Influence of hemodynamics on myocardial cell cardiac index and its molecular mechanism

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Abstract: With the improvement of people's living standards and change of lifestyles, the morbidity of the cardiovascular and cerebrovascular diseases (cardiovascular and cerebrovascular diseases, CCVD), especially the atherosclerosis (atherosclerosis, AS), presents a rising tendency year by year. The injury and inflammatory reaction of endothelial cells is one of the important factors causing AS. However, its molecular mechanism still needs further studying. This paper will discuss the influence of hemodynamics on the cardiac muscle cells cardiac index and its molecular mechanism. the Human umbilical vein endothelial cells (Human umbilical vein endothelial cells, HUVEC) were cultivated and separated and processed by 100ng Lipopolysaccharide (Lipopolysaccharide, LPS) to simulate the injury and inflammation state of the vascular endothelial cells. The hemodynamic state was simulated by the Parallel-Plate Flow Chamber in laboratory. And the MTT was adopted to detect HUVEC growth and the flow cytometry (flow cytometry, FCT) to detect HUVEC apoptosis. And the cardiac index was tested by RT-PCR and western blot. And the cell apoptosis caused by LPS was tested when the cardiac index was over-expressed and reduced. LPS could inhabit HUVEC growth and lead to its apoptosis. Hemodynamics (16dyn/cm^2) could reduce HUVEC growth inhibition and apoptosis caused by LPS. And the dose-dependent LPS reduced HUVEC cardiac index, while when it was processed by the hemodynamics (16dyn/cm^2), the HUVEC cardiac index would increase. And the over-expression of the cardiac index could inhabit the cell apoptosis caused by LPS, and the interference technology was adopted to deal with the cardiac index, which could enhance the cell apoptosis caused by LPS. Hemodynamics could inhabit the HUVEC apoptosis caused by LPS, which might be one of the reasons causing AS, through enhancing the cardiac index.

Keywords: Hemodynamics; LPS; HUVEC; Cell growth; Cell apoptosis; Cardiac index; AS.

INTRODUCTION

Atherosclerosis (AS) is a common vascular disease at home and abroad. With the improvement of people's living standards and change of lifestyles, the morbidity and mortality of AS presents a rising tendency year by year (Heuslein *et al*., 2015; Li *et al*., 2015). The current researches said that the incidence of AS started from the internal walls of the diseased artery blood vessel, in which the glyco-components and fatty components gathered here causing vascular calcification and hyperplasia of fibrous tissue, then it spread to the middle-level vessel causing thrombus or bleeding and finally blocking the artery and cellangionecrosis, or ischemia of the tissues and organs (Klingberg *et al*., 2015; Kim *et al*., 2015; Qin *et al*., 2015). Since the lipid complex gathering on the artery internal walls takes on the athero-claybank, this disease is named AS. However, its molecular mechanism is still need further studying (Lorenz *et al*., 2015).

The occurrence and development of AS was closely related to the diabetes, hyperlipidemia, hypertension, smoking and sex from a large number of laboratory findings and epidemiological analysis (Raasch *et al*., 2015; Lee *et al*., 2015). AS was a chronic inflammatory reaction. When there occurred fiber crack in the injured artery internal wall and vascular smooth muscle, the body would develop inflammatory reaction to prevent from the further damage (Polidoro *et al*., 2013; Panieri*et al*., 2010). The body will recovery if the inflammatory reaction be removed immediately, on the contrary, if it goes on, atherosclerotic lesions will develop gradually (Marampon *et al*., 2013).

Vascular endothelial cells are located between the blood vessels and blood, with the influence of hemodynamics for a long-term. And the hemodynamics with different intensity, frequency and direction will have a different influence on the vascular endothelial cells. And the hemodynamicsin the artery endothelial cells is larger than the other vascular endothelial cells (Roy *et al*., 2013; He *et al*., 2015). Considered that AS often develops in the diverged outlet of the main artery, the bending and narrow areas, the abnormal influence of hemodynamics on the vascular endothelial cells is one of the most important reasons, and the inflammatory state and cell apoptosis may be the starting and critical procedure of AS (Freese *et al*., 2014; Ségaliny *et al*., 2015; Li *et al*., 2015). Therefore, this paper will explore the effect of

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hemodynamics on the regulation of vascular endothelial cell growth and apoptosis and its molecular mechanism.

