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Effects of amlodipine combined with atorvastatin on Th17/Treg imbalance and vascular microcirculation in hypertensive patients with atherosclerosis

A double-blind, single-center randomized controlled trial

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Abstract

Objective: Helper T cells 17 (Th17) and regulatory T cells (Treg), as CD4+T lymphocyte subsets, play an important role in the process of atherosclerosis. However, there are few studies on the regulation and efficacy of atorvastatin combined with amlodipine on Th17/Treg balance in hypertension combined with carotid atherosclerosis. Therefore, this study aims to verify the efficacy and immunomodulatory effects of atorvastatin combined with amlodipine in the treatment of hypertension combined with carotid atherosclerosis.

Methods: A total of 260 patients with hypertension and carotid atherosclerosis were randomly divided into atorvastatin or combined treatment group. Inflammatory factors and Th17 and Treg levels were detected by enzyme-linked immunosorbent assay and flow cytometry. The messenger ribonucleic acid expression of retinoic acid receptor-related orphan receptor gamma and forkhead spiral transcription factor were detected by real-time quantitative polymerse chain reaction.

Results: We found that the total effective rate in the treatment group was significantly higher than that in the control group. The levels of whole blood high shear viscosity, whole blood low shear viscosity, plasma specific viscosity and fibrin content in the 2 groups were significantly decreased after treatment, and the combined group was significantly lower than the control group (all $P < .05$). The improvement of endothelial function in the treatment group was also significantly higher than that in the control group (all $P < .05$). In addition, we found that there were statistically significant differences in Th17 percentage, Treg percentage and Treg/Th17 between the treatment group and the control group ($P < .05$). The messenger ribonucleic acid levels of retinoic acid receptor-related orphan receptor gamma and forkhead spiral transcription factor showed the same trend. Further detection of Th17-related inflammatory factors showed that the expression of interleukin (IL)-17, IL-6, IL-23 and tumor necrosis factor-α in the treatment group was significantly decreased, which was better than that in the control group (all $P < .05$).

Conclusion: These data indicate that amlodipine combined with atorvastatin can improve Th17/Treg imbalance, vascular endothelial function and efficacy in patients with hypertension and atherosclerosis.

Abbreviations: Fh = fibrin content, Foxp3 = forkhead spiral transcription factor, H_2S = hydrogen sulfide, IL = interleukin, LDL = low-density lipoprotein, mRNA = messenger ribonucleic acid, Nbh = whole blood high shear viscosity, Nbl = whole blood low shear viscosity, NO = nitric oxide, Np = plasma specific viscosity, PCR = polymerse chain reaction, ROR-γt = retinoic acid receptor-related orphan receptor gamma, Th17 = helper T cells 17, TNF = tumor necrosis factor, Treg = regulatory T cells.

Keywords: amlodipine, atherosclerosis, atorvastatin, hypertension, Th17/Treg

1. Introduction

Atherosclerosis is the most common and important type of arteriosclerosis, and it is also an important pathological basis of atherosclerotic cardiovascular disease.[1,2] Hypertension is one of the main risk factors for atherosclerosis, and atherosclerosis are causal and mutually reinforcing, and ultimately lead to target organ dysfunction.[3] With the development of

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social economy and the aging of the population, the incidence of cardiovascular disease caused by hypertension and atherosclerosis in China is increasing year by year.[3] Statistics from the World Health Organization show that in 2012, the number of deaths from cardiovascular diseases worldwide was 17 million, accounting for 46% of the deaths from chronic diseases, of which 9.4 million died from hypertension complications, accounting for 7% of the total disease burden (disability-adjusted years).[4] As a result, hypertension has become the leading cause of the global disease burden. At present, there are about 11 million coronary heart disease patients in China. A survey and study included 3513 in patients with coronary heart disease in 52 tertiary hospitals in 7 major cities in my country, and the results showed that 70.5% of them had hypertension. According to statistics, 50% of myocardial infarctions are related to hypertension.[5–8] Therefore, it is of great significance to find effective therapeutic drugs for patients with hypertension and carotid atherosclerosis to reduce the burden of disease and improve life treatment.

