The effects of pentoxifylline administration on NFKB P50 transcription factor expression

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Abstract

BACKGROUND: Pentoxifylline has anti-inflammatory properties and could suppress some inflammatory processes including tumor necrosis factor-alpha (TNF-α) production. We assessed the effects of a two-month administration of pentoxifylline on nuclear factor-kappa B (NFκB) pathways in patients with coronary artery disease (CAD) in which inflammatory pathways, especially NFκB transcription factors, have a critical role.

METHODS: A double-blind randomized placebo-controlled study design was used. Forty CAD patients were randomized to either 2 months of pentoxifylline treatment (1200 mg/day) (n = 20) or placebo treatment (n = 20). Blood samples were obtained just before and after two months of treatment. P50 protein concentration in peripheral blood mononuclear cells (PBMCs) was measured by Enzyme Linked ImmunoSorbent Assay (ELISA) method.

RESULTS: P50 concentration did not significantly change during two months of pentoxifylline administration.

CONCLUSION: Longer pentoxifylline administration is needed to see its favorable effects on NFκB family elements.

Keywords: Coronary Artery Diseases, Inflammation, NFκB, Pentoxifylline.

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Introduction

Ischemic conditions due to atherosclerosis and its complications such as coronary artery syndrome (CAS) are the leading cause of death in developed countries. Conventional cardiovascular risk factors include cigarette smoking, hypertension, hypercholesterolemia, diabetes and obesity. Treatment of these risk factors is the goal of primary prevention. However, the absence of conditional risk factors does not completely guaranty individuals to be free from cardiovascular diseases (CADs) and new risk factors have been identified, including markers of inflammatory origin.[1,2] Inflammation plays an important role in almost all stages of atherosclerosis progression and atherosclerotic plaque vulnerability to rupture. Leukocytes infiltrated in atherosclerotic plaques of unstable patients secret matrix-degrading enzymes and thrombogenic substances, resulting in plaque rupture and local thrombosis and subsequent clinical events, such as acute coronary and cerebrovascular syndromes (unstable angina, myocardial infarction, sudden death and stroke). [3,4] Interestingly, rupture in vulnerable plaques often occurs with even less than 50% stenosis. Conversely, plaques from asymptomatic patients or patients with stable symptoms demonstrate thick fibrous caps, small lipid cores and significantly fewer inflammatory cells. Stable plaques usually increase symptoms after plaque stenosis to greater than 70%. Significant reduction in flow to the myocardium is induced by these large plaque lesions, resulting in the typical symptoms of stable angina pectoris.[5,6] Therefore, both plaque rupture and plaque stenosis are vastly influenced by inflammatory process. As these processes lead to cardiovascular events and even death, their inhibition could be a target for prevention and treatment of CVDs. Furthermore, it was reported that drugs such as atorvastatin,[7-9] simvastatin[10,11] and

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ezetimibe,[12] which have obvious favorable effects on CAD patients, also have immunomodulatory properties that could be an additional mechanism for their effects.

Nuclear factor-kappa B (NFκB) is a family of transcription factors including p50 and p65. Translocation of p50 to the nucleus is considered as a response of cells to inflammatory mediators such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1). The canonical pathway of NFκB activation that involves p50 is activated in human atherosclerosis and results in selective upregulation of major proinflammatory and prothrombotic mediators.[13]

Several studies[14,15] used peripheral blood mononuclear cells (PBMCs) as reporters of drug response to the immune system. Circulating mononuclear cells are suitable to study the atherosclerosis process. They are also accessible surrogate cells to investigate the immune system and atherosclerosis.[16]

Pentoxifylline has been shown to have favorable effects on components of the immune system.[17] Impaired differentiation and maturation of human monocyte-derived dendritic cells have been reported after pentoxifylline administration.[18] Pentoxifylline 800 mg daily for one month decreased C-reactive protein (CRP) and total leukocyte count.[19] Decreased C-reactive protein and TNF-α concentrations were reported in a randomized placebo-controlled study after administration of pentoxifylline 1200 mg daily for 6 months in patients with acute coronary syndrome (ACS) compared to the control group.[20]

Thus, we hypothesized that the NFκB system could be downregulated by pentoxifylline in patients with atherosclerosis. Therefore, we decided to compare p50 expression levels in PBMCs between a control group and a group treated with pentoxifylline. It was the first time that the effects of pentoxifylline on NFκB system have been evaluated in a clinical study.