Cardiac index is a kind of NAD-dependent sirtuin-4 protein, mainly regulating the activity of the pyruvate dehydrogenase (Reed *et al*., 2014). It had been verified in some researches that the expression level of cardiac index was regulated by hemodynamics. Cardiac index was also involved in the damage repair, playing a key role in various injury signal transmission, and making a rapid response to the external changes (Wong *et al*., 2014). This paper will explore the influence of hemodynamic on vascular endothelial cell cardiac index and its potential molecular mechanisms. Therefore, the HUVEC will be cultured and separated and processed by LPS to simulate the injury and inflammatory reaction. And then HUVEC were dealt with the hemodynamics to study its influence.

MATERIAL AND METHODS

The test reagent

Dulbecco Modified Eagle Medium (Dulbecco Modified Eagle Medium, DMEM) and the calf serum were bought from the BD Company in the United States. The MTT (3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) used to detect the cell growth was purchased from the Santa Cruz Company of the United States. The polyclonal antibody of the mouse anti-human cardiac index protein, and the monoclonal antibody of the mouse anti-human and horse radish per oxidase coupled with Goat anti-mouse IgG were all bought from the Sigma Company of the United States. And the siRNA of the cardiac index and the siRNA of the control group were designed and compounded by the Shanghai Gene Pharma Co., Ltd., with the sequence of 5'AGGCATATCCAGG CACCAAGGCAGGC3' and 5'GGCAGCACCTGCTCC AGGAAAAGGCA3';5'CACCAAGAGCAGCATGGAG TCCAGGC3' and 5'GCACTGAGCTCCAGGAGCACA AGGCA3' respectively. The regent kit of caspase-3 used to detect the cell apoptosis and the FITC-Annexin-V used to test the Phosphatidylserine (Phosphatidylserine, PS) eversion were bought from the Beyotime Biotechnology Co., LTD.

Culture and isolation of HUVEC

The culture and isolation of myocardial cells were conducted according to the previous reports (Fede *et al*., 2015). And the specific methods are as follows: take the fresh umbilical cord postpartum 5h with the length of 10cm to 15cmfrom the Obstetrics and Gynecology Department of the xx hospital; put them into the PBP at 37°Cto wash out the blood and other liquids completely. One end of the umbilical cord was put into the 10mL 0.1% pancreatic enzyme digestive solution and the other into the water bath at 37°C for 30min. And added the digestive juice into the beaker with 5mL calf serum. And the umbilical cord was put into the PBS to wash for 3 times. And the digestive juice and cleaning solution were mixed up and centrifuged at the speed of 8000r/min for 8min to remove the supernate. And DMEM was used to resuspend the cell deposition. And the cell suspension was put into the cell culture vessel with 1.5% agar. And cultured the cells in the cell incubator at 37°C with 5%CO_2 and changed the medium every24h. When the cells grew into the fusion state, taking up the full of the bottom single-layer, they were washed with the PBS to remove the serum, then added into the 0.1%pancreatic enzyme digestive juices at 37°C for 2min. Then the cell suspension was collected and centrifuged at 8000r/min for 8min with the supernatant removed. In the last, they were put into the DMEM to re-suspend and deposed and passage with the ratio of 1:4 for 2 days for the test.

The establishment of flow device and processing of the HUVEC

This paper adopted the hemodynamic through the plate flow chamber in the laboratory. And the building of the plate flow chamber was according to the method in the report (Baeyens *et al*., 2014). And its concrete structure was as follows: its upper layer and the lower layer were consists of organic glass. At the two ends of the lower-layer were two small buffer room, for flowing in and flowing out respectively. And in the middle layer was a glass slide, which could be taken out and inserted in freely, used for culturing cells. The whole flat flow chamber system was made up with a liquid tank, a valve, a computer, a medical silicone hose, a constant flow pump, and a flow chamber. And its working principle are as follows: the perfusion fluid flew from the constant flow pump, passing through the silicone hose into the flow chamber. And the cells in the middle layer would be processed by the flow shear, and finally the perfusion fluid flew back into the liquid tank, forming a closed circulation system. All the parts were kept in the cell culture box, with the temperature of 37°C and 5% CO_2.

