doi.org/10.36721/PJPS.2024.37.4.REG.777-784.1

Effects of atorvastatin and ezetimibe on CD147, HIF-1, MMP-2 and VEGF in carotid atherosclerotic plaque under the guidance of IVUS

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Abstract: Atherosclerosis (AS), as the main pathophysiological basis of coronary heart disease, can develop into carotid atherosclerotic plaque (CAP) through intimal inflammation, necrosis, fibrosis and calcification. However, there are few reports on the clinical drug selection of CAP. The aim of this study was to explore the effects of atorvastatin and ezetimibe on CD147, HIF-1, MMP-2 and VEGF in CAP under the guidance of IVUS, so as to provide basis for CAP of the best drug. 32 male New Zealand rabbits were divided into the control group, the model group, the atorvastatin group and the ezetimibe group randomly. The levels of serum LDL-C and MMP-2 have a significant decrease in atorvastatin group and ezetimibe group ($P<0.05$). The level of serum CD147 has a significant decrease in ezetimibe group ($P<0.05$). The average OD value of HIF-1 in atorvastatin group decreased significantly ($P<0.05$). The relative expression of CD147 and VEGF decreased significantly in atorvastatin group ($P<0.05$). There were different degrees of fibrous plaque and lipid plaque in model group, atorvastatin group and ezetimibe group. There exists a significant decline of CD147, HIF-1, MMP-2 and VEGF by atorvastatin in plaque, but the effect of ezetimibe is not obvious.

Keywords: Atorvastatin, ezetimib, atherosclerosis, inflammation, fibrosis.

INTRODUCTION

Coronary atherosclerotic heart disease is regard as a kind of most common cardiovascular disease, as coronary heart disease (CHD), that causes lumen stenosis caused by the formation of coronary atherosclerotic plaque or acute thrombosis secondary to plaque rupture and erosion, resulting in insufficient myocardial blood supply and eventually clinical symptoms, including acute coronary syndrome (ACS) as well as stable coronary heart disease (SCAD). ACS has a critical onset and is the first cause of death in the world (Vedanthan et al., 1975). As the main pathophysiological basis of CHD, atherosclerosis (AS) is a lipvvoprotein driven disease, which eventually develops into atherosclerotic plaque through intimal inflammation, necrosis, fibrosis and calcification.

Abundant researches have confirmed that extra cellular matrix metalloproteinase inducer (EMMPRIN/ CD147) and its induced matrix metalloproteinase (MMP) emphasized in the process of atherosclerosis, including from the beginning of atherosclerosis to the formation of obvious plaque, plaque rupture and thrombosis (Newby, 2008; Schmidt, 2008; Wang, 2016). The existence of hypoxia in plaque and caused to the high expression of hypoxia inducible factor (HIF) in macrophage aggregation area can generate endothelial cell dysfunction, angiogenesis, proliferation and inflammation through a variety of ways (Sluimer, 2008; Khan, 2018). As a cytokine promoting angiogenesis, HIF regulated vascular endothelial growth factor (VEGF) is considered to be related to neovascularization in atherosclerotic plaque Macrophage aggregation and plaque hemorrhage are closely related. It was found that CD147 can regulate VEGF receptor (VEGFR) system through paracrine and promote angiogenesis through direct effect on endothelial cells (Bougatef, 2009).

Atorvastatin is an inhibitor for hydroxymethylglutaryl coenzyme A (CoA) reductase, which can not only reduce low density lipoprotein cholesterol (LDL-C), triglyceride (TG), anti-inflammation and improve endothelial function (Schonbeck, 2004), but also animal experiments have found that it may down regulate the expression of CD147 through COX-2/PGE2 pathway and reduce plaque vulnerability (Liang, 2017). Ezetimibe, as an inhibitor of intestinal cholesterol absorption, has been widely used in clinic and can evade the risk of atherosclerotic cardiovascular disease (ASCVD) (Tsujita, 2015).

We established a rabbit carotid atherosclerotic plaque model in this study to explore the expression of HIF-1, CD147, VEGF and MMP-2 in atherosclerotic plaque, to probe the effects of ezetimibe and atorvastatin on the expression of various proteins and to analyze the correlation between CD147 and the expression of HIF-1, VEGF and MMP-2 in plaque, to further understand the occurrence and development mechanism of coronary atherosclerotic plaque and provide new ideas for clinical treatment of ASCVD.

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MATERIALS AND METHODS

Main experimental materials and reagents

32 ordinary grade 3 months age male New Zealand rabbits, with an average weight of (2.9±0.2) kg and they were purchased from Beijing fangyuanyuan experimental animal farm. Production license: SCXK (Jing) 2014-0012 and Ethics committee approval No. 201721. The rabbits were kept in the Experimental Animal Center of Peking University First Hospital and could eat and drink freely.

Automatic biochemical analyzer (XR220 Pluse) was from XinRui medical equipment Technology Co., Ltd. Rabbit anti-HIF-1 polyclonal antibody, mouse anti-MMP-2 monoclonal antibody and mouse anti-VEGFA monoclonal antibody were from Abcam, USA. Anti-CD147 Antibodies was from Aviva Systems Biology, USA. Elisa kits for CD147, HIF-1α, VEGF and MMP-2 were from Beijing Biosco Biotechnology. Upright fluorescence microscope and heating system for paraffin embedding were from Leica, Germany. Ezetimibe was from MSD Pharmaceutical Co., Ltd., USA. Atorvastatin was from Pfizer Pharmaceuticals Co., Ltd., USA. High-fat diet was from Kee-Aid Feed Co., Ltd., Beijing [License No: SCXK (Jing) 2005-0007]. Intravascular ultrasound system H749iLab220C270 was from Boston Scientific, USA. DSA digital subtraction angiography machine was from SIEMENS, Germany. Runthrough guide wire was from Abbott Medical Devices, USA.