Atorvastatin is a clinically commonly used lipid-lowering drug for the treatment of cardiovascular and cerebrovascular diseases.[9] Its mechanism of action is to inhibit the rate-limiting enzyme hydroxyglutaryl coenzyme A reductase in the early stage of cholesterol synthesis.[10] At the same time, it can increase the expression of low-density lipoprotein (LDL) receptors in the liver cell membrane, reduce the levels of cholesterol and lipoprotein, and increase the uptake and catabolism mechanism of LDL by increasing the number of LDL receptors on the surface of liver cells.[11] In recent years, studies have confirmed that atorvastatin has good curative effect in improving ventricular myocardial function, vascular endothelial cell function and myocardial fibrosis.[12–14]

Helper T cells 17 (Th17) and regulatory T cells (Treg) are a group of $CD4^+$T lymphocyte subsets discovered in recent years that are different from Th1 and Th2 cells, and play an important role in the process of inflammation and atherosclerosis.[15,16] Treg cells in human immune-mediated diseases and their findings suggest that an acquired defect in forkhead spiral transcription factor (Foxp3) expression may be a contributing factor. Treg cells play an important role in the formation of immunological self-tolerance and thereby in the prevention of autoimmunity. Moreover, Foxp3 expression has been shown to be reduced in individuals who develop inflammatory neurologic diseases, and reduced functionality of Treg cells is associated with upregulation of RORγt. Treg cells in human immune-mediated diseases and their findings suggest that an acquired defect in Foxp3 expression may be a contributing factor. Treg cells play an important role in the formation of immunological self-tolerance and thereby in the prevention of autoimmunity. Moreover, Foxp3 expression has been shown to be reduced in individuals who develop inflammatory neurologic diseases, and reduced functionality of Treg cells is associated with upregulation of RORγt.[17] At present, a large number of studies have shown that T helper cells play an important role in human diseases, including neuroimmune diseases.[18,19] However, the current research on cardiovascular disease is still insufficient. Interleukin (IL)-22 and IL-23, as regulators of atherosclerosis, can inhibit the expansion of pro-atherosclerotic microbiota and avoid atherosclerosis caused by microbiota and inflammation. It plays a regulatory role in the occurrence and development of sclerosis. Amlodipine is a dihydropyridine calcium channel antagonist, which can effectively inhibit the transfer of calcium ions into cardiomyocytes and smooth muscle cells, and play an important role in inhibiting vascular smooth muscle contraction, dilating blood vessels, and improving blood flow.[20–23] At the same time, the drug can improve myocardial microcirculation, increase blood oxygen supply level, and improve cardiac function while stabilizing blood pressure. In addition, other studies have shown that amlodipine also plays an important role in immune regulation.[24,25] However, there are few studies on the regulation of Th17/Treg balance and the efficacy of atorvastatin combined with amlodipine in hypertension complicated with carotid atherosclerosis. Therefore, this study designed a single-center clinical randomized controlled trial to verify the efficacy and immune regulation of atorvastatin combined with amlodipine in the treatment of hypertension complicated with carotid atherosclerosis.

2. Materials and methods

2.1. Clinical sample

The sample size of this study was calculated with reference to the clinical sample calculation method of Chow S et al referring to our preliminary clinical pre-experiment. This trial uses the indicators of $\alpha = 0.05$, $1-\beta = 0.80$ and $\kappa = 1$ to estimate the sample size, then each group needs 108 cases, and considering the 20% increase in loss to follow-up, each group needs 130 cases, and a total of research subjects are required 260 cases.

This study is a double-blind, randomized, controlled trial of efficacy assessors and data analysts, and will be conducted in Sichuan Corps Hospital of Chinese People's Armed Police from January 2020 to June 2021. The patients will be randomly assigned in a 1:1 ratio to a treatment group (n = 130) and a control group (n = 130). Inclusion criteria: Essential hypertension; meet the diagnostic criteria of hypertension: systolic blood pressure ≥ 140 mm Hg (1 mm Hg = 0.133 kPa), diastolic blood pressure ≥ 90 mm Hg; echocardiography showed ventricular myocardial hypertrophy, diastolic blood pressure interventricular septum thickness ≥ 10 mm. Meet the diagnostic criteria for atherosclerosis: the patient was found to have plaque in the carotid artery after color Doppler ultrasonography, and the intima thickness was ≥1.2 mm; the patient was found to be positive by coronary angiography. The patient can communicate in complete language. Exclusion criteria: Patients with secondary hypertension; those who have taken hypolipidemic, antihypertensive drugs, statins, anti-inflammatory drugs, steroid hormones, etc in the near future; patients who are allergic to the drugs in this test; patients with liver and kidney function, brain function, and cardiac dysfunction; and patients who could not complete this study. All patients signed informed consent. The research protocol and the entire research process were approved and supervised by the Ethics Committee of Sichuan Corps Hospital of Chinese People's Armed Police.