Processing of the HUVEC

HUVEC separated from human umbilical cord were inoculated into the middle flow chamber. After HUVEC were adherent to the walls, being processed by hemodynamics (16dyn/cm^2) for 24h (Fan *et al*., 2015). After the test finished, HUVEC were digested and collected for the analysis of cell activity and apoptosis.

Grouping

There were four groups in this experiment, they were: the HUVEC static culturing group, the HUVEC static culturing +LPS group, the HUVEC hemodynamic (16dyn/cm^2) group and the HUVEC hemodynamic (16dyn/cm^2)+ LPS group.

MTT

The experiment was conducted according to the past reports (Doddaballapur *et al*., 2015), and the HUVEC cell growth of each group was detected.

Transfection

The cardiac index siRNA and the control siRNA sequences were designed and compounded as: 5'AGGCA TATCCAGGCACCAAGGCAGGC3' and 5'GGCAGCA CTGCTCCAGGAAAAGGCA3';5'CACCAAGAGCAG CATGGAGTCCAGGC3' and 5'GCACTGAGCTCCAG GAGCACAAGGCA3'. And the HUVECs iRNA were transfected according to liposome transfection method.

Flow cytometer detection of cell apoptosis

The Flow cytometer detection instrument was used according to the specification in Beyotime Biological Technology Co., LTD to test the phosphatidylserin-eeversion in HUVEC cells in each group (De Franceschi *et al*., 2015). Specific methods were as follows: Mix up the cell suspension, binding Buffer with FITC-Annexin V and FITC-Annexin V reagent according to the proportion of 250:50:1; then incubate for 30 min keeping away from light; in the last, detect the cell reaction liquid by Flow cytometer detection instrument with the emission wavelength of 488nm and absorption wavelength of 624nm.

Caspase 3 activity detection

The HUVEC apoptosis in each group was detected by the caspase 3 activity detection kits produced by the Beyotime Biological Technology Co., LTD. And the specific methods were as follows: collect HUVEC cells in each group, suspend them into the cell lysisbuffer for 30min. Then add Ac-DEVD-pNA (5mm) into the cell lysis buffer and mix them up at 37°C to incubate for 30min. Then detect the absorbance value at 492nm by Micro plate reader. And the light absorptive value in the blank control group was taken as the reference, then the corresponding caspase-3 values in the HUVEC cell lysis buffer in other groups symbolized the corresponding activity of caspase-3.

RT-PCR

The HUVEC RNA in each group were extracted with the conventional method (Fontijn *et al*., 2014; Martin *et al*., 2014). And the cardiac index of HUVEC was detected with RT-PCR according to the specification in the regent kit produced by the Beyotime Biological Technology Co., LTD. And the cardiac index expression level was tested by detecting PCR production with sepharose gel. And Image J software was used to analyze the gray value in the electrophoretic band of the sepharose gel. And the cardiac index expression value was the Ratio of the gray value of the cardiac index to the gray value of the reference Actin.

Western blot

The protein levels of the cardiac index were detected by western blot according to the conventional method (Li *et al*., 2014). And the specific methods were as follows: Add cell lysis solution into the HUVEC cell suspension in each group and process the specimen of the cell lysis solution with the SDS-PAGE and Western blot. Then take the anti-cardiac-index-protein (1:1000) as the antibody and the anti-Actin (1:1000) as the control for 2h incubation at room temperature. And then use the Goat polyclonal Secondary Antibody to Mouse after washing-up for 2h incubation at room temperature. And it was better to use developing and fixing to analyze the cardiac index of HUVEC. And Image J software was used to analyze the gray value in the electrophoretic band of the sepharose gel. And the cardiac index expression value was the Ratio of the gray value of the cardiac index to the gray value of the reference Actin.

STATISTICS ANALYSIS

SPSS 13.0 statistical software was adopted to analyze all the data. And the data were expressed by the Mean ±standard deviation. One-way analysis of variance was used to compare the HUVEC of each group. And $P<0.05$ was statistically significant.

RESULTS

LPS inhabiting HUVEC growth

The cell growth of HUVEC was inhabited significantly after processed by 100ng LPS, compared with the control group (P=0.0086) as shown in fig. 1.

