Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi: 10.1016/S1995-7645(14)60027-4

Correlation of restenosis after rabbit carotid endarterectomy and inflammatory cytokines

Jun-Jun Liang[1], Wei Xue[1], Li-Zhi Lou[1], Cheng Liu[1], Zhao-Fen Wang[2], Qing-Guo Li[3], Shao-Hua Huang[4*]

[1]*Department of Cardiovascular Thoracic Surgery, Anqiu People's Hospital, Anqiu 262100, China*
[2]*Department of Medical Service, Anqiu People's Hospital, Anqiu 262100, China*
[3]*Tianjin Cerebral Hospital, Tianjin 300060, China*
[4]*Department of Neurosurgery, Anqiu People's Hospital, Anqiu 262100, China*

ARTICLE INFO

Article history:
Received 10 December 2013
Received in revised form 15 January 2014
Accepted 15 February 2014
Available online 20 March 2014

Keywords:
Inflammatory factors
Carotid endarterectomy
Restenosis after angioplasty

ABSTRACT

Objective: To establish rabbit model of restenosis after carotid endarterectomy surgery, and to study tissue inflammatory cytokines (TNF-α, IL-6) involved in restenosis. **Methods:** A total of 32 rabbits were randomly divided into two groups: model group and control group. The right common carotid artery in rabbits was damaged by carotid endar terectomy in model group. The tissues were harvested at different time points respectively, the pathological changes of the vascular wall after operation were observed at different time points. The changes of expression of tissue vascular wall inflammatory cytokines (TNF-α, IL-6) at different time points after the surgery was observed by RT-PCR, and the changes of serum inflammatory cytokines (TNF-α, IL-6) were detected by ELISA. **Results:** The new intima appeared after 7 days of the injury and reached the peak on 28 d which is uneven and significantly thicker than the control group (P<0.01). The tissue inflammatory cytokines (TNF-α, IL-6) were significantly increased after the rabbit common carotid artery injury, which was significant difference compared with normal control group (P<0.05). **Conclusions:** The tissue inflammatory factors significantly increase after the rabbit carotid artery injury, which suggests the mutual concurrent effects of inflammatory cytokines can result in the proliferation of vascular restenosis.

1. Introduction

At present, restenosis after angioplasty become the limitation for the clinical application of carotid endarterectomy (CEA). However, the mechanism of restenosis is still unclear and the prevention is very difficult. Genesis and development of restenosis after the CEA, changes of the related indicators during the restenosis development process and treatment becomes a clinical research hotspot[1-3]. Recent studies suggest that inflammatory cytokines play important role in the arterial injury intimal hyperplasia. In this study, we observed the changes of the expression of tissue inflammatory factors, study the pathophysiological mechanisms of vascular proliferation restenosis, and explore the prevention and treatment of restenosis after angioplasty.

2. Materials and methods

2.1. Animals and model establishment

A total of 32 healthy adult New Zealand white rabbits of either sex were selected, weighing 2 800 to 3 200 g. All animals were purchased from XX University Experimental Animal Center, and were kept at isolation cabinet under 12-h light/dark cycle, 40% to 50% humidity, 24 ℃-26 ℃. Animals were fed with autoclaved water and high fat diet for one week. A total of 30 rabbits were randomly divided into two groups: normal group (n=16), model group (n=16). Normal group rabbits were fed with high fat diet for 4 weeks and received the intravenous anesthesia with pentobarbital

*Corresponding author: Shao-Hua Huang, M.M., Associate Chief Physician, Department of Neurosurgery, Anqiu People's Hospital, Anqiu 262100, China.
Tel: 15653670639
E-mail: sdaqhsh@163.com
Foundation project: It is supported by Shandong Provincial Health Department scientific researching fund projects 2005HW116.

sodium, draped in a sterile manner. They were opened layers by layers and the right common carotid artery was isolated. Without special intervention, they had adequate hemostasis, and then were sutured layers by layers. Model group rabbits were fed with high fat diet for 4 weeks, and the right common carotid artery was isolated. The artery was blocked in both ends with the artery occlusion. Longitudinal incision of the carotid was made and the intima and plaques was peeled. Residual vascular intimal flap was removed completely, vessel suture and adequate hemostasis was performed. Then they were sutured layers by layers. Ten thousand U/kg/d penicillin sodium was intramuscularly injected 3 d after surgery; the rabbits were sacrificed after 8 weeks of high-fat diet.