2.2. Randomization principles

This study adopts the principle of stratified randomization. Number of stratification: According to the age range of the collected patients, age is stratified, and the number of layers is 3. Number of experimental groups: divided into 2 groups, namely the experimental group and the control group. Distribution ratio: Distribution at a ratio of 1:1. The random number table method was used to divide the stratified personnel into groups, among which the odd number was group A, and the even number was group B (operators and executives do not know the specific situation of group AB). When implementing the randomization concealment process, it should be operated by a third-party person who is not related to the clinical trial. This process should be true, not fake, and operate strictly in accordance with the random number table. Completion of the above steps can ensure good randomization concealment.

2.3. Treatment methods

The patients in both groups were treated with conventional antihypertensive and hypoglycemic treatments, and lifestyle interventions such as controlling sodium salt intake, reasonable

diet, controlling body weight, quitting smoking and drinking, and enhancing exercise. On this basis, the control group was given atorvastatin (Pfizer Pharmaceutical Co., Ltd, New York, NY, H20051408): 40 mg/d, once/d, orally. On the basis of the control group, the combined group was given amlodipine (Zhejiang Kangle Pharmaceutical Co., Ltd, Wenzhou, China, H20083685, 5 mg) orally, 5mg/d, once/d. The patients in both groups were continuously treated for 6 months and then the related indicators were measured.

2.4. Efficacy evaluation criteria

Significantly effective: After treatment, the patient's blood pressure returned to normal, and the area of atherosclerotic plaque was reduced by >1/2; Effective: After treatment, the patient's blood pressure was basically normal, and the area of atherosclerotic plaque was reduced by <1/2; Ineffective: After treatment, the patient's blood pressure did not return to normal, and the atherosclerotic plaque and area did not decrease or increase. The total effective rate = (the number of markedly effective cases + the number of effective cases)/ the total number of cases × 100%.

2.5. Laboratory measurements

Total cholesterol, triglyceride, high-density lipoprotein cholesterol, and LDL cholesterol were detected by automatic biochemical analyzer (Hitachi 7180, Hitachi Co., Ltd, Tokyo, Japan). instrument manual. Blood flow indicators include whole blood high shear viscosity (Nbh), whole blood low shear viscosity (Nbl), plasma specific viscosity (Np) and fibrin content (Fh), using LBYN6-A automatic cleaning rotary viscometer (Beijing Prism Instruments Co., Ltd, Beijing, China) assay. Furthermore, carotid color Doppler ultrasound (PHILIPS SONOS 7500, PHILIPS Co., Ltd, Amsterdam, Netherlands) was used to evaluate the plaque status including intima-media thickness and plaque volume.

2.6. Flow cytometry analysis

5 mL of fasting cubital venous blood was collected in the morning, and peripheral blood mononuclear cells were extracted with Ficoll lymphocyte separation medium, prepared into a single-cell suspension, stored in a −80°C refrigerator overnight, and stored in liquid nitrogen. Isolated peripheral blood mononuclear cells were taken and 50μL of tube and cell suspension was detected. Then CD3-PE and CD4-FITC monoclonal antibodies were added respectively, and IgG1-PE and IgG-FITC were added for incubation at room temperature and dark for 30 minutes. 100 μL fixing solution was added and dark for 15 minutes. After washing with phosphate buffered saline, 100μL breaking solution was added and PE-IL-17 monoclonal antibody was added at the same time. The proportion of Th17 cells was measured by Beckman flow cytometry (Beckman Co., Ltd, Bria, CA). First gated according to the physical properties of the lymphocytes (forward and lateral scattering), then plotted a second gate according to the immunofluorescence properties of the gated cells. Forward SCattering-side scatter dot plot was used to gate the cells, and then the lymphocytes were gated to analyze the percentage of CD3 + CD4 + IL-17A + cells.[26] In addition, we used the same method as above to add CD25-FITC, CD4-FITC monoclonal antibody marker, and finally PE-Foxp3 antibody to detect the proportion of Treg cells (CD4 + CD25 + Foxp3 + T cells) by flow cytometry.

2.7. Real-time quantitative polymerse chain reaction (PCR)

Plasma retinoic acid receptor-related orphan receptor gamma (ROR-γt) and forkhead wing helix transcription factor (Foxp3) messenger ribonucleic acid (mRNA) expressions were detected by real-time PCR. Blood samples were taken from each group, and total RNA was extracted by TRIzol method; first-strand complementary deoxyribonucleic acid was synthesized; and a fluorescence quantitative PCR reaction system was constructed. The reaction conditions were 95°C for 2 minutes, 95°C for 20 seconds, 60°C for 30 seconds, and 68°C for 55 seconds (40 cycles). ROR-γt sense: 5'-CCTGGGCTCCTCGCCTGACC-3' and antisense: 5'-TCTCTCTGCCCTCAGCCTTGCC-3'. Foxp3 sense: 5'-CTGGCAAATGGTGTCTGCAAGT-3' and antisense: 5'-TGGCACCCAGCACAATGAA-3'. Using GAPDH as an internal reference, the $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression levels of ROR-γt and Foxp3 mRNA.