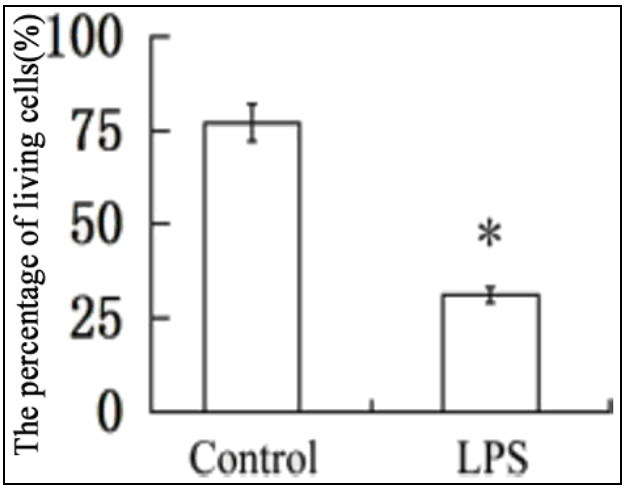

Fig. 1: LPS inhabiting HUVEC growth
Note: *the difference was significant compared with the control group.

Cell apoptosis caused by LPS

There was significant PS extroversion in the group processed by LPS, compared with the groups without LPS (P=0.012) as shown in fig. 2.

There was notable caspase-3 activation in HUVEC after being processed by LPS compared with the groups without LPS (P=0.0052). Shown in fig. 3. These findings showed that LPS could cause HUVEC cell apoptosis.

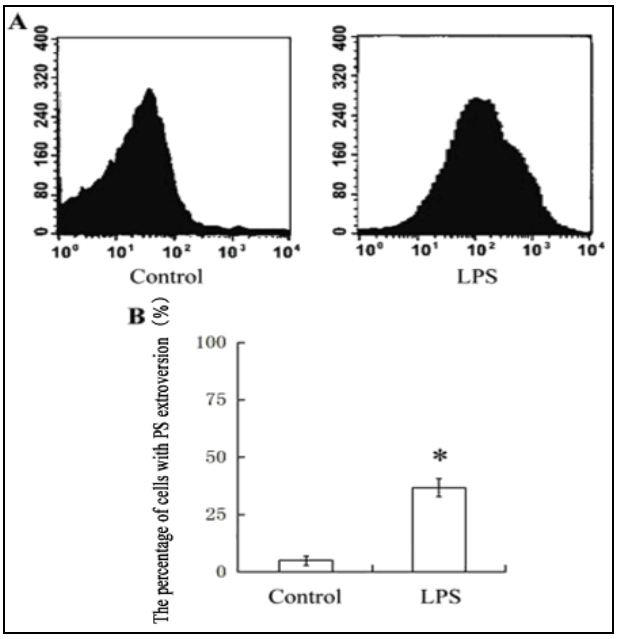

Fig. 2: PS extroversion in HUVEC caused by LPS
Note: *the difference was significant compared with the control group.

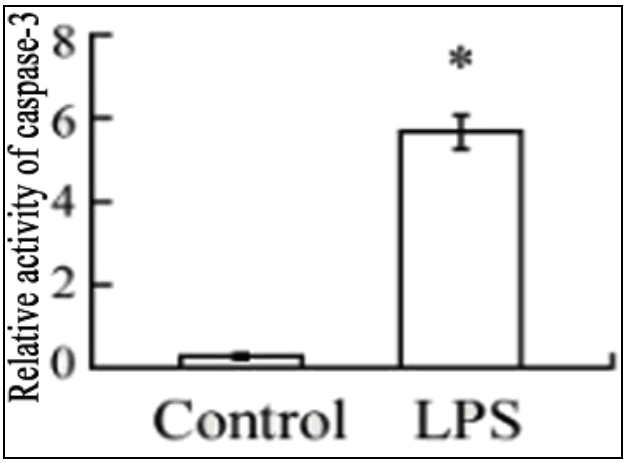

Fig. 3: LPS causing the activity of caspase-3 in HUVEC cells
Note: *the difference was significant compared with the control group.

Hemodynamics (16dyn/cm^2) reducing the HUVEC growth inhibition and apoptosis caused by LPS

As shown in fig. 4 and fig. 5, hemodynamics did not influence the HUVEC growth and apoptosis. However, it significantly reduce the HUVEC growth and apoptosis caused by LPS, indicating that it could be against the toxicity of LPS, making the HUVEC keep the normal function in the toxicity condition.