Model establishment and dosing method

Group A was fed with normal diet all the time and groups B, C and D were fed with high-fat diet (including 21% fat and 0.15% cholesterol). 4 weeks later, rabbits of groups B, C and D were received a right carotid artery liquid nitrogen frostbite surgery and the atheromatous plaque model was established by continuing high-fat diet until week 12. At the week 13, atorvastatin (2.5mg/kg·d) was given to the C group and ezetimibe (2.0mg/kg·d) to the group D until the week 16. Groups A and B were given with the same amount of saline.

Serum biochemical analysis

At the 1st, 12th and 16th weeks, 5mL blood was collected in the blood collection vessel through the ear vein and placed at the room temperature for 2 hours and then centrifuged at 3000 rpm last 20 min for the sake of the supernatant. The supernatant was reserved in the refrigerator at -80ºC for testing and the full-automatic biochemical analyzer was used to detect low density lipoprotein cholesterol (LDL-C).

Enzyme linked immunosorbent assay (ELISA) method

The levels of CD147, MMP-2, HIF-1 and VEGF in serum were measured at week 12 and 16. The antibodies were coated in 96 wells plate and the antibodies of different concentrations of 50μL standard samples were added to the micropores. No sample was added to the blank hole and 100μL was added to both the standard hole and the sample hole and incubated at 37ºC for 1 hour. Remove the sealing film, remove the coating liquid and dry, fill the washing liquid in each hole and discard after standing for half a minute. Repeat the above steps 5 times before drying. 50μL reagent A and B were added to each well in turn, mixed evenly by shaking and prevented from color development at 37ºC for 15 minutes. Add 50μL to each hole termination solution. The absorbance (OD 450 nm) of the sample was measured by enzyme calibrator. The ordinate is the standard concentration, and the abscissa is the OD value. Draw the standard curve, find the OD value of the sample according to the curve, and then multiply the dilution multiple to get the actual concentration of the samples.

Histopathological examination (HE) of carotid artery

After the arterial tissue was fixed by formaldehyde, and embedded, the cross section of the blood vessel was sectioned continuously for 4μm. The slices were put into water after conventional dew axing, successively staining with configured Weigert's iron hematoxylin, Ponceau red, aniline blue staining solution. Then the slices were dehydrated with ethanol, transparent with xylene and sealed with neutral gum. The pathological changes of the arterial tissue were observed under an optical microscope, and the relative area of carotid plaque (plaque area/lumen area) was calculated by image analysis software (Image Pro Plus 6.0). For samples of each tissue, the three slices with the largest plaque area (with a distance of 10 slices) were selected and the average value was used for statistical analysis.

Immunohistochemical (IHC) staining

Paraffin slices were baked for 1 hour. Then the slices were dew axed by xylene, dehydrated with gradient alcohol, repaired with citrate buffer at pH 6.0, soaked and washed with PBS immersion. After that, hybridization of primary and secondary antibodies, DAB coloration, hematoxylin counterstaining, dehydration, transparent and seals with neutral gum were carried out successively. 400x magnified images were collected and analyzed by image analysis software (as above). The mean optical density values (OD value, IOD/AREA) of positive expression for CD147, MMP-2, HIF-1α and VEGF were calculated for semi-quantitative analysis.

Western Blot analysis

20 mg of carotid tissue was weighed and put it into a centrifuge tube, adding 200ml of lysate, then the mixture homogenized on ice and lysed for 30min. After that, it was centrifuged at 12000 rpm last 15 min for the sake of the supernatant. Using BCA quantitative method to determine the protein concentration and the protein was prepared to 5mg/ml with protein loading buffer, loading 10ul per well. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel was prepared and

protein gel electrophoresis loading buffer with 5 times the volume of the sample was added according to the protein quantification result, then they were denatured at 95ºC for 10 min. After that, electrophoresis was performed at 8v/cm. The protein bands isolated on the gel were transferred wetly to the PVDF solid support and then PVDF solid support was incubated and detected with unlabeled primary antibody and horseradish peroxidase labeled secondary antibody. The image J image analysis system was used for gray scanning. The gray level ratios of CD147, MMP-2, HIF-1α, VEGF and internal reference β-actin were used as the relative expression of each target protein.

Carotid intravascular ultrasound (IVUS) examination

The DSA and the intravascular ultrasound system were opened, and the rabbit number was entered. Then the 4F puncture sheath was used to enter the femoral artery and the carotid angiography was finished under the 4F guide catheter. Runthrough was selected as the guide wire to connect the ultrasound catheter, then completing the image collection.

STATISTICAL METHODS

SPSS software 25.0 was used for statistical analysis. Blood lipid LDL-C, CD147, MMP-2, HIF-1 and VEGF levels were showed by $\bar{x}\pm s$ and all data were tested for normality. One-Way ANOVA was used to compare the mean of each group. Bonferroni was used for homogeneous variance, and Dunnett's T3 was used for uneven variance to compare. Pearson correlation analysis was used for bivariate correlation analysis. $P < 0.05$ was considered statistically significant.

