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Beneficial clinical effects of grape seed proanthocyanidin extract on the progression of carotid atherosclerotic plaques

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Abstract

Background Atherosclerotic plaques indicate the occurrence of ischemia events and it is a difficult task for clinical physicians. Grape seed proanthocyanidin extract (GSPE) has been reported to exert an antiatherogenic effect by inducing regression of atherosclerotic plaques in animal experimental studies. In this study, the antiatherogenic effect of GSPE has been investigated in clinical use. **Methods** Consecutive 287 patients diagnosed with asymptomatic carotid plaques or abnormal plaque free carotid intima-media thickness (CIMT) were randomly assigned to the GSPE group (n = 146) or control group (n = 141). The patients in the GSPE group received GSPE 200 mg per day orally, while patients in the control group were only enrolled in a lifestyle intervention program. Carotid ultrasound examination was performed at baseline and 6, 12, 24 months during follow-up. Mean maximum CIMT (MMCIMT), plaque score, echogenicity of plaques and ischemic vascular events were recorded. **Results** As anticipated, after treatment, GSPE resulted in significant reduction in MMCIMT progression (4.2% decrease after six months, 4.9% decrease after 12 months and 5.8% decrease after 24 months) and plaque score (10.9% decrease after six months, 24.1% decrease after 12 months and 33.1% decrease after 24 months) for the primary outcome, while MMCIMT and plaque score were stable and even increased with the time going on in control group. The number of plaques and unstable plaques also decreased after treatment of GSPE. Furthermore, the carotid plaque can disappear after treatment with GSPE. The incidence rate for transitory ischemic attack (TIA), arterial revascularization procedure, and hospital readmission for unstable angina in GSPE group were statistically significant lower (P = 0.02, 0.08, 0.002, respectively) compared with the control group. **Conclusions** GSPE inhibited the progression of MMCIMT and reduced carotid plaque size in GSPE treated patients, and with extended treatment, the superior efficacy on MMCIMT and carotid plaque occurred. Furthermore, the GSPE group showed lower rates of clinical vascular events.

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Keywords: Atherosclerosis; Carotid plaque; Grape seed proanthocyanidin extract

1 Introduction

Atherosclerosis is the main underlying cause of ischemic vascular diseases and is predicted to become the leading cause of global mortality by 2050.[1,2] Atherosclerosis is a systemic disease and the carotid atherosclerosis may reflect atherosclerosis elsewhere in the body, including coronary artery, cerebral artery, renal artery and so on.[3] Studies have shown that carotid plaque, particularly thick plaque and irregular plaque, is a risk factor for vascular events, especially stroke.[4,5] So, early detection and intervention with atherosclerosis is crucial, for it can significantly reduce the incidence, morbidity and mortality of the ischemic vascular disease.

The 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins), as a part of the conservative treatment of patients with carotid atherosclerosis, has been cited by the National Cholesterol Education Project.[6] However, according to guidelines recommendations, only when the lipid level was beyond the normal level, is lipid-lowering therapy with statins recommended. Studies reported that a clinically significant proportion of patients with the multiple lipid measurement in the normal range had serious coronary atherosclerosis and plaque formation by coronary angiography.[7] Moreover, the dispute on the adverse effects of statins is becoming more and more drastic. How to deal with this question is a difficult task for clinical physicians.

Researchers paid more attention to this issue. Yamakoshi, *et al*.[8] found that grape seed proanthocyanidin extract (GSPE) can significantly reduce atherosclerotic plaque for-

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mation in the wall of the aortic arch and the thoracic aorta using the New Zealand rabbit atherosclerosis model, so, it was reasoned that GSPE may provide a new means in the prevention and treatment of atherosclerosis. A series of studies were conducted using GSPE to demonstrate its cardioprotective ability in animals.[8–10] GSPE can protect endothelial function, resist myocardial oxidative stress damage, inhibit low density lipoprotein oxidation and anti-inflammation. Additionally, our previous animal experiment has demonstrated that GSPE can slow down the development of atherosclerotic plaques. However, clinical trials about the effect of GSPE on atherosclerotic plaques have not been reported. In this study, we examined the effect of GSPE on carotid intima-media thickness (CIMT) and carotid plaque in clinical use.

