

Pentoxifylline Decreases Serum Level of Adhesion Molecules in Atherosclerosis Patients

Amir Hooshang Mohammadpour^{1,2}, Homa Falsoleiman³, Jamal Shamsara², Ghazaleh Allah Abadi⁵, Ramin Rasooli² and Mohammad Ramezani^{*2,4}

¹Dept. of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Science, Mashhad, Iran; ²Pharmaceutical Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran; ³Cardiovascular Research Center, Ghaem Hospital, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; ⁴Nanotechnology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Received 9 March 2013; revised 11 August 2013; accepted 13 August 2013

ABSTRACT

Background: Inflammation is involved in development, progression, and complications of atherosclerotic disease. Clinical studies have indicated that the level of monocyte chemoattractant protein 1 (MCP-1), IL-18, and adhesion molecules correlates with the severity of atherosclerosis and can predict future cardiovascular events. Experimental studies have shown pentoxifylline (PTX) reduces these factors in animal models. The purpose of the present pilot study was to evaluate effect of PTX on a group of inflammatory biomarkers in patients with coronary artery disease (CAD). **Methods:** Forty patients with angiographically documented CAD, who fulfilled inclusion and exclusion criteria, were entered in the double-blind, randomized, pilot clinical study. The patients were randomly given PTX (400 mg three times daily) or placebo (3 tab/day) for 2 months. Serum concentrations of MCP-1, IL-18, intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule 1 (VCAM-1) were measured before and at the end of intervention by enzyme-linked immunosorbent assay. **Results:** Our study showed that the serum levels of ICAM-1 and VCAM-1 was decreased in the study population after two-month treatment ($P < 0.05$). **Conclusion:** Based on the results of our pilot study, administration of PTX in CAD patients significantly decreases adhesion molecules levels. *Iran. Biomed. J. 18 (1): 23-27, 2014*

Keywords: Atherosclerosis, Inflammation, Pentoxifylline

INTRODUCTION

Cardiovascular disease (CVD) continues to lead as a principal cause of death in developed countries. The vast majority of CVD-related deaths are attributed to a disease of arterial blood vessels, known as atherosclerosis [1].

The current view of atherosclerosis emphasizes molecular and cellular components of the immune system. The activation of immune system during inflammation decreases plaque stability and provoke its rupture, thus exposes highly pro-coagulant mediators to the blood stream and results in thrombosis and its subsequent clinical symptoms [1]. Hence, immune mechanisms that regulate integrity and stability as well as formation of atherosclerotic plaques have gained considerable attention among vascular biologists [2].

Traditional cardiovascular risk factors include smoking, hypertension, hypercholesterolemia, diabetes

and obesity. However, the absence of the traditional risk factors does not completely protect from the disease and new risk factors have been identified, including markers of calcifications such as receptor activator of nuclear factor kappa-B ligand/osteoprotegerin ratio [3] and molecules involved in inflammation such as intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), monocyte chemoattractant protein 1 (MCP-1) and IL-18 [4].

ICAM-1, CD54 (cluster of differentiation 54), is a transmembrane protein of the immunoglobulin superfamily. The first stage of leukocyte-endothelial interaction is mediated by the weaker nonspecific selectin molecules, including P, E, and L selectins. Inflammatory mediator responses will upregulate the expression of ICAM-1, thereby increasing the adhesive nature of leukocytes and endothelial cells [1, 4, 5].

* Corresponding Author; E-mail: ramezanim@mums.ac.ir

VCAM-1 is a protein, which mediates the adhesion of lymphocytes, monocytes, eosinophils, and basophiles to vascular endothelium. It also functions in leukocyte-endothelial cell signal transduction, and it may play a role in the development of atherosclerosis [1, 2, 4-6].

MCP-1 is a chemokine, which plays an important role in the recruitment and activation of monocytes. Indeed, circulating MCP-1 levels are elevated in patients with acute myocardial infarction and in those with unstable angina. Production of MCP-1 and macrophage accumulation are also observed after coronary angioplasty, indicating that MCP-1 expression may be related not only to instability of atheromatous plaques, but also to the formation of restenotic lesions [7].

