Asian Pacific Journal of Microbiology Research (AJMR)
DOI : http://doi.org/10.26480/ajmr.01.2017.15.17

ISSN: 2329-8251 (Print)
ISSN: 2329-826X (Online)

THE EFFECT OF AEROBIC EXERCISE ON EXPRESSIVE LEVEL OF VCAM-1 AT APOE-DEFICIENT MOUSE AORTA

Sunyan[1], Hao Xuan-ming[2]*, Geng Qing-qing[2], Li Jin-huan[2], Zhang Yan-yan[2], Ren qi[2]

[1]School of P.E East China JiaoTong University NanChang JiangXi 330013 China
[2]Department of Sports Science South China Normal University College City GuangZhou 510006 China

ARTICLE DETAILS

Article History:

Received 12 November 2017
Accepted 12 December 2017
Available online 1 January 2018

ABSTRACT

ApoE-deficient mice was used as AS model to approach the effect of aerobic exercise on the expressive level of VCAM-1 on apoE-deficient mouse aorta. METHODS: 20 apoE-deficient mice were randomly divided into control group (CG) and exercise group (EG, swimming, 90min/d, 6d/w, 10 wks), the expressive level of VCAM-1 on aorta were tested after 10 weeks by immunohistochemistry. RESULTS: Compared with CG, the expressive level of VCAM-1 on the aorta of EG were significantly lower ($P<0.01$). Conclusion: This result suggested that aerobic exercise improved the local inflammation in apoE-deficient mouse aorta.

KEYWORDS

apoE-deficient mice; aerobic exercise; atherosclerosis; VCAM-1.

1. INTRODUCTION

Many Since the 90s of last century, the inflammatory mechanisms of arteriosclerosis(AS) have been widely recognized by researchers. Current theory suggests that atherosclerosis is initially began with the activation of vascular endothelial cells by physical factors (blood flow shear stress, pulling, spasm) or chemical factors (high blood lipids, hypoxia, free radical generation, proinflammatory cytokines).The activated endothelial cells express cell adhesion molecules in cell membrane, attracting monocytes and lymphocytes in the blood adhesion to the vessel wall. Monocytes and lymphocytes gradually migrated to the endothelium by the chemokines of intima, resulting in the vascular intima and tube wall damage. At the sametime, macrophages and smooth

muscle cells change to foam cells by swallowing large amounts of blood lipids . And then smooth muscle cells and fibroblasts proliferate, secrete collagen and extracellular matrix.It makes the arterial intima become thicken and harden, and at the end causing the formation of atherosclerotic plaque. Vascular endothelial inflammation state become the core mechanism of AS and researchers are paying close attention to the problem. Based on a study, the inflammatory mechanism of AS become the new target of treating AS [1,2]. The study use apoE-deficient mice as animal models for AS and take the expression of VCAM-1 on vascular intima as the index, using immunohistochemical method to observe the effects of aerobic exercise on vascular wall inflammatory state .

2. MATERIALS AND METHODS

2.1 Animals

20 male 7-8 weeks old apo-E gene deficient mice were randomly divided to sedentary control group(SCG) and exercise group.(EG),10 mice per group.Mice were fed the “western type diet” feed,in which cholesterol content 0.15%,fat content 21%. Mice were Sub-housed, only 3-5 per cage, free diet.Temperature was controlled between 18-26 ℃, humidity 50%, natural light. Animal breeding cages and drinking water bottles were regularly disinfected. The animal room is regularly disinfected with ultraviolet light.

2.2 Exercise Model

Exercise group carried out the adaptive training at the first week, and then swimming for 10 weeks,90m/t ,1t/1d,6t/w. Water temperature maintained at 34 ± 2 ℃. Sedentary control group did not exercise.The other conditiongs were equal.

2.3 Derived Method

After the last swimming bout ,mice were fasted overnight and harvested after 24 hours. After the mice were sacrificed, quickly open the chest and abdominal cavity, separate the entire aorta from the arch of the aorta, remove connective tissue, tinfoil wrapped, preservate the entire aorta in liquid nitrogen. The above operation process is conducted in an ice bath .

2.1 The Immunohistochemical Detection Method of VCAM-1 on Aortic Wall

Thoracic aorta was embedded by OCT.Frozen section continuously per 8μm.Take 1 section from every 5 sections.Take 4 sections of every mouse to do immunohistochemistry.