2.2. Reagents and primers

Standard rabbit diet, 2% cholesterol, 6% peanut oil, 4% paraformaldehyde, 2% glutaraldehyde, hematoxylin, eosin, etc. were provided by the laboratory.

Fat rabbit diet: mixed standard rabbit formulation+2% cholesterol +6%peanut oil and made into small pellets. TNF-α, IL-6 rabbit anti-mouse monoclonal antibody, TNF-α, IL-6 immunohistochemistry kit and TNF-α, IL-6 ELISA kits were purchased from Wuhan Boster Biological Engineering Co., Ltd. TNF-α, IL-6 transcription kits were purchased from Qiagen Corporation. The primers of TNF-α, IL-6 and β-actin were designed by the ABI software prism2.0 (Table 1).

2.3. Methods

2.3.1. Drawing materials

Blood was collected from ear marginal veins 1 d, 4 d, 1 week, 2 weeks, 3 weeks, 4 weeks, 6 weeks, 8 weeks postoperative respectively. They were centrifuged and the supernatant was stored at -80 ℃. After blood collection, two rabbits were injected with over-dose anesthetic and sacrificed. The carotid artery was exposed and 1 cm of artery was excised during the operation then divided into two sections. One blood vessel was immersed in 4% paraformaldehyde for immunohistochemical staining of vascular tissue, the other section was frozen at -80 ℃ refrigerator a for the TNF-α and IL-6 mRNA detection.

2.3.2. ELISA detection and RT-PCR

The serum cytokines TNF-α and IL-6 were measured by sandwich ELISA.

The blood samples were collected, total RNA was extracted by TRIzol. The reverse transcription kit of the Qiagen company was used to conduct reverse transcription, total system was 20 μL (Table 2).

They were centrifuged after mixing, and the process was as follow: 95 ℃ 5 min, *Taq* DNA polymerase was added after 1 cycle; 94 ℃ 1 min, 63 ℃ (TNF) or 65 ℃ (IL-6) 45 s and 72 ℃ 45 s, 35 cycles; 72 ℃ extension for 5 min. The agarose gel electrophoresis and photograph was performed. Quantity One software was used to analyze the light intensity of each band. β-actin expression was used as control to calculate and compare the relative expression levels of each factor.

2.3.3. Immunohistochemical assay

The blood vessel was embedded in paraffin, dewaxed, and added with the first antibody (TNF-α, IL-6 antibody, both diluted 1:100). Horseradish peroxidase (HRP)-labeled secondary antibody was added and incubated. PBS instead of primary antibody was used as a negative control. Five (200 ×) visions were randomly selected, the color range was divided into four grades: Color range <1/4 of the vision as 1; Color range 1/4-1/2 of the vision as 2; Color range 1/2-3/4 of the vision as 3; Color range >3/4 of the vision as 4;

Table 1
PCR detection of gene and primers.

Gene	Sequence 5′→3′	Fragment size (bp)
TNF-α	Upstream: ACCCTCACACTCAGATCATCTTCT	422
	Downstream: CAGATTGACCTCAGCGCTGAGTTG	
IL-6	Upstream: GTCTATACCACTTCACAAGTCGGA	441
	Downstream: TTGGATGGTCTTGGTCCTTAGCCA	
β-actin	Upstream: AGGGAAATCGTGGGTGACATCAAA	478
	Downstream: ACTCATCGTACTCCTGCTTGCTGA	

Table 2
Reverse transcription reaction system.

System components	Volum (μL)	Final concentration
10×Buffer RT	2	1×
dNTP Mix (5 mM each dNTP)	2	0.5 mM each dNTP
Random primer (50 ng/mL)	1	
RNase inhibitor (10 units/μL)	1	10 units (per reaction)
Sensiscipt reverse transcriptase	1	
RNase-free water	Variable	
Experimental samples RNA	Variable	<50 ng (per reaction)
Total volume	20	

2.4. Statistical analysis

The data was analyzed with SPSS 13.0 software. t-test, χ^2 examination and the Spearman rank correlation analysis were applied. $P<0.05$ was considered as statistical significant difference.

3. Results

3.1. TNF-α, IL-6 level

TNF-α, IL-6 expression were significantly increased in model group and reached the highest after two weeks. Compared with the control group, each monitoring point were statistically significant after 8 weeks ($P<0.05$) (Table 3).