IL-18 is a member of the IL-1 cytokine family. It was initially discovered as a potent inducer of IFN- γ in TH1 cells and natural killer cells. IL-18 induces the expression of additional inflammatory mediators, including IL-1 β , IL-6, TNF- α , IFN- γ , IL-8, MCP-1, MIP-1 α , VCAM-1 and ICAM-1 [2, 4-8].

Pentoxifylline (PTX) is a methylxanthine derivative and a nonselective phosphodiesterase inhibitor which exhibits its anti-inflammatory properties in many experimental and clinical studies [9-12]. Experimental studies have shown that PTX reduces inflammation in atherosclerosis [13-16]. Clinical studies have demonstrated that it has a possible role in atherosclerosis treatment, and it has been found to be safe [9, 17, 18]. Regarding the anti-inflammatory effect of PTX, we hypothesized that it could suppress inflammatory pathways involved in atherosclerosis progression. Furthermore, because of its documented safety, it could be a reliable choice in a clinical trial.

In the present pilot study, we evaluated the effect of PTX on serum levels of ICAM-1, VCAM1, MCP-1, and IL-18 in atherosclerotic patients.

MATERIALS AND METHODS

Patients. This study was approved by the Ethics Committee of Mashhad University of Medical Sciences (Mashhad, Iran). Forty patients with coronary artery disease (CAD), admitted for drug therapy, signed a consent form prior to entry into the study. All patients had established atherosclerosis and enrolled in the study between July 2009 and November 2010. A cardiovascular specialist defined CAD through angiographic findings. The study was double-blinded. Patients were randomized into two equal groups. Blood was obtained from patients before and two months after either PTX (400 mg three times daily) or placebo administration. Demographic data, drug history, post

medical history, familial history, and cardiovascular risk factors were recorded for each patient. The inclusion and exclusion criteria were as follow: Patients who were treated with angiotensin converting enzyme inhibitors inhibitors, statins (except few patients who needed these medications in the very late of this study) or immunosuppressive drugs were excluded. Patients who had established chronic disease, including diabetes, renal, or hepatic diseases were also excluded from the study.

Sample collection and storage. Blood sampling was performed before and two months after either PTX or placebo administration. Plasma was collected using EDTA as an anticoagulant and centrifuged at 1,000 \times g at 2-8 $^{\circ}$ C for 15 minutes within 30 minutes of collection. The samples were aliquoted and stored at -70 $^{\circ}$ C. ELISA was chosen as the method for determining the serum level of biomarkers (Human IL-18 ELISA and Quantikine Human sVCAM-1 Immunoassay Kits as well as Quantikine Human CCL2/MCP-1 Immunoassay and Quantikine Human sICAM-1/CD54 Immunoassay [all from R&D systems, USA]). Each assay was calibrated using biomarker standard curves following the manufacturer's instructions.

Statistical analyses. Results were presented as mean \pm SEM. Differences in plasma concentrations between patients and controls were calculated with the independent sample t-test. Chi-square test and paired t test were used in patient characteristics data analysis. $P < 0.05$ were considered significant. All analyses were performed with SPSS software 11.5.

RESULTS

Patient characteristics. Demographics and traditional cardiovascular risk factors and clinical characteristics of the study population are shown in Table 1.

Evaluation of PTX effect on serum concentration of adhesion molecules. The results showed that PTX reduced serum levels of adhesion molecules in the study group compared with those of the placebo group significantly ($P < 0.0001$) (Fig. 1A and 1B and Table 2).

Evaluation of PTX effect on serum concentration of MCP-1 and IL-18. The results showed that PTX did not reduce serum levels of MCP-1 and IL-18 in the study group compared with those of placebo group significantly (Fig. 1C and 1D and Table 2).

