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Original Article

Celastrol may have an anti-atherosclerosis effect in a rabbit experimental carotid atherosclerosis model

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Abstract: Background: Celastrol may have an anti-atherosclerosis effect. This study aimed to investigate if celastrol had an anti-AS effect using a rabbit experimental carotid atherosclerosis model. Methods: Forty male Japanese white rabbits were divided into the sham group (normal diet), the model group (high fat diet), the group treated with celastrol (high fat diet) and the group treated with atorvastatin (high fat diet) randomly. The rabbits fed a high fat diet underwent balloon injury of the right common carotid artery and were treated with dimethyl sulfoxide (DMSO) (the model group, 3.5 ml/kg/d), celastrol and its dissolvent DMSO (the celastrol group, 1 mg/kg/d and 3.5 ml/kg/d) and atorvastatin and its dissolvent DMSO (the atorvastatin group, 2.5 mg/kg/d and 3.5 ml/kg/d) for 12 weeks by gavage. Results: The ratio of the plaque area and the arterial wall cross-section area in the celastrol group was significantly less than the model group ($P < 0.001$), and there was no significant difference compared with the atorvastatin group. The serum level of LDL-C of the celastrol group was significantly lower than the model group ($P = 0.014$), and there was no significant difference compared with the atorvastatin group. The expression of VEGF in the celastrol group was significantly less compared with the model group ($P = 0.014$), whereas the expression of VEGF in the atorvastatin group and the model group showed no significant differences. Conclusion: Our findings suggest that celastrol effectively reduced the plaque ratio, decreased the serum levels of LDL and downregulated the expression of VEGF, suggesting an anti-AS effect of celastrol.

Keywords: Celastrol, carotid artery disease, balloon injury, low-density lipoprotein cholesterol, vascular endothelial growth factor

Introduction

Cerebrovascular disease (70% ischemic stroke) has become one of the leading causes of morbidity and mortality in China and has an increasing incidence each [1], which may be caused by several factors, including the uncontrolled risk factors, the aging population, the unhealthy lifestyles and the unknown complicated pathologies of stroke. According to the guidelines for the primary prevention of stroke, there are many well-documented and modifiable risk factors for stroke. Of the modifiable risk factors, the presence of an atherosclerotic stenotic lesion in the extracranial internal carotid artery or carotid bulb has a close association with the incidence and recurrence of ischemic stroke [2]. Carotid atherosclerosis (CAS) can lead to a direct infarction at the narrowest part of the constricted vessel, or an unstable plaque can have an unpredictable sudden breakage, rupture, fissure or ulceration that leads to platelet activation, thrombosis, shedding and the obstruction of the distal vasculature [3].

It is well accepted that atherosclerosis (AS) is a multifocal, smoldering, chronic immunoinflammatory disease whose pathogenesis involves imbalanced lipid metabolism and a maladaptive immune response leading to a chronic inflammation of the arterial wall [4], in which endothelial cells, leukocytes and intimal smooth muscle cells are the major cellulous players [5]. Low-density lipoprotein (LDL) [6], C-reactive

protein (CRP) [4], matrix metalloproteinases (MMPs) [7] and vascular endothelial growth factor (VEGF) [8] are considered to be some important molecular players.

Tripterygium wilfordii is a medicinal plant that has been used in the treatment of inflammatory and rheumatic diseases for thousands of years in China because of the plant's profound effects on detumescence, acesodyne, activating blood circulation and removing stasis [9]. Celastrol, one of the effective constituents of tripterygium wilfordii, has been identified to inhibit the function of proteasomes, to activate the heat shock response and to have several molecular targets that are mostly centered on the inhibition of IKK-NF-κB signaling [10]. Several studies have demonstrated that celastrol has anti-cancer and anti-inflammatory activities and can inhibit platelet activation [11]. The anti-inflammatory activities of celastrol and the inhibition of platelet activation by celastrol might limit the occurrence and progression of AS. Celastrol might inhibit the atherogenic migration of vascular smooth muscle cells (VSMC) within atherosclerotic lesions [12] and could inhibit vasculogenesis by suppressing the expression of VEGF in some other systems such as the locomotor system [13], which delays the progression of AS and maintains the stability of the AS plaque. Few studies have demonstrated the anti-AS effects of celastrol using an animal experimental CAS model.

It is widely approved that a high-cholesterol diet after the balloon injury of carotid arteries is one of the ways to form a rabbit CAS model. Thus, we focus on whether celastrol has an anti-AS effect using this rabbit experimental CAS model.

Materials and methods

Animals

This animal study was approved by the Animal Care and Use Committee of Wenzhou Medical College. All animal care was in accordance with the "Guide for the Care and Use of Laboratory Animals" (Office of Science and Health Reports CPRR/NIH 1996). Forty Japanese white rabbits aged four months and weighing between 2.0 to 2.5 kg were provided by and housed at the Experimental Animal Center of Wenzhou Medical College (Wenzhou, China). A high fat diet containing 2% cholesterol and 6% peanut was produced by the Jiangsu Province Synergistic Biological Engineering (Nanjing, China).

Grouping and modeling

Forty Japanese white rabbits were divided into the sham group, the model group, the group treated with celastrol and the group treated with atorvastatin using the simple random method. During the entire experiment, the rabbits in the sham group were fed a regular diet (120 g per day) and the others were fed a high fat diet (120 g per day). All of the rabbits were given free access to water.

At the end of the first week, after being anesthetized with sodium pentobarbital (22.5 mg/kg, intravenous injection), the rabbits fed a high fat diet underwent balloon injury of the right common carotid artery according to the previously described method [14]. The 3.0-mm balloon catheter was gently inflated and retracted, and it was pulled repeatedly three times in each rabbit. The rabbits in the sham group underwent the other steps of the operation except for the balloon injury. A post-operation muscular injection of ampicillin [50 mg/(kg*d), five days] was given to prevent infection.

After the operation, the rabbits were treated with dimethyl sulfoxide (DMSO) [the model group, 3.5 ml/(kg*d), Chinese agent of Sigma-Aldrich, Shanghai, China], celastrol and its dissolvent DMSO [the celastrol group, 1 mg/(kg*d) and 3.5 ml/(kg*d), Pi & Pi Technology Inc, Guangzhou, China] and atorvastatin and its dissolvent DMSO [the atorvastatin group, 2.5 mg/(kg*d) and 3.5 ml/(kg*d), Dalian Factory of Pfizer Inc, Dalian, China], respectively for 12 weeks by gavage.

Histology and immunohistochemistry

At the end of week 13, after the blood collection, all of the rabbits were sacrificed by an overdose of intravenous sodium pentobarbital. The right common carotid arteries were quickly removed and transferred to 10% formalin fixation. Each specimen was embedded in paraffin and was sectioned into intermittent uniform 5-μm thick cross-sections for hematoxylin-eosin (HE) or immunohistochemical staining.

The HE stained specimens were observed with an inverted fluorescence microscope (Leica,

Table 1. Serum levels of several risk factors for atherosclerosis at week 0[a]

Factors	Groups				*F*	P_0
	Sham (n = 9)	Model (n = 9)	Celastrol (n = 8)	Atorvastatin (n = 10)		
FBG (mmol/L)	5.73 ± 1.59	6.11 ± 1.18	6.03 ± 1.14	5.97 ± 0.92	0.157	0.924
TC (mmol/L)	1.24 ± 0.33	1.28 ± 0.41	1.13 ± 0.37	1.38 ± 0.32	0.744	0.534
TG (mmol/L)	0.66 ± 0.20	0.65 ± 0.22	0.70 ± 0.21	0.75 ± 0.28	0.325	0.807
HDL-C (mmol/L)	0.43 ± 0.19	0.43 ± 0.17	0.38 ± 0.10	0.45 ± 0.11	0.300	0.825
LDL-C (mmol/L)	0.41 ± 0.16	0.45 ± 0.19	0.40 ± 0.14	0.46 ± 0.14	0.358	0.784
CRP (ng/mL)	7.75 ± 2.19	6.87 ± 1.94	7.59 ± 2.11	7.26 ± 2.06	0.313	0.816
MMP9 (ng/mL)	41.7 ± 10.7	39.5 ± 11.2	38.4 ± 10.5	43.6 ± 12.0	0.382	0.766

[a]The data are given as the mean ± SD. No significant difference was found among these groups. FBG, fasting blood glucose; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CRP, C-reactive protein; MMP9, matrix metalloproteinase 9.

