Original Article
Probucol combined with valsartan in diabetic nephropathy: a 52-week, randomized, double-blind clinical trial

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Abstract: Treatment of diabetic nephropathy (DN) remains challenging. This randomized, double-blind clinical trial assessed the efficacy and safety of probucol therapy regimen that combined valsartan in patients with DN. 88 patients with DN at screening were randomized to a treatment group (750 mg/day probucol combined with 160 mg/day valsartan) or a control group (160 mg/day valsartan plus placebo) and followed for 52 weeks. The primary efficacy outcome was the magnitude of change in 24 h urinary protein level relative to the baseline. Secondary outcomes included the magnitude of change in eGFR and serum creatinine relative to the baseline. At baseline, the two groups in any measured clinical variable were comparable. Following 52 weeks of therapy, the magnitude of changes of 24-h urinary protein relative to the baseline were -400.85 (-2283.56, 489.98) mg and +693.87 (-1029.38, 3654.20) mg for the treatment and the control group, respectively (P = 0.017). Effects in eGFR (P = 0.296) and serum creatinine (P = 0.491) were comparable between the two groups. The treatment group was more effective in reducing total cholesterol (P = 0.009), low-density lipoprotein cholesterol (P = 0.040), and high-density lipoprotein cholesterol (P = 0.001) levels compared with control group. A trend towards significance was observed for the difference in serum and urinary Lipid peroxidation product malondialdehyde and Total antioxidant capacity levels between the two groups. Probucol combined with valsartan was more effective in reducing proteinuria. Additional benefits for eGFR and serum creatinine were not observed at the 52 weeks follow up in two group patients. However, the long-term effect needs further investigation.

Keywords: IgA nephropathy, probucol, valsartan, anti-oxidation, randomized controlled study

Introduction

Diabetes mellitus is an increasingly prevalent disease around the globe. Diabetic nephropathy (DN) is a major complication of diabetes mellitus and a leading cause of end-stage renal disease, accounting for 35 to 40% of all new cases that require dialysis therapy worldwide [1]. Despite the availability of many therapeutic strategies, current treatment of DN still remains a major challenge.

A great many of previous studies have demonstrated that multiple factors are involved in the evolution of DN, including metabolic disturbance, renal haemodynamics abnormity, chronic inflammation and the oxidative stress [2-4]. Rectification of metabolic disturbance with intensive insulin treatment [5, 6] is a basic strategy in the treatment of DN. Angiotensin converting enzyme inhibitors (ACEI) and angiotensin II receptor blockers (ARB) are internationally accepted drugs in the treatment of DN by improving renal haemodynamics, resulting in attenuated albuminuria [7-9]. Though these current measurements are widely used in clinical setting, their renoprotective effects are not enough to prevent completely the progression

Figure 1. Patient flow diagram.

[19, 20]. Moreover, interestingly, recent clinical trial indicates that probucol reduce urinary protein, prevent the progression of DN, and extend the interval to initiation of hemodialysis therapy in type 2 diabetic patients with clinical albuminuria [21, 22]. However, ACEI or ARB medication as a basis was not involved in these clinical trials. In addition, these clinical trials are single center study, therefore, more clinical evidences from multiple centers around world and more effective therapy regimens for DN are needed. ACEI and ARB has definite protective role in the development and progress of DN [7-9]. A previous study in the rat have indicated that addition of the antioxidant probucol to angiotensin II type I receptor antagonist fully arrested progressive mesangioproliferative glomerulonephritis [23]. Thus, we hypothesized that antioxidant potential of probucol combination with ARB therapy regimen would be more effective in decreasing 24 h urinary protein level, eGFR and serum creatinine than a single agent in DN. To confirm our hypothesis and safety of probucol therapy regimen that combined valsartan (an ARB) for DN, we conducted a multicenter, double-blind clinical trial. The primary results of which are presented in this report. Secondary outcomes included the magnitude of change in eGFR and serum creatinine relative to the baseline.