The steps of immunohistochemistry:

1. Wash 3 times with PBS,3mins/time;

2. Wash 10-20 mins with 30% Hydrogen peroxide solution.Inactivate endogenous peroxidase;

3. Wash 3 times with PBS,3mins/time;

4. Wash 10-20 mins with 10% Bovine serum albumin. Blocking antigen;

5. Add first antibody by appropriate dilution .Incubate 20-30mins under room temperature;

6. Wash 3 times with PBS,3mins/time;
7. Add HRP. Incubate 5mins under room temperature;
8. Wash 3 times with PBS,3mins/time;
9. Add DAB.Control the reactive time under microscope;
10. Wash with double distilled water;
11. Dyeing 2-5mins with hematoxylin;
12. Wash with double distilled water;
13. Differentiate several seconds with 1% hydrochloric alcohol;
14. Wash with double distilled water;
15. Dehydrate with alcohol of different concentration;
16. Make transparent with xylene;
17. Seal up the section with neutral resins.Observe under microscope.

Use PBS replacing first antibody as negative control.VCAM-1 antibody was diluted 1:1800.Take photos under microscope Calculate the positive dyeing area using Leica Qwin image analysis software.Average value was taken to be compared.

Concrete steps as follow:

1. Take the tissue section under the microscope(40×).
2. Chose a fixed window(768×576) on view.
3. Measure the positive reaction product area then devided by the total area and calculate the area ratio.

We chosed 4 sections from every aorta and 5 unoverlap views were chosed from every section. Statistics Use Leica Qwin image analysis software to collect data from the results of immunohistochemistry. Excel statistical software is used for data analysis. Data are given as Means ± SE .Deviation among the groups were test by using independent sample T test.

3. RESULTS

It showed that the local vascular intima of SCG was dyed deeply.There are also positive expression on the surface of lipid deposit,but there are not obvious positive expression on the core of lipid deposit.Under the vascular intima,there are scattered positive expression (Figure1).On the contrary,there are only punctuate and linear positive expression in EG (Figure2). Compared with SCG, the positive expression area of EG was significantly small (area ratio 0. 247±0. 065 vs 0.558±0. 083 , Table1).

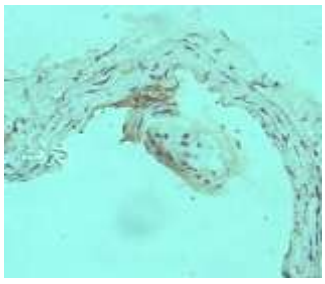

Figure 1: The VCAM-1 expression of SCG

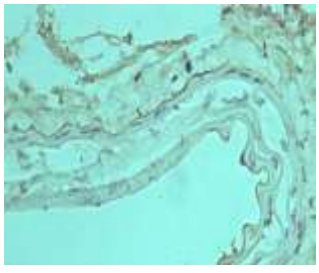

Figure 2: The VCAM-1 expression of EG

Table 1: Comparison of positive area of VCAM-1 on aortic wall between the two groups

	SCG(n=10)	EG(n=10)
VCAM-1	0. 558±0. 083	0. 247±0. 065*

* indicates P<0. 01 between SCG and EG

4. DISCUSSION

Almost more than 80 years ago, researchers began to use animal models to study the mechanism of atherosclerosis. Previous research often caused animal atherosclerosis by using the short time high fat, high cholesterol (often dozens of times of normal diet) diet. However, this is acute process and is different from the chronic process of human AS. High fat, high cholesterol diet can cause the death of the animal. The success rate is not high. Recent years, along with the gene targeting technology, researchers developed a variety of congenital arterial sclerosis mouse model. ApoE-deficient mice is the most popular one. A study in 1992 showed apo-E gene deficient mice almost simultaneously breeds successfully by two different laboratories using gene targeting technology [3,4]. The serum total cholesterol content of apo-E gene deficient mice can reach 500mg/dl (normal 60-90mg/dl),and mainly in VLDL and chylomicrons. The early pathological changes of AS -- fatty streaks can emerge in the aortic arch of Apo-E gene deficient mice at 6-8 weeks of age. At the same time, macrophages begin to aggregate in endothelial.

Lipid-rich foam cells can be observed in endometrium about 10 weeks of age. At about 15 weeks of age, lipid plaque which contain smooth muscle cells and foam cells can be observed. The composite patches which contain the fibrous cap and necrotic core are visible 20 weeks of age. The AS process of apoE-deficient mice is very similar with that of human. According to the experiment, ApoE-deficient mice become the very suitable animal model to study the mechanism of human AS [5,6]. Based on a research, VCAM-1 was successfully cloned in 1989 [7]. VCAM-1 can express in endothelial cells, epithelial cells and macrophages. VLA-4（ very late antigen-4）is its specific ligand. VLA-4 only express in mononuclear cells in blood,not in neutrophils.So VCAM-1 selectively mediates the adhesion of blood monocyte and endothelial cell.