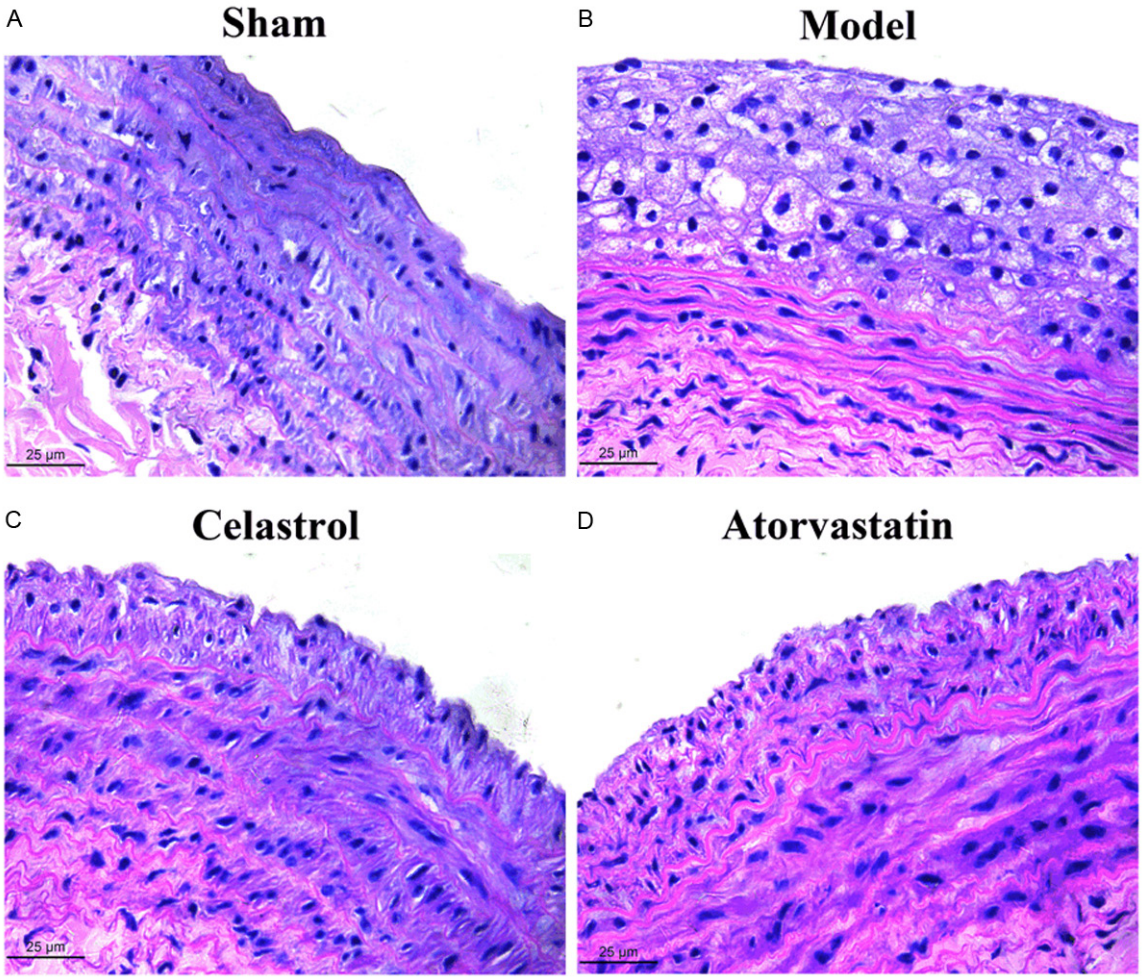

Figure 1. Histological Changes of Carotid Aortic AS plaque in Rabbits (HE staining, ×400). Notes: A: Sham group; B: Model group; C: Celastrol group; D: Atorvastatin group.

Germany). After the morphological observation of AS lesions, the sizes of the plaques and the arterial wall cross-sections were calculated using the Image-Pro® Plus (IPP) software.

Immunostaining was performed with the primary antibody of a mouse monoclonal antibody against rabbit VEGF [Anti-VEGF antibody (JH1210), distributor of Abcam, Shanghai,

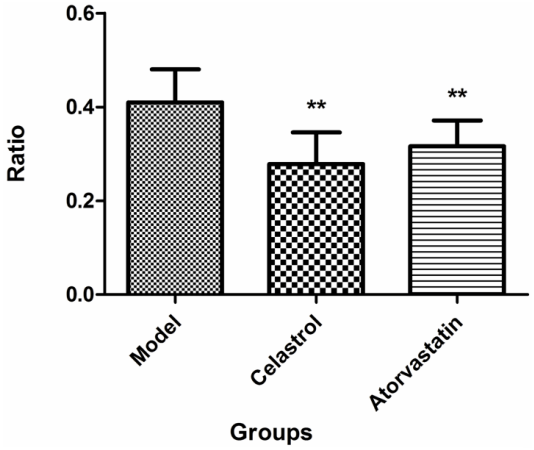

Figure 2. The ratio of the plaque area and the arterial wall cross-section area. **Compared with model group $P < 0.01$.

China] and the mouse specific HRP/DAB (ABC) Detection IHC Kit (distributor of Abcam, Shanghai, China) according to the manufacturer's protocol. The mean optical density (MOD) for VEGF (the brown-yellow granular area) in the plaque area was calculated using the IPP software.

The operators and recorders of the histology and immunohistochemistry were blinded to the grouping information of each sample.

Blood collection and ELISA

Blood samples were obtained through an ear vein at week 0 and obtained again through the heart before sacrifice. All of the rabbits fasted overnight before the collection. The fasting blood glucose (FBG), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured in the clinical laboratory of our hospital, and the serum levels of CRP and MMP9 were measured by the enzyme-linked immunosorbent assay (ELISA) using the ELISA kit for rabbits (Uscn Life Science Inc, Houston, USA), according to the manufacturer's protocol.

Statistical analysis

The statistical analysis was performed using the SPSS18.0 software package. The data are presented as the mean ± standard deviation (SD). ANOVA with a LSD post-hoc test among sub-groups was used to compare the measurement data between the four groups. A P value of less than 0.05 was considered statistically significant.

Results

From weeks 0 to 13, four rabbits were excluded from the study for failing to survive to week 13. These rabbits did not show signs of trauma, paralysis or infection and were considered to have experienced normal death.

There were no statistically significant differences among the sham group, the model group, the group treated with celastrol and the group treated with atorvastatin in the serum levels of FBG, TC, TG, HDL-C, LDL-C, CRP and MMP9 at week 0, as summarized in **Table 1**.

At the end of week 13, the pathological examinations showed that the sham group had no AS plaques, no foam cells and no lipidoses, and the model group had typical AS, including AS plaque formation, lipidoses, foam cell deposition, inflammatory cell infiltration. The two treatment groups had AS plaques. There were some differences among these groups in terms of plaque morphology, including a smaller AS plaque area, less lipidoses, less foam cell deposition, less inflammatory cell infiltration (**Figure 1**). The ratio of the plaque area and the arterial wall cross-section area in the celastrol group was significantly less than the model group ($P < 0.001$, **Figure 2**), and there was no significant difference between the celastrol group and the atorvastatin group on the ratio of the plaque area and the arterial wall cross-section area ($P = 0.227$, **Figure 2**). Compared with the model group, the severity of the lesions in the treatment groups was lighter.

Compared with the sham group, the model group and the two treatment groups had higher serum levels of TC, TG, HDL-C, LDL-C, CRP at the end of week 13 (**Table 2**). The serum levels of LDL-C and TC of the celastrol group were significantly lower than the model group ($P = 0.014$ and < 0.001, respectively, **Table 2**), and there were no significant difference in the serum levels of LDL-C and TC between the celastrol group and the atorvastatin group ($P = 0.578$ and 0.779, respectively, **Table 2**).