Materials and Methods

Patients

This study was approved by the Ethics Committee of Mashhad University of Medical Sciences, Iran. Forty patients with CAD, admitted for drug therapy, signed a consent form prior to entering the study. All patients had established atherosclerosis and were enrolled in the study between July 2009 and November 2010. A cardiovascular specialist defined CAD. Diagnosis of stable angina was done based on either clinical assessment alone or in case of uncertainty, clinical assessment plus diagnostic testing (anatomical testing for obstructive coronary artery disease). The study was double-blinded. Patients were randomized to two equal groups. To achieve balanced group sizes, restricted randomization (blocked randomization type) was used. Blood samples were obtained from the patients before and two months after either pentoxifylline or placebo administration. Demographic data, laboratory data, drug history, post medical history, familial history and cardiovascular risk factors were recorded for each patient. Patients who were treated with angiotensin-converting enzyme (ACE) inhibitors, statins (except few patients who needed these medications in the last phases of this study) or immunosuppressive drugs were excluded. Patients who had chronic disease including diabetes, renal or hepatic diseases were also excluded from the study.

PBMCs isolation

PBMC isolation was done by centrifugation of the whole blood through a density gradient.[14,21,22] In this method, the whole blood was layered onto a sterile density gradient separation medium containing sodium diatrizoate at a predetermined density of 1.0770 g/ml at 22°C (CEDARLANE, Ontario, Canada). Centrifugation at room temperature at 500 rpm for 15 minutes results in the separation of PBMCs at the blood-separation medium interface while the other white blood cells (WBCs) and red blood cells (RBCs) pass through the interface and collecting at the bottom of the tube. The obtained PBMCs were transferred to a new tube and washed with sterile phosphate-buffered saline (PBS) to remove any possible contaminating separation medium. A hypotonic aqueous medium [RBC lysis buffer (Sigma-Aldrich, Germany)] was used to lyse any possible contaminating RBCs. The PBMCs were pelleted by centrifugation and washed with PBS. Finally, the PBMCs were transferred to a microcentrifuge tube and centrifuged briefly to form a tight pellet. Any remaining liquid was completely removed and the resulting "pellet" was frozen at –70°C until use.

Enzyme Linked ImmunoSorbent Assay (ELISA) for NFκB measurement

Cayman's NFκB (human p50) transcription factor assay (Cayman Chemical Company, USA) kit for the p50 assay was used in this study. It is a non-radioactive, sensitive method for detecting specific transcription factor DNA binding activity in nuclear extracts and whole cell lysates. A specific double stranded DNA (dsDNA) sequence containing the NFκB response element had been immobilized on to the bottom of wells of a 96 well plate. NFκB contained in a nuclear extract bound specifically to the NFκB response element. NFκB (p50) was

detected by addition of specific primary antibody directed against NFκB (p50). A secondary antibody conjugated to horseradish peroxidase (HRP) was added and the absorbance was read out at 450 nm.

Statistical analyses

Results are presented as mean [SEM (standard error of the mean)]. Differences between patients and controls and different groups of patients or controls were calculated by the two-tailed paired or unpaired student's t-test as appropriate. A $P < 0.05$ was considered as statistically significant. All analyses were performed with $SPSS_{11.5}$ (IBM Corporation, New York, USA).

Results

Demographic and clinical characteristics of the study population are shown in tables 1 and 2. As it is seen, there were no significant differences between the two groups ($P > 0.05$) except in case of chest pain ($P < 0.05$).

Table 1. Patient characteristics

	Placebo group (n = 20)	**Pentoxifylline group (n = 20)**
Age (years)	53.62 (9.19)	55.46 (7.69)
Sex M/F (%)	85/15	75/25
Smoking (%)	50	30
Hypertension (%)	30	30
Hyperlipidemia (%)	25	25
Chest pain (%)	55	25
Involved in coronary artery bypass graft surgery (%)	45	30
Involved in percutaneous coronary intervention (%)	30	55
Plaque in left anterior descending-coronary artery (%)	75	75
Plaque in right coronary artery (%)	15	40
Plaque in circumflex coronary artery (%)	10	45
Triple vessel coronary artery disease (%)	25	15
Chronic obstructive pulmonary disease (%)	10	10
Hypothyroidism (%)	10	0
Ejection fraction	51.46 (8.33)	52.33 (8.63)
Myocardial infarction (%)	30	40

Table 2. Drugs administered for each group

Administered drugs	**Placebo group (n = 20)**	**Pentoxifylline group (n = 20)**
Metoprolol	40	25
Propranolol	25	10
Atenolol	30	70
Nitrocontin	45	40
Diltiazem	15	10
Hydrochlorothiazide	10	10
Isosorbide dinitrate	0	15
Clopidogrel	0	15
Captopril	0	10
Enalapril	15	15
ASA	90	100
Statins	15	25

All the values are expressed as percentage

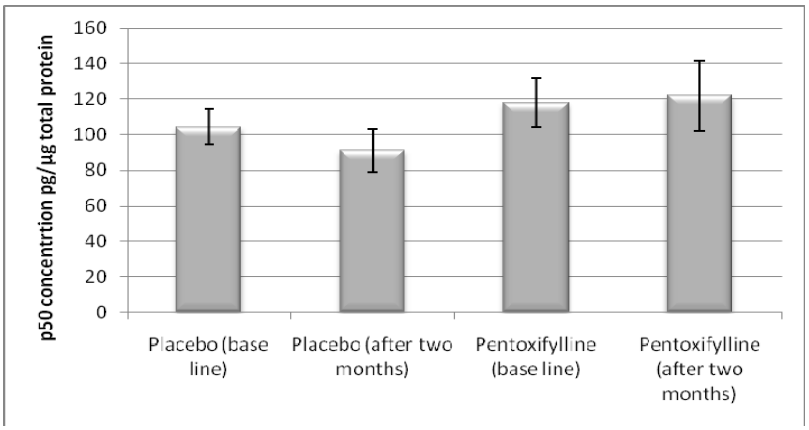

Figure1. Mean (SEM) p50 protein levels in PBMCs of placebo and pentoxifylline groups

Expression of p50 protein in PBMCs did not significantly change [117.8 (0.4) vs. 121.8 (20) pg/µg total protein] after two months of treatment with pentoxifylline determined by ELISA method described previously (Figure 1).

Discussion

Our results indicate that two months pentoxifylline administration did not induce any significant changes in expression of p50 protein of PBMCs.

As pentoxifylline has shown immunomodulatory activity in various populations, especially patients with cardiovascular diseases, we selected it for our study. A double-blind, prospective, placebo-controlled study on ACS patients have showed that pentoxifylline administration (1200 mg/day) for 6 months decreased TNF-α and CRP and inhibited the increase of interleukin 12 (IL-12). It also inhibited interleukin-10 (IL-10) (an anti-inflammatory cytokine) reduction.[20] Likewise, decreased CRP levels were reported in hypertensive diabetic patients after the administration of pentoxifylline (800 mg/day) for 2 months.[19] Moreover, hypercholesterolemic atherosclerosis development in rats was reduced by pentoxifylline probably via decreasing platelet-activating factor (PAF) and reactive oxygen species (ROS) levels.[23] Pentoxifylline can decrease TNF-α, interleukin-12 (IL-12) and interleukin-18 (IL-18) levels in monocyte-derived dendritic cells and suppress their maturation.[18] It was suggested that the cardioprotective effects of pentoxifylline against ischemia and reperfusion injury may be due to reductions in the activation of NFκB and the production of TNF-α content.[24] Pentoxifylline has been suggested to downregulate p65 expression in cultured rat vascular smooth muscle cells.[25]

NFκB activation involves p65 and p50. It is activated in human atherosclerosis and results in selective upregulation of both major proinflammatory and prothrombotic mediators.[13] Furthermore, the correlation between circulating levels of NFκB in patients with unstable angina and plasma oxidized low-density lipoprotein (LDL) levels was demonstrated.[26] We tested the effects of the pentoxifylline administration on NFκB system in our study. In contrast with some of the abovementioned studies that have shown the favorable effects of pentoxifylline on NFκB system components, we could not find any significant results.

Conclusion

As previously published results proved the favorable effects of pentoxifylline on inflammatory mediators involving in atherosclerosis and ACS progression, longer pentoxifylline administration is needed to see its favorable effects on NFκB family elements.

Conflict of Interests

Authors have no conflict of interests.

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