Material and methods

Design overview

This prospective, randomized, multicenter double-blind clinical trial compared probucol therapy regimen that combined valsartan to valsartan plus placebo for the treatment of DN. The study protocol was registered at Clinical Trials. gov (registration no.: NCT00655330). The study was approved by ethics committee of Guangdong General Hospital, and all participants provided written informed consent. The study was
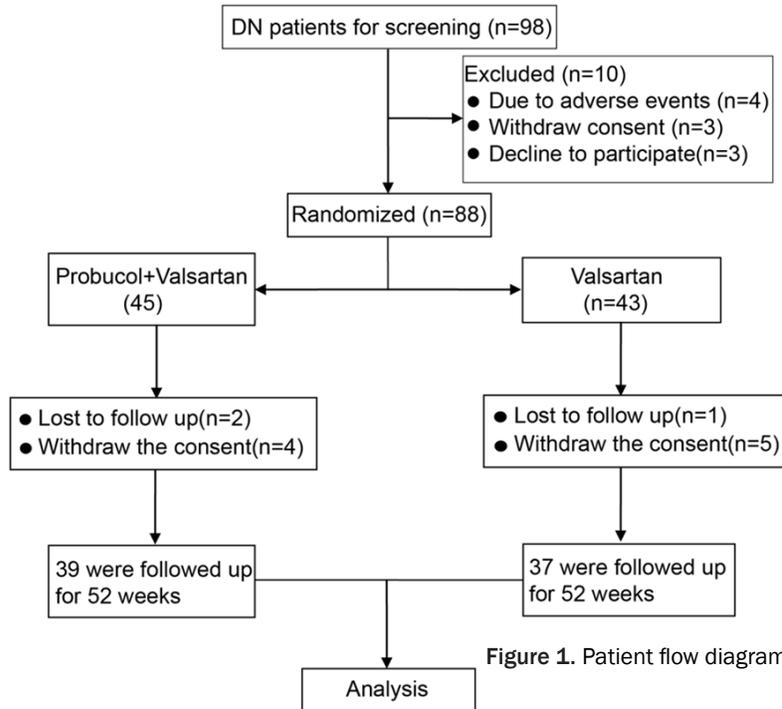
of DN [1]. Therefore, it has become urgent to provide new insights into therapeutic strategies for diabetic nephropathy. In recent years, more attention has been paid to the anti-oxidative treatments, which have been found to delay the progression of DN [10-12]. Indeed, several *in vitro* and *in vivo* studies have shown DN amelioration by managing the hyperglycemia-induced oxidative stress, inflammation, and lipid accumulation [13-15]. All these suggested that rectification of metabolic disturbance and renal hemodynamics combined with anti-oxidative treatments may provide new strategies for comprehensive treatment to improve the therapeutic efficacy in patients with DN.

Probucol is a compound with two phenolic groups, which has been used for years as an anti-hyperlipidemic agent. For this reason, it is often prescribed for the treatment of hypercholesterolemia and xanthoma [16]. Over the last several years, intensive studies have shown that this drug is not only a lipid-lowering drug but also has anti-oxidant and anti-inflammatory properties [17]. It acts as a potent oxygen radical scavenger and can efficiently prevent tissue and organ damage caused by oxidative stress [18]. *In vitro* and *in vivo* studies have demonstrated that the antioxidant potential of probucol is effective in preventing atherosclerosis

performed in accordance with the declaration of Helsinki and principles outlined in the "Guidelines for Good Clinical Practice" International Conference on Harmonisation(ICH) Tripartite Guideline (January 1997). The flow chart of the study was shown in **Figure 1**.

Study population

A total of 98 type 2 diabetes patients (according to World Health Organization criteria) with clinical albuminuria (urinary albumin excretion >300 mg/g Cr) [24] were enrolled in the study. These patients were recruited from 5 renal centers across China from October 2008 to January 2014. The inclusion criteria were: age of 18-70 years; 24-h urinary protein of 1.0-3.0 g; serum creatinine no more than 265.2 μmol/L; no treatment with an ACEI, ARB, anti-oxidant, lipid-lowering drug in at least previous 4 weeks. Patients were excluded if they had received a diagnosis of type 1 diabetes or non-diabetic renal disease, including bilateral renal-artery stenosis. Patients with any of the following were excluded: acute kidney injury, crescentic glomerulonephritis, obstructive nephropathy, pregnancy, tumor, active gastrointestinal ulcer coronary heart disease, cardiomyopathy, serious arrhythmia, cerebrovascular disease, and active infection (including tuberculosis). Written informed consent was not signed and patients who did not comply with the research program were also excluded.