The immunohistochemical and imaging analyses showed that the sham group had no plaque, so the positive area percentage could not be

Table 2. Serum levels of several risk factors for atherosclerosis at the end of week 13[a]

Factors	Groups				*F*	P_0
	Sham (n = 9)	Model (n = 9)	Celastrol (n = 8)	Atorvastatin (n = 10)		
FBG (mmol/L)	7.33 ± 2.64	7.23 ± 1.96	6.32 ± 3.31	6.81 ± 3.39	0.216	0.885
TC (mmol/L)	2.65 ± 0.34	37.48 ± 6.48**	26.6 ± 5.29$^{**,\Delta\Delta}$	27.24 ± 4.85$^{**,\Delta\Delta}$	84.4	< 0.001
TG (mmol/L)	1.96 ± 0.24	3.11 ± 0.31**	3.09 ± 0.30**	2.94 ± 0.34**	30.2	< 0.001
HDL-C (mmol/L)	0.46 ± 0.13	6.03 ± 1.80**	4.76 ± 1.80**	5.71 ± 1.56**	27.3	< 0.001
LDL-C (mmol/L)	0.44 ± 0.20	20.43 ± 6.65**	14.02 ± 4.62$^{**,\Delta}$	15.36 ± 5.89$^{**,\Delta}$	25.6	< 0.001
CRP (ng/mL)	7.27 ± 2.07	15.10 ± 3.07**	12.66 ± 4.07**	13.50 ± 3.35**	10.2	< 0.001
MMP-9 (ng/mL)	39.2 ± 10.2	39.9 ± 10.2	40.5 ± 11.6	45.7 ± 13.1	0.659	0.583

[a]The data are given as the mean ± SD. $^{**}P < 0.01$ compared with the Sham group. $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$ compared with the model group. No significant difference was found between the group treated with celastrol and the group treated with atorvastatin.