2.8. Enzyme-linked immunosorbet assays

The peripheral blood samples of each treatment group were collected, and the serum was collected after centrifugation, and IL-1β (Abcam Co., Ltd, Cambridge, England, ab217608), IL-2 Abcam, ab100566), IL-6 (Abcam, ab178013), IL-10 (Abcam, ab185986), IL-17 (Abcam, ab119535), IL-23 (Abcam, ab64708), tumor necrosis factor (TNF)-α (Abcam, ab181421), transforming growth receptor-β1 (Abcam, ab100647), interferon-γ(Abcam, ab174443), high-sensitivity C-reactive protein (Shuhua Biology, China, SH1297), monocyte chemotactic protein-1 (Abcam, ab179886) and intercellular cell adhesion molecule (ICAM)-1 (Abcam, ab174445) were detected by enzyme-linked immunosorbet assays. For specific steps, refer to the instruction manual of the kit (Hangzhou Lianke Biotechnology Co., Ltd, Hangzhou, China).

2.9. Hydrogen sulfide (H_2S) and nitric oxide (NO) detection

In this study, total NO assay kit (Beyotime, China, S0023) and endogenous H_2S assay kit (Nanjing jiancheng Bioengineering, China, A146-1) were used to detect the content of NO and H_2S in blood samples. All operations were performed according to the reagent manufacturer's procedures.

2.10. Statistical analyses

Categorical variables were estimated as frequencies with proportions, while continuous variables as mean ± standard deviation. Chi-squared tests or *t* tests were used to compare the differences in the data between the treatment and control groups according to demographic and baseline variables. Paired *t* tests were used to compare the effects between the treatment and control groups based on the primary and secondary outcome measures. The level of statistical significance was set at $P < .05$.

3. Results

3.1. Baseline clinical data of patients in observation group and control group

All eligible patients were randomized into control and treatment groups. During the follow-up period, no patients withdrew, died, or dropped out of the study for other unknown reasons. Ultimately, 260 patients (130 in the control group and 130 in the treatment group) completed the trial and were used for statistical analysis. There were no significant differences in age, gender, body mass index, systolic blood pressure, diastolic blood pressure, total cholesterol, triglyceride, high-density lipoprotein cholesterol, LDL cholesterol, fasting plasma glucose and intima-media thickness between the 2 groups (all $P > .05$, Table 1).

Table 1

Baseline clinical characteridtics.

	Control group (n = 130)	Treatment group (n = 130)	χ^2/*t* value	*P* value
Gender (male)	67 (51.54%)	70 (53.85%)	0.14	.71
Age (yr)	64.83 ± 6.82	65.39 ± 7.32	0.64	.52
BMI (kg/m^2)	23.45 ± 3.18	22.98 ± 4.36	0.99	.32
SBP (mm Hg)	163.37 ± 14.85	161.51 ± 15.56	0.60	.55
DBP (mm Hg)	101.80 ± 6.39	100.49 ± 5.57	1.64	.10
TC (mmol/L)	3.99 ± 0.79	4.15 ± 0.89	1.53	.13
TG (mmol/L)	1.42 ± 0.36	1.50 ± 0.45	1.58	.11
HDL-C (mmol/L)	1.22 ± 0.30	1.19 ± 0.24	0.89	.37
LDL-C (mmol/L)	2.38 ± 0.61	2.51 ± 1.00	1.27	.21
IMT (mm)	1.54 ± 0.24	1.48 ± 0.31	1.75	.08

BMI = body mass index, DBP = diastolic blood pressure, HDL-C = high-density lipoprotein cholesterol, IMT = intima-media thickness. LDL-C = low-density lipoprotein cholesterol, SBP = systolic blood pressure, TC = total cholesterol, TG = triglyceride.

3.2. Analysis of the effective rate of the 2 groups of patients in the treatment

First, we analyzed the treatment effect of the 2 groups, and found that the disease symptoms of the 2 groups were significantly improved after treatment and the difference was statistically significant ($P < .05$). In addition, we found that the marked efficiency in the combined treatment group (93.85%) was higher than that in the control group (84.62%) (χ^2 value = 8.65, $P < .05$) (Table 2).

3.3. Changes in blood pressure and blood lipid levels in the 2 groups

Furthermore, Student *t* test was used to analyze the blood pressure and blood lipids of the 2 groups before and after treatment.

Table 2

Analysis of the effective rate of the 2 groups of patients in the treatment.