2 Methods

2.1 Patients

This research was designed as an open-label, single-center, prospective randomized controlled study over 24 months. Between October 2008 and June 2011, patients were enrolled with diagnosed asymptomatic carotid artery plaques or abnormal CIMT based on carotid ultrasonography performed at the B-mode ultrasonic room of Qilu Hospital of Shandong University.

Basic entry criteria for the study were as follows: (1) carotid ultrasound examination: presence of carotid plaque or abnormal CIMT of between 0.9 and 1.2 mm; (2) lipid profile: low density lipoprotein (LDL) ≤ 3.12 mmol/L, total cholesterol (TC) ≤ 5.2 mmol/L; (3) no lipid-lowering treatment within the past six months. Exclusion criteria included severe cardiomyopathy, acute coronary syndrome, hepatic dysfunction, end-stage renal failure (serum creatinine ≥ 117 mmol/L), prior carotid endarterectomy, and/or patients who did not agree to participate in the present study.

Finally, a sample of 287 patients was enrolled in the study. All subjects provided written informed consent before enrollment and the study protocol was approved by the institutional ethics committee.

2.2 Treatment

Eligible participants were randomized to either control group (n = 141) or GSPE group (n = 146) by using random, permuted blocks within strata. In the control group, all patients were enrolled in a lifestyle intervention including dietary modification permitting a total ingestion of calories by standard body weight of 25 kCal and an individualized home exercise program of a minimum 150 min per week of moderate-intensity physical activity. The patients in the GSPE group received oligomeric proanthocyanidin 200 mg per day (100 mg bid) in addition to the same lifestyle intervention. The patients in GSPE group took two GSPE capsules twice a day. One kind of GSPE capsule made in Tianjin Jianfeng Natural Product Research and Development Company contains 50 mg oligomeric proanthocyanidin.

2.3 Carotid B-mode ultrasound

High-resolution B-mode ultrasonography of carotid artery was performed with an ultrasound scanner (iE33, Philips) equipped with a linear array 5-MHz to 7-MHz transducer. The ultrasound procedure has been described in previous studies.[3,5,6,11]

The patients were examined in the supine position, with their head turned slightly to the contra-lateral side. The common, internal, and external carotid arteries were carefully identified by combining B-mode ultrasonography and color-Doppler duplex examination in the anterior oblique, lateral, and posterior oblique planes. All data were collected in 12 segments: the near (intimal-luminal surface) and far (medial- adventitial) walls of the distal common (1 cm proximal to dilation of the carotid bulb), the bifurcation (1cm proximal to the flow divider), and the proximal internal (1 cm section of the internal carotid artery immediately distal to the flow divider) left and right carotid artery.

Carotid ultrasound examination was performed at baseline and 6, 12, 24 months after treatment. Each ultrasound examination was performed as an independent study. All carotid ultrasound scans were performed by a single trained and certified sonographer, without knowledge of the previous results and subjects' clinical information. At each visit, three single measurements of CIMT were carried out aimed at recording the maximum CIMT for each of the 12 carotid artery segments. Mean maximum CIMT (MMCIMT), defined as the average of the segment maximum CIMTs across the 12 carotid arterial segments, was used for further statistical analysis. The presence of an atherosclerotic plaque was determined as a localized protrusion of the vessel wall into the lumen with the thickness >1.2 mm. The maximum carotid plaque thickness (MCPT, millimeters) was the greatest actual axial thickness of the plaque and was analyzed in the form of plaque score which was the sum of MCPT of all plaques. Plaque echogenicity was characterized according to the criteria of the European Carotid Plaque Study Group.[12] Echogenicity was classified as strong (echo-rich), intermediate, or weak (low echogenicity or echolucency). In our study, we defined the weak and intermediate echogenic plaque as the vulnerable plaque and the strong echogenic plaque was considered as stable plaque.