LPS dose-dependence reducing HUVEC cardiac index level

LPS dose-dependence reduced PICCONA and protein level of cardiac index in HUVEC significantly, compared with the control group without the LPS in fig.6 and fig. 7.

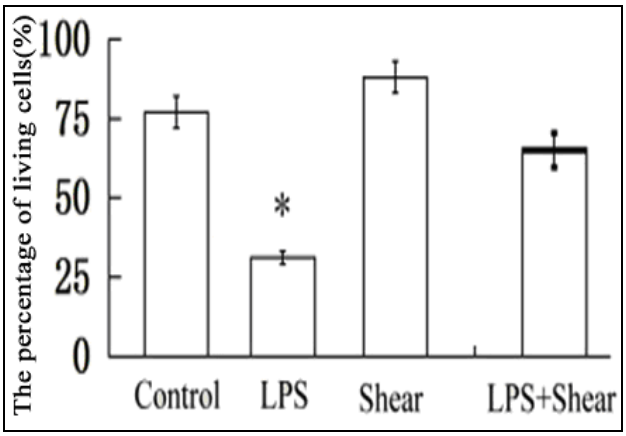

Fig. 4: Hemodynamics (16dyn/cm^2) significantly reducing the HUVEC growth caused by LPS
Note: *the difference was significant compared with the control group.

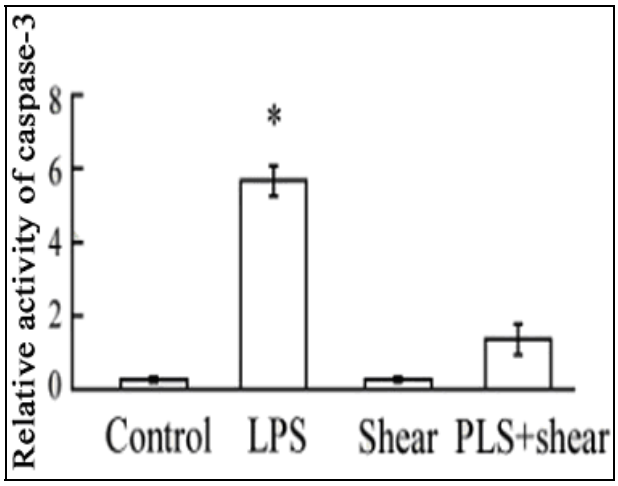

Fig. 5: Hemodynamics (16dyn/cm^2) significantly reducing the HUVEC apoptosis caused by LPS
Note: *the difference was significant compared with the control group

Hemodynamics (16dyn/cm^2) increasing cardiac index in HUVEC

As shown in fig. 8 and fig. 9 the cardiac index in HUVEC processed by hemodynamics (16dyn/cm^2) increased significantly, compared with the control group. And the difference was significant (P=0.0083).

Over-expression cardiac index inhabiting cell apoptosis caused by LPS

The cardiac index increased after using the over-expression technology, and the cell apoptosis evoked by LPS was inhabited significantly (P=0.0064), shown in the activity detection data of the caspase-3 in fig. 10.

The reduction of the cardiac index processed by the interfering technology enhancing cell apoptosis evoked by LPS

The reduction of the cardiac index processed by the interfering technology enhanced cell apoptosis evoked by

LPS (P=0.0064), shown in the activity detection data of the caspase-3 in fig. 11.

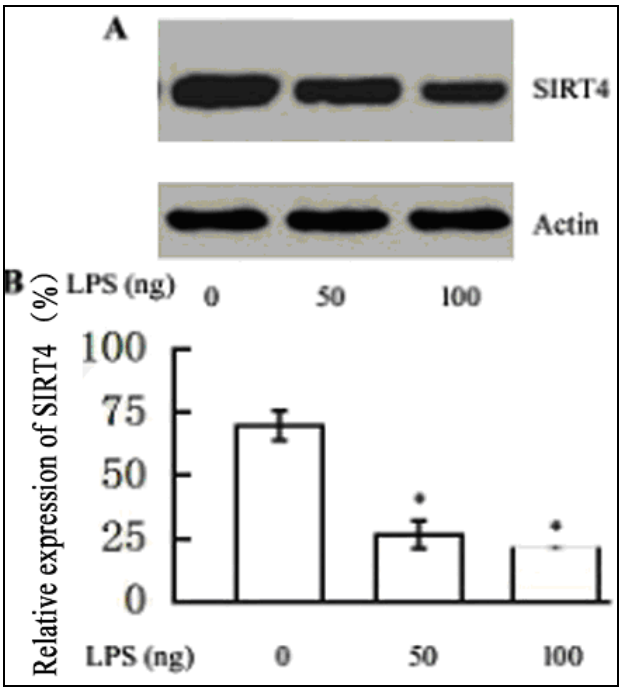

Fig. 6: LPS dose-dependent reducing PICCONA level of cardiac index in HUVEC

Note: *the difference was significant compared with the control group.