Group	Ineffective	Effective	Significantly effective	Total effective rate
Control group (n = 130)	20 (15.38%)	48 (36.92%)	62 (47.69%)	110 (84.62%)
Treatment group (n = 130)	8 (6.15%)	40 (30.77%)	82 (63.08%)	122 (93.85%)
χ^2 value				8.65
P value				**.01**

We found that both groups could effectively improve the symptoms of hypertension. But as we expected, the improvement was more significant in the treatment group compared to the control group ($P < .05$). In the following detection of blood lipid indexes, we also found that although both groups improved after treatment, the effect of the treatment group was significantly better than that of the control group ($P < .05$). This indicated that the curative effect of amlodipine on hypertension and hyperlipidemia was significantly increased after the addition of amlodipine (all $P < .05$, Table 3).

3.4. The blood flow level and endothelial function of the 2 groups of patients before and after treatment

Vascular microcirculation and endothelial function run through the whole process of thrombus formation, plaque expansion, and lipid accumulation in hypertensive patients. Therefore, we used Student *t* test to analyze the blood flow level and endothelial function of the 2 groups of patients. Before treatment, there was no significant difference in the levels of Nbh, Nbl, Np and Fh between the 2 groups (all $P > .05$). Compared with before treatment, the levels of Nbh, Nbl, Np and Fh in the 2 groups were significantly decreased after treatment (all $P < .05$), and the combined group was significantly lower than that in the control group (all $P < .05$), as shown in Table 4. The detection of endothelial function showed that there was no significant difference in the levels of NO, H_2S and ICAM-1 between the 2 groups before treatment (all $P > .05$). Compared with before treatment, the levels of NO and H_2S in the 2 groups were significantly increased after treatment (both $P < .05$), and the treatment group was

Table 3

Changes in blood pressure and blood lipid levels in the 2 groups.

Category	Treatment	Control group (n = 130)	Treatment (n = 130)	*t* value	*P* value
SBP (mm Hg)	Before therapy	163.37 ± 14.85	161.51 ± 15.56	0.60	.55
	After therapy	130.66 ± 11.57*	119.74 ± 11.33*†	7.69	**.00**
DBP (mm Hg)	Before therapy	101.80 ± 6.39	100.49 ± 5.57	1.64	.10
	After therapy	78.22 ± 5.33*	73.24 ± 4.30*†	8.29	**.00**
TC (mmol/L)	Before therapy	3.99 ± 0.79	4.15 ± 0.89	1.53	.13
	After therapy	3.32 ± 0.30*	3.12 ± 0.23*†	6.03	**.00**
TG (mmol/L)	Before therapy	1.42 ± 0.36	1.50 ± 0.45	1.58	.11
	After therapy	1.23 ± 0.15*	1.03 ± 0.10*†	12.65	**.00**
HDL-C (mmol/L)	Before therapy	1.22 ± 0.30	1.19 ± 0.24	0.89	.37
	After therapy	1.19 ± 0.21*	1.35 ± 0.36*†	4.38	**.00**
LDL-C (mmol/L)	Before therapy	2.38 ± 0.61	2.51 ± 1.00	1.27	.21
	After therapy	2.12 ± 0.16*	1.73 ± 0.11*†	22.90	**.00**

DBP = diastolic blood pressure, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, SBP = systolic blood pressure, TC = total cholesterol, TG = triglyceride.
* versus Before therapy $P < .05$, † versus Control group $P < .05$.

Table 4

The blood flow level and endothelial function of the 2 groups of patients before and after treatment.

Category	Treatment	Control group (n = 130)	Treatment group (n = 130)	*t* value	*P* value
Nbh (mPa.S)	Before therapy	6.17 ± 1.23	6.21 ± 1.00	0.29	.77
	After therapy	5.80 ± 1.01*	5.18 ± 0.91*,†	5.20	**.00**
Nbl (mPa.S)	Before therapy	9.69 ± 1.33	9.72 ± 1.29	0.18	.85
	After therapy	8.70 ± 1.33*	7.38 ± 1.30*,†	8.09	**.00**
Np (mPa.S)	Before therapy	2.13 ± 0.31	2.15 ± 0.21	0.61	.54
	After therapy	1.92 ± 0.35*	1.49 ± 0.32*,†	10.34	**.00**
Fh (g/L)	Before therapy	3.95 ± 0.36	3.92 ± 0.43	0.61	.54
	After therapy	3.78 ± 0.25*	3.45 ± 0.18*,†	12.21	**.00**
NO (μmol/L)	Before therapy	84.92 ± 10.01	85.10 ± 11.02	0.14	.89
	After therapy	103.02 ± 12.21*	130.74 ± 11.76*,†	18.64	**.00**
H_2S (μmol/L)	Before therapy	24.92 ± 3.30	25.22 ± 2.24	0.86	.39
	After therapy	34.21 ± 3.01*	41.11 ± 3.36*,†	17.44	**.00**
ICAM-1 (μmol/L)	Before therapy	302.22 ± 38.32	300.05 ± 44.21	0.42	.67
	After therapy	251.52 ± 28.95*	208.16 ± 24.95*,†	12.94	**.00**