In 1991,a researcher detected the VCAM-1 in endothelial cells of atherosclerotic lesions of Watanable heritable hyperlipidemic rabbit for the first time [8]. Subsequent studies found that there have VCAM-1 expression in endothelial cells, smooth muscle cells and macrophages of human atherosclerotic lesions [9]. There have very high level expression of VCAM-1 in atherosclerotic lesions of apoE-deficient mice. At the same time,there have only very low level expression of VCAM-1 in normal mice. It found in further research that the expression intensity of VCAM-1 is associated with serum cholesterol levels [10].

In order to study the exact effect of VCAM-1 in AS, the researchers observed VCAM-1 expression in abdominal aortic wall of 13 patients with immunohistochemical method .It was found that there have VCAM-1 expression whatever in endothelial cells, intima and subendometrial tissue,and where the AS is more serious there have higher VCAM-1 expression. This finding makes people have reason to believe that VCAM-1 expression in endothelial cells attracting monocyte adhesion in arteriosclerosis played a crucial role in the pathogenesis of AS [11].

There are rare reports about the effects of aerobic exercise on VCAM-1 expression in arteriosclerotic vascular wall .The previous researchers focus on the effects of exercise on the soluble VCAM-1 in blood. A researcher fed rabbit with high cholesterol diet and let the rabbit do aerobic treadmill exercise simultaneously. After 6 weeks, the VCAM-1 immunohistochemical results in rabbit thoracic aorta of control group showed strong positive, whereas the exercise group expression was decreased significantly [12].

The experimental results showed that there are high level expression of VCAM-1 in aortic wall of apoE-deficient mice and 10-week aerobic exercise can obviously down-regulate its expression level.It suggests that aerobic exercise has anti-inflammatory and anti-arteriosclerosis effects by interventing the adhesion of aortic endothelial cells and blood monocytes .

In conclusion, our work found that there are high expression level of VCAM-1 in aortic wall of apoE-deficient mice by immunohistochemical method and 10-week aerobic exercise can significantly down-regulate the expression of VCAM-1.It suggests that aerobic exercise can improve the local inflammatory state of aortic wall and improve the AS .

REFERENCES

[1] Ross, R. 1999. Atherosclerosis - an inflammatory disease. The New England Journal of Medicine, 340: 115-26.

[2] Libby, P. 2002. Inflammation in Atherosclerosis, Nature, 420: 868-874

[3] Plump, A.S., Smith, J.D., Hayek, T. 1992. Severe Hypercholesterolemia and Atherosclerosis in Apolipoprotein E-deficient Mice Created by Homologous Recombination in ES Cells. 71: 343-353

[4] Coleman, R., Hayek, T., Keidar, S. 2006. A Mouse Model for Human Atherosclerosis: Long-term Histopathological Study of Lesion Development in the Aortic Arch of Apolipoprotein E-deficient (E0) Mice. Acta Histochemica. 108: 415-424.

[5] Karen, S.M., Leitersdorf, E. 2004. Atherosclerosis in the Apolipoprotein E–Deficient Mouse. A Decade of Progress. Arteriosclerosis, Thrombosis and Vascular Biology, 24: 1006-1014.

[6] Osborn, L., Hession, C., Tizard, R. 1989. Direct Expression Cloning of Vascular Cell Adhesion Molecule-1, a Cytokine-induced Endothelial Protein that Binds to Lymphocytes, 59 (6): 1203-1211.

[7] Cybulsky, M.I, Gimbrone, M.A Jr. 1991. Endothelial Expression of a Mononuclear Leukocyte Adhesion Molecule During Atherogenesis. Science. 251: 788-791.

[8] O'Brien, K.D., Allen, M.D., McDonald, T.O. 1993. Vascular Cell Adhesion Molecule-1 is Expressed in Human Coronary Atherosclerotic Plagues: Implications for the Mode of Progression of Advanced Coronary Atheroscterosis, Journal of Clinical Investigation, 92 (2): 945-951.

[9] Poston, RN., Haskard, D.O., Coucher, J.R. 1992. Expression of Intercellular Adhesion Molecule-1 in Atherosclerotic Plaques. The American Journal of Pathology, 140: 665-673.

[10] Per, H.U., Schmidt, A., Roessner, A. 1996. Expression of the Adhesion Molecules ICAM-1, VCAM-1, and ELAM in the Arteriosclerotic Plaque. General and Diagnostic Pathology. 141 (5): 289-294.

[11] Yang, A.L., Jen, C.J., Chen, H.I. 2003. Effects of High-cholesterol Diet and Parallel Exercise Training on the Vascular Function of Rabbit Aortas: a Time Course Study. Journal of Applied Physiology, 95 (3)