Table 3
Serum inflammatory cytokines TNF-α, IL-6 levels at different time points..

Time	Control group		Model group	
	TNF-α (ng/mL)	IL-6 (pg/mL)	TNF-α (ng/mL)	IL-6 (pg/mL)
1 d	0.7±0.2	1.8±0.2	1.5±0.4*	2.3±0.2*
4 d	0.7±0.2	1.8±0.1	2.4±0.5*	7.5±0.5*
1 week	0.7±0.2	1.8±0.2	4.6±1.1*	55.6±3.2*
2 weeks	0.7±0.2	1.8±0.2	6.2±1.8*	65.7±3.7*
3 weeks	0.7±0.2	1.8±0.1	5.3±1.4*	51.4±3.3*
4 weeks	0.7±0.2	1.8±0.2	3.5±0.8*	41.3±2.5*
6 weeks	0.7±0.2	1.9±0.2	2.9±0.6*	19.5±1.2*
8 weeks	0.8±0.2	1.9±0.2	2.3±0.5*	8.6±0.6*

Note: * Compared with control group, $P<0.05$.

3.2. TNF-α, IL-6 mRNA expression of the vascular wall in two groups

After 1 day of the injury, the intimal injury was not yet been repaired, and no tissue was detected. After 4 days of the injury, intimal appeared and was gradually thickened. The expression in the model group was higher than control group. In the intimal, the TNF-α expression reached the peak after two weeks, then decreased gradually. In the media, the TNF-α expression of the model group was significantly increased after l day and reached the peak after two weeks, then decreased gradually. In the adventitia, the TNF-α expression of the model group was significantly increased after l day and reached the peak after one week, then decreased gradually. IL-6 expression was similar to the TNF-α expression. In the intimal, the IL-6 expression reached the peak after two weeks, then returned to the baseline. In the media, the IL-6 expression of the model group was significantly increased after l day and reached the peak after two weeks, then returned to the baseline. In the adventitia, the IL-6 expression of the model group was significantly increased after l day and reached the peak after one week, then returned to the baseline (Table 4, 5; Figure 1, 2).

After 1 day of the injury, the intimal injury was not yet been repaired, a small amount of positive staining cells were found in the media and the adventitia. After 4 days of

Table 4
TNF-α mRNA expression in each layer of the artery wall of the two groups.

Time	Intimal		Media		Adventitia	
	Model group	Control group	Model group	Control group	Model group	Control group
1 d	0	0.3±0.1	1.6±0.7*	0.9±0.1	1.0±0.5*	0.5±0.1
4 d	0.5±0.2*	0.3±0.1	2.3±1.1*	0.9±0.1	3.5±1.4*	0.5±0.1
1 week	2.5±1.4*	0.3±0.1	4.5±2.0*	0.9±0.1	4.4±1.8*	0.5±0.1
2 weeks	4.3±2.6*	0.3±0.1	5.6±1.8*	0.9±0.1	2.2±1.0*	0.5±0.1
3 weeks	3.7±1.9*	0.3±0.1	3.6±1.4*	0.9±0.1	1.5±0.6*	0.5±0.1
4 weeks	2.2±1.1*	0.3±0.1	3.2±1.3*	0.9±0.1	0.8±0.2*	0.5±0.1
6 weeks	1.0±0.4*	0.3±0.1	2.4±1.1*	0.9±0.1	0.6±0.1	0.5±0.1
8 weeks	0.8±0.3*	0.3±0.1	1.8±0.8*	0.9±0.1	0.5±0.1	0.5±0.1

Note: * Compared with control group, $P<0.05$.

Table 5
IL-6 mRNA expression in each layer of the artery wall of the two groups.

Time	Intimal		Media		Adventitia	
	Model group	Control group	Model group	Control group	Model group	Control group
1 d	0	0.3±0.1	1.3±0.4*	0.7±0.1	1.5±0.8*	0.3±0.1
4 d	1.1±0.4*	0.3±0.1	2.7±1.0*	0.7±0.1	4.3±1.9*	0.3±0.1
1 week	3.5±1.5*	0.3±0.1	3.9±1.7*	0.7±0.1	4.5±2.0*	0.3±0.1
2 weeks	4.4±2.8*	0.3±0.1	4.8±2.3*	0.7±0.1	3.4±1.5*	0.3±0.1
3 weeks	3.4±1.7*	0.3±0.1	3.5±1.4*	0.7±0.1	2.5±1.0*	0.3±0.1
4 weeks	2.4±1.0*	0.3±0.1	2.8±1.1*	0.7±0.1	1.6±0.9*	0.3±0.1
6 weeks	1.4±0.6*	0.3±0.1	2.2±1.0*	0.7±0.1	1.0±0.6*	0.3±0.1
8 weeks	1.0±0.4*	0.3±0.1	1.7±0.6*	0.7±0.1	0.7±0.5*	0.3±0.1

Note: * Compared with control group, $P<0.05$.

the injury, intimal appeared and a small amount of positive cells appeared, the positive staining cells were increased in the media and the adventitia. TNF-α expression reached the peak after 4 days in the intimal and the adventitia, and it reached the peak after 1 week in the media, then returned to the baseline. IL-6 expression reached the peak after one week in the intimal and the media, the adventitia, then returned to the baseline (Table 6, 7).

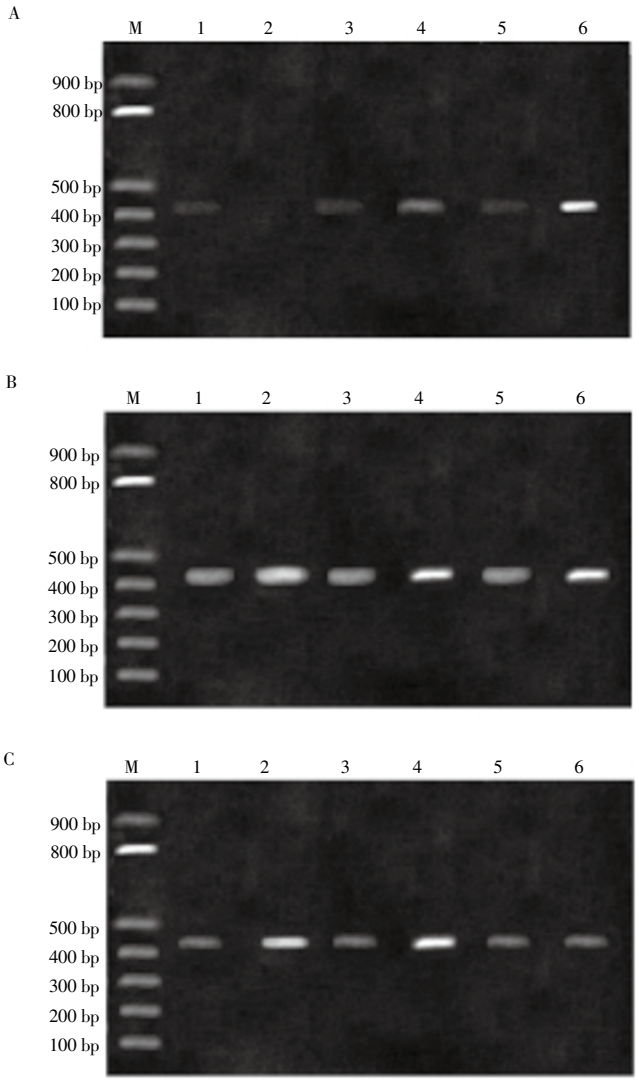

Figure 1. TNF-α mRNA expression at each time of the two groups.
A: intima; B: media; C: adventitia.
1,3,5: the control group. 2,4,6: the model group.

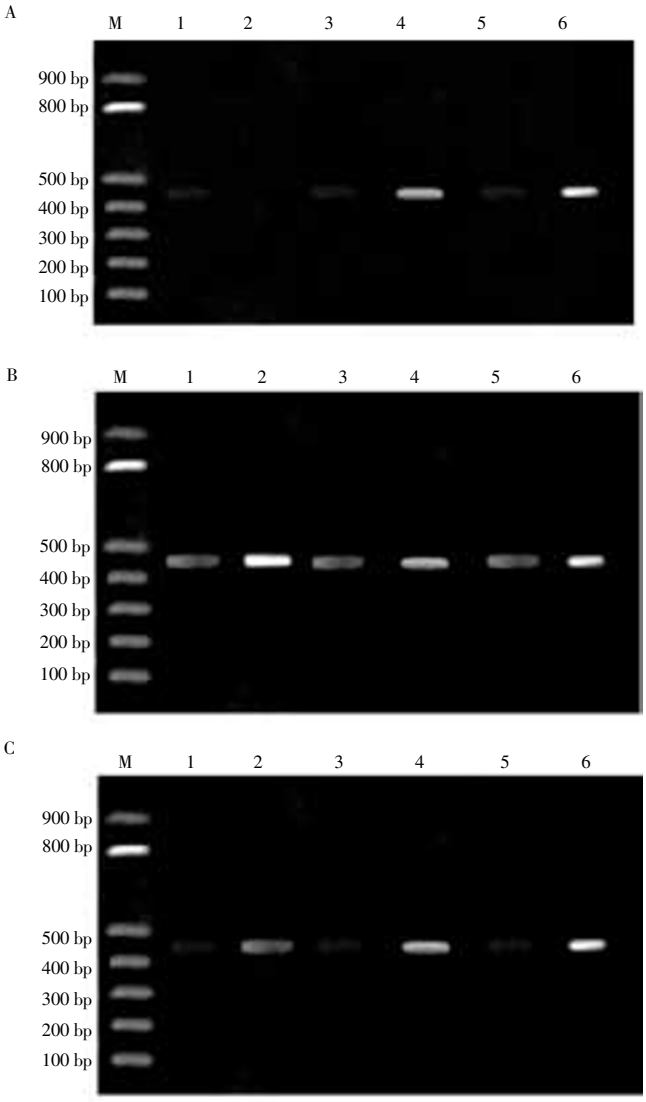

Figure 2. IL-6 mRNA expression at each time of the two groups.
A: intima; B: media; C: adventitia.
1,3,5: the control group. 2,4,6: the model group.

3.4. Correlation analysis of TNF-α and IL-6 mRNA expression in the vascular wall tissue

According to the Spearman correlation analysis of TNF-α and IL-6 mRNA expression in the vascular wall tissue, IL-6 and TNF-α expression was positively correlated (r=0.734 in

Table 6
TNF-α protein expression in each layer of the artery wall of the two groups.

Time	Intimal		Media		Adventitia	
	Model group	Control group	Model group	Control group	Model group	Control group
1 d	0	1.3±0.1	1.9±0.7*	1.3±0.1	1.5±0.5*	1.3±0.1
4 d	3.5±0.5*	1.3±0.1	2.8±1.0*	1.3±0.1	3.5±0.5*	1.3±0.1
1 week	2.5±0.4*	1.3±0.1	3.8±0.2*	1.3±0.1	3.2±0.8*	1.3±0.1
2 weeks	2.3±0.4*	1.3±0.1	3.6±0.4*	1.3±0.1	2.6±1.0*	1.3±0.1
3 weeks	2.0±0.3*	1.3±0.1	2.8±1.2*	1.3±0.1	2.4±1.0*	1.3±0.1
4 weeks	1.8±0.3*	1.3±0.1	2.6±1.4*	1.3±0.1	1.8±0.8*	1.3±0.1
6 weeks	1.8±0.4*	1.3±0.1	1.8±1.1*	1.3±0.1	1.6±0.5	1.3±0.1
8 weeks	1.5±0.3*	1.3±0.1	1.6±1.0*	1.3±0.1	1.5±0.5	1.3±0.1

Note: * Compared with control group, P <0.05.

Table 7
IL-6 protein expression in each layer of the artery wall of the two groups.

Time	Intimal		Media		Adventitia	
	Model group	Control group	Model group	Control group	Model group	Control group
1 d	0	1.1±0.1	1.5±0.7*	1.1±0.1	1.7±0.6*	1.1±0.1
4 d	1.5±0.6*	1.1±0.1	2.8±1.0*	1.1±0.1	3.5±0.5*	1.1±0.1
1 week	3.5±0.5*	1.1±0.1	3.6±0.4*	1.1±0.1	3.0±1.0*	1.1±0.1
2 weeks	3.2±0.8*	1.1±0.1	2.8±1.2*	1.1±0.1	2.4±0.8*	1.1±0.1
3 weeks	2.4±0.6*	1.1±0.1	2.5±1.0*	1.1±0.1	2.2±0.7*	1.1±0.1
4 weeks	2.0±0.5*	1.1±0.1	2.2±0.8*	1.1±0.1	1.6±0.9*	1.1±0.1
6 weeks	1.4±0.5*	1.1±0.1	2.0±0.6*	1.1±0.1	1.4±0.6*	1.1±0.1
8 weeks	1.2±0.4*	1.1±0.1	1.5±0.4*	1.1±0.1	1.1±0.4	1.1±0.1

Note: * Compared with control group, P <0.05.

intimal, r=0.678 in meida, r=0.785 in advebtitia, all P <0.05).

4. Discussion

Currently CEA is one of the important treatment for extracranial carotid artery stenosis. However, because the intimal self-healing can cause restenosis, the postoperative stenosis rate is up to 30%. At present, it is reported that the restenosis is caused by various tissue components. So prevention of restenosis is difficult in clinical[4,5]. In this study, we established restenosis rabbit model and analyzed the relationship between TNF-α, IL-6 and postoperative restenosis, then explore the pathogenesis of endarterectomy restenosis.

Inflammatory mediators play important roles in the process of the initiation and progression of the vascular injury. Moderate reaction is benefit to the vascular repair, but the overreaction can lead to vascular stenosis. TNF-α, IL-6 are important members of the complex network of inflammatory cytokine[6]. TNF-α is a multi-directional inflammatory mediators, which was almost not expressed in the normal vessel wall, but the expression was increased significantly in the vascular injury. In previous studies, it is reported that TNF-α plays the role in accelerated vascular smooth muscle cell migration, and it can also promote the expression of a variety of growth factors and cytokines. Restenosis is the vascular healing response to injury, while TNF-α produces a positive effect in the process of vascular remodeling[7-10]. Studies suggest that the hyperplasia reaction was significantly slowed after intimal injury of the TNF-α gene knockout rat model, so that TNF-α plays an important role in the process of arterial injury intimal hyperplasia[10].

The results of this study also showed that TNF-α expression in the serum and tissues were significantly increased in the intimal injury model group. It was significantly increased after 1 day, and reached the peak after 1-2 weeks, then returned to the baseline levels. IL-6 is a cytokines which produced by monocyte/macrophage cells, the expression increased after stent implantation. The increased expression is a series of water fall effects which can activate different substances, induced the increase of other cytokines, growth factors and mitogens[11-13]. The results of this study showed that the IL-6 expressions in the serum and tissues were significantly increased in the intimal injury model group. It was significantly increased after 1 day, reached the peak after 1-2 weeks, then returned to the baseline levels. Studies have shown that knocking IL-6 gene also can reduce the degree of stenosis due to vascular remodeling.

Studies suggest that IL-6 can promote intimal smooth muscle hyperplasia by activating STAT3 and other ways. Current data indicate that the proliferation smooth muscle in the media of vascular plays a vital role in the intima migrate. Its development process includes the following five aspects: thrombus formation phase, inflammatory reaction period phase, the proliferation of vascular smooth muscle cells phase, extracellular matrix formation and vascular remodeling phase[14-17]. According to the Spearman correlation analysis of TNF-α and IL-6 mRNA expression in the vascular wall tissues, the IL-6 and TNF-α expression was positively correlated (P<0.05). It is considered that the TNF-α and IL-6 expression were synergy to promote the further development of inflammation, which result in the excessive proliferation and migration of intimal vascular, as well as the lipid infiltration and the migration of adventitial fibroblast to the intima, thus constitute the basic pathological changes of restenosis. Although the whole process of the development mechanism is still unclear, the degree of intimal hyperplasia can be suppressed by knocking out inflammatory cytokine gene or inhibit the inflammatory response. This may suggest the endometrial response and inflammation are closely related[18].

In summary, after vascular injury, TNF-α and IL-6 play

important roles in repairing the vascular intimal. However, there is a correlation between them. It can promote more release of inflammatory mediators and lead to excessive vascular intimal repair, then lead to vascular stenosis. Therefore, inhibition of inflammatory cytokines is a new therapeutic approach to suppression of angiogenesis. But its exact mechanism and the regulation of mutual relations are still not yet very clear, and it need further study.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Li JM, Eslami MH, Rohrer MJ. Interleukin18 binding protein (IL-1 β) inhibits neointimal hyperplasi-a after balloon injury in an at-hero sclerotic rabbit model. *Vasc Surg* 2008, **47**(5): 1048-1057.

[2] Feng P, Yu XM, Huang YX, Jin HY. Study on the correlations beteen serum C-reactive protein (CRP) and carotid artery intima-media thickness (IMT) in the patients with impaired glucose tolerance (IGT). *Zhejiang Clin Med J* 2008; **10**(1): 21-22.

[3] Yang M, Zhou JG, Cao HT, Cao XB. The establishment of the experimental animal model of atherosclerosis and evaluation. *Med Equipm* 2010; **23**(6): 35-36.

[4] Yu XY, Wei L, Li XG, Liu Y, Ban X, Cao HL. Dynamic variation of macrophage in atherosclerotic plaques in rabbit. *J Chin Pract Diagn Ther* 2010; **24**(5): 436-439.

[5] Zhang CB, Zhu J, Zhang QY, Wu JJ, Shen BH. Relationship between expression of Cyr1 TNF-α, IL-6, IL-1β and restenosis of injured carotid artery. *J Shanghai Jiaotong Univ (Med Ed)* 2008; **28**(11): 1410-1415.

[6] Agrawal NK, Maiti R, Dash D, Pandey BL. Cilostazol reduces inflammatory burden and oxidativestress in hypertensive type 2 diabetes mellitus patients. *Pharmacol Res* 2007; **56**(2): 118-123.

[7] Wang B, Ren J, Ooi LL, Chong SS, Lee CG. Dinucleotide repeats negatively modulate the promoter activity of CYR61 and is unstable in hepatocellular carcinoma patients. *Oncogene* 2005; **24**(24): 3999-4008.

[8] Panutsopulos D, Arvanitis DL, Tsatsanis C, Papalambros E, Sigala F,. Spandidos DA. Expression of heregulin in human coronary atherosclerotic lesions. *Vascular Res* 2005; **42**(6): 463-474.

[9] Zheng CH, Liu QM, Zhou SH, Li XP, Shen XQ, Cao DL, et al. The inhibition of atorvastatin on aldose reductase expression and intimal hyperplasia in the intervention rats with restenosis. *Chin J Arterioscler* 2006; **14**(6): 487-490.

[10]Wei F, Wang YX. The study on restenosis after coronary artery angiplaty. *J Inner Mongolia Med Coll* 2006; **1**(28): 67-71.

[11]Honold J, Lehmann R, Heeschen C, Walter DH, Assmus B, Sasaki K, et al. Effects of granulocyte colony simulating factor on functional activities of endothelial progenitor cells in patients with chronic ischemic heart disease. *Arterioscler Thromb Vasc Biol* 2006; **26**(10): 2238-2243.

[12]Suh W, Kim KL, Choi JH, Lee YS, Lee JY, Kim JM, et al. C-reactive protein impairs angiogenic functions and decreases the secretion of arteriogenic chemo-cytokines in human endothelial progenitor cells. *Biochem Biophys Res Commun* 2004; **321**(1): 65-71.

[13]Laufs U, Wassmann S, Czech T, Münzel T, Eisenhauer M, Böhm M, et al. Physical inactivity increases oxidative stress, endothelial dysfunction, and atherosclerosis. *Arterioscler Thromb Vasc Biol* 2005; **25**(4): 809-814.

[14]Aggarwal A, Schneider DJ, Terrien EF, Gilbert KE, Dauerman HL. Increase in interleukin-6 in the first hour after coronary stenting: an early marker of the inflammatory response. *Thromb Thrombolysis* 2003; **15**(1): 25-31.

[15]Takaoka M, Uemura S, Kawata H, Imagawa K, Takeda Y, Nakatani K, et al. Inflammatory response to acute myocardial infarction augments neointimal hyperplasia after vascular injury in a remote artery. *Arterioscler Thromb Vasc Biol* 2006; **26**(9): 2083-2089.

[16]Danenberg HD, Fishbein I, Ep stein H, Waltenberger J, Moerman E, Mönkkönen J, et al. Systemic depletion of macrophages by liposomal bisphosphonates reduces neointimal formation following balloon-injury in the rat carotid artery. *Cardiovasc Pharmacol* 2003; **42**(5): 671-679.

[17]Schober JM, Lau LF, Ugarova TP, Lam SCT Identification of a novel integrin α M β 2 binding site in CCN1 (CYR61), a matricellular protein expressed in healing wounds and atherosclerotic lesions. *J Biological Chem* 2003; **278**(28): 25808-25815.

[18]Funayama H, ishikawa SE, Kubo N, Yasu T, Saito M, Kawakami M. Close association of regional interleukin-6 levels in the infarct-releted culprit coronary artery with restenosis in acute myocardiol infarction. *Circ J* 2006; **70**(4): 426-429.