RESULTS

Table 1 showed the blood lipid level of rabbits. The concentration of LDL-C in model group, atorvastatin group and ezetimibe group increased significantly at 12 weeks ($P<0.05$) and at 16 weeks, the concentration of LDL-C in atorvastatin group and ezetimibe group decreased significantly ($P<0.05$).

Table 2 and table 3 showed the concentrations of CD147, MMP-2, HIF-1 and VEGF in rabbits at week 12 and 16 by ELISA reagent method. At 12 weeks, the levels of MMP-2 in the model group, the atorvastatin group and the ezetimibe group increased significantly ($P<0.05$). At 16 weeks, the levels of MMP-2 in atorvastatin group and ezetimibe group decreased significantly ($P<0.05$) and the concentration of CD147 in ezetimibe group decreased significantly ($P<0.05$).

The results of HE staining (fig. 1) in carotid arteries showed smooth intima, no plaque formation and arranged neatly smooth muscle cells in the middle layer in the control group. In the model group, stable and unstable masses were observed and the intima was not smooth. The lumens were significantly narrowed, in which 2 rabbits had mild stenosis and 6 rabbits had moderate to severe stenosis, even occlusion. The proliferation of the medial smooth muscle cells was obvious and thrombus was found on the surface of the plaques in two rabbits. A thin fibrous cap, a large lipid core and a large number of macrophage infiltration were used to characterize the plaques, meanwhile in some plaques, immature small blood vessels could be seen. In the atorvastatin and ezetimibe group, typical stable plaques could be observed and the lumen is slightly narrowed. No obvious macrophage infiltration was observed in the plaques, and only a small amount of smooth muscle cells proliferated under partial plaque coverage.

Figs. 2-5 and table 4 showed that immunohistochemical staining in carotid tissues of rabbits in each group. Compared to the control group, the mean OD values of CD147, HIF-1α and MMP-2 in the model group increased significantly ($P<0.05$), while the mean OD value of VEGF had no statistical difference ($P>0.05$). Compared to the model group, the mean OD value of HIF-1α decreased significantly in the atorvastatin group ($P<0.05$), and the mean OD values of CD147, MMP-2, and VEGF had no statistical difference ($P>0.05$). In addition, there do not exist significant difference in the mean OD values of CD147, MMP-2, HIF-1α and VEGF in ezetimibe group ($P>0.05$).

The results of Western blot analysis were shown in table 5 and fig. 6-7 and the expressions of CD147, MMP-2, HIF-1α and VEGF in carotid tissue of each group and the correlation between the expressions of each protein. Compared to the control group, CD147, HIF-1α, MMP-2 and VEGF were expressed significantly increased in the model group ($P<0.05$). Compared to the model group, CD147 and VEGF were expressed significantly reduced in the atorvastatin group ($P<0.05$). According to the Pearson correlation analysis results, there exist a positive correlation between the expression of HIF-1α, MMP-2 and the expression of CD147 ($P<0.01$), as well as a positive correlation between the expression of HIF-1α and the expression of VEGF ($P<0.01$).

Fig. 8 showed Carotid IVUS imaging results that the arterial intima of the rabbits in the control group was smooth and no atherosclerotic plaque formation was observed, while fibrous plaques with various degrees were found in the model group, atorvastatin group and ezetimibe group. Types of plaques such as lipid plaques and calcified plaques did not show severe stenosis, plaque rupture, or thrombosis. No other types of plaques were observed, such as lipid plaques and calcified plaques and no serious stenosis, plaque rupture and thrombosis was observed.

Table 1: Comparison of LDL-C concentration in serum of rabbits (n=8, x±s)

Groups	Week 1st (mmol/L)	Week 12th (mmol/L)	Week 16th (mmol/L)
Control group	2.18±0.26	2.28±0.49	2.07±0.23
Model group	2.40±0.45	3.43±0.38[a]	3.25±0.44[a]
Atorvastatin group	2.29±0.29	3.67±0.46[a]	2.28±0.65[b]
Ezetimibe group	2.00±0.26	3.58±0.35[a]	2.73±0.25[b]

Table 2: Comparison of concentrations of CD147, MMP-2, HIF-1α and VEGF in serum of Rabbits at week 12 (n=8, x±s)

Group	CD147 (ng/ml)	MMP-2 (pg/ml)	HIF-1α (ng/ml)	VEGF (pg/ml)
Control group	57.58±5.88	3.63±1.16	2.30±0.21	50.32±8.91
Model group	58.63±4.92	7.62±1.34[a]	2.36±0.32	50.35±13.87
Atorvastatin group	61.90±2.58	7.57±1.49[a]	2.60±0.23	46.62±11.69
Ezetimibe group	54.77±6.81	6.83±1.40[a]	2.48±0.26	49.82±11.82

Table3: Comparison of concentrations of CD147, MMP-2, HIF-1α and VEGF in serum of Rabbits at week 16 (n=8, x±s)

Group	CD147 (ng/ml)	MMP-2 (pg/ml)	HIF-1α (ng/ml)	VEGF (pg/ml)
Control group	57.52±5.94	3.49±0.90	2.54±0.22	43.39±13.12
Model group	60.68±4.01	7.67±1.29[a]	2.39±0.38	48.36±10.13
Atorvastatin group	57.73±3.52	3.93±1.31[b]	2.47±0.30	51.61±8.61
Ezetimibegroup	53.20±5.45[b]	4.98±2.05[b]	2.44±0.31	44.61±9.07

Note: Compared with control group, [a] P<0.05; Compared with model group, [b] P<0.05.

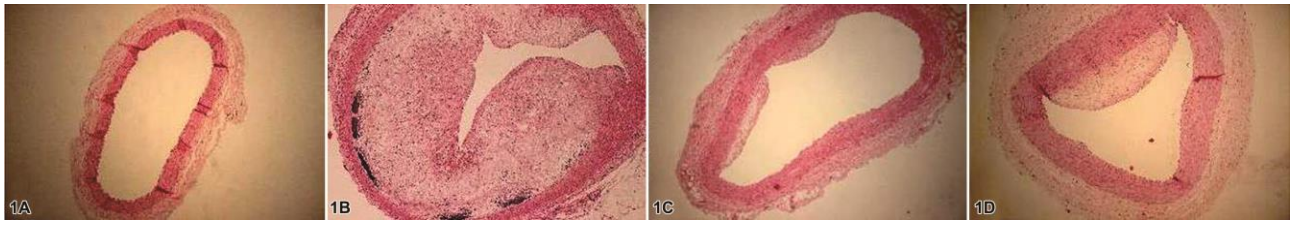
Table 4: Mean OD values of CD147, MMP-2, HIF-1α and VEGF in carotid arteries of rabbits in each group (n=8, x±s)

Group	CD147	HIF-1	MMP-2	VEGF
A	0.055±0.019	0.086±0.032	0.051±0.012	0.064±0.017
B	0.090±0.028[a]	0.140±0.044[a]	0.120±0.035[a]	0.056±0.024
C	0.080±0.021	0.095±0.027[b]	0.093±0.035	0.091±0.056
D	0.099±0.029	0.119±0.016	0.106±0.049	0.109±0.048

Table 5: Relative expression of CD147, MMP-2, HIF-1α and VEGF in carotid arteries of rabbits in each group (x±s)

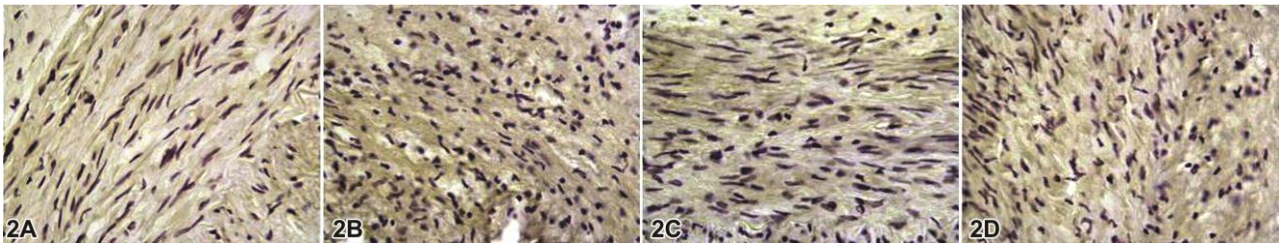
Group	CD147	MMP2	HIF-1α	VEGF
A	0.19±0.05	0.17±0.08	0.19±0.12	0.14±0.03
B	0.53±0.10[a]	0.48±0.21[a]	0.44±0.15[a]	0.43±0.10[a]
C	0.28±0.13[b]	0.29±0.11	0.32±0.17	0.29±0.03[b]
D	0.35±0.54	0.41±0.22	0.44±0.14	0.30±0.08

Note: A: the control group, B: the model group, C: the atorvastatin group, D: the ezetimibe group; Compared to the control group, [a] P < 0.05; Compared to model group, [b] P < 0.05.



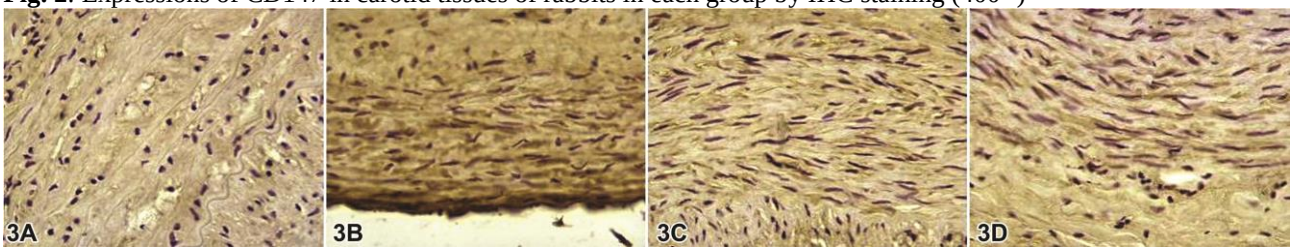
Note: Fig. 1A: control group, 1B: model group, 1C: atorvastatin group, 1D: ezetimibe group. Fig. 1A showed no plaque formation; fig. 1B showed atherosclerotic plaque formation and the lumen was almost occluded; the formation of atherosclerotic plaque was also observed in fig. 1C and 1D.

