L)	−5.43 (−6.63, −2.53)†	−4.06 (−4.68, −1.40)†	0.031*
Total cholesterol (mmol/L)	−1.52 (−2.18, −1.24)†	−0.67 (−0.90, 0.20)	<0.001*
Triglyceride (mmol/L)	−0.49 (−0.99, −0.39)†	0.02 (−0.34, 0.14)	<0.001*
UAER (μg/min)	−106.99 (−121.29, −85.55)†	−69.38 (−86.89, −51.86)†	<0.001*
SCr (μmol/L)	−3.33 (−11.02, 2.15)†	2.31 (0.13, 11.03)†	0.003*
Ccr (mL/min)	−9.22 (−13.42, −5.82)†	−13.44 (−15.55, −11.94)†	0.091
Serum type IV collagen (ng/ml)	−38.96 (−52.00, −31.21)†	−29.01 (−41.06, −21.69)†	0.091
ANP (ng/L)	−67.26 (−86.29, −37.87)†	−46.51 (−51.75, −30.01)†	0.009*
ET-1 (ng/L)	−57.25 (−78.80, −35.56)†	−28.74 (−51.53, −14.17)†	0.003*
VEGF (pg/ml)	−14.45 (−104.40, −3.10)†	34.18 (−30.53, 54.21)	0.004*

Values are expressed as median (interquartile range). p-values are based on Mann-Whitney U test. *Significant differences between zishentongluo and benazepril groups. †Significant differences between baseline and last visit (Wilcoxon Signed Ranks test). Abbreviations: HbA1c, glycated hemoglobin; FBG, fasting blood glucose; UAER, urinary albumin excretion rate; SCr, serum creatinine; Ccr, endogenous creatinine clearance rate; ANP, atrial natriuretic peptide; ET-1, endothelin; VEGF, vascular endothelial growth factor.

Following 36 weeks of treatment, there were significant reductions from baseline in serum levels of HbA1c levels in both the ZSTL and benazepril groups (both $p < 0.001$) (Table 2). The reduction in HbA1c levels was significantly greater in the ZSTL group compared with the benazepril group (4.29% vs. 3.26%; $p = 0.016$).

After 36 weeks of ZSTL therapy, there were significant reductions from baseline in all the secondary endpoints (Table 2), whereas for benazepril there were significant reductions from baseline in only FBG, UAER, SCr, Ccr, VI-C, ANP, and ET-1. Changes from baseline were significantly different between ZSTL and benazepril for FBG, TC, TG, UAER, SCr, ANP, ET-1, and VEGF ($p < 0.05$). In fact, TG, SCr and VEGF serum levels increased following benazepril therapy. There was no difference between treatment groups in serum levels of Ccr and serum type IV collagen ($p = 0.091$ each) after 36 weeks of treatment.

Discussion

In patients with early-stage DN, an oral administration of ZSTL twice daily for 36 weeks was more effective compared with benazepril in reducing HbA1c serum levels as well as lowering FBG, TC, TG, UAER, SCr, ANP, ET-1, and VEGF serum levels. These findings suggest that ZSTL is superior to benazepril in improving the metabolic and renal functioning of patients with early-stage DN, in part, by possibly modifying ANP, ET-1, and VEGF.

Sustained hyperglycemia damages renal function and structure resulting in albuminuria, which in turn, leads to proteinuria and irreversible renal damage (Cao and Cooper, 2011). In addition to hyperglycemia, abnormalities in lipid metabolism are known to exacerbate DN (Rosario and Prabhakar, 2006). In the present study, both ZSTL and benazepril conferred a reduced hypoglycemia; however, this effect was greater with ZSTL treatment than with benazepril. Moreover, ZSTL, in contrast to benazepril, significantly reduced total cholesterol and triglyceride levels from baseline. These findings suggest that ZSTL combined with oral hypoglycemic agents is superior to benazepril in improving glucose and lipid metabolism in patients with early-stage DN, and thereby, may be more effective in delaying the progression of renal failure.

Early-stage DN is characterized by microalbuminuria, which, if not treated, progresses to overt proteinuria and the development of ESRD (Cao and Cooper, 2011). In fact, UAER is commonly used as an early marker of renal damage. Although the UAER was reduced in both treatment groups compared to pre-treatment levels, the reduction in UAER was significantly greater with ZSTL compared with benazepril. These findings suggest that ZSTL is superior to benazepril in reducing urinary albumin excretion in patients with early-stage DN. ZSTL also appeared to result in greater renal function improvement than benazepril treatment; ZSTL induced significant decreases from baseline in SCr while benazepril actually increased SCr serum levels. Taken together, these findings suggest that ZSTL is more effective than benazepril in improving not only glucose and lipid metabolism, but also preventing, if not reversing, the progression of renal damage and improving renal function in patients with early-stage DN.

While the mechanisms involved in the pathogenesis and progression of DN are very complex, it is known that DN occurs as a result of cross-talk between metabolic and hemodynamic factors (Cooper, 2001). The activation of various vasoactive hormonal pathways has also been implicated in the pathogenesis of DN (Zietse *et al.*, 1997; Cao and Cooper, 2011; Cooper, 2001). Indeed, in patients with DN, plasma and urinary ET-1 levels are elevated and associated with reduced renal function, increased blood pressure, and albuminuria (Zanatta *et al.*, 2008; Gupta *et al.*, 2011). This can be explained by the fact that ET-1 reduces renal blood flow and glomerular filtration by inducing renal vasoconstriction, which promotes the onset and progression of renal disease and glomerular damage by promoting mesangial cell hypertrophy, extracellular matrix accumulation, and basement membrane thickening (Zanatta *et al.*, 2008). There may interplay between ET-1 and ANP in the development of DN (Yu *et al.*, 2002). In particular, one study reported that patients with DN presented with elevated ET-1 and ANP levels compared to those of the normal control group, and these elevations in ET-1 and ANP were significantly and positively correlated with FBG and TG (Yu *et al.*, 2002). In the present study, we demonstrated that ET-1 and ANP levels were significantly lowered by ZSTL and benazepril treatment in patients with early-stage DN ($p < 0.05$). The reduction in ET-1 and ANP after ZSTL treatment was significantly greater than that achieved with benazepril treatment ($p < 0.05$), suggesting ZSTL may be more efficacious than benazepril in reducing serum levels of these two factors.

Endothelial dysfunction is a major mechanism underlying chronic microvascular complications in both diabetic and non-diabetic individuals (Karalliedde and Gnudi, 2011).

In fact, endothelial dysfunction was shown to parallel microalbuminuria in DN (Karalliedde and Gnudi, 2011). During sustained hyperglycemia, various hemodynamic pathways, independently and or in conjunction with metabolic pathways, activate various growth factors, such as VEGF (Cooper, 2001; Cao and Cooper, 2011). VEGF has been implicated in the pathogenesis of DN by mediating endothelial dysfunction (Khamaisi *et al.*, 2003). In the present study, treatment with ZSTL induced a significant reduction in VEGF serum levels of patients with early-stage DN while benazepril increased VEGF levels, although this increase from baseline did not reach significance. Our findings suggest that ZSTL may inhibit the progression of DN by lowering VEGF serum levels.

In conclusion, our findings suggest that an oral administration of ZSTL twice daily for 12 weeks is more effective than benazepril in improving not only glucose and lipid metabolism, but also preventing, if not reversing, the progression of renal damage and improving renal function in patients with early-stage DN. Additionally, it appears that these beneficial effects of ZSTL may be, in part, due to its modulating effects on ET-1, ANP, and VEGF. Together, these findings suggest that ZSTL is an effective traditional Chinese medicine for patients with early-stage DN.

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