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Expression of Toll-Like Receptor 4, Tumor Necrosis Factor-Alpha, Matrix Metalloproteinase-9 and Effects of Benazepril in Patients with Acute Coronary Syndromes

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Abstract

Objectives: The study aims to explore the relationship between expressions of toll-like receptor 4 (TLR4) on peripheral blood monocytes, serum tumor necrosis factor-alpha (TNF- α) and matrix metalloproteinase-9 (MMP-9) in patients with acute coronary syndromes (ACS), and to investigate the possible mechanisms of Benazepril stabilizing atherosclerosis plaques.

Methods: 70 patients selected were randomly divided into Benazepril treatment group (35 patients) and regular treatment group (35 patients). Meanwhile, Stable angina pectoris (SAP) group of 32 patients and control group of 22 patients were also set up. With the help of flow-cytometry, expressions of TLR4 on peripheral blood monocytes of the four groups were analyzed and compared to show differences, correlations and changes of the above mentioned indicators. The concentration of TNF- α and MMP-9 in serum were measured by enzyme linked immunosorbent assay (ELISA).

Results: (1) Expressions of TLR4, levels of TNF- α and MMP-9 were increased and the rate was rising from the control group, to SAP group and then to ACS group. All these indicators in ACS group are significantly higher than those in other groups ($P < 0.05$). (ACS versus SAP, control; all ($P < 0.05$)). (2) Multi-linear regression analysis indicates that there was a positive correlation between the expression level of TLR4 and serum levels of TNF- α and MMP-9 in patients with ACS ($P < 0.01$). (3) There is no significant differences between the expression level of TLR4 and serum levels of TNF- α and MMP-9 in Benazepril treatment group and regular treatment group before treatment ($P > 0.05$) while they all fell after treatment ($P < 0.05$). In addition, all the indicators decreased more greatly than the regular treatment group.

Conclusions: TLR4 on peripheral blood monocytes and serum TNF- α and MMP-9 in patients with coronary arteriosclerosis disease may be effective markers of the vulnerable plaque. Benazepril can inhibit over-expression of TLR4 and reduce serum levels of TNF- α and MMP-9, thus stabilize the vulnerable plaques and improve the condition of the patients with ACS.

Keywords: acute coronary syndromes, toll-like receptor 4, tumor necrosis factor-alpha, matrix metalloproteinase-9, benazepril

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Introduction

Acute coronary syndromes (ACS) is often related with vulnerable atherosclerosis plaque rupture, the formation of thrombus and subsequent influence in blood flow of coronary arteries. Early diagnosis and treatment of vulnerable plaques exert a very important part in the prevention and reduction of further rupture and reduction of clinical emergent accidents. Recent studies have shown that the formation and development of atherosclerosis are at least partly transmitted through toll-like receptor 4 (TLR4)/nuclear factor κ B (NF- κ B) pathway. TLR4 cell signal transmission finally activate NF- κ B, induce monocytes/macrophage to produce immunity inflammation cytokines (such as TNF- α), and adhesion molecules, which lead to plaque rupture. Current medicine research in treating vulnerable atherosclerosis plaque focuses mainly on statins. It is not clear whether angiotensin converting enzyme inhibitors (ACEI) can stabilize plaques by inhibiting signal transduction pathways of inflammatory cells toll-like receptor 4 (TLR4). Based on the observation of expression of TLR4 on the peripheral blood monocytes, serum TNF- α and MMP-9, this experiment aims to obtain reliable serum inflammatory indicators, which indicate unstable atherosclerosis plaque. This article will explore the possibilities of treating ACS with ACEI by showing effects of such medicine treatment on inflammatory indicators.

Methods

Objects

(1) We selected 70 ACS patients (42 UAP and 28 AMI), including 41 males and 29 females. We divided them into two groups, respectively regular treatment group and Benazepril treatment group. The 35 members of the regular treatment group consists of 21 males and 14 females with an average age of 58.9 ± 12.1 , while the Benazepril treatment group involves 22 males and 13 females with and average age of 61.2 ± 13.1 . The regular treatment group use similar medical management (such as nitric acid ester, aspirin, beta-blocker, statin and low molecule heparin). The Benazepril treatment group use Benazepril apart from these regular medicines. (2) 32 stable angina pectoris (SAP) patients of 22 males and 10 females have an average age of 56.8 ± 13.6 . The diagnosis of UAP, AMI and SAP conforms to the standard set by guideline of American College of Cardiology/The American Heart

Association (ACC/AHA) with a further confirmation of coronary angiography. Exclusion criteria included acute infection, malignancy, autoimmunity diseases, vascular diseases, severe liver or kidney diseases or operations and injuries within one week. (3) Control group comprises patients without a history of high blood pressure, diabetes or coronary heart disease. After checking with electrocardiogram (ECG), myocardium enzymology and coronary coronary arteriography, these 22 patients of 15 males and 7 females do not have coronary heart disease.