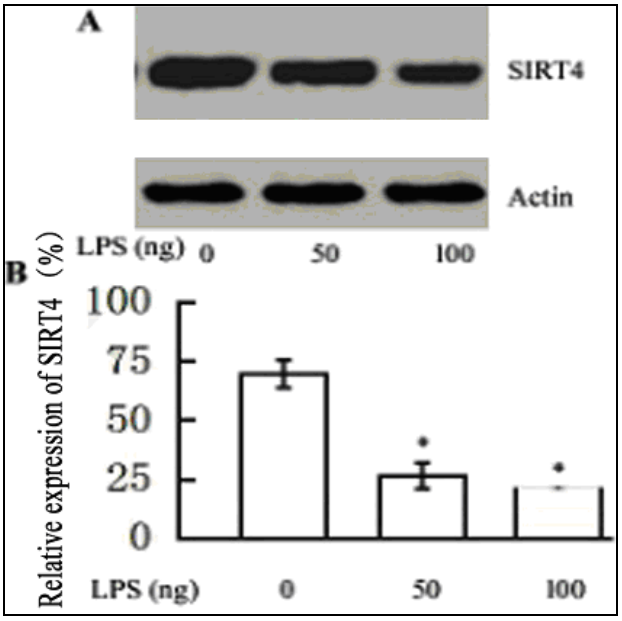

Fig. 7: LPS dose-dependent reducing protein level of cardiac index in HUVEC

Note: *the difference was significant compared with the control group.

DISCUSSION

With the improvement of people's living standard and changes of lifestyle, the incidence rate of cardiovascular and cerebrovascular diseases, especially AS presents with a rising trend year by year. The injury and inflammation reaction of the Endothelial cells is one of the important factors evoking AS (Chien *et al*., 2014). However, the molecular pathogenesis of AS remains to be further research (Claes *et al*., 2014). This study discussed the effects of fluid hemodynamic on myocardial cells and its molecular mechanism.

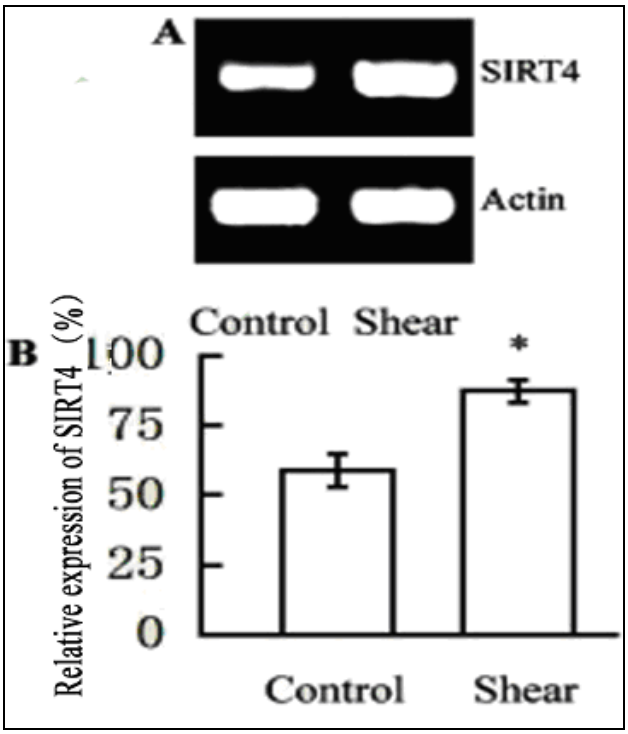

Fig. 8: Hemodynamics (16dyn/cm^2) increasing cardiac index in HUVEC

Note: *the difference was significant compared with the control group

There were 4 innovation points in this article. First, this paper found out that LPS could inhibit HUVEC growth, and cause HUVEC apoptosis. Second, the findings in this paper showed that hemodynamics (16dyn/cm^2) could significantly reduce HUVEC growth and apoptosis caused by LPS. Third, LPS could reduce cardiac index while hemodynamics (16dyn/cm^2) could increase the level. Fourth, the results in this paper showed that over-expression cardiac index could inhibit cell apoptosis caused by LPS; while when the cardiac index was reduced by the interference technique could increase cell apoptosis caused by LPS. These findings suggested that hemodynamics could inhabit the HUVEC apoptosis caused by LPS by increasing the cardiac index, which might be one of reasons causing AS.