All 98 eligible patients were screened before formal enrollment. For screening, patients were treated with 160 mg/day valsartan for 4 consecutive weeks, during which blood pressure, serum potassium, serum creatinine, and cough were monitored. After 4 weeks, patients who had serum potassium less than 5.5 mmol/L, an increase in serum creatinine less than 30%, and without intolerable side effects related to valsartan therapy were enrolled. At the end of 4 weeks, 88 patients had no intolerable adverse effects, complied with the treatment, and were formally enrolled.

Randomization and interventions

A computer-generated list that was maintained by a third party not involved in the conduct of the study was used for randomization. Sequentially numbered, concealed envelopes containing group assignment were provided to the investigators. Investigators were unaware of the randomization schedule when recruiting patients, and both investigators and patients were blinded during the follow-up period. After eligible patients provided written informed consent, investigator opened the envelopes in sequence and patients were randomly assigned to the probucol combined valsartan therapy group (750 mg/day probucol combined with 160 mg/day valsartan, n = 45) or valsartan plus placebo group (160 mg/day, n = 43).

Patients were followed up at week 2 and 4, and then every 6-8 weeks until 52 weeks. All patients were treated by a protein restriction diet. The total protein intake was 0.8 g/kg/day, and total energy intake was 30 kcal/kg/day. Mild dietary sodium restriction limited to 90 mmol/d was advised. Blood glucose was controlled by sulfonylureas (glibenclamide and tolbutamide), a-glucosidase, pioglitazone, and insulin to reduce HbA1c to less than 6.5%. When the target blood pressure (BP) of 130/80 mmHg was not achieved, a α-adrenergic antagonist was administered; if blood pressure was still not controlled, a β-adrenergic antagonist was added. Diuretics and calcium antagonists were used only temporarily if necessary.

Patient monitoring

At study entry, complete medical histories were taken and physical examinations were performed for all patients. Initial clinical and laboratory results were sent to the coordinating center. Follow-up patient examinations and measurements of BP, serum creatinine (Scr); blood urea nitrogen (BUN); 24-hour urinary albumin, 24-hour urinary protein, urine albumin to creatinine ratio, estimated glomerular filtration rate (eGFR; estimated with the Cockcroft-Gault equation), Blood routine examination; total cholesterol (CHOL), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C); serum albumin (ALB); and electrocardiogram (ECG). The results of echocardiography examination were obtained at admission and at the end of the study. Also, first morning urinalysis, liver function, including total protein (TP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TBIL), direct bilirubin (DBIL) and serum potassium were collected and analyzed at the local center at each scheduled visit. To reduce variability, serum and urine of oxidative stress indicators including superoxide

Table 1. Baseline characteristics of patients in the probucol combined with valsartan group and the valsartan plus placebo group

Variables	Probucol plus valsartan (n = 45)	Valsartan plus placebo (n = 43)	P
Sex (M/F) n	29/16	23/20	0.24
Age (yr) Mean (range)	56.1 (39~77)	54.8 (37~69)	0.47
Body Mass Index (kg/m^2)	24.07±3.40	24.88±3.60	0.39
Duration of DN, months	110.1±125.7	112.2±90.0	0.95
SBP (mmHg)	138.90±16.69	142.51±19.74	0.40
DBP (mmHg)	79.79±9.64	82.44±12.10	0.34
HGB (g/L)	114.27±20.74	112.58±18.31	0.69
PLT (×10^9/L)	245.15±67.25	248.81±82.40	0.82
Scr (μmol/L)	118.35±38.20	125.54±49.49	0.46
BUN (mmol/L)	9.38±8.21	8.43±3.91	0.50
Blood glucose (mmol/L)	8.77±4.24	8.90±4.66	0.89
HbA1c1 (%)	8.63±2.41	7.99±2.05	0.22
eGFR (ml/min 1.73 m^2)	57.93±26.59	57.68±30.27	0.97
24-h urinary protein (mg/24 h)	2869.00 (1696.63, 3987.00)	2468.28 (1581.67, 4047.25)	0.58
24-h urinary albumin (mg/24 h)	2340.00 (1376.20, 3114.50)	1669.30 (1071.50, 2875.65)	0.06
Urinary albumin/creatinine ratio (mg/g Cr)	260.50 (168.18, 401.62)	242.00 (144.93, 469.59)	0.95
Serum albumin (g/L)	32.00 (24.50, 35.80)	30.60 (27.60, 35.50)	0.89
Serum potassium (mmol/L)	4.04±0.52	3.97±0.54	0.51
ALT (U/L)	25.04±27.99	19.78±9.09	0.26
AST (U/L)	21.37±7.30	23.62±8.12	0.19
Total protein (g/L)	58.00±10.49	59.97±8.49	0.35
TBIL (mmol/L)	10.48±2.82	10.98±4.14	0.53
DBIL (mmol/L)	2.76±1.14	3.27±1.34	0.07
CHOL (mmol/L)	5.81±1.91	5.57±2.27	0.60
TG (mmol/L)	1.44 (0.99, 2.11)	1.37 (0.99, 2.01)	0.99
HDL-C (mmol/L)	1.32±0.43	1.26±0.60	0.59
LDL-C (mmol/L)	3.07±1.28	2.99±1.38	0.77