Table 1. Patient characteristics and drugs administered for them

Parameters, drugs and intervention (%)	Placebo group (n = 20)	Pentoxifylline group (n = 20)
Age (year)	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 CABG	45	30
Involved in PCI	30	55
Plaque in LAD	75	75
Plaque in RCA	15	40
Plaque in Cx	10	45
TVD	25	15
Hypothyroidism	10	0
Ejection fraction	51.46 ± 8.33	52.33 ± 8.63

There is no significant ($P > 0.05$) difference between two groups after statistical analysis (Chi-square/student's *t*-test) except in case of chest pain ($P < 0.05$); CABG, coronary artery bypass graft surgery; PCI, percutaneous coronary intervention; LAD, left anterior descending-coronary artery; RCA, right coronary artery; Cx, circumflex coronary artery; TVD, triple vessel disease

DISCUSSION

Our results indicate that two-month PTX administration decreases both ICAM-1 and VCAM-1 levels in patients with CAD. However, it did not induce any significant changes in expression of IL-18 and MCP-1 proteins.

Without any doubt, inflammation plays a crucial role in virtually all stages of atherosclerotic process. Recent researches have related plaque formation to both dyslipidemia and inflammation [10]. Leukocyte recruitment and inflammatory cytokines expression may be characteristics of early stages of atherosclerosis. Our results are consistent with other studies suggesting inflammation suppression properties of PTX in various inflammatory disorders. A prior study has reported the inhibitory effect of PTX on ICAM-1 and VCAM-1 [19]. Cultured monocytes isolated after oral application of PTX expressed decreased amounts of ICAM-1 compared with monocytes collected prior to oral PTX administration. Moreover, Northern blot analysis revealed reduced amounts of ICAM-1 mRNA in monocytes derived from volunteers after oral PTX

