

Clinical study of double dose of valsartan combined with tacrolimus in treatment of diabetic nephropathy

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Abstract. – **OBJECTIVE:** To investigate the clinical effect of double dose of valsartan combined with tacrolimus in the treatment of diabetic nephropathy (DN).

PATIENTS AND METHODS: A total of 86 cases diagnosed with DN were selected from October 2013 to October 2014 in Zaozhuang Municipal Hospital, China. The study was approved by our hospital Ethics Committee and written consent was obtained from patients and their family members. Patients were randomly divided into three groups according to the sequence of admission, group A (conventional dose of valsartan group, n = 28 cases), group B (double dose of valsartan group, n = 29 cases) and group C (double dose of valsartan combined with tacrolimus group, n = 29). Clinical effects were compared by analyzing the renal function tests after 8 weeks.

RESULTS: 24h urine protein, serum creatinine level of patients in group B and group C were significantly lower than that of group A. Those in group C was much lower. The glomerular filtration rates were significantly higher for group B and C than that of group A, and those in group C were much higher. The difference is statistically significant ($p < 0.05$). High-sensitivity C-reactive protein (hs CRP) and adiponectin levels of patients in group B and C were significantly lower than that of group A and those in group C were much lower. The difference is statistically significant ($p < 0.05$). The high mobility group protein 1 (HMGB1) and renal tubular and interstitial damage index (TDI) of patients in B and C groups were significantly lower than those in the A group, and those in C group were significantly lower. The difference was statistically significant ($p < 0.05$). The clinical effective rates of patients in group B and C were significantly higher than that in group A, and those of group C were much higher. The difference is statistically significant ($p < 0.05$). The recurrence rates of patients in group B and group C were significantly lower than those of group A and those in group C were much lower. The difference is statistically significant ($p < 0.05$). Patients in three groups showed no obvious drug complications.

CONCLUSIONS: Double dose of valsartan combined with tacrolimus treatment of DN patients can improve clinical symptoms, reducing inflammation, inhibiting or even reversing the interstitial fibrosis, which will improve the curative effect and reduce the recurrence, as to provide a new theoretical basis for the clinical treatment of the disease.

Key words:

Double dose of valsartan, Tacrolimus, Diabetic nephropathy, High-sensitivity C-reactive protein, Leptin, High mobility group box-1 protein, Renal tubule matter damage index.

Introduction

Diabetic nephropathy (DN) is the renal complication of uncontrolled diabetes mellitus, it is the clinical IV stage. Pathological changes occur in the renal tissue. The 24h urine protein excretion is greater than 0.5 g, accompanied by hypertension and decreased glomerular filtration rate. The course is irreversible. Most of cases will develop to the final end-stage renal disease (ESRD), which needs long-term dialysis or a kidney transplant treatment, bringing heavy economic and mental burden for individuals, families and society. More studies have shown that¹, the kidney renin-angiotensin-angiotensin system (RAS) activity increases under high blood sugar condition. Angiotensin II (AngII) synthesis is enhanced. AngII not only causes abnormal renal hemodynamics but also acts as an important cytokines in stimulating renal collagen synthesis and expression. The double dose of valsartan has effect of reducing protein and reversing renal fibrosis process, which is independent of antihypertensive effect². Tacrolimus is an analogue of the immune inhibitor rapamycin. Study shows that³ its renal protective effect is superior to rapamycin and

other immunosuppressive agents and has high safety. Previous studies were limited to the basic theory. This study will try to prove the rationality of the theory through the clinical trial.

Patients and Methods

Patients

A total of 86 patients diagnosed with DN were selected over a period of 12 months from October 2013 to October 2014 in our hospital. All patients were in line with the 1998 WHO diabetes mellitus diagnostic criteria, i.e.: (1) A long history of diabetes, general in 10 years or so. (2) Continued proteinuria is more than or equal to 0.5. (3) It can be accompanied by hypertension, renal insufficiency, edema, low protein hyperlipidemia and blood lipid disorders. (4) Diagnosis depends on pathological analysis.

Inclusion criteria: (1) Age ≥ 18 , < 75 . (2) Consistent with the diagnosis criteria for DN. (3) Treatment for the first time.

Exclusion criteria: (1) Other causes of proteinuria, such as nephrotic syndrome, lupus nephritis. (2) Pregnancy, autoimmune disease, accompanied with serious heart, liver, kidney and other organ dysfunction, severe anemia. Hypoproteinaemia cannot be corrected. Serious diabetic complications, such as ketosis acid poisoning. (3) Serious adverse reactions of patients caused by valsartan and tacrolimus, poor compliance, those who refused the study, etc.

The study was approved by our hospital Ethics Committee and patients and their family members. Patients were randomly divided into three groups according to the sequence of admission, group A (conventional dose of valsartan group, $n = 28$ cases), group B (double dose of valsartan group, $n = 29$ cases) and group C (double dose of valsartan combined with tacrolimus group, $n = 29$).

Group A had 16 male and 12 female between age group of 38 to 69 years, average of (53.4 ± 10.5) years old. The duration of diabetes was 8-13 years with the average of (11.3 ± 1.6) years. Group B had, 17 males and 12 females, aged from 39 to 72 years with the average of (54.3 ± 11.2) years. The duration of diabetes was 7.5-14.5 years with the average of (12.6 ± 1.3) years. Group C had, 16 males and 13 females, aged from 36 to 73 years old with the average of (53.9 ± 13.4) years. The duration of diabetes was 8.5-15.6 years with the average of (13.7 ± 1.6) years. There was no significant difference in gender, age and duration of diabetes between the 3 groups ($p > 0.05$).

Methods

The treatment given to the patients was based on the practice guidelines which consisted: All patients were treated with correct hypoglycemic drugs and insulin treatment for the treatment of diabetes on the basis of practice guidelines and regular monitoring of blood glucose and blood pressure. Diet, sodium and water uptake were controlled. Symptomatic treatments such as supplementary protein, diuretic, erythropoietin hormone treatment of anemia were given. Patients in group A were treated with the routine dose of valsartan (Novartis, Beijing, China), 80 mg qd. Patients in group B were treated with double dose of valsartan, 160 mg qd. Patients in group C received treatment of double dose of valsartan combined with tacrolimus (Fujisawa, Japan) $0.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, oral 1h before dinner, and medicines were regulated according to concentration changes in plasma. 5-10 ng/ml is appropriate. The clinical effects of the medication were compared after 8 weeks.

Observation index

Comparative analysis of 24h urine protein, the serum creatinine and glomerular filtration rate difference, the difference of high-sensitivity C-reactive protein (hs CRP) and adiponectin levels, difference of high mobility group box-1 (HMGB1) levels and renal tubule matter and interstitial damage index (TDI), difference of clinical efficiency and the recurrence rate were done.

In the early morning, about 5 ml blood was taken from the elbow vein blood. The serum creatinine and hs-CRP were measured by chemiluminescence method, and ELISA method was used to determine the serum adiponectin.

For patients who had renal puncture indication were conducted with renal biopsy pathology after the consent of patients and their families. The biopsy was conducted with immunofluorescence staining, of which specific fluorescent antibody markers will be directly added to the antigen. After dyeing, wash way excess fluorescent antibody those did not participate in the reaction. Dry at room temperature after mounting. Examination with microscopic was done. Bio-MIAS 2011 the image analysis system was applied for detecting the expression of HMGB1 under 400 times Olympus (model bx50, Tokyo, Japan) optical microscope. Five visual fields were randomly selected and each field selected five positive areas. Each specimen was calculated of the average optical density value using target measurement. The score of total deviation index (TDI) is calculated based

on the renal interstitial pathological change index, such as renal interstitial fibrosis, vacuolar degeneration, inflammatory cell infiltration, etc., including renal tubular epithelial cell vacuolar degeneration, renal tubular ectasia, renal tubular atrophy, red blood cell tube type, tube type protein, edema, interstitial fibrosis, interstitial cells infiltration. The 8 weeks index score was recorded as 0, 1, 2, 3 according to the severity of each index. Then calculate the average value of each score and get the score of renal tubular interstitial injury index.

Judgment standard for clinical effect: *Complete remission* means blood glucose decrease to normal, 24h urinary protein quantitative <0.3 g and normal serum albumin, normal serum creatinine continued for six months without recurrence. *Effective* means normal blood glucose. 24 h urinary protein decreased by 50%, but still higher than the 0.3 g. Serum albumin is normal. Creatinine can increased slightly, lasted less than half a year. It can have a relapse. *Invalid* means that various indicators are not controlled, even with progress.

Statistical Analysis

Data was analyzed and processed using the software package SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Quantitative data was shown as (means±standard deviation). Comparison between groups was shown as variance analysis. Count data was shown as (%). Comparison between groups was shown with χ^2 test. $p < 0.05$ means that the differences was statistical significant.

Results

Comparison of 24h urine protein, serum creatinine and glomerular filtration rate

Before treatment, 24h urine protein, the level of serum creatinine and glomerular filtration rate

for the patients in the three groups were compared. The difference was not statistically significant ($p > 0.05$). 24h urine protein for patients in three groups after treatment was decreased. Those in group A decreased more than those of group B and C. The difference was statistically significant ($p < 0.05$). After treatment, serum creatinine and glomerular filtration rate in group A have no change. While, creatinine levels in both group B and C was lower and glomerular filtration rate was higher. The changes in group C were more obvious that those in group B. The differences were statistically significant ($p < 0.05$) (Table I).

Comparison of hs-CRP, adiponectin, HMGB1 and TDI

Before treatment, hs CRP and adiponectin levels for patients in three groups were compared. The differences were not statistically significant ($p > 0.05$). These indicators of patients in all groups were lower after treatment. Those in group B and group C were significantly lower than those in group A. Indicators in group C were much lower. The differences were statistically significant ($p < 0.05$). 23 cases in the group A were conducted with renal biopsy, 24 cases in group B, 25 cases in group C. HMGB1 and TDI levels in group B and C were significantly lower than those in group A. Those in group C were significantly lower. The differences were statistically significant ($p < 0.05$) (Table II).

Comparison of clinical efficacy and recurrence rate

The clinical effective rates of patients in group B and C were significantly higher than those in group A. Those in group C were much higher. The difference is statistically significant ($p < 0.05$); The recurrence rates in group B and group

Table I. Comparison of 24h urine protein, serum creatinine and glomerular filtration rate.

Group	24h urine protein (g)		Serum creatinine (imol/L)		Glomerular filtration rate (ml/min)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Group A	5.3±1.2	3.8±0.7	122.5±23.6	106.8±24.8	71.3±5.9	74.5±4.7
Group B	5.2±1.3*	1.5±0.4#	134.3±25.7*	86.7±29.5#	69.5±5.4*	78.3±4.6#
Group C	5.4±1.5*	0.8±0.2#	146.9±26.9*	62.5±17.7#	68.7±5.2*	85.5±4.5#
F	0.527	4.268	0.159	4.827	0.936	5.041

Note: Group A, conventional dose valsartan group. Group B, double dose valsartan group. Group C, double dose valsartan combined with tacrolimus group. *represents that compared with that of Group A, $p > 0.05$. # represents that compared with that of Group A, $p < 0.05$. * $p > 0.05$ statistically non significant, # $p < 0.05$ statistically significant

Table II. Comparison of hs-CRP, adiponectin, HMGB1 and TDI.

Group	hs-CRP (mg/L)		Adiponectin (mg/L)		HMGB1	TDI
	Before treatment	After treatment	Before treatment	After treatment		
Group A	9.2±1.5	7.4±0.8	24.5±5.7	18.7±4.3	96.7±12.6	3.6±0.5
Group B	9.3±1.4*	5.2±0.6#	25.6±5.6*	16.4±4.7#	75.8±15.8#	1.5±0.4#
Group C	9.5±1.3*	4.3±0.4#	26.7±5.2*	12.3±4.2#	62.4±14.3#	0.8±0.2#
F	0.849	5.823	0.735	5.749	6.105	6.534

*represents that compared with that of Group A, $p > 0.05$. # represents that compared with that of Group A, $p < 0.05$. * $p > 0.05$ statistically non significant, # $p < 0.05$ statistically significant

C were significantly lower than those of group A and those in group C were much lower. The differences were statistically significant ($p < 0.05$). All patients in three groups had no obvious drug complications (Table III).

Discussion

The mechanism of DN is complex, which is not fully understood. Studies have shown that [4], the occurrence and development of DN is the result of comprehensive action of multiple factors under high glucose environment, of which sugar metabolic disorders, renal hemodynamics change, cytokines factors such as AngII, serum VEGF, IGF-1, HGF, TNF- α , GTGF, etc. and genetic background factors play very important role. The most important pathological changes in the late DN were renal fibrosis, and it is very difficult for clinical treatment after fibrosis [5].

Ang II can not only promote higher blood pressure, also directly stimulate the proliferation of mesangial cells, inducing generation of transforming growth factor β and plasminogen activator inhibitor-1, which will result in the accumulation of extracellular matrix. Valsartan, as

a potent antagonist of angiotensin II receptor-1, can block the effect of angiotensin II on any of the sources from the receptor level. The study shows that Valsartan has protective effect on diabetic nephropathy and has the effect of anti-fibrosis⁶. Its mechanism is related to the renin-angiotensin system (RAS). Recent studies⁷⁻⁸ has indicated that it can significantly increase the strength of urine protein and protect kidney function, but not increase the blood pressure. Its related mechanism is still under further study. In this study, 24h urinary protein and serum creatinine level of group B were lower than those of group A, and the glomerular filtration rate increased in group A.

Many pro-inflammatory cytokines, chemotaxis factor, adhesion molecules and growth regulating factor can interact and crosslink with each other, participating and expanding a cascade of inflammation, promoting the DN. So, the effect of micro inflammation in DN has been paid more and more attention. Hs-CRP is a sensitive marker of systemic inflammatory response. Study pointed out that the Hs-CRP play an important role in the occurrence and development of DN. Its level can be gradually increased accompanied with the increase of proteinuria in

Table III. Comparison of clinical efficacy and recurrence rate [Cases(%)].

Group	Cases	Complete remission	Effective	Invalid	Clinical efficiency rate	Recurrence rate
Group A	28	5	9	14	14 (50.0)	11 (39.3)
Group B	29	9	13	10	22 (75.9) #	4 (13.8) #
Group C	29	11	13	5	24 (82.8) #	3 (10.3) #
X2					6.851	6.847

#represents that compared with that of Group A, $p < 0.05$. # $p < 0.05$ statistically significant

different stages of DN. It is positively correlated with the degree of kidney damage. Adiponectin is a recently discovered type of insulin sensitization, which is a kind of cell factor that is secreted by mature adipocytes. It can prevent atherosclerosis and reduce the inflammatory reaction through regulation of endothelial function, immune function, glucose and lipid metabolism. Adiponectin levels in different stages of DN were found⁹⁻¹⁰ to be correlated with the levels of urinary protein and the degree of renal damage.

HMGB1 (high-mobility group box 1) is a kind of nuclear protein, widely distributed in mammals. It can be specific involved in genes replication, transcription and cell differentiation regulation and also related with cell apoptosis, inflammatory reaction and immune function disorder and tumor cell proliferation. Lipopolysaccharide (LPS) and pre inflammatory factor TNF, IL-1, INF- γ , etc., released by Gram negative bacteria under Endotoxemia can induce monocyte/ macrophages actively secreting HMGB1 and necrotic tissue cells can also be passive release of HMGB1. HMGB 1 release can further promote inflammatory cells releasing a lot of pre-inflammatory factor to initiate the inflammatory cascade. Many authors¹¹⁻¹³ pointed that HMGB 1 has the potential to become an important target for anti-inflammatory and preventing tissue damage.

Tacrolimus is an immunosuppressive agent, belonging to the macrolide, which can inhibit the expression of cytokines. It has advantages such as low toxicity, no renal toxicity, etc. in the treatment of chronic kidney disease. Tacrolimus can significantly inhibit the infiltration of inflammatory cells in the kidney, especially lymphocytes and macrophages in patients with diabetic nephropathy. This effect is likely to be one of the mechanisms by which tacrolimus inhibits the proliferation of renal cell proliferation and inhibits the proliferation of B lymphocytes and T lymphocytes. Tacrolimus was also able to inhibit the release of inflammatory cytokines and chemokines in kidney such as Monocyte Chemoattractant Protein-1 (MCP-1), RANTES (regulated on activation, normal T cell expressed and secreted) and IL-8, and fractaline. These substances are to accelerate diabetic nephropathy renal inflammatory process¹⁴⁻¹⁵.

Through the research, it is known that 24h quantitative urinary protein and serum creatinine levels in group C were significantly lower than those of group B. The glomerular filtration rate was significantly higher than that in group B.

Hs-CRP and adiponectin levels group C were significantly lower than those in group B. HMGB1 and TDI was significantly lower than those of group B. The clinical efficiency of group C was significantly higher than that in group B. The recurrence rate was significantly lower than that in group B. The differences were statistically significant.

Conclusions

A double dose of valsartan combined with tacrolimus treatment for patients in DN stage can improve clinical symptoms and reduce inflammation, inhibiting or even reversing the interstitial fibrosis, improving the curative effect, reducing the recurrence, as to provide a new theoretical basis for the clinical treatment of the disease.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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