Fig. 1: HE staining results of carotid arteries in each group (100×)



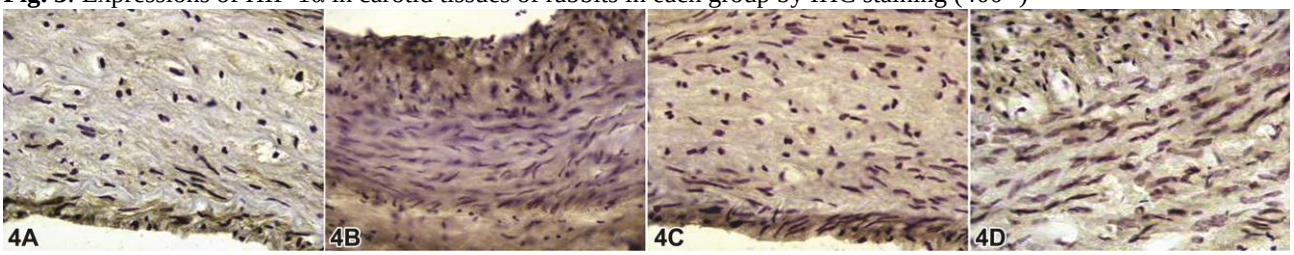
Note: Fig. 2A: control group, 2B: model group, 2C: atorvastatin group, 2D: ezetimibe group.

Fig. 2: Expressions of CD147 in carotid tissues of rabbits in each group by IHC staining (400×)



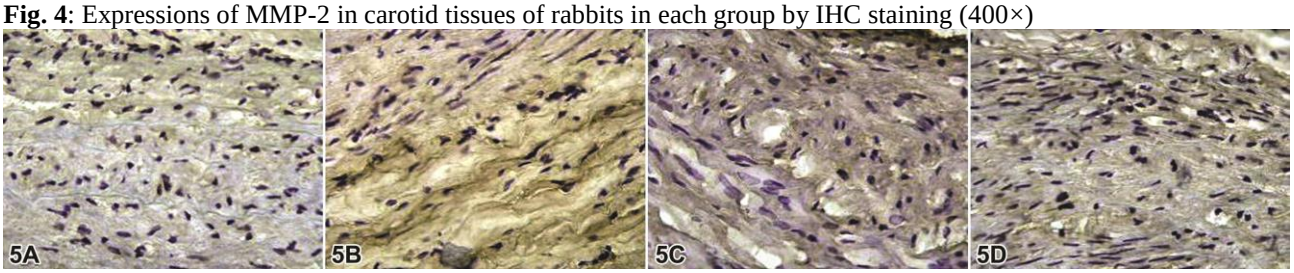
Note: Fig. 3A: control group, 3B: model group, 3C: atorvastatin group, 3D: ezetimibe group.

Fig. 3: Expressions of HIF-1α in carotid tissues of rabbits in each group by IHC staining (400×)



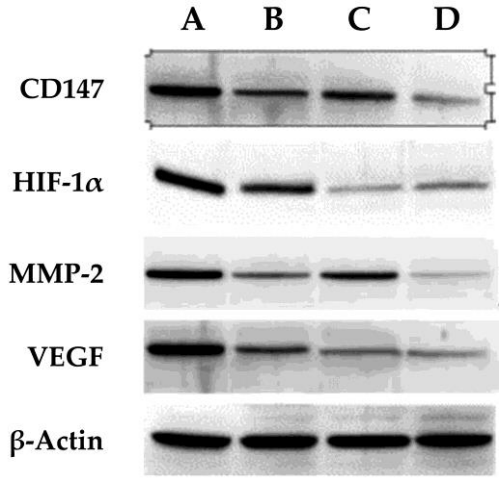
Note: Fig. 4A: control group, 4B: model group, 4C: atorvastatin group, 4D ezetimibe group.

Fig. 4: Expressions of MMP-2 in carotid tissues of rabbits in each group by IHC staining (400×)



Note: Fig. 5A: control group, 5B: model group, 5C: atorvastatin group, 5D ezetimibe group.

Fig. 5: Expressions of VEGF in carotid tissues of rabbits in each group by IHC staining (400×)



Note: A: model group, B: atorvastatin group, C: ezetimibe group, D: control group.