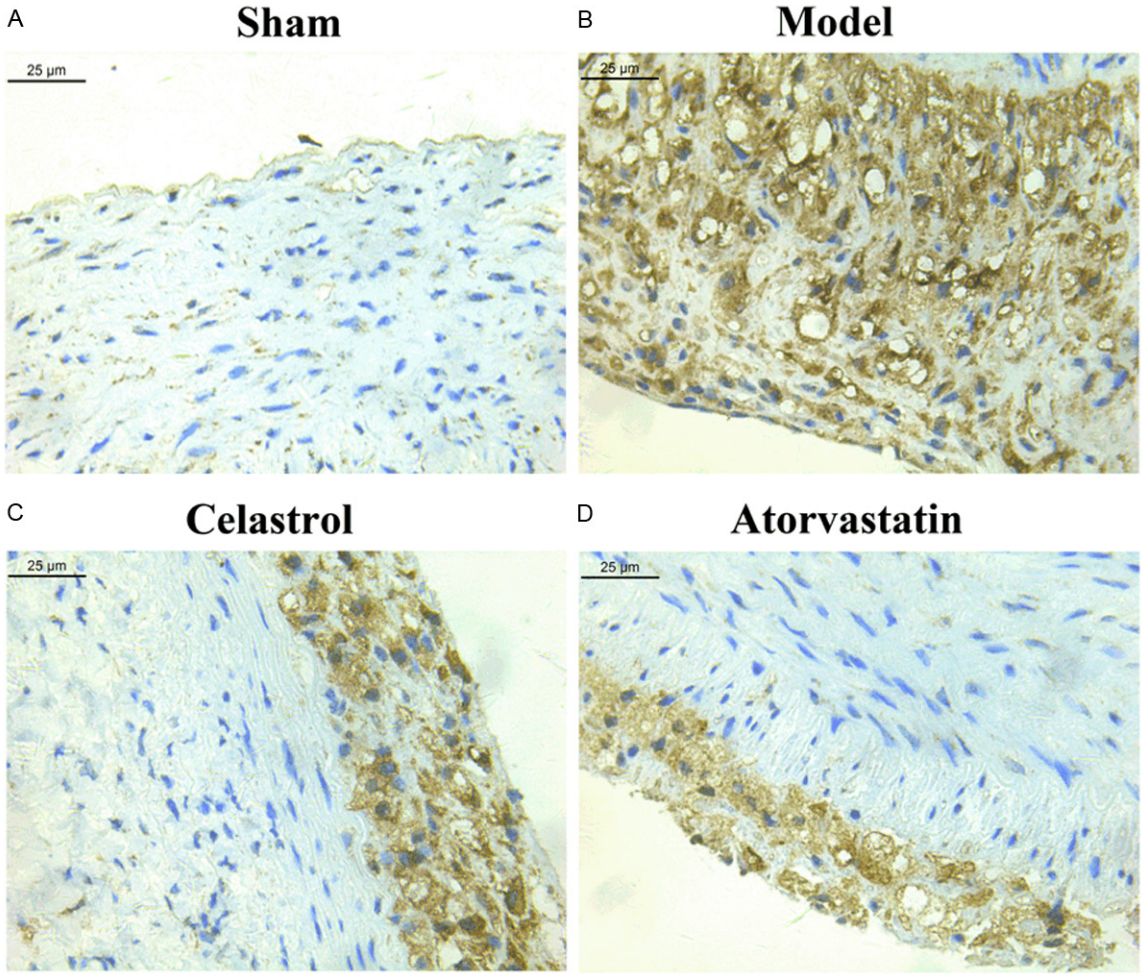

Figure 3. Expression of VEGF Positive Area in AS plaque (Immunohistochemical staining, ×400). Notes: A: Sham group; B: Model group; C: Celastrol group; D: Atorvastatin group.

calculated (**Figure 3**). The expression of VEGF in the celastrol group was significantly lower (P = 0.014, **Figure 4**) compared with the model group, and the expression of VEGF in the atorvastatin group and the model group showed no significant differences (P = 0.857, **Figure 4**).

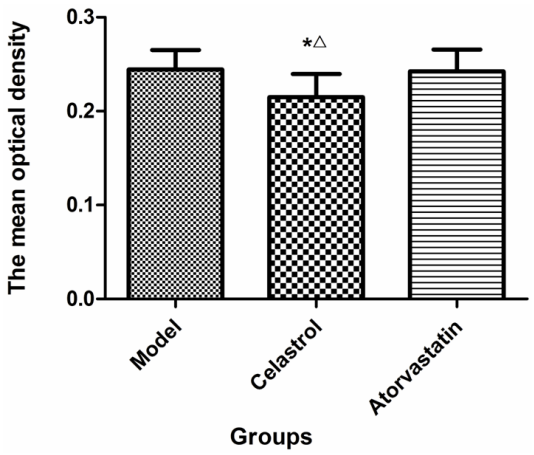

Figure 4. The mean optical density of three groups. *Compared with the model group $P < 0.05$, △Compared with the atorvastatin group $P < 0.05$.

Discussion

In the present study, we investigated the relationship between celastrol and CAS in a rabbit model. We found that celastrol effectively reduced the plaque size in experimental CAS and decreased the serum level of LDL and downregulated the expression of VEGF in CAS plaques.

Numerous studies have shown that CAS has a close relationship with ischemic stroke. CAS plaque rupture is the most frequent cause of arterial thrombosis, accounting for approximately 90% of thrombosed plaques in stroke patients [15]. CAS has a close relationship with atherosclerosis of the coronary arteries and acute coronary syndrome [16, 17]. Because atherosclerosis is a systemic condition [18], the severity of CAS represents the extent of systemic atherosclerosis to some degree. Inhibiting the occurrence and development of CAS reduces the morbidity and the recurrence rate of ischemic stroke and means suppressing systemic atherosclerosis, including atherosclerosis of the coronary arteries and acute coronary syndrome.