According to the latest study, vascular endothelial cell injury is the starting and key factor during the incidence and development of the CVD, heart disease, infection, shock, acute lung injury and trauma etc. (Heuslein *et al*., 2015). LPS is the important substance causing various

pathological processes and also a proper reagent simulating injury *in vitro*. Therefore, this paper adopted LPS to process HUVEC to simulate the injury state. Endotoxins, especially LPS are the key substances causing shock and inflammation in mammal. And under normal circumstances, LPS can induce inflammation in the white blood cells and vascular smooth muscle cells, if the inflammation cannot be removed by the body it can cause cell apoptosis and necrosis, eventually leading to the vascular endothelial cells injury. Therefore, the model of HUVEC culture *in vitro* is suitable, also consistent with previous studies (Wong *et al*., 2014). The findings in this paper showed that LPS could lead to HUVEC apoptosis significantly. Based on this, we discussed the co-regulation of HUVEC by hemodynamics and LPS. And the results showed that hemodynamics could inhabit the cell growth inhibition and cell apoptosis caused by LPS, which helped the further study of molecular mechanism of HUVEC cell regulation by hemodynamics.

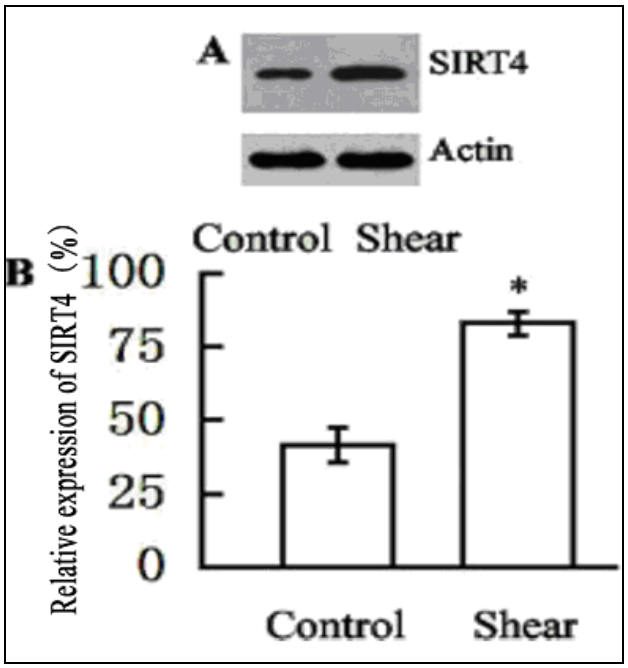

Fig. 9: Hemodynamics (16dyn/cm^2) increasing the protein level of cardiac index in HUVEC

Note: *the difference was significant compared with the control group.

The cardiac blood index expression level is regulated by the hemodynamics, which have shown in some researches. In addition, cardiac blood index is a kind of sirtuin-4 NAD-depending, mainly regulating the activity of pyruvate dehydrogenase (Reed *et al*., 2014). Cardiac blood index is also involved in the wound repair. This paper firstly discussed the influence of hemodynamics on cardiac index expression level of vascular endothelial cells. And the cardiac index expression level would increase when being processed by the Hemodynamic (16dyn/cm^2). And the over-expression of cardiac index would inhabit the cell apoptosis caused by LPS and the reduce of cardiac index processed by interference technique could increase cell apoptosis caused by LPS. These data further supported the regulation function of hemodynamic on endothelial cells, consistent with the previous results. Meantime, this paper further showed that hemodynamics may inhabit cell toxicity and inflammation caused by LPS through directly or indirectly regulating cardiac index. This study provided theoretical basis for hemodynamics regulating vascular endothelial cell inflammatory state.