Fig. 6: Western blot analysis of protein expression

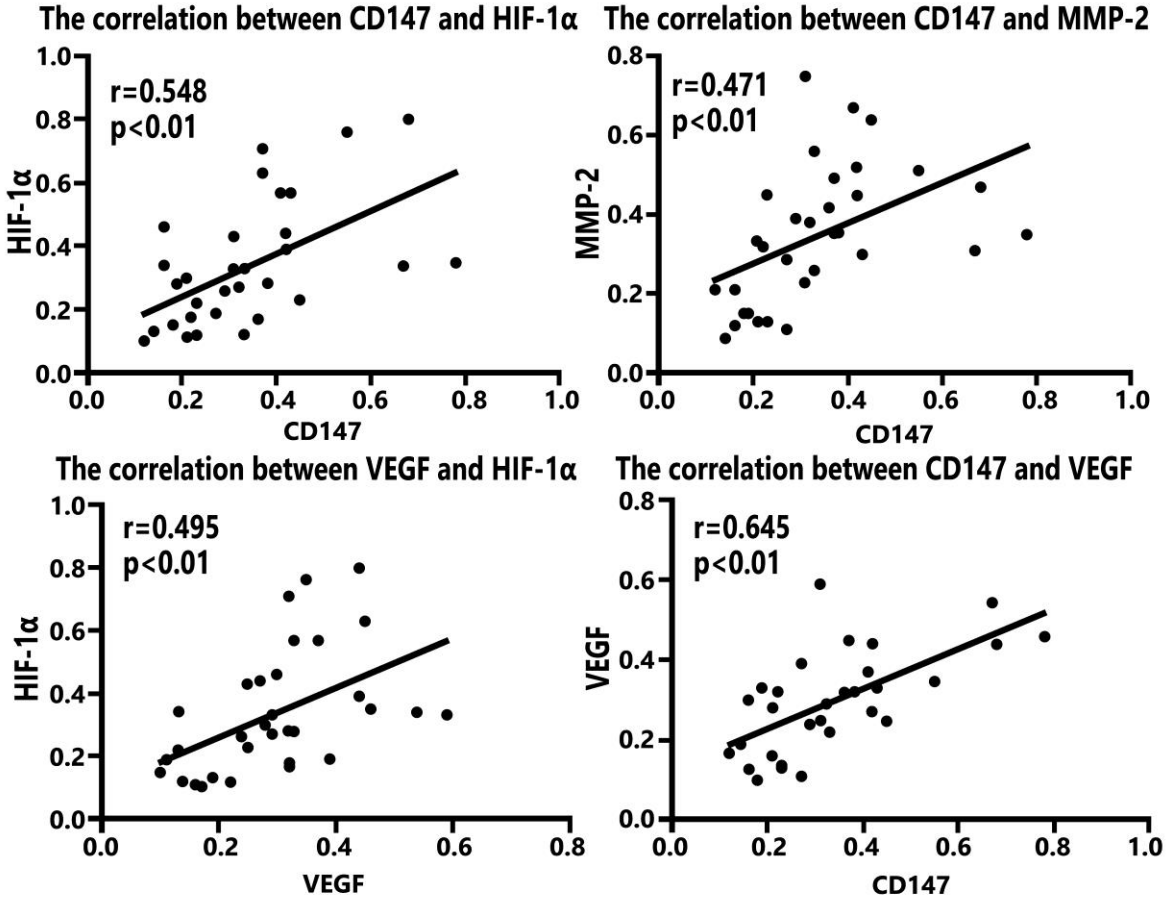
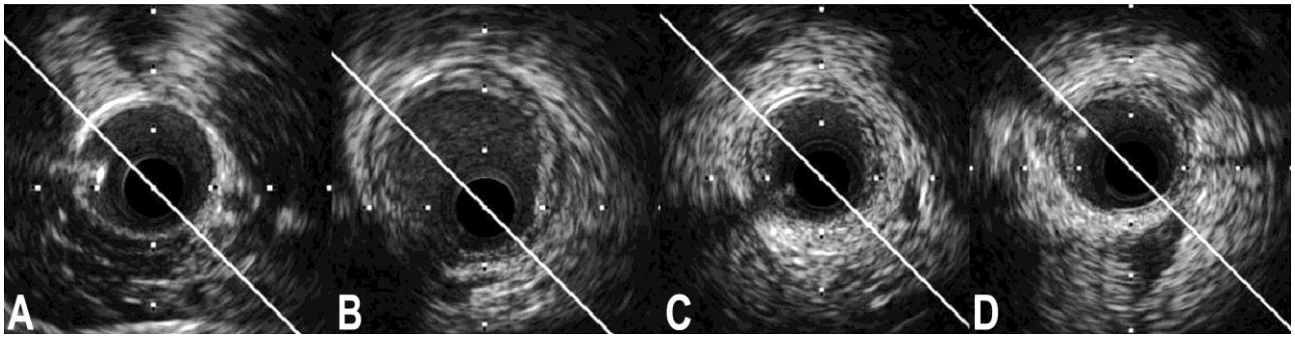


Fig. 7: The correlations between the expressions of MMP-2, HIF-1α and CD147, and HIF-1α and VEGF according to western blot results



Note: A: the control group, B: the model group, C: the atorvastatin group, D: the ezetimibe group.
Fig. 8: IVUS imaging results

DISCUSSION

Atherosclerosis is a chronic inflammatory disease, which forms atherosclerotic plaque and may lead to coronary artery thrombosis, which is suddenly life-threatening. There are many mechanisms of plaque formation and rupture (Bentzon, 2014), including inflammation, endothelial injury, calcification and fibrosis. Therefore, it is very necessary to explore the occurrence and development mechanism of atherosclerotic plaque for the purpose to prevent and treat cardiovascular disease.

CD147, as a receptor molecule expressed on the surface of cell membranes, is a member of the immunoglobulin superfamily. It can play a role in cardiovascular disease by binding with various ligands or receptors such as E-selectin, platelet glycoprotein VI, cyclophilin A (CyPA), cyclophilin B and apolipoprotein D (von Ungern-Sternberg, 2018). Especially when combined with CyPA, CD147 can increase the stress response of monocytes and macrophages to inflammation. It can induce the chemotaxis migration of monocytes to the intima and plaques by chemokines and can induce the transformation

of monocytes into macrophages and foam cells (Yurchenko, 2002; Nigro, 2011). It can also increase plaque vulnerability by degrading extra cellular matrix by up-regulating MMPs (MT1-MMP, MMP-9), increasing the secretion of macrophage colony-stimulating factor (M-CSF) and promoting the occurrence of local inflammation (Seizer, 2010; Yoon, 2005). The important reasons for plaque progression and rupture are intra-plaque hemorrhage and neovascularization. Neovascularization mainly originates from the nourished vessel of adventitia. Because of the lack of supporting cells, the new blood vessels in atherosclerotic plaques are fragile and highly permeable, which may lead to local exosmosis of plasma protein and red blood cells and this may be the pathological basis for the progression of atherosclerotic plaques and the occurrence of rupture and hemorrhage (Sluimer, 2009; Kolodgie, 2003; Takaya, 2005; Sun, 2012). A study has found that the expression of HIF-1 α in carotid and femoral endarterectomy specimens is closely related to the presence of macrophages and plaques with large lipid core and its expression is strongly positively related to its important downstream regulator VEGF (Vink, 2007), which fully shows that HIF-1α and VEGF play an important role in promoting the progression of atherosclerosis and the formation of unstable plaques.