Fh = fibrin content, H_2S = hydrogen sulfide, ICAM-1 = intercellular cell adhesion molecule-1, Nbh = whole blood high shear viscosity, Nbl = whole blood low shear viscosity, NO = nitric oxide, Np = plasma specific viscosity.

* versus Before therapy $P < .05$, † versus Control group $P < .05$.

significantly higher than the control group (both $P < .05$); the levels of ICAM-1 in the 2 groups were significantly decreased, and the treatment group was significantly lower than the control group ($P < .05$), see Table 4.

3.5. Comparison of Treg/Th17 cell levels

Inflammatory responses caused by imbalanced Th17/Treg cell ratios play an important role in the progression of atherosclerosis, as found in studies. Therefore, we detected the expression and proportion of related cells in the peripheral blood of the 2 groups of patients after treatment, and found that there was no significant difference in the percentage of Th17 cells, the percentage of Treg cells and the ratio of Treg/Th17 between the treatment group and the control group before treatment ($P > .05$). After 6 months of treatment, the percentage of Th17 cells in the 2 groups decreased compared with those before treatment ($P < .05$), while the percentage of Treg cells and the ratio of Treg/Th17 increased ($P < .05$). The difference was statistically significant ($P < .05$). After 6 months of treatment, there were statistically significant differences in Th17 percentage, Treg percentage and Treg/Th17 between the treatment group and the control group ($P < .05$), as shown in Table 5.

At the same time, we detected the mRNA expression levels of ROR-γt (Th17-related gene) and Foxp3 (Treg-related gene) and found the same trend as above (Fig. 1). Taking these data together, we found that the combined treatment of lamidipine and atorvastatin significantly improved the Th17/Treg imbalance, which may be one of the reasons for its remarkable efficacy.

3.6. Effects of 2 treatments on inflammation levels

In order to test our hypothesis, we first examined the regulation effects of the 2 treatment groups on the expression of classical inflammatory factors and found that both groups could significantly inhibit the inflammatory response after treatment. However, the inhibitory effect of the treatment group was significantly better than that of the control group (all $P < .05$, Table 6).

Further, we detected Th17-related inflammatory factors and found that the treatment group could significantly reduce the expression of IL-17, IL-6, IL-23, and TNF-α, which was better than that of the control group (all $P < .05$, Table 7).

However, the detection of relative anti-inflammatory factors in Treg found that the elevated levels in the treatment group were also significantly higher than those in the control group (all $P < .05$, Table 8).

Table 5

Comparison of Treg/Th17 cell levels.

Category	Treatment	Control group (n = 130)	Treatment group (n = 130)	*t* value	*P* value
Th17 (%)	Before therapy	1.65 ± 0.53	1.61 ± 0.58	0.58	.56
	After therapy	1.49 ± 0.46*	1.28 ± 0.43*,†	3.80	**.00**
Treg (%)	Before therapy	4.48 ± 0.67	4.51 ± 0.78	0.33	.74
	After therapy	5.70 ± 0.58*	6.49 ± 0.72*,†	8.09	**.00**
Treg/Th17	Before therapy	3.20 ± 2.05	3.32 ± 1.83	0.50	.62
	After therapy	4.31 ± 1.84*	6.14 ± 4.93*,†	9.74	**.00**

Th17 = helper T cells 17, Treg = regulatory T cells.

* versus Before therapy $P < .05$, † versus Control group $P < .05$.

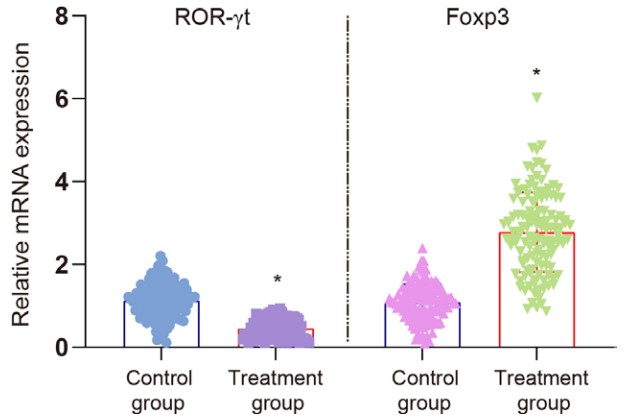

Figure 1. The mRNA expression levels of ROR-γt (Th17-related gene) and Foxp3 (Treg-related gene) in different treatment group. * versus Control group $P < .05$. Foxp3 = forkhead spiral transcription factor, mRNA = messenger ribonucleic acid, ROR-γt = retinoic acid receptor-related orphan receptor gamma, Th17 = helper T cells 17, Treg = regulatory T cells.