Blood sampling and laboratory determinations

Blood was drawn in the sitting position from the antecubital vein respectively at the day of admission and 4 weeks after treatment between 8:00 and 9:00 Am in the fasting state. All medications were withdrawn for at least 12 h. The blood samples were immediately cooled on ice. 1 ml of the blood is anti-coagulated by potassium ethylene diamine tetraacetic acid (EDTA-K₂), Serum was obtained by subsequent centrifugation at 3000 g for 10 min. All samples were stored at -75°C until assayed.

(1) Flow cytometer (FCM) is used to measure expressions of TLR4 on the peripheral blood monocytes. 100 ul whole blood anti-coagulate with EDTA-K₂ was obtained, added 20 ul hTLR4 antibody (mouse anti-human monoclonal antibody, clone HTA125, IgG2a, Biolegend, San Diego, America) conjugated with PE to it and mixed them, Cells were incubated with hTLR4 antibody for 30 minutes under room temperature, then fixed it up and broken the membrane, After cells were washed with staining buffer (PBS containing 0.1% BSA and 0.1% sodium azide), and with sham control, analysis by Flow cytometry. (Coulter Company, McLean America). (2) TNF- α and MMP-9 were determined by commercially available enzyme-linked immunosorbent assay (TPI company, Houston, Texas Area, America).

Statistics

The Statistical Package for the Social Science (SPSS for windows 12.0) was employed for statistical analysis. Continuous variables are expressed as mean \pm SD ($\bar{x} \pm S$). The differences between groups adopted a repeated measures ANOVA while the correlation was analyzed with multiple linear regression analysis. Statistical significant was defined at a P value of <0.05 .



Results

Subject profile

Table 1 present the clinical characteristics of the subjects investigated. The differences between ACS, SAP and control subjects in gender, age, Body Mass Index (BMI), percentage of smokers, DM and the level of LDL-C were not significant ($P > 0.05$). There is no significant differences in gender, age, BMI, percentage of smokers, high blood pressure, DM and the level of LDL-C between regular treatment group and Benazepril treatment group ($P > 0.05$).

The changes of expressions of TLR4 on peripheral blood monocytes, TNF- α and MMP-9 in ACS, SAP and control groups. As shown in Table 2, expressions of TLR4 and levels of TNF- α and MMP-9 were significantly increased in ACS group ($P < 0.05$) (Table 2).

The correlation between TLR4 and TNF- α , MMP-9 in ACS patients

Multiple linear regression analysis showed that there was a positive correlation between the expression level of TLR4 and serum levels of TNF- α and MMP-9 in patients with ACS ($P < 0.01$).

Comparison of the expression level of TLR4 and serum levels of TNF- α and MMP-9 before and after treatment. There is no significant difference between the expression level of TLR4 and serum levels of TNF- α and MMP-9 in Benazepril treatment group and regular treatment group before treatment ($P > 0.05$). While these indicators all fell after treatment ($P < 0.05$). In addition, all of them decreased more greatly in Benazepril treatment group than in the regular treatment group. (Table 3).

Discussion

Recent studies have shown that the formation and development of atherosclerosis are at least partly transmitted through toll-like receptor 4 (TLR4)/nuclear factor κ B (NF- κ B) pathway. TLR4 cell signal transmission finally activate NF- κ B, induce monocytes/macrophage to produce immunity inflammation cytokines (such as TNF- α), and adhesion molecules, which lead to coronary heart disease.^{1,2} Methe reported that the increase of expressions of TLR4 on peripheral blood monocytes in patients with ACS had correlation with the stability of plaques.³ The results of study by Ishikawa indicated expression of both systemic and local plaque TLR4 may be one of the mechanisms responsible for the pathogenesis of AMI.⁴ Pro-inflammatory cytokine plays an important part in the pathology process of the ACS. Inflammatory cytokine can activate the secretion of matrix metalloproteinases (MMPs) and lead to the instability and vulnerability of plaques. Cytokine is a multifunctional medium of inflammation and immunoreaction, which was secreted and released by local inflammation cells. Its autocrine and paracrine affect the function of vascular wall cells. Studies showed that vascular smooth muscle cell stimulated by IL-1 and TNF- α not only increased the synthetic secretion of MMP-1, but also activated MMP-2 and MMP-9 in the form of zymogen and started to synthesize and secrete MMP-3.^{1,5} Although the activity of MMPs is inhibited by its tissue inhibitors of metalloproteinase (TIMP), normally vascular smooth muscle cells will secrete a tiny amount of TIMP-1 and TIMP-2 for modulation and cytokine which does nothing to its secretion. The net result is that MMPs has advantage over the degradation of extracellular matrix (ECM), the intensity of

Table 1. Characteristics of subject (s). ($\bar{x} \pm s$).