In this study, the MMP-2 concentrations in the serum of rabbits in the model group, atorvastatin group and ezetimibe group were significantly increased than that in the control group at 12 weeks. We speculated that the serum level of MMP-2 may be used as a marker of chronic inflammation during atherosclerosis progression. After 4 weeks of intervention with atorvastatin and ezetimibe, the serum concentration of MMP-2 and LDL-C decreased significantly, which was consistent with the lipid-lowering and anti-inflammatory effects of statins. Ezetimibe may slow the progression of atherosclerosis caused by lipid deposition in the intima by reducing LDL-C level. Study on serum levels of CD147 found that serum levels of CD147 in patients with transient ischemic attack (TIA) and acute cardiac infarction (AMI) were significantly increased compared to healthy controls (Xu, 2018). Another found indicated that serum levels of CD147 in patients with myocardial infarction (ST-segment elevation myocardial infarction [STEMI] or non-ST elevated myocardial infarction [NSTEMI]) was significantly increased than that in stable coronary artery disease (SCAD) patients or healthy patients, while there do not exist statistical difference between STEMI and NSTEMI patients, SCAD and healthy control patients (Akkus, 2016). It was preliminarily confirmed that the increase in serum level of CD147 reflected the sudden change of the disease. According to the WB and IHC results, the expressions of CD147, HIF-1α, MMP-2 and VEGF in atherosclerotic plaques were significantly increased. Besides, there exist a positive correlation between the expressions of MMP-2 and HIF-1α and the expression of CD147 and another positive correlation between the expression of VEGF and HIF-1α, which confirmed that CD147 could not only induce the increase of MMP activity and expression, but also indirectly increased the expression of VEGF by up regulating the expression of HIF-1α. All of these accelerated the progression of AS and the end stage of the progression led to the occurrence of plaque instability, rupture and hemorrhage and acute thrombosis (Haifeng, 2021).

CONCLUSION

Atorvastatin could delay the progression of AS by reducing the expression of CD147, MMP-2, HIF-1α and VEGF in plaques. Ezetimibe didn't have a plaque stabilization effect.

REFERENCES

Akkus MN, Ormam A, Seyis S, Baran Ç, Gorur A and Bilen MN (2016). Plasma EMMPRIN levels in acute myocardial infarction and stable coronary artery disease. *Clin. Invest. Med.,* **39**(3): E79-E87.

Bougatef F, Quemener C, Kellouche S, Naïmi B, Podgorniak MP, Millot G, Gabison EE, Calvo F, Dosquet C, Lebbé C, Menashi S and Mourah S (2009). EMMPRIN promotes angiogenesis through hypoxia-inducible factor-2alpha-mediated regulation of soluble VEGF isoforms and their receptor VEGFR-2. *Blood*, **114**(27): 5547-5556.

Bentzon JF, Otsuka F, Virmani R and Falk E (2014). Mechanisms of plaque formation and rupture. *Circ. Res.,* **114**(12): 1852-1866.

Fang SM, Zhang QH and Jiang ZX (2009). Developing a novel rabbit model of atherosclerotic plaque rupture and thrombosis by cold-induced endothelial injury. *J. Biomed. Sci.,* **16**: 39.

Kolodgie FD, Gold HK, Burke AP, Fowler DR, Kruth HS, Weber DK, Farb A, Guerrero LJ, Hayase M, Kutys R, Narula J, Finn AV and Virmani R (2003). Intraplaque hemorrhage and progression of coronary atheroma. *N. Engl. J. Med.,* **349**(24): 2316-2325.

Khan MI, Rath S, Adhami VM and Mukhtar H (2018). Hypoxia driven glycation: Mechanisms and therapeutic opportunities. *Semin Cancer Biol.,* **49**: 75-82.

Liang X, Yang LX, Guo R, Shi Y, Hou X, Yang Z, Zhou X and Liu H (2017). Atorvastatin attenuates plaque vulnerability by downregulation of EMMPRIN expression via COX-2/PGE2 pathway. *Exp. Ther. Med.,* **13**(3): 835-844.

Newby AC (2008). Metalloproteinase expression in monocytes and macrophages and its relationship to atherosclerotic plaque instability. *Arterioscler. Thromb. Vasc. Biol.,* **28**(12): 2108-2114.

Nigro P, Satoh K, O'Dell MR, Soe NN, Cui Z, Mohan A, Abe J, Alexis JD, Sparks JD and Berk BC (2011).

Cyclophilin A is an inflammatory mediator that promotes atherosclerosis in apolipoprotein E-deficient mice. *J. Exp. Med.,* **208**(1): 53-66.

Schmidt R, Bültmann A, Fischel S, Gillitzer A, Cullen P, Walch A, Jost P, Ungerer M, Tolley ND, Lindemann S, Gawaz M, Schomig A and May AE (2008). Extracellular matrix metalloproteinase inducer (CD147) is a novel receptor on platelets, activates platelets, and augments nuclear factor kappaB-dependent inflammation in monocytes. *Circ. Res.,* **102**(3): 302-309.

Sluimer JC, Gasc JM, van Wanroij JL, Kisters N, Groeneweg M, Sollewijn Gelpke MD, Cleutjens JP, van den Akker LH, Corvol P, Wouters BG, Daemen MJ and Bijnens AP (2008). Hypoxia, hypoxia-inducible transcription factor and macrophages in human atherosclerotic plaques are correlated with intraplaque angiogenesis. *J. Am. Coll. Cardiol.,* **51**(13): 1258-1265.

Sluimer JC, Kolodgie FD, Bijnens AP, Maxfield K, Pacheco E, Kutys B, Duimel H, Frederik PM, van Hinsbergh VW, Virmani R and Daemen MJ (2009). Thin-walled microvessels in human coronary atherosclerotic plaques show incomplete endothelial junctions relevance of compromised structural integrity for intraplaque micro vascular leakage. *J. Am. Coll. Cardiol.,* **53**(17): 1517-1527.

Schonbeck U and Libby P (2004). Inflammation, immunity and HMG-CoA reductase inhibitors: Statins as antiinflammatory agents? Circulation, **109**(21 Suppl 1): I18-I26.

Seizer P, Schonberger T, Schott M, Lang MR, Langer HF, Bigalke B, Krämer BF, Borst O, Daub K, Heidenreich O, Schmidt R, Lindemann S, Herouy Y, Gawaz M and May AE (2010). EMMPRIN and its ligand cyclophilin A regulate MT1-MMP, MMP-9 and M-CSF during foam cell formation. *Atherosclerosis,* **209**(1): 51-57.

Sun J, Underhill HR, Hippe DS, Xue Y, Yuan C and Hatsukami TS (2012). Sustained acceleration in carotid atherosclerotic plaque progression with intraplaque hemorrhage: A long-term time course study. *JACC Cardiovasc Imaging,* **5**(8): 798-804.

Tsujita K, Sugiyama S, Sumida H, Shimomura H, Yamashita T, Yamanaga K, Komura N, Sakamoto K, Oka H, Nakao K, Nakamura S, Ishihara M, Matsui K, Sakaino N, Nakamura N, Yamamoto N, Koide S, Matsumura T, Fujimoto K, Tsunoda R, Morikami Y, Matsuyama K, Oshima S, Kaikita K, Hokimoto S and Ogawa H (2015). Impact of dual lipid-lowering strategy with ezetimibe and atorvastatin on coronary plaque regression in patients with percutaneous coronary intervention: The multicenter randomized controlled PRECISE-IVUS *Trial. J. Am. Coll. Cardiol.,* **66**(5): 495-507.

Takaya N, Yuan C, Chu B, Saam T, Polissar NL, Jarvik GP, Isaac C, McDonough J, Natiello C, Small R, Ferguson MS and Hatsukami TS (2005). Presence of intraplaque hemorrhage stimulates progression of carotid atherosclerotic plaques: A high-resolution magnetic resonance imaging study. *Circ.,* **111**(21): 2768-2775.

Vedanthan R, Seligman B and Fuster V (2014). Global perspective on acute coronary syndrome: A burden on the young and poor. Circ. Res., **114**(12): 1959-1975.

Vink A, Schoneveld AH, Lamers D, Houben AJ, van der Groep P, van Diest PJ and Pasterkamp G (2007). HIF-1 alpha expression is associated with an atheromatous inflammatory plaque phenotype and upregulated in activated macrophages. *Atherosclerosis,* **195**(2): e69-e75.

Von Ungern-Sternberg S, Zernecke A and Seizer P (2018). Extracellular matrix metalloproteinase inducer EMMPRIN (CD147) in cardiovascular disease. *Inter. J. Molecular Sci.,* **19**(2): 507.

Wang H, Bu L, Shu F, Bai Y, Xue F, Shi S and Sun D (2021). Molecular mechanism of biofilm locator protein kinase Dbf2p-related kinase 1 in regulating innate immune response to interleukin 17-induced viral pneumonia. *Bioengineered,* **12**(2): 10335-10344.

Wang QM, Wang H, Li YF, Xie ZY, Ma Y, Yan JJ, Gao YF, Wang ZM and Wang LS (2016). Inhibition of EMMPRIN and MMP-9 expression by Epigallocatechin-3-Gallate through 67-kDa Laminin receptor in PMA-induced macrophages. *Cell Physiol. Biochem.,* **39**(6): 2308-2319.

Xu B, Wu C, Wu W, Tan Y, Sun N, Cui J, Lu S, Wang J and Cen S (2018). Study of serum CD147 level in patients with transient ischemic attack and CD147 expression in atherosclerotic plaque. *J. Cardiovasc. Transl. Res.,* **11**(4): 285-291.

Yoon YW, Kwon HM, Hwang KC, Choi EY, Hong BK, Kim D, Kim HS, Cho SH, Song KS and Sangiorgi G (2005). Upstream regulation of matrix metalloproteinase by EMMPRIN; extra cellular matrix metalloproteinase inducer in advanced atherosclerotic plaque. Atherosclerosis, **180**(1): 37-44.

Yurchenko V, Zybarth G, O'Connor M, Dai WW, Franchin G, Hao T, Guo H, Hung HC, Toole B, Gallay P, Sherry B and Bukrinsky M (2002). Active site residues of cyclophilin A are crucial for its signaling activity via CD147. *J. Biol. Chem.,* **277**(25): 22959-22965.