Note. Values are expressed as mean ± SD or Mean (range) or Median (25th, 75th). SBP, systolic blood pressure; DBP, diastolic blood pressure; HGB, hemoglobin; PLT, Blood platelet; Scr, serum creatinine; BUN, Blood urea nitrogen; HbA1c1, Hemoglobin A1C; eGFR, estimated glomerular filtration rate, estimated with the Cockroft-Gault equation; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin, DBIL, direct bilirubin; CHOL, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

dismutase (SOD), lipid peroxidation product malondialdehyde (MDA) and total antioxidant capacity (T-AOC) were measured in one center (Guangdong general hospital) using the corresponding assay kits following the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute Biotech, Jiangsu, China). All clinical and laboratory results were recorded on case report forms, forwarded to the coordinating center, and entered for data processing. Study drug compliance was calculated by taking the amount of drug ingested divided by the amount the patient should have ingested and multiplied by 100%. Safety assessments included histories and physical exams, laboratory tests and adverse events.

Study outcomes

The primary efficacy outcome was the magnitude of change in 24-h urinary protein level relative to the baseline. The secondary outcome included the degree of change in eGFR and serum creatinine as compared with the baseline, or the development of end-stage renal disease that required renal replacement therapy or death during the study period.

Statistical analysis

Results are expressed as mean ± SD or Median (25th, 75th percentiles) for continuous data and as percentages for categorical variables.

Table 2. Change of clinical indicators of the patients in the probucol plus valsartan group and valsartan plus placebo group at the 52 weeks follow-up

Change of variables (Δ)	Probucol plus valsartan (n = 39)	Valsartan plus placebo (n = 37)	P Value
Urinary protein (mg/24 h)[a]	-400.85 (-2283.56, 489.98)	+693.87 (-1029.38, 3654.20)	0.017[a]
Scr (μmol/L)[a]	+13.15 (5.68, 44.75)	+10.35 (-4.25, 43.50)	0.491
eGFR (ml/min/1.73 m²)[a]	-10.11 (-15.98, -5.58)	-7.37±(-20.76, 3.33)	0.296
SBP (mmHg)[b]	-7.44±17.86	-9.51±15.96	0.595
DBP (mmHg)[b]	-2.67±7.34	-4.12±15.03	0.597
TC (mmol/L)[a]	-0.67 (-2.26, 0.03)	+0.36 (-0.53, 1.53)	0.009
TG (mmol/L)[a]	0.08 (-0.83, 0.69)	0.08 (-0.44, 0.66)	0.758
HDL-C (mmol/L)[a]	-0.31 (-0.53, 0.10)	-0.03 (-0.17, 0.21)	0.001
LDL-C (mmol/L)[b]	-0.40±1.42	0.30±1.45	0.040

Note. Values are expressed as mean ± SD or Median (25th, 75th percentiles). eGFR, estimated glomerular filtration rate, estimated with the Cockroft-Gault equation; SBP, systolic blood pressure; DBP, diastolic blood pressure; Scr, serum creatinine; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol. Δ Change of variables relative to the baseline. [a]Based on two-sided rank sum test. [b]Based on two-sided student's t-test.
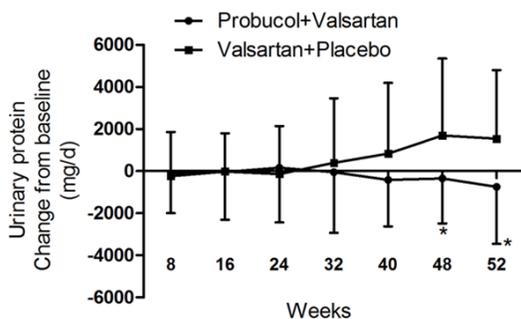
Figure 2. Change of urinary protein in the treatment group (probucol combined with valsartan) and the control group (valsartan plus placebo) during follow up periods.