Variable	ACS group (n = 70)	Benazepril group (n = 40)	Regular treatment group (n = 30)	SAP group (n = 32)	Control group (n = 22)
Age (yrs)	59.9 \pm 11.9	61.2 \pm 13.1	58.9 \pm 12.1	56.8 \pm 13.6	58.6 \pm 8.3
Male/female ratio	41/29	21/14	22/13	22/10	15/7
BMI (kg/m ²)	23.9 \pm 2.6	24.1 \pm 2.7	23.5 \pm 2.6	25.3 \pm 1.9	24.0 \pm 2.7
Smokers	24	14	10	7	11
Hypertension	30	17	13	10	0
history of DM	14	6	5	4	1
LDL-C (mmol/L)	2.0 \pm 0.6	2.1 \pm 0.5	2.2 \pm 0.4	1.9 \pm 0.5	1.8 \pm 0.4

Abbreviations: BMI, body mass index; ACS, acute coronary syndromes; SAP, stable angina pectoris; DM, diabetes mellitus; LDL-C, low density lipoprotein cholesterol.



Table 2. Comparison of TLR4 on peripheral blood monocytes, TNF- α and MMP-9 between patients with ACS, SAP and control subjects. ($\bar{x} \pm s$).

	TLR4 (%)	TNF- α (pg/ml)	MMP-9 (ng/L)
ACS group (n = 70)	80.3 \pm 11.45* [▲]	17.53 \pm 6.62* [▲]	14.25 \pm 5.63* [▲]
SAP group (n = 22)	60.35 \pm 15.5	10.26 \pm 2.83	2.31 \pm 1.91
Control group (n = 32)	51.3 \pm 10.92	9.15 \pm 2.30	1.86 \pm 0.97

Notes: * $P < 0.01$, vs. control group; [▲] $P < 0.01$ vs. SAP group.

Abbreviations: TLR4, toll-like receptor 4; TNF- α , tumor necrosis factor-alpha; MMP-9, matrix metalloproteinase-9; ACS, acute coronary syndromes; SAP, stable angina pectoris.

fiber head cap weakens and plaques grow vulnerable. This study shows that the expression level of TLR4, serum levels of TNF- α and MMP-9 indicate a rising tendency from control group to SAP group and then to ACS group. All these indicators in the last group (ACS) is significantly higher than those in the first two ($P < 0.05$). It is also found out that the expression level of TLR4 has a positive correlation with serum levels of TNF- α and MMP-9, which affirms the correlation between them from serology. The rise of TLR4, TNF- α and MMP-9 is closely related to ACS, which may be applied as a serology indicator in treatment of patients with coronary heart disease. However, the sample in this study is limited and it needs further experiment to prove.

Angiotensin II (ang. II) can affect various vascular system factors which lead to the instability of plaques. It causes malfunction of endothelium and arteriosclerosis plaques. It also induces expressions of vascular cell adhesion molecular 1 and contributes to the attachment of monocyte to endothelial cell and produces oxygen-derived free radicals. All these will bring about pre-inflammatory state and instability of plaques.⁶ ACE inhibitors or angiotensin II receptor antagonists are useful in changing it. The subsection analysis SECURE of HOPE study indicates that Ramipril

of 10 mg can greatly lower the thickness of carotid endarterium. TREND study show that Quinapril can bring down the contraction reaction of blood vessel to the inducement of acetylcholine. Our past clinical research also proved that ACEI preparation Fosinopril can inhibit NF- κ B signal path, inflammatory cytokine TNF- α and MMPs/TIMPs.⁷ The results of this study show that ACEI preparation Benazepril can remarkably reduce TLR4, TNF- α and MMP-9. It is estimated that ACEI can stabilize vulnerable plaques of atherosclerosis and reduce the number of acute cardiovascular events by inhibiting the over-expression of TLR4 and reduce serum levels of TNF- α and MMP-9.

Conclusions

TLR4 on peripheral blood monocytes and serum TNF- α and MMP-9 in patients with coronary arteriosclerosis disease may be effective markers of the vulnerable plaque. Benazepril can inhibit over-expression of TLR4 and reduce serum levels of TNF- α and MMP-9, thus stabilize the vulnerable plaques and improve the condition of the patients with ACS.

Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under

Table 3. Comparisons of the expression level of TLR4 and serum levels of TNF- α and MMP-9 before and after treatment. ($\bar{x} \pm s$).

	TLR4 (%)		TNF- α (pg/ml)		MMP-9 (ng/L)	
	Before	After treatment	Before	After treatment	Before	After treatment
Regular group (n = 40)	79.63 \pm 10.87	61.25 \pm 9.66*	16.79 \pm 6.13	12.75 \pm 5.98*	14.16 \pm 5.36	3.97 \pm 1.11*
Benazepril group (n = 30)	81.22 \pm 11.91	52.29 \pm 8.47* [≥]	18.02 \pm 6.71	9.86 \pm 5.04* [≥]	14.35 \pm 5.97	2.13 \pm 1.01* [≥]

Notes: * $P < 0.05$ vs. before treatment group; [≥] $P < 0.05$ vs. regular treatment group. See Table 2 for abbreviations.



consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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