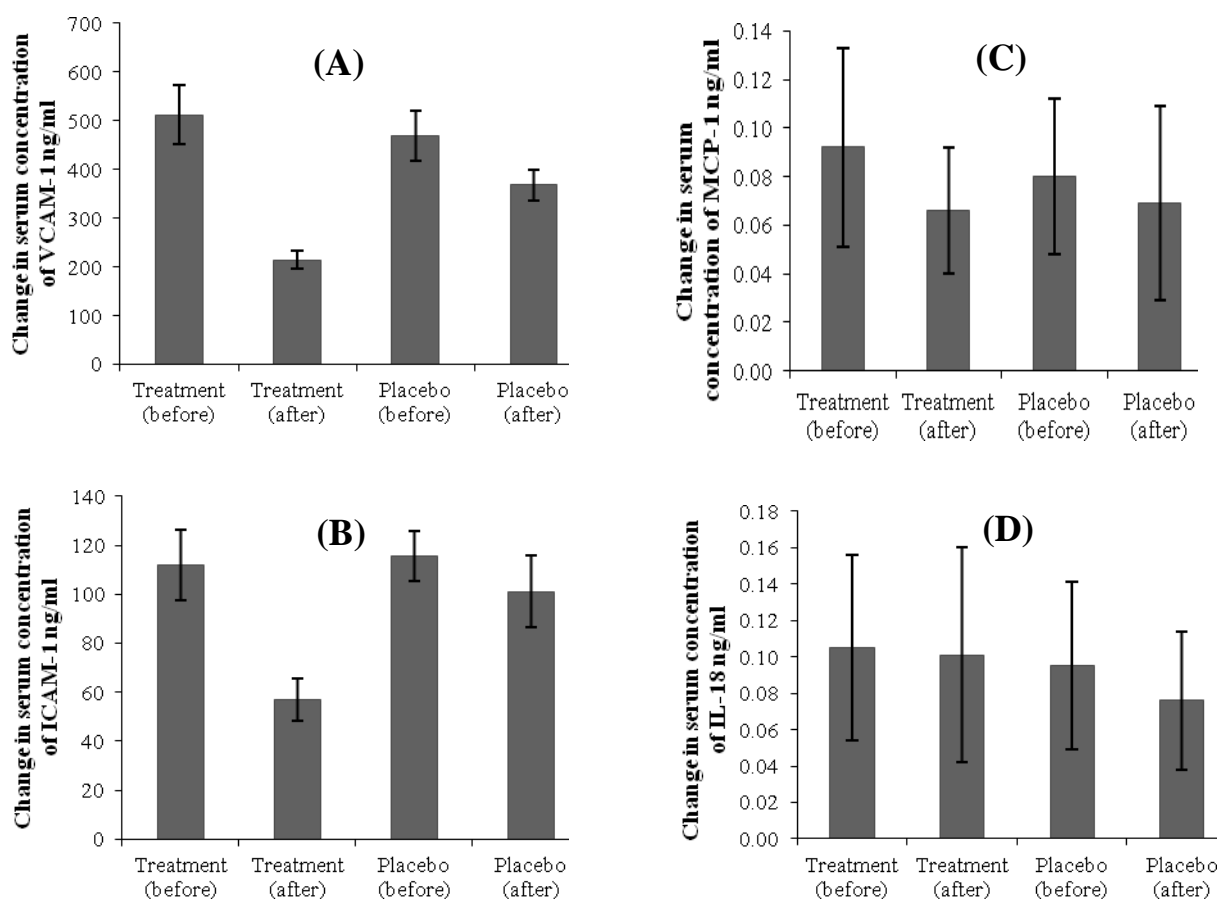


Fig. 1. Mean variations in measured inflammatory components. Mean variations in (A) VCAM-1 ($P = 0.0001$), (B) ICAM-1 ($P = 0.0001$), (C) MCP-1 ($P = 0.4$), and (D) IL-18 ($P = 0.3$) concentration before and after treatment in two groups. mean ± SD; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1, and MCP, monocyte chemoattractant protein 1

Table 2. Comparison of change in serum concentrations before and after either the drug or placebo administration

Inflammatory component ¹	Study group ² (ng/ml)	Control group (ng/ml)	P value ³
VCAM-1	-298.27 ± 39.0610	-100.29 ± 10.8200	0.0001
ICAM-1	-55.13 ± 6.6500	-14.3 ± 2.9300	0.0001
MCP-1	-0.026 ± 0.0168	-0.0207 ± 0.0110	0.4
IL18	-0.0375 ± 0.0145	-0.0194 ± 0.0175	0.3

1, Serum concentration changes (concentration after administration-concentration before administration); 2, mean ± SD; 3, two independent sample test

treatment compared with monocytes isolated before oral PTX administration [12]. In our study, the inhibitory effects of PTX administration on ICAM-1 were also confirmed. Other studies have also shown the inhibitory effects of PTX on ICAM-1 and MCP-1 in various pathological conditions. PTX completely inhibited the surface expression of ICAM-1 and the production of IL-8 and MCP-1 by cytokine-activated epithelial cells of airway [10]. In another study [13], continuous intravenous administration of PTX was successful in blunting the increase of soluble adhesion molecules such as VCAM-1 and ICAM-1 in critically ill patients undergoing continuous veno-venous hemofiltration. Infusion of PTX decreased the synthesis of ICAM-1 and VCAM-1 in patients with necrotizing pancreatitis and respiratory complications [14]. These findings further support the idea that PTX has inhibitory effects on ICAM-1 and VCAM-1.

On the other hand, besides our study, there are several studies on the effects of PTX on inflammatory biomarkers in CVD that support the benefits of PTX administration in CVD. Regarding to the effect of PTX on decreasing MCP-1, Pei *et al.* [15] have reported that taking 200 mg/day PTX by congestive heart failure patients could reduce the serum levels of MCP-1. A double-blind, prospective, placebo-controlled study on acute coronary syndrome patients have shown that PTX administration (1200 mg/day, for 6 months) decreased TNF- α and C-reactive protein. It also inhibited decrease of IL-10 (an anti-inflammatory cytokine) and increase of IL-12 [16]. TNF- α inhibitory property of PTX has reduced the vascular impairment in insulin resistance [17]. Decreased C-reactive protein level after administration of PTX (800 mg/day, for 2 months) has been reported in hypertensive diabetic patients [18]. Development of hypercholesterolemic atherosclerