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Original Article

The effects of alprostadil combined with α-lipoic acid in the treatment of senile diabetic nephropathy

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Abstract: Objective: This study aimed to investigate the clinical efficacy of alprostadil combined with α-Lipoic acid (α-LA) in the treatment of senile diabetic nephropathy (SDN) and the combination's effect on the serum chemerin and neutrophil gelatinase-associated lipocalin (NGAL) expressions. Methods: Seventy-six patients with diabetic nephropathy (DN) admitted to our hospital from March 2018 to October 2019 were recruited as the research cohort. Among them, 36 patients who were administered alprostadil monotherapy were placed in the control group (CG), and the remaining 40 patients administered α-LA in addition to the alprostadil were placed in the research group (RG). The treatment effectiveness rate, the incidence of adverse reactions, and the changes in the renal function indexes (BUN, SCr, UAER) and the blood glucose indexes (FPG, 2hPG) were compared between the two groups. Results: The total effective rate in the RG was significantly higher than the total effective rate in the CG ($P < 0.05$). The renal function and blood glucose indexes dropped significantly after the treatment ($P < 0.001$). The chemerin and NGAL levels were significantly reduced in both groups after the treatment ($P < 0.05$), and the chemerin and NGAL expressions in the RG were significantly lower than they were in the CG ($P < 0.05$). Conclusion: Alprostadil combined with α-LA is better than alprostadil monotherapy in the treatment of DN because it can improve the effectiveness rate, reduce the blood glucose, and improve the renal function while effectively reducing patients' serum chemerin and NGAL levels.

Keywords: α-Lipoic acid, alprostadil, chemerin, diabetic nephropathy, NGAL

Introduction

With a growing elderly population, the incidence of chronic diseases, such as diabetes, increases yearly, a major challenge to the public healthcare system [1]. Diabetes is a common clinical endocrine and metabolic disease. According to data from the International Diabetes Federation [2], there were about 415 million adults worldwide aged 20-79 years with diabetes in 2015, and the number is expected to reach 642 million by 2040, with the prevalence rising from 8.8% to 10.4%. Diabetes is a long-course disease, and if it is not treated in time, problems, such as microvascular lesions, caused by the continuous development of the disease can lead to a variety of complications [3]. Among them, diabetic nephropathy (DN) is a high-risk complication ignited by progressive diabetes. Its clinical manifestations mainly include albuminuria and hyperglycemia, which lead to a continuous decline in patients' renal function, and it has become the second major cause of end-stage renal disease, seriously affecting patient health and even endangering patients' lives [4, 5]. Therefore, the early diagnosis and treatment of the disease are particularly important. Currently, the pathogenesis of DN is not yet fully understood [6], except that it is related to glomerular hemodynamic changes and an increased production of cytokines [7, 8]. According to the research results of Gyurászová et al. [9], oxidative stress is related to the pathogenesis of DN, and it can damage renal tissue, promote inflammation, and lead to further tissue damage. A typical natural prostaglandin drug, alprostadil can effectively dilate the renal blood vessels and directly act on the glomerular artery and its smooth muscle after spasms caused by a rise in blood sugar, but its effect on the disease-induced oxidative stress response is insignificant [10]. On the other hand, α-lipoic acid (α-LA) [11] is a strong antioxidant that can improve vascular endothelial

function, reduce the excretion of proteinuria, alleviate renal fibrosis, protect residual renal function, and delay the progression of the disease. At present, there are few published studies on the combination therapy of alprostadil and α-LA in the treatment of SDN at home and abroad. Thus, this paper analyzes the efficacy of alprostadil combined with α-LA in the treatment of senile diabetic nephropathy (SDN) and further investigates its effect on the expression of serum adipocytokine chemerin and neutrophil gelatinase-associated lipocalin (NGAL).

Materials and methods

Clinical data

This study included 76 patients with DN admitted to our hospital from March 2018 to October 2019 as research participants. Among them, the 36 patients who were administered alprostadil were placed in the control group (CG), and the remaining 40 patients who were administered α-LA in addition to the alprostadil were classified as the research group (RG). This experiment was approved by the Medical Ethics Committee of Sichuan Provincial People's Hospital (XJTU2AF2018LSY-07), and all the enrolled participants signed the informed consent forms.

Inclusion and exclusion criteria

The inclusion criteria included patients no less than 60 years old who were diagnosed with DN through a clinical examination. The exclusion criteria included patients under 60 years old, patients with other metabolic diseases, patients with other malignancies, patients who had received relevant treatment before this study, patients with poor treatment compliance, patients with drug allergies, patients with incomplete clinical data, and transferred patients.

Methods

Treatment methods: All the patients underwent routine treatment, such as diet and insulin injections. In the CG, 5-10 μg of alprostadil injections (Tide Pharmaceutical Co., Ltd., Beijing, China; State Drug approval document number, H10980023) were added to 100 ml of normal saline for intravenous drips administered once a day. In addition to the drug administered to the CG, the RG added 250-500 mg of α-LA injections (Xinbai Pharmaceutical Co., Ltd., Nanjing, China; state drug approval document number, H20093235) with 250 ml of normal saline administered using an intravenous drip once a day. The treatment lasted for one month.

Measuring the serum chemerin and NGAL expressions: Fasting venous blood (5 ml) was obtained from all the patients and stored in a refrigerator at low temperature. After 60-minute coagulation, the blood was centrifuged at 4°C at 1000 × g for 20 minutes, and the obtained serum was cryopreserved. The serum NGAL and chemerin expressions in the two groups were measured using enzyme-linked immunosorbent assays. The NGAL kits (article no. HZ-NGAL-p) were purchased from Huzheng Industrial Co., Ltd., Shanghai, China, and the Chemerin kits (article no. CSB-E10398h) were obtained from the Shanghai Hengfei Biotechnology Co., Ltd., Shanghai, China. The quantification process was carried out in strict accordance with the kits' instructions.

Measuring the renal function and blood glucose indexes: Peripheral venous blood (5 ml) was collected from the patients in the two groups before and after the treatment and centrifuged for examination. Renal function indexes: the urea nitrogen (BUN) and serum creatinine (SCr) levels were measured using an automatic biochemical analyzer, and the urinary albumin excretion rate (UAER) was determined using radioimmunoassays. Blood glucose indexes: the fasting plasma glucose (FPG) and the 2 h postprandial blood glucose (2hPG) levels were measured using an automatic blood glucose detector.

Outcome measures

Clinical efficacy: markedly effective: The clinical signs and symptoms disappeared or significantly improved in the patients, the blood glucose level recovered, and the 24-hour urinary protein quantification or serum creatinine (SCr) levels returned to normal or improved by more than 30%. Furthermore, the clinical signs and were symptoms improved in the patients, the blood glucose returned to normal, and the 24-hour urinary protein quantification or SCr improved by 10%-29%. However, the clinical symptoms and renal function of the patients

Table 1. Comparison of the clinical data between the two groups (n [%])

	Research group (n=40)	Control group (n=36)	t/X^2	*P*
Age (years old)	73.49±9.37	73.25±9.16	0.113	0.911
Gender			0.002	0.961
Male	22 (55.00)	20 (55.56)		
Female	18 (45.00)	16 (44.44)		
BMI	23.16±2.65	23.27±2.88	0.173	0.863
Course of diabetes (year)	7.20±2.31	7.09±2.24	0.210	0.834
Family history of diabetes			0.407	0.524
Yes	9 (22.50)	6 (16.67)		
No	31 (77.50)	30 (83.33)		
Place of residence			0.237	0.627
Urban	28 (70.00)	27 (75.00)		
Rural	12 (30.00)	9 (25.00)		
Smoking			0.499	0.480
Yes	8 (20.00)	5 (13.89)		
No	32 (80.00)	31 (86.11)		
Drinking			0.337	0.562
Yes	10 (25.00)	7 (19.44)		
No	30 (75.00)	29 (80.56)		

Table 2. Comparison of the clinical efficacy between the two groups (n [%])

	Research group (n=40)	Control group (n=36)	X^2	P
Markedly effective	22 (55.00)	11 (30.56)		
Effective	15 (37.50)	16 (44.44)		
Ineffective	3 (7.50)	9 (25.00)		
Total effective rate	37 (92.50)	27 (75.00)	4.364	0.037

did not improve or may have even gotten worse. The total effective rate is computed by (markedly effective + effective)/total cases × 100%. With regard to the renal function indices, the BUN, SCr, and UAER changes before and after the treatment were compared between the two groups. In the analysis and comparison of the blood glucose indices, the FPG and 2hPG levels before and after treatment were compared between the two groups. The adverse reactions of the two groups were compared, and the changes in the chemerin and NGAL levels in the two groups before and after treatment were recorded.

Statistical methods

In this study, the statistical analysis of the data was processed using SPSS 24.0 (Shanghai Yuchuang Network Technology Co., Ltd.), and the required pictures were plotted using GraphPad Prism 5. The counting data were expressed in the form of a percentage (%), and chi-squared tests were used for the intergroup comparisons. The measurement data were expressed as the mean ± standard deviation, and t-tests were used for the intergroup comparisons. $P < 0.05$ indicated a statistically significant difference.

Results

Comparison of the clinical data

There were no significant differences in the clinical data, such as ages, sexes, BMIs, course of diabetes, family history of diabetes, residence, smoking, or drinking between the two groups ($P > 0.05$), indicating comparability (**Table 1**).

Comparison of the clinical efficacy

In the comparison of the treatment effects of the two groups, it was found that the total effective rate of treatment in the RG (92.50%) was significantly higher than the total effective rate of treatment in the CG (75.00%) (**Table 2**).

Comparison of the renal function and blood glucose indexes

After the treatment, the BUN, SCr, and UAER decreased significantly in both groups, and their levels in the RG were statistically lower than their levels in the CG ($P < 0.001$) (**Table 3**).

There was no significant difference with regard to the FPG and 2hPG levels between the two groups before the treatment ($P > 0.05$), but their levels were significantly reduced after the treatment ($P < 0.001$), and the post-treatment FPG and 2hPG levels in the RG were significantly lower compared to the FPG and 2hPG levels in the CG ($P < 0.05$) (**Table 4**).

Table 3. Comparison of the renal function indexes between the two groups

Groups	BUN (mmol/L)		SCr (μmol/L)		UAER (μg/min)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
RG (n=40)	7.84±1.21	4.03±0.67*	129.43±10.33	73.37±7.54*	125.21±7.35	48.32±4.41*
CG (n=36)	7.67±1.18	5.45±0.88*	131.25±10.89	94.25±8.38*	122.76±7.11	82.95±5.83*
t	0.619	7.961	0.748	11.430	1.474	29.380
p	0.538	< 0.001	0.451	< 0.001	0.145	< 0.001

Note: *indicates P < 0.001 compared with before the treatment.

Table 4. Comparison of the blood glucose indexes between the two groups before and after the treatment (mmol/L)

Groups	FPG		2hPG	
	Before treatment	After treatment	Before treatment	After treatment
RG (n=40)	9.48±2.25	6.23±1.05#	13.42±3.17	9.25±1.73#
CG (n=36)	9.52±2.41	7.58±1.39#	13.61±3.43	10.34±2.04#
t	0.075	4.806	0.251	2.520
P	0.941	< 0.001	0.803	0.014

Note: #indicates P < 0.001 compared with before the treatment.

Table 5. Comparison of the adverse reactions between the two groups (n [%])

	Research group (n=40)	Control group (n=36)	χ^2	*P*
Phlebitis	2 (5.00)	1 (2.78)		
Rash	1 (2.50)	0 (0.00)		
Gastrointestinal reaction	2 (5.00)	1 (2.78)		
Dizziness and head bilges	1 (2.50)	2 (5.56)		
Incidence	6 (15.00)	4 (11.11)	0.251	0.617

Comparison of the adverse reactions and the serum chemerin and NGAL levels

In terms of the incidence of adverse reactions, there was no significant difference between the RG (15.00%) and the CG (11.11%) (P > 0.05) (**Table 5**).

After the different treatment were administered, the chemerin and NGAL levels in the two groups were significantly reduced (P < 0.05), and the levels were significantly lower in the RG than they were in the CG (P < 0.05) (**Figure 1**).

Discussion

The prevalence of diabetes is a growing global public health problem [12]. DN is a serious microvascular complication in diabetic patients, which is characterized by glomerulosclerosis caused by abnormal glucose metabolism, and it is also the leading cause of death in DN [13]. In addition to the microproteinuria status, which can last for many years but is easily ignored by patients, there is no obvious clinical manifestation in the early stage of the disease. With progression, this disease can gradually develop into renal function decline, systemic edema, and even end-stage renal failure, which is a prevalent cause of death among diabetic patients [14, 15]. According to published data [16], more than 20%-40% of diabetic patients are likely to develop chronic kidney disease. Other evidence has shown that DN increases the total 10-year mortality rate of diabetic patients by at least six times [17]. The pathogenesis of DN is complicated, but it is mainly associated with hyperglycemia and hemorheological abnormalities [18]. Conventional clinical treatment methods mainly include blood glucose control and a reasonable diet, but it is difficult to achieve the ideal treatment effect. Alprostadil is a new treatment, and the main component is prostaglandin E1. Zhang et al. [19] found that it can inhibit platelet aggregation and thrombosis by dilating the renal blood vessels, reducing the urinary protein, and improving renal function by increasing the blood flow in the kidneys as well as glomerular filtration rate. It is commonly used in the clinical treatment of DN. However, it is difficult to achieve the best clinical effect with alprostadil alone.

On the other hand, α-LA is a strong antioxidant, and it can inhibit lipid peroxidation, increase

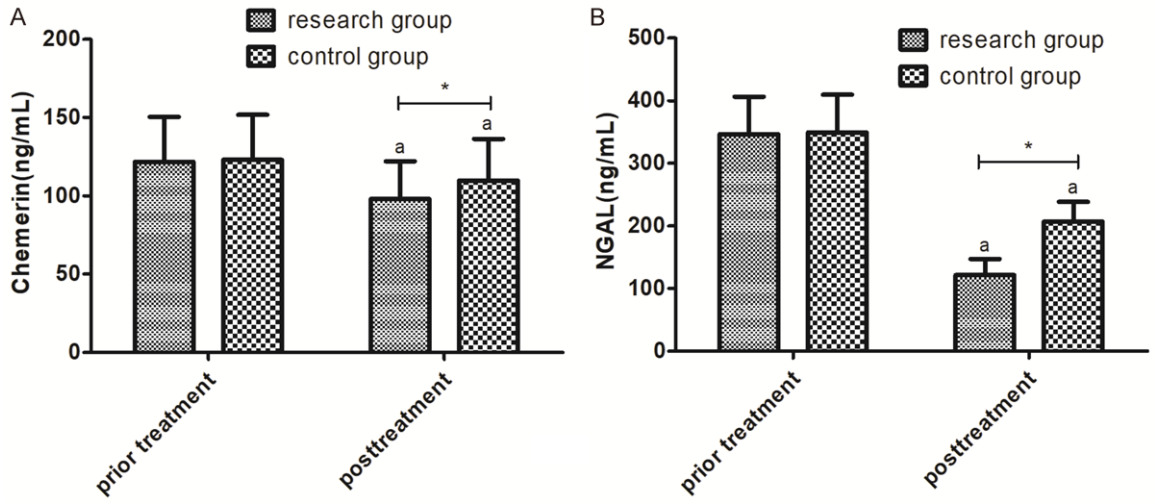

Figure 1. Comparison of the serum chemerin and NGAL levels between the two groups before and after the treatment. A: Comparison of the chemerin levels between the two groups before and after the treatment; B: Comparison of the NGAL levels between the two groups before and after the treatment; [a]indicates $P < 0.05$ compared with before the treatment; [*]indicates $P < 0.05$.

microvascular blood flow, and protect vascular endothelial function [20]. As a chemotactic secretory protein with a molecular weight of 16 kU, chemerin affects the metabolism of adipocytes. Some studies have found that [21] chemerin can regulate the sensitivity of adipose tissues to insulin and then participate in the occurrence and development of diabetes. NGAL is a type of lipocalin that is widely distributed in the human body; it is a biomarker for acute renal injury, which aggravates the damage on the glomerular filtration membrane by inducing the activation of neutrophil enzymes, resulting in the emergence of urinary protein and abnormal renal function [22]. By comparing the clinical efficacy of alprostadil combined with α-LA and alprostadil monotherapy in the treatment of SDN, this paper proves the application value of the combination therapy in the treatment of SDN and its effect on the serum chemerin and NGAL expressions.

The results of this experiment demonstrated that the total effective rate of the patients in the RG treated with alprostadil combined with α-LA was significantly higher compared to the CG treated with alprostadil alone. After the treatment, the renal function indexes (BUN, SCr, UAER) and the blood glucose indexes (FPG, 2hPG) in the RG were remarkably lower than they were in the CG, suggesting that alprostadil combined with α-LA is better than alprostadil monotherapy for the treatment of SDN. The reason behind it, we speculate, may be that α-LA can enhance the hypoglycemic effect and has antioxidant and anti-inflammatory effects [23]. Because α-LA is protective of islet β cells, it can protect islet cells from being attacked by free radicals [24]. In addition, the key role of α-LA is its antioxidant activity due to its ability to scavenge and inactivate free radicals. In particular, research has pointed out that α-LA possesses a therapeutic effect in reducing the blood sugar levels of diabetic patients, a finding consistent with the results of this experiment and that can be mutually corroborated [25]. Meanwhile, inflammation is considered to be one of the potential processes in the development of kidney disease in diabetic patients [26]. Gurley SB et al. [27] pointed out that the genes that control the inflammatory response, triggered by hyperglycemia and the activation of the renin-angiotensin system, may be early determinants of susceptibility to DN. α-LA possesses anti-inflammatory and neuroprotective properties and can reverse corneal sensitivity and nerve damage in type 2 diabetic rats [28]. Furthermore, the adverse reactions of the two groups were compared in the course of the treatment. It was found that the incidence of adverse reactions in the two groups was low, indicating that the combination therapy was also safer than monotherapy. Finally, we compared the differences in the serum chemerin and NGAL levels between the two groups of patients before and after the treatment. It was

found that the pretreatment serum chemerin and NGAL levels showed no significant difference, while after administering different treatment measures, the chemerin and NGAL levels statistically dropped in both groups, and those in the RG were significantly lower than those in the CG, indicating that the combination therapy was more effective than the monotherapy in delaying disease progression. It is hypothesized that alprostadil and α-LA jointly regulate the expression of chemerin and NGAL in DN patients, thus playing a certain therapeutic role.

However, due to the limited conditions, this study has several shortcomings. For example, the research cohort was small, and the population was relatively homogeneous, so the influencing mechanism of alprostadil and α-LA on the serum chemerin and NGAL in DN patients still needs further research and discussion. In future studies, we will perform a long-term follow-up investigation on the research participants and continually improve our experiments.

In summary, alprostadil combined with α-LA is better than alprostadil monotherapy for the treatment of DN, as it can effectively improve the treatment efficiency of patients, reduce the blood sugar, improve renal function, and decrease the serum chemerin and NGAL expressions.

Disclosure of conflict of interest

None.

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