Statistical analysis was performed using the statistical package SPSS for Windows Ver. 17.0 (SPSS, Inc., Chicago, USA). Descriptive analysis was used for evaluation of the general characteristics of patients and a chi square test or a student's t-test was used to compare baseline parameters of the two groups. A repeated-measure analysis of variance (ANOVA), student's t-test or the rank sum test was used to compare parameters of the two groups before and after treatment. A chi square test was used to compare the incidence of adverse effects of the two groups. All statistical tests were 2 sided, with P less than 0.05 considered significant.

Results

Baseline characteristics

Between October 2008 and January 2014, a total of 98 patients with type 2 diabetic nephrop-

athy were initially screened. After screening, 88 of these patients were deemed eligible and 76 patients ultimately completed the study. Among these 88 patients, 52 males, 36 females. The 88 patients were randomly divided into 2 groups: the treatment group (probucol combined with valsartan, n = 45) and the control group (valsartan plus placebo, n = 43). **Figure 1** shows patient flow diagram of the treatment and control groups. The median age was 56.1 (range 39-77) years in the treatment group and 54.8 (37~69) years in the control group. There were no significant differences between the two groups in blood pressure, Scr, 24-h urinary protein excretion, or any of the other parameters listed in **Table 1**.

Primary and secondary outcomes

During the study period, in the treatment group, there was a decrease in proteinuria with protein from 2869.00 (1696.63, 3987.00) mg at baseline to 1926.37 (962.96, 4034.25) mg at 52 weeks, but not reach significant difference (P = 0.103), In the control group, there was slight decrease in proteinuria with protein from 2468.28 (1581.67, 4047.25) mg at baseline to 2416.00 (1381.80, 7115.00) mg at 52 weeks (P = 0.989). Conversely, absolute changes of 24-h urinary protein at 52 weeks relative to the baseline were -400.85 (-2283.56, 489.98) and +693.87 (-1029.38, 3654.20) for the treatment group and the control group, respectively (**Table 2**, P = 0.017). The profile of decrease of proteinuria for the two groups is listed in **Figure 2**. The magnitude of change of proteinuria in the treatment and control groups were nearly
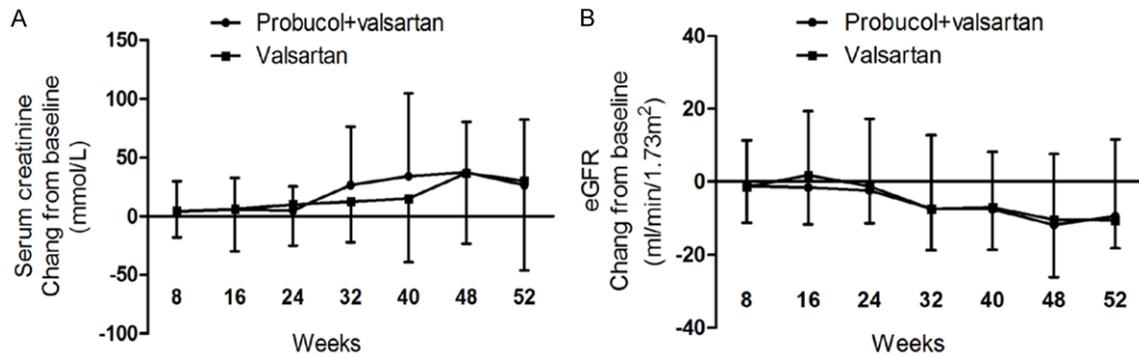
Figure 3. Change of eGFR and serum creatinine in the treatment group (probucol combined with valsartan) and the control group (valsartan plus placebo) during follow up periods.

Table 3. Change of oxidative stress indicators at 52 weeks follow-up

Change of variables (Δ)	Probucol plus Valsartan (n = 39)	Valsartan plus Placebo (n = 37)	P value[a]
Serum			
MDA (nmol/ml)	-3.75 (-6.25, -0.00)	+0.60 (-0.18, 4.65)	0.000
SOD (nmol/ml)	+4.40 (-7.05, 10.63)	+3.60 (-15, 10.15)	0.819
T-AOC (nmol/ml)	+5.70 (0.90, 9.25)	-1.60 (-9.60, 1.25)	0.003
Urine			
MDA (nmol/ml)	-1.60 (-4.20, 0.20)	+0.90 (-1.68, 2.68)	0.004
SOD (nmol/ml)	+3.55 (-1.70, 8.23)	+1.35 (-9.65, 9.50)	0.452
T-AOC (nmol/ml)	+4.25 (-4.23, 10.75)	-3.15 (-6.55, 2.10)	0.037

MDA, Malondialdehyde; SOD, superoxide dismutase;T-AOC, Total antioxidant capacity. Data are presented as Median (25th, 75th percentiles) due to extreme skewness. Δ Change of variables relative to the baseline. [a]Based on two-sided rank sum test.

serum creatinine as compared with the baseline. 4 patients [10.0%] in the treatment group, 7 [18.9%] in the control group had increased serum creatinine more than 30% as compared with the baseline (P = 0.264). At 52 weeks of follow-up, the change of serum creatinine was +13.15 (5.68, 44.75) μmol/L and +10.35 (-4.25, 43.50) μmol/L in the treatment group and the control group, respectively (P = 0.491). **Figure 3A** lists change of serum creatinine across time between the treatment group and control group.

similarly from baseline to 24 weeks. Proteinuria decreased in the treatment group from 32 to 52 weeks. However, for the control group, there is no decline trend for proteinuria, which increased from 32 to 52 weeks.

Over time, the eGFR declined in both groups (**Figure 3B**); the eGFR slope was -0.84 ml per minute per 1.73 m^2 per month in the treatment group and -0.61 ml per minute per 1.73 m^2 per month in the control group, respectively. There was no significant difference in treatment effect on the decline in the eGFR between the treatment group and control group at the 52 weeks follow-up. (**Table 2**, P = 0.296). **Figure 3B** lists eGFR across time for the treatment and control groups.

The mean serum creatinine in the treatment and control groups were comparable to the baseline levels (**Table 1**). During 52 weeks follow-ups, 3 patients [7.5%] in the treatment group, 2 [5.4%] in the control group had doubling

Changes in blood pressure and serum lipids

At baseline, the mean blood pressure was comparable in both groups (**Table 1**). At the 52 weeks follow-up, the change of systolic pressure (SBP) was -7.44±17.86 mmHg in the treatment group and -9.51±15.96 mmHg in the control group compared with baseline levels. The change of diastolic pressure (DBP) was -2.67± 7.34 mmHg and -4.12±15.03 mmHg in the treatment group and control group relative to the baseline. These changes in blood pressure were comparable (**Table 2**, SBP, P = 0.595; DBP, P = 0.597).

At the 52 weeks follow-up, the mean plasma total cholesterol was 5.81±1.91 mmol/L in the treatment group and 5.57±2.27 mmol/L in the control group (P = 0.60). Changes of plasma total cholesterol at 52 weeks relative to the

Table 4. Adverse events of patients in the treatment group (probucol plus valsartan) and control group (valsartan plus placebo) at baseline and at study completion (52 weeks)

Variables	Treatment group		Control group	
	Before	After	Before	After
ALT (U/l)	25.04±27.99	25.68±23.54	19.78±9.09	23.11±9.82
AST (U/l)	21.37±7.30	24.27±7.92	23.62±8.12	22.25±4.82
Serum potassium (mmol/l)	4.04±0.52	4.42±0.54*	3.97±0.54	4.46±0.47*
Prolonged QT interval (n)	0	1	0	0

Note. *$P<0.05$, before vs. after.

baseline were -0.67 (-2.26, 0.03) and +0.36 (-0.53, 1.53) mmol/L for the treatment group and the control group, respectively (**Table 2**, P = 0.009). Decrease of HDL-cholesterol at 52 weeks relative to the baseline were -0.31 (-0.53, 0.10) mmol/L and -0.03 (-0.17, 0.21) mmol/L for the treatment group and the control group, respectively (**Table 2**, P = 0.001). A trend towards significance was also observed for the difference in LDL-cholesterol between the two groups (0.40±1.42 and 0.30±1.45 mmol/L, P = 0.040). There was no significant difference in changes of triglycerides [0.08 (-0.83, 0.69) and 0.08 (-0.44, 0.66) mmol/L, P = 0.758] at 52 weeks relative to the baseline between the two groups (**Table 2**).