Celastrol has anti-inflammatory activities and could inhibit platelet activation, the migration of VSMC and the expression of VEGF, which might limit the occurrence and progression of AS. We investigated the relationship between celastrol and carotid atherosclerosis in rabbits receiving a high-cholesterol diet after the balloon injury of the carotid arteries. Our data show that compared with the model group, celastrol effectively reduced the plaque size ($P < 0.001$), and there was no statistical significance between the celastrol group and the atorvastatin group ($P = 0.227$), indicating that celastrol played a remedial role in CAS and had a similar function compared with atorvastatin, one of the statins that is used in the treatment of AS. The significant effect of atorvastatin on AS and atherosclerotic progression has been demonstrated by clinical trials and mainly depends on its cholesterol-lowering-dependent effects and anti-inflammatory actions [19, 20]. Our data show that the intervention of atorvastatin reduced the plaque size and significantly lowered the serum levels of lipids (TC and LDL-C mainly), which is consistent with existing studies.

It is universally acknowledged that LDL plays a central role in the pathogenesis of AS, and a high serum LDL level is a risk factor for AS [6]. Our data show that compared with the model group, celastrol decreased the serum levels of LDL ($P = 0.014$), which might partly explain the mechanism of how celastrol affects the pathogenesis of AS. According to Li GQ et al. [21], celastrol suppresses NF-κB-mediated MMP9 expression in rheumatoid fibroblast-like synoviocytes, which indicates that celastrol could suppress the expression of MMP9. MMP9 is a key proteinase that plays a central pathologic role in stroke by degrading ECM substrates. The degradation of ECM substrates causes a loss of ECM components, leading to the thinning of a plaque's fibrous cap, exposing the lipid-filled core, making the plaque more unstable [22]. Our data shows no significant difference in the serum level of MMP9 between the model group and the celastrol group.

The expression of VEGF is significantly increased in AS plaques, especially in the foam cells, and plays an important role in the development of the early stage of AS. The enhanced expression of VEGF is at least associated with hypoxia and inflammation, the two processes that AS is subject to [8]. The angiogenesis in AS plaques, the migration of vascular smooth muscle cells and the regulation of fatty acid uptake into endothelial cells mediated by VEGF contribute to atherogenesis. VEGF-induced vasculogenesis could be inhibited by celastrol's effect on angiogenesis and metastasis of

tumor, indicating celastrol's suppressive action on VEGF [13, 23]. Our data show that the VEGF expression in CAS plaque was suppressed by celastrol, which enhances the evidence of celastrol's anti-AS action and presents a new way to explain the mechanism.

We investigated the relationship between celastrol and CAS in a rabbit balloon injury model. Our findings suggest that celastrol effectively reduced plaque size, decreased the serum levels of LDL and downregulated the expression of VEGF in CAS plaque in experimental CAS, which suggested an anti-AS effect of celastrol. Our study has some limitations, including the few biomarkers studied, the limited sample size and the unclear mechanism of celastrol. More studies are needed to focus on the association between celastrol and AS and to focus on its potential mechanism.

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Disclosure of conflict of interest

None.

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References

[1] Neurology Committee of the Chinese Medical Association for cerebrovascular disease secondary prevention guidelines for ischemic stroke. Chinese secondary prevention guidelines for ischemic stroke and transient ischemic attacks 2010. Chin J Neurol 2010; 43: 154-160.

[2] Goldstein LB, Bushnell CD, Adams RJ, Appel LJ, Braun LT, Chaturvedi S, Creager MA, Culebras A, Eckel RH, Hart RG, Hinchey JA, Howard VJ, Jauch EC, Levine SR, Meschia JF, Moore WS, Nixon JV, Pearson TA. Guidelines for the primary prevention of stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. Stroke 2011; 42: 517-584.

[3] Fisher M, Paganini H, Martin A, Cosgrove M, Toole JF, Barnett HJ, Norris J. Carotid Plaque Pathology: thrombosis. Ulceration, and stroke Pathogenesis. Stroke 2005; 36: 253-257.

[4] Weber C, Noels H. Atherosclerosis: current pathogenesis and therapeutic options. Nat Med 2011; 17: 1410-1422.

[5] Falk E. Pathogenesis of atherosclerosis. J Am Coll Cardiol 2006; 47: C7-C12.

[6] Galkina E, Ley K. Immune and inflammatory mechanisms of atherosclerosis. Annu Rev Immunol 2009; 27: 165-197.