4. Discussion

This study investigated the efficacy of amlodipine/atorvastatin in combination with carotid atherosclerotic hypertension and the role of Th17/Treg dysfunction in a single-center randomized controlled clinical trial. Our data showed that: the efficacy of combination therapy was significantly higher than that of atorvastatin alone. Combination therapy can promote blood flow, improve microcirculation, and endothelial function.

Table 6

Expression levels of classical inflammatory factors.

Category	Treatment	Control group (n = 130)	Treatment group (n = 130)	*t* value	*P* value
IL-1β (pg/mL)	Before therapy	141.19 ± 74.79	139.29 ± 68.76	0.21	.83
	After therapy	68.46 ± 25.71*	45.18 ± 20.81*†	8.03	**.00**
IL-2 (pg/mL)	Before therapy	138.54 ± 56.24	139.25 ± 48.72	0.11	.91
	After therapy	58.70 ± 11.43*	47.38 ± 11.30*†	8.09	**.00**
IFN-γ (pg/mL)	Before therapy	43.35 ± 12.57	45.56 ± 12.65	1.41	.16
	After therapy	21.92 ± 5.35*	18.69 ± 3.22*†	5.90	**.00**
hsCRP (pg/mL)	Before therapy	11.95 ± 4.36	10.92 ± 5.65	1.65	.10
	After therapy	6.78 ± 1.25*	4.65 ± 1.00*†	15.17	**.00**
MCP-1 (pg/mL)	Before therapy	76.22 ± 19.52	77.66 ± 18.75	0.61	.54
	After therapy	33.68 ± 8.65*	30.45 ± 7.18*†	4.29	**.00**

hsCRP = high-sensitivity C-reactive protein, IFN = interferon, IL = interleukin, MCP = monocyte chemotactic protein.

* versus Before therapy $P < .05$, † versus Control group $P < .05$.

Table 7

Expression levels of Th17-related inflammatory factors.

Category	Treatment	Control group (n = 130)	Treatment group (n = 130)	*t* value	*P* value
IL-17 (pg/mL)	Before therapy	30.18 ± 11.23	28.21 ± 12.15	1.36	.18
	After therapy	21.80 ± 4.61*	18.18 ± 5.91*†	5.51	**.00**
IL-6 (pg/mL)	Before therapy	27.45 ± 10.33	28.72 ± 11.19	0.95	.34
	After therapy	20.70 ± 7.58*	17.28 ± 6.34*†	3.14	**.00**
IL-23 (pg/mL)	Before therapy	213.18 ± 76.46	218.51 ± 84.75	0.53	.59
	After therapy	111.72 ± 45.35*	97.69 ± 23.38*†	3.95	**.00**
TNF-α (pg/mL)	Before therapy	62.13 ± 21.22	61.38 ± 20.43	0.29	.77
	After therapy	43.78 ± 8.25*	32.65 ± 7.18*†	11.60	**.00**

IL = interleukin, Th17 = helper T cells 17, TNF = tumor necrosis factor.

* versus Before therapy $P < .05$, † versus Control group $P < .05$.

Table 8

Expression levels of Treg-related inflammatory factors.

Category	Treatment	Control group (n = 130)	Treatment group (n = 130)	*t* value	*P* value
IL-10 (pg/mL)	Before therapy	11.17 ± 5.23	12.21 ± 6.00	1.49	.14
	After therapy	14.80 ± 3.61*	17.22 ± 3.58*†	5.43	**.00**
TGF-β1 (pg/mL)	Before therapy	896.73 ± 182.76	932.75 ± 193.29	1.54	.12
	After therapy	988.70 ± 172.57*	1035.38 ± 203.45*†	2.00	**.04**

IL = interleukin, TGF = transforming growth receptor, Treg = regulatory T cells.

* versus Before therapy $P < .05$, † versus Control group $P < .05$.