2.4 Study end point

The predefined primary end point of this study was the

change of MMCIMT, plaque score, and stability of plaques. The stability of carotid plaque is presented in the form of percentage of vulnerable plaque which is the number of vulnerable plaque divided by total number of carotid plaque. Additional end point included a composite of clinical vascular events during 24 months follow-up, including hospital readmission for unstable angina, myocardial infarction, stroke, TIA, an arterial revascularization procedure (percutaneous coronary revascularization or coronary bypass surgery) and cardiac death.

2.5 Statistical analysis

Numerical data are expressed as mean ± SD. The significance of the differences in various parameters within each group between baseline and after treatment was tested via analysis of variance for repeated measurement data. The significance of the differences between groups in these parameters was tested by an unpaired Student's *t*-test. Categorical data were summarized as percentages and compared using Chi-square analysis. All analyses were carried out by SPSS 15.0 software. All *P* values were two-tailed and statistical significance was set at $P < 0.05$.

3 Results

3.1 Baseline clinical characteristics

Baseline clinical characteristics of the patients are shown in Table 1. All 287 patients completed the protocol. No noteworthy adverse effects were reported. There was no significant difference in all baseline demographic parameters between the two groups (Table 1). In total, 67 patients with no ultrasound evidence of atherosclerotic plaque had only abnormal CIMT (control group: $n = 36$, GSPE group: $n = 31$) in this study.

3.2 GSPE inhibited the progression of MMCIMT

As shown in Figure 1, after treatment, GSPE resulted in significant reduction in MMCIMT progression. Six months after treatment, MMCIMT decreased by 0.047 ± 0.026 mm in GSPE group ($P = 0.32$) while MMCIMT remained stable in the control group (increased by 0.008 ± 0.014 mm, $P = 0.42$). GSPE induced progressive MMCIMT regression over 12 months in GSPE group (reduction of 0.056 ± 0.037 mm, $P < 0.05$) and a continued reduction can also be observed after treatment of 24 months (reduction of 0.066 ± 0.028 mm, $P < 0.01$). However, in the control group, MMCIMT increased by 0.018 ± 0.016 mm after 12 months and 0.045 ± 0.021 mm after 24 months ($P < 0.05$).

At the start of the study, there were not significantly differences in MMCIMT values between the two groups as

Table 1. Demographics and baseline characteristics of 287 patients.

	GSPE group ($n = 146$)	Control group ($n = 141$)	*P*
Age (yr)	69.7 ± 15.0	72.9 ± 10.9	0.14
Men	68 (69.4%)	66 (70.2%)	0.88
BMI	23.58 ± 2.96	24.01 ± 3.12	0.16
Systolic BP (mmHg)	132.39 ± 16.76	131.23 ± 17.61	0.509
Serology (mmol/L)			
TG	1.66 ± 0.72	1.68 ± 0.45	0.87
TC	5.02 ± 0.47	4.96 ± 0.62	0.24
LDL-C	2.64 ± 0.56	2.67 ± 0.76	0.56
HDL-C	1.43 ± 0.42	1.41 ± 0.54	0.237
Carotid ultrasound			
Total number of plaque (n)	186	193	/
Unstable plaque	154 (82.8%)	149 (77.2%)	0.17
MMCIMT (mm)	1.132 ± 0.281	1.120 ± 0.324	0.61
Plaque score (mm)	3.557 ± 0.512	3.593 ± 0.147	0.26
Combined disease			
Diabete mellitus	32 (21.9%)	28 (19. 9%)	0.67
Hypertension	25 (17.1%)	31 (22.0%)	0.30

Values presented as mean ± SD or n (%) unless other indicated. Baseline characteristics were similar and well-balanced between treatment groups. BMI: body mass index; BP: blood pressure; GSPE: grape seed proanthocyanidin extract; HDL-C: high density lipoprotein-cholesterol; LDL-C: low density lipoprotein-cholesterol; MMCIMT: mean maximum carotid intima-media thickness; TC: total cholesterol; TG: triglyceride.

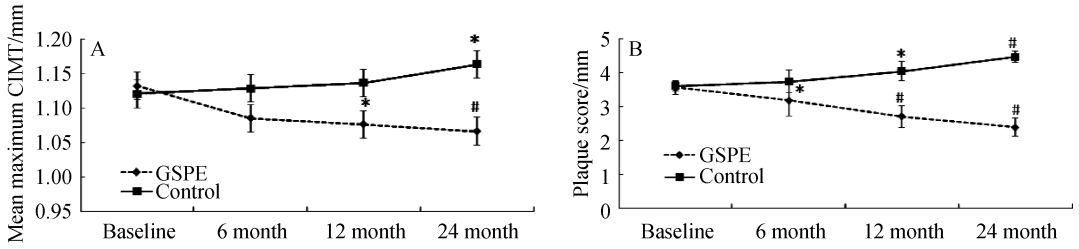

Figure 1. Compared with baseline within groups. *$P < 0.05$, #$P < 0.01$. Serial mean maximum CIMT (A) and plaque score (B) measurements at baseline and 6, 12 and 24 months during treatment with GSPE 240 mg per day or control group. CIMT: carotid intima-media thickness; GSPE: grape seed proanthocyanidin extract.

shown in Figure 2, while at six month follow-up, significant differences of MMCIMT between the groups were observed, and with long time treatment, the differences become more pronounced ($P < 0.01$ at the 12th and 24th month).

3.3 GSPE attenuated development of carotid plaques

In Figure 1, the plaque score measurements at 6, 12 and 24 months for the two experimental groups are shown in detail. As expected, GSPE resulted in a significant reduction in plaque score. After treatment of six months, the plaque score had a statistically significant reduction of 0.386 mm in GSPE group ($P < 0.05$) and had a continued regression with time (reduction of 0.856 mm, $P < 0.01$ at the 12th month; reduction of 1.178 mm, $P < 0.001$ at the 24th month). However, in the control group, the plaque score was stable after six months and showed a statistically significant increase after 12 and also 24 months (increase of 0.442, 0.864 mm; $P < 0.05$, $P < 0.01$, respectively, for the 12th and 24th month). The plaque thickness progressively decreased in the GSPE group, which was significantly different from the results in the control group (Figure 2).

The percentage of vulnerable plaque was similar between groups at baseline (Table 1, $P = 0.17$). Six months after treatment, the total number of plaque decreased by 13 and the number of unstable plaques decreased by 22 in the GSPE group, while the two parameters remained stable in control group. After treatment of 12 months, the change in the GSPE group compared with baseline was statistically different. In the control group, the total number of plaque increased by 21 and the number of unstable plaque increased by 18. After treatment of 24 months, 39 plaques disappeared and unstable plaques reduced by 54 in the GSPE group; and in the control group, the total number of plaque increased by 38 and the number of unstable plaque increased by 33, the difference became significantly greater between groups (Figure 3).

Figure 4 shows the representative high-resolution B-mode ultrasound images of carotid plaques, indicating the effects of GSPE on carotid atherosclerotic plaques. The thickness of plaque was shown by an arrow in the regular B-mode image of carotid atheroma. Images A, B, C and D represent the regression process of the same carotid plaque of one person at different times. The thickness of the plaque in image A, B and C was 3.13 mm, 2.33 mm and 1.73 mm, respectively, while the plaque disappear after 24-month treatment. The CIMT of carotid artery is 0.8 mm in image

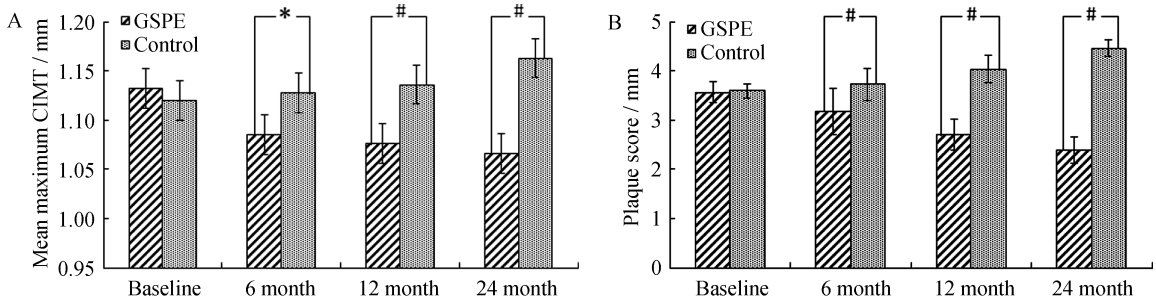

Figure 2. Compared between groups at the same time point. *$P < 0.05$, #$P < 0.01$. Histogram shows distribution of serial mean maximum CIMT (A) and plaque score (B) comparison between groups at baseline and 6, 12 and 24 months. CIMT: carotid intima-media thickness; GSPE: grape seed proanthocyanidin extract.

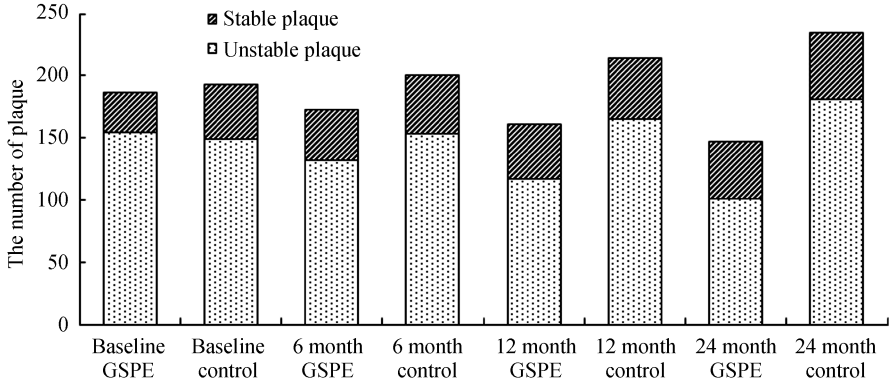

Figure 3. Histogram shows the change of number of total carotid plaques and unstable carotid plaques. GSPE: grape seed proanthocyanidin extract.

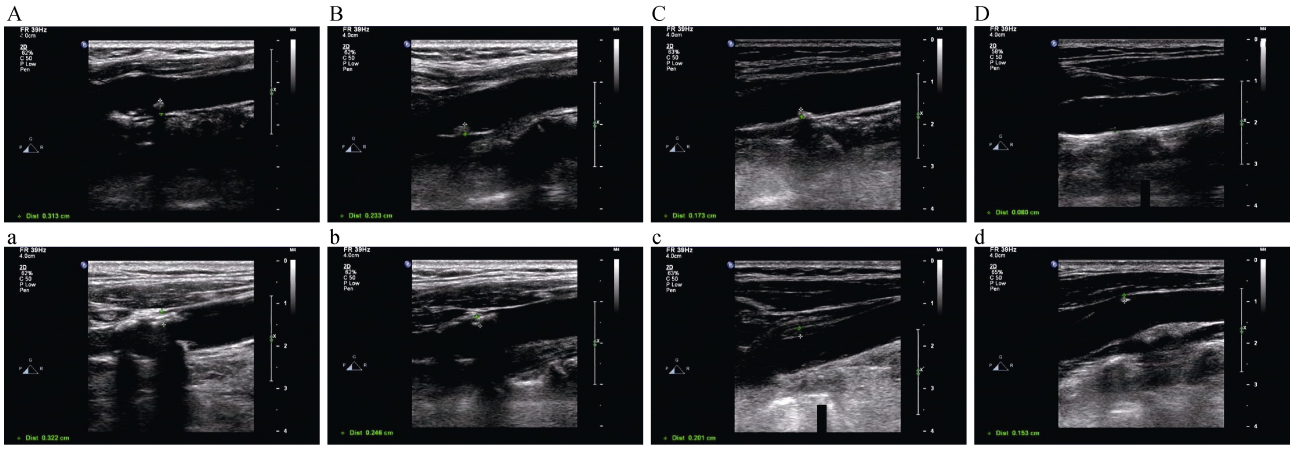

Figure 4. The representative high-resolution B-mode ultrasound images of carotid plaques. Images A, B, C and D represent the regression process of the same carotid plaque of one person at different times. Images a, b, c and d represent the regression process of another person. (A/a: baseline, B/b: 6 months, C/c: 12 months, D/d: 24 months).

D. Images a, b, c and d represent the regression processes of another person, the thickness of the plaque in image a, b, c and d was 3.22 mm, 2.46 mm, 2.01 mm and 1.53 mm, respectively. The thickness of the plaque became increasingly smaller with the time going on after treatment of GSPE.

3.4 GSPE decreased ischemic vascular events

After a 24-month follow-up, 50 patients suffered from ischemic vascular events (17.4%) and no patients suffered from cardiac death in any group (Table 2). As shown in Table 2, the incidence rate of myocardial infarction and stroke in GSPE group is not statistically different from that in the control group (P = 0.54 in both). The incidence rate for TIA, arterial revascularization procedure and hospital readmission for unstable angina in GSPE group were statistically significant low (P = 0.02, 0.08, 0.002, respectively, for comparison between groups) compared with control group. After treatment with GSPE, the incidence of ischemic vascular events statistically significantly decreased.

Table 2. The major clinical vascular events after treatment of 24 months.

	Cardiac death	Myocardial infarction	Stroke	TIA	Rehospitalization for unstable angina	An arterial revascularization procedure	Total events
GSPE	0	1 (9.09%)	1 (9.09%)	3 (27.27%)	4 (36.36%)	2 (18.18%)	11
Control	0	2 (5.13%)	2 (5.13%)	11 (28.21%)	17 (43.59%)	7 (17.59%)	39
P*	/	0.54	0.54	0.02	0.002	0.08	< 0.001

Data are presented as n or n (%). *GSPE group *vs.* control group. The percentage refers to an event in the proportion of all events. GSPE: grape seed proanthocyanidin extract; TIA: transitory ischemic attack.

4 Discussion

Atherosclerosis is the important pathologic basis of cerebral- and cardio-vascular disease. Atherosclerosis, and it's devastating complications of cerebral and myocardial infarction and gangrene of the extremities, is the leading cause of death worldwide.[13] It is reported that there are more than 20 million people experience a sudden cardiac event (acute coronary syndromes and/or sudden cardiac death) every year, and a large portion of this population has no prior symptom.[14] Drugs are the foundation of treatment for atherosclerotic diseases, which can prevent the occurrence of restenosis after interventional therapy and the new generation of narrowing in other parts. However, the clinical use of statins is recommended for the patients with abnormal lipid profile. And the reports on the adverse effects of statins, especially liver damage, is becoming more drastic. The urgent demand for prevention and treatment of atherosclerosis remains unresolved, especially in the significant proportion of patients who had serious atherosclerosis and plaque formation with multiple lipid measurement in the normal range.

It is well established that atherosclerosis is a bland proliferative process.[15] Endothelial denuding injury led to platelet aggregation and release of platelet-derived growth factor that would trigger the proliferation of smooth muscle cells in the arterial intima, and form the nidus of the athero-

sclerotic plaque. Extensive atherosclerosis may be associated with increased blood thrombogenicity, and vulnerable plaques are much more thrombogenic than stable ones. Previous studies have showed that proanthocyanidin inhibited thrombogenesis *in vivo* and inhibited ADP-induced platelet aggregation and arterial thrombus formation, enhance platelet-derived NO release, modulate the activity of some enzymes systems including cyclooxygenase and lipooxygenase and decrease superoxide production.[16,17] In our study, GSPE showed a great effect on plaque stability, the mechanism may involve direct protective effect on platelets and blood vessels.

In previous animal experiments and clinical trials, GSPE have been reported to possess a variety of potent properties including anti-oxidant, anti-inflammation, anti-tumor and so on.[8–10,16–19] A series of experiments confirmed that the antioxidant activity of GSPE is 20 times greater than vitamin C, 50 times that of vitamin E and the antioxidant activity is closely related to the effect of scavenging free radicals and inhibiting enzymes activity.[19] Recent research has shown that OX-LDL was related to the development of atherosclerotic cardiovascular disease. Several animal experiments show that GSPE can inhibit LDL oxidative modification.[9] One study of GSPE on diabetic rats also showed that GSPE increased the expression of catalase and partly contributed to reducing the aortic lesions in diabetes mellitus (DM), supported that GSPE can prevent aortic atherosclerosis from developing in a hamster atherosclerosis model,[10] observations similar to our results.

In this study, we choose MMCIMT and plaque score as the ultrasound indices to observe the effect of the intervention of GSPE on atherosclerosis and predict the incidence rate of ischemic vascular events. To determinate the lesion properties and degree, carotid artery ultrasound provide a simple, non-invasive, cost-effective and easily reproducible approach for visualizing and quantifying atherosclerotic lesions. On the same hand, there was evidence which proved that the mean maximum estimate was a more precise estimate of an individual's atherosclerosis or cardiovascular risk status.[20,21] Separate characterization of plaque and IMT is prudent in order to derive better information on vascular risk.[22] In present study, we observed that GSPE could statistically decrease plaque thickness after treatment of six months, and with the long time treatment, the superior efficacy on carotid plaque occurred.

Furthermore, the composition of the plaque is one of the key factors affecting the risk of ischemic events in patients with carotid atherosclerosis. Research suggested plaque stabilization is a promising clinical strategy to prevent cardiovascular complications in patients with established atherosclerosis disease.[21,23] In our study, after 24-month treatment, there was no further increase in plaque in the GSPE group, but also unstable plaques reduced to fifty-four and thirty-nine plaques disappear.

This is the first report of the effects of GSPE on carotid plaque and CIMT, and it presents a valuable method to prevent the progression of atherosclerotic plaque and protect the patients without high risks. One important point should also be mentioned, that in our study, vascular events significantly decreased compared with the control group after 24-month treatment with GSPE. Meanwhile, no noteworthy adverse effects were reported.

4.1 Conclusions

GSPE can inhibit the development of CIMT, regress the carotid plaque and promote the stabilization of carotid plaque, and as the treatment of prolonged, the anti-atherosclerotic effect of GSPE becomes more apparent. Finally, after 24-month treatment, GSPE can reduce the cardiovascular event significantly. In the light of our results, we can speculate that GSPE could be an effective therapeutic candidate for the primary prevention programs for the asymptomatic patient with atherosclerotic lesion. Future *in vivo* studies are required to investigate the mechanism.

4.2 Limitations

The small number of enrolled patients is the first limitation of the present study. Previous studies of the relationship between plaque growth and cardiovascular risk factors have required thousands of patients,[24] but the statistical power of our study is relatively low. The second limitation is that we could not determine the mechanism of plaque regression. A longitudinal prospective study in a larger number of patients would be required to show an improved outcome.

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