Changes in oxidative stress indicators

At baseline, the serum median MDA, SOD and T-AOC were 8.20 (5.50, 11.50), 65.00 (27.25, 77.08), 14.30 (8.75, 34.20) nmol/ml in the treatment group and 6.85 (4.25, 11.68), 55.90 (32.13, 70.48) and 18.60 (10.20, 34.20) nmol/ml in the control group (P = 0.446; P = 0.459; P = 0.731, respectively). The urine median MDA, SOD and T-AOC levels at baseline were also comparable in both groups [(7.25 (4.87, 9.43) vs. 5.70 (3.80, 7.25), P = 0.446; 62.20 (44.43, 73.18) vs. 57.00 (24.00, 73.95), P = 0.651; 26.10 (10.25, 43.93) vs. 18.40 (9.83, 26.03), P = 0.104)]. Changes of serum MDA, SOD and T-AOC at 52 weeks relative to the baseline are listed in **Table 3**. The magnitude of changes was significant in serum and urine median MDA and T-AOC at 52 weeks relative to the baseline between the two groups, respectively (**Table 3**). A statistically significant change of serum and urinary SOD levels relative to the baseline was not observed between the two groups.

Adverse events

Adverse events are listed in **Table 4**. There were no significant differences in the baseline levels of AST and ALT with the levels at the 52 weeks follow-up, indicating they did not have evident liver toxicity. In the treatment group, the ECGs of 1 patients indicated prolonged QT interval. There was significant difference between the baseline level of serum potassium and the level at the 52 weeks follow-up in each group, however, significant difference in level of serum potassium was not observed between the treatment and control group at the 52 weeks of therapy.

Discussion

The aim of present multi-center and placebo-controlled study is assess the effect of an anti-oxidant (probucol) in combination with an ARB (valsartan) on the progression of DN. In this study, 88 patients with type 2 DN were officially enrolled and 76 patients ultimately completed the study. During 52 weeks follow-up, urinary protein, serum creatinine, and eGFR were evaluated. Blood glucose control, blood pressure control and the amount of protein intake are known factors affecting the progression of DN. During 52 weeks follow up, no significant differences in these factors were observed between the probucol plus valsartan group and valsartan plus placebo group. Therefore, the subject population was appropriate for evaluation of the effect.

At the 52 weeks follow-up, our results showed there was significant difference in the change of reducing 24-h urinary protein relative to baseline level between probucol plus valsartan group (-400.85) and valsartan plus placebo group (+693.87) (P = 0.017), however, we didn't found a statistically significant change in serum creatinine (+13.15 vs. +10.35, P = 0.491) and eGFR (-10.11 vs. -7.07, P = 0.296) between the two groups, respectively. In addition, we also noted that the decrease of eGFR in patients was faster than typically observed in average DN patients. This probably due to the fact that

most patients had increased risk for rapid progression. As listed in **Table 1**, mean serum creatinine level was 118.35±38.20 and 125.54± 49.49 (µmol/L) in probucol plus valsartan group and valsartan plus placebo group, respectively, so they can be regarded as advanced DN.

Oxidative stress plays a pivotal role in the initiation and progression of DN [10, 25]. Combined therapy with antioxidants and anti-inflammatory agent may lead to satisfactory results. Probucol, a mild cholesterol-lowering agent with antioxidant and anti-inflammatory properties, has been shown to reduce atherosclerosis in previous clinical trials [17, 26]. In recent years, some investigators reported that probucol also prevented the progression of early-stage DN and suppressed urinary type 4 collagen excretion [21, 27]. Recently, Endo et al also demonstrated that probucol suppressed the increase in serum creatinine and urinary protein and delayed hemodialysis in DN patients [21, 22]. Studies in this field are limited and there are no enough data from human studies. ACE inhibitor and ARB are also well known to prevent the progression of DN [7-9]. However whether combination therapy of probucol and ACE inhibitor or ARB has additive or synergistic effect to prevent DN is still unclear. Based on this problem, we conducted this multicenter, placebo-controlled, randomized clinical trial. Similarly, in this study, our data also indicated that during the 52 weeks of therapy, there are marked difference in absolute change of 24-h urinary protein relative to baseline level in probucol plus valsartan group compared to valsartan plus placebo group (P = 0.017). However, at 52 weeks follow up, we didn't found a statistically significant change in the level of 24-h urinary protein in probucol treatment group and control group compared to baseline levels, respectively, although there are decreased trend of 24-h urinary protein in each group (Median 2869.00 vs. 1926.37 mg P = 0.103; Median 2468.28 vs. 2416.00 mg, P = 0.989). We speculated this probably due to the fact that the recruited number of patients is small. Contrary to previous studies mentioned above, our results indicated that patients given probucol combined with valsartan had no significant differences in the change of serum creatinine and eGFR compared to patients with valsartan plus placebo therapy. This lack of significant difference may