Combination therapy can significantly improve Th17/Treg imbalance and inhibit inflammatory response. Our data provide a new clinical option for the treatment of hypertensive patients with carotid atherosclerosis. Hypertension is a common clinical cardiovascular disease, which is closely related to the dysfunction of vascular endothelial cells.[27] It is accompanied by the decline of endothelial relaxation and contraction regulation, which is easy to cause endothelial cell damage, while vascular endothelial cell dysfunction can cause capillary dysfunction.[28] The decrease in density can lead to myocardial ischemia in severe cases, which in turn leads to the occurrence of other symptoms.[29] Studies have shown that the factors that trigger high blood pressure mainly include family inheritance, high mental stress, unreasonable diet, long-term smoking and drinking, and excessive intake of high-sugar and high-fat substances.[29] Atherosclerosis refers to the thickening of the arterial wall and the lack of corresponding elasticity, which is mainly characterized by the deposition of lipids in the subintima of the carotid artery, accompanied by the proliferation of smooth muscle cells and the proliferation of fibrous matrix components, thereby gradually forming carotid atherosclerosis.[30,31] Hardening, eventually manifesting as plaques. Hypertension complicated with atherosclerosis is a systemic manifestation that seriously affects the health of patients.

It is well known that both hypertension and dyslipidemia share common mechanisms leading to atherothrombosis. Atorvastatin is a clinically commonly used lipid-lowering drug for the treatment of cardiovascular and cerebrovascular diseases. It inhibits the synthesis of cholesterol and at the same time increases the expression of LDL receptors in the liver cell membrane, reduces the levels of cholesterol and lipoprotein, and increases liver LDL uptake and catabolism are increased by the number of cell surface LDL receptors.[10] Amlodipine is a dihydropyridine calcium channel antagonist, which can effectively inhibit the transfer of calcium ions into cardiomyocytes and smooth muscle cells. Our results showed that the combined treatment group after adding amlodipine was significantly better than the single treatment group in improving blood pressure, blood lipids, and overall disease efficacy. In addition, the combination group also significantly improved the patient's blood flow level and endothelial function.[20–23] They effectively improved the patient's microcirculation, which may be one of the reasons for its improved efficacy.

There is currently evidence that amlodipine can improve the immune level of patients with some diseases.[20–23] However, inflammatory responses are involved in the formation of atherosclerotic plaques.[32] The expression levels of hs-CRP and classical inflammatory factors can reflect the fragility of plaques and the degree of inflammation, and can be used as important indicators of the occurrence and development of the disease.[33] Our results showed that the anti-inflammatory effect of the combined treatment group was significantly better than that of atorvastatin alone. In addition, this combination also significantly improved Th17/Treg dysfunction. Th17 cells and Treg cells are a group of T lymphocyte subsets that regulate and inhibit each other.[34] Th17 cells are named for their specific secretion of IL-17, and they can also secrete other inflammatory mediators such as IL-22, which play a major role in promoting atherosclerosis.[35] IL-17 can recruit neutrophils to aggregate to the site of inflammation, and promote the secretion of TNF-α, IL-1β, IL-6 and other inflammatory factors by mononuclear macrophages. Fibroblasts stimulate the secretion of IL-8, IL-6, matrix metalloprotinase-3, and monocyte chemotactic protein-1 through natural killer-κB and mitogen-activated protein kinase pathways, and participate in the inflammatory response of atherosclerosis.[36] Treg cells are a kind of negative regulating immune cells, which have 2 functions of immune function and immune suppression, and play an important role in maintaining the normal immune function of the body and preventing excessive immune activation. Treg cells can inhibit the progression of atherosclerosis by secreting negative inflammatory factors such as transforming growth receptor-β and IL-10, downregulating the expression levels of the above-mentioned inflammatory mediators. Both animal experiments and human studies have shown that there is an imbalance in the ratio of Th17/Treg cells in patients with atherosclerosis, which is positively correlated with the degree of lesions.[37] At present, the role of Th cells in immune system regulation has been paid attention to in neuropathic and autoimmune diseases.[38–41] However, the role of Th17 and Treg cells in cardiovascular diseases and their influence on the current therapeutic mechanism are still scarce. And that's one of the innovations of our research. Our results show that the combined treatment group can significantly improve the imbalance of Th17/Treg, which provides a new idea for the combined treatment of amlodipine and atorvastatin in patients with hypertension and carotid atherosclerosis. However, our study has some limitations. We still lack relevant factorial design data to explore the effect form after the combination of the 2 drugs. In addition, we still need multi-center data to confirm the results of this study at multiple levels and combine relevant molecular biology experiments to provide a basis for the development of new therapeutics such as targeted therapy.

5. Conclusion

Taken together, our data suggest that amlodipine combined with atorvastatin can ameliorate Th17/Treg imbalance and improve vascular endothelial function in patients with hypertension and atherosclerosis. By increasing the therapeutic effect after the combination of drugs, this discovery will be conducive to the subsequent promotion and development of new therapies.

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