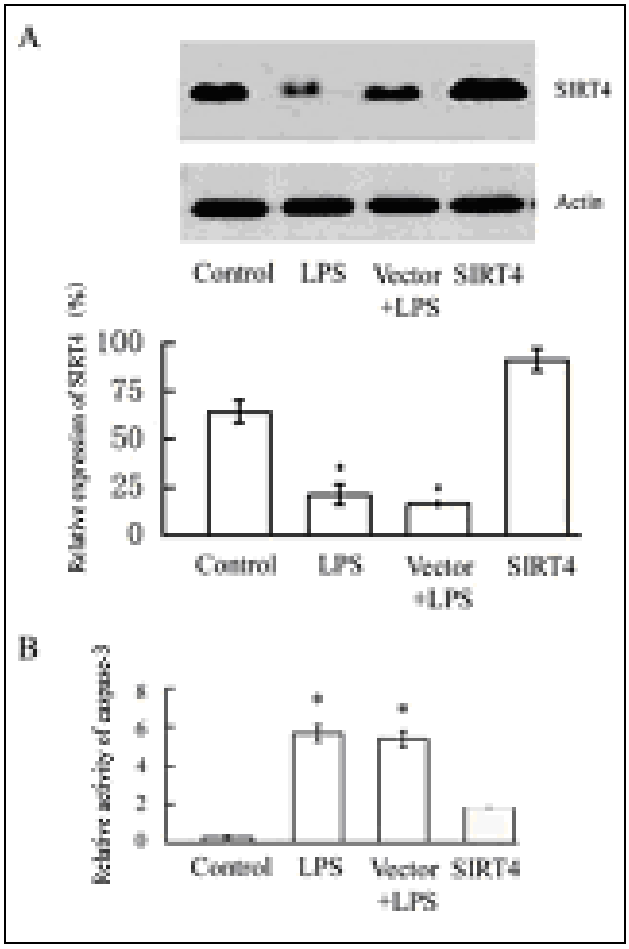

Fig. 10: over-expression cardiac index inhabiting cell apoptosis evoked by LPS

Note: *the difference was significant compared with the control group.

And there were three shortcomings in this article. First, there was no specific method of how hemodynamics regulated vascular endothelial cells by controlling cardiac index. Second, this paper only discussed experiments *in vitro*, instead building a model of AS in rats from the overall level. Third, there were no clinical specimens of AS to further validate the results in this paper.

CONCLUSION

These findings in this paper showed that hemodynamics could inhibit HUVEC apoptosis caused by LPS through

increasing cardiac index level. And cell apoptosis may be one of factors causing AS.

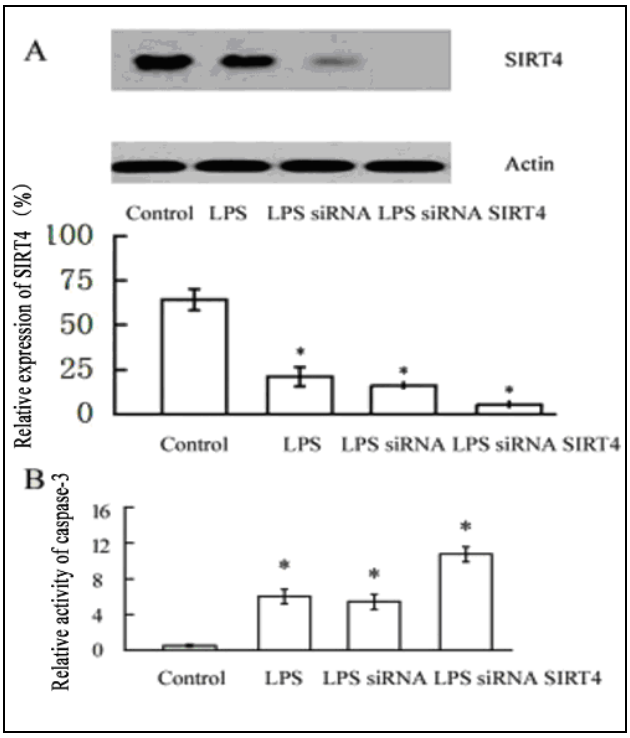

Fig. 11: The reduction of the cardiac index processed by the interfering technology enhancing cell apoptosis evoked by LPS

Note: *the difference was significant compared with the control group.

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