[7] Moore KJ, Tabas I. Macrophages in the pathogenesis of atherosclerosis. Cell 2011; 145: 341-355.

[8] Greenberg DA, Jin K. Vascular endothelial growth factors (VEGFs) and stroke. Cell Mol Life Sci 2013; 70: 1753-61.

[9] Liu Z, Ma L, Zhou GB. The main anticancer bullets of the Chinese medicinal herb, thunder god vine. Molecules 2011; 16: 5283-5297.

[10] Salminen A, Lehtonen M, Paimela T, Kaarniranta K. Celastrol: Molecular targets of Thunder God Vine. Biochem Biophys Res Commun 2010; 394: 439-442.

[11] Hu HY, Straub A, Tian Z, Bassler N, Cheng J, Peter K. Celastrol, a triterpene extracted from Tripterygium wilfordii Hook F, inhibits platelet activation. Cardiovasc Pharmacol 2009; 54: 240-250.

[12] Kang SW, Kim MS, Kim HS, Kim Y, Shin D, Park JH, Kang YE. Celastrol attenuates adipokine resistin-associated matrix interaction and migration of vascular smooth muscle cells. J Cell Biochem 2013; 114: 398-408.

[13] Huang S, Tang Y, Cai XD, Peng XS, Liu XM, Zhang LJ, Xiang YC, Wang DC, Wang X, Pan T. Celastrol inhibits vasculogenesis by suppressing the VEGF-induced functional activity of bone marrow-derived endothelial progenitor cells. Biochem Biophys Res Commun 2012; 423: 467-472.

[14] Meng L, Lv B, Zhang S, Yv B. In vivo optical coherence tomography of experimental thrombosis in a rabbit carotid model. Heart 2008; 94: 777-80.

[15] Spagnoli LG, Mauriello A, Sangiorgi G, Fratoni S, Bonanno E, Schwartz RS, Piepgras DG, Pistolese R, Ippoliti A, Holmes DR. Extracranial thrombotically active carotid plaque as a risk factor for ischemic stroke. J Am Med Assoc 2004; 292: 1845-1852.

[16] Shetty S, George P, Venkatesha BM, Alva J. A study to correlate carotid intima thickness by B-mode ultrasonography in patients documented with coronary artery disease. Heart Views 2011; 12: 157-160.
[17] Wang Q, Zeng Y, Wang Y, Cai JM, Cai YQ, Ma L, Xu X. Comparison of carotid arterial morphology and plaque composition between patients with acute coronary syndrome and stable coronary artery disease: a high-resolution magnetic resonance imaging study. Int J Cardiovasc Imaging 2011; 27: 715-726.
[18] Jashari F, Ibrahimi P, Nicoll R, Bajraktari G, Wester P, Henein MY. Coronary and carotid atherosclerosis: Similarities and differences. Atherosclerosis 2013; 227: 193-200.
[19] Bybee KA, Lee JH, O'Keefe JH. Cumulative clinical trial data on atorvastatin for reducing cardiovascular events: the clinical impact of atorvastatin. Curr Med Res Opin 2008; 24: 1217-1229.
[20] Rubba P. Effects of atorvastatin on the different phases of atherogenesis. Drugs 2007; 67: 17-27.
[21] Li GQ, Zhang Y, Liu D, Qian YY, Zhang H, Guo SY, Sunagawa M, Hisamitsu T, Liu YQ. Celastrol inhibits interleukin-17A-stimulated rheumatoid fibroblast-like synoviocyte migration and invasion through suppression of NF-κB-mediated matrix metalloproteinase-9 expression. Int Immunopharmacol 2012; 14: 422-431.
[22] Golledge J, Siew DA. Identifying the carotid 'high risk' plaque: is it still a riddle wrapped up in an enigma? Eur J Vasc Endovasc Surg 2008; 35: 2-8.
[23] Huang L, Zhang Z, Zhang S, Ren JH, Zhang RG, Zeng H, Li QF, Wu G. Inhibitory action of Celastrol on hypoxia-mediated angiogenesis and metastasis via the HIF-1α pathway. Int J Mol Med 2011; 27: 407-415.