be partially due to the relatively *short follow-up period* and a smaller number of cases.

Recent studies have showed that oxidative stress markers such as MDA [28] and 8-oxodG (oxo-2'-deoxyguanosine) [29] increased in patients with DN, and antioxidant enzymes SOD [30] and total antioxidant capacity (T-AOC) [31] decreased in diabetic mouse or DN patients. Probucol is an antioxidant agent. Endo *et al*. have reported that probucol reduced urinary 8-hydroxy-2'deoxyguanosine (8-OHdG) [32]. In the present study, our results indicated that the magnitude of decreased serum MDA relative to baseline was significant in probucol plus valsartan group compared to valsartan plus placebo group (P = 0.000). In addition, we also found a statistically significant change in serum T-AOC level relative to baseline between the two groups (P = 0.003). Similar results are also obtained for urinary MDA and T-AOC between the two groups. Thus, we speculate that the antioxidant action of probucol might be involved in its decreased effect on proteinuria.

Probucol possesses also cholesterol-lowering effects as well as antioxidant. In this study, there were significant difference in the magnitude of decreased CHOL, LDL-C and HDL-C relative to baseline level between combination therapy group and valsartan plus placebo group, respectively. However, the change of TG level relative to baseline was not different between the two groups. These observational effects were agreement with previous studies [17, 21]. Previous clinical trials also demonstrated that lowering cholesterol and LDL-C with probucol or statin regimens were able to decrease proteinuria and to improve renal function [17, 33]. Therefore, we cannot rule out the probability of urinary protein reduction in this study is due from the effect of probucol in improving serum lipids.

A previous study showed the effectiveness of a combined ACE inhibitor and an angiotensin II receptor antagonist administered valsartan at a dose of 80-160 mg/day [34]. In the present study, we administered valsartan at a dose of 160 mg/day. Our results also indicated that 160 mg/day valsartan combined with probucol was a safe treatment for DN. Only 1 patients developed abnormal ECG (prolonged QT interval), and the patient recovered after treatment discontinuation. All patients maintained normal

liver function, no patients had serum potassium more than 5.5 mmol/L. Although there were marked differences in serum potassium in both groups before and after treatment, respectively, the levels of serum potassium was not more than 5.5 mmol/L, meanwhile, this significance was not observed between the two groups at the 52 weeks of therapy. These indicated that both therapies are safe for treating DN patients.

Several limitations of this study must be taken into account. Firstly, whether the dose of probucol in our study is sufficient to prevent long-term development of oxidative damage, especially for advanced DN patients with elevated Scr and massive proteinuria. The dose of probucol (750 mg/day) was below the maximal tolerable dose [35], and this may have led to reduced therapeutic efficacy. Secondly, Chinese patients only be focused and a very small sample size. Thirdly, short follow-up period. Therefore, further trials with a larger sample sizes, longer follow-up as well as well-designed, are needed to verify whether such a strategy can provide additional benefits.

Conclusion

Taken together, here, for the first time, we evaluated the efficacy and safety of valsartan combined with probucol for treatment of patients with DN. Our study indicates that probucol (750 mg/day) in combination with valsartan (160 mg/day) was more effective in reducing the 24-h urinary protein than valsartan therapy at 52 weeks follow up in DN patients, and both therapies are safe for treating DN patients. A statistically significant change was not found in serum creatinine and eGFR in probucol combined valsartan group compared to valsartan alone. However, the long-term effect needs further investigation.

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Disclosure of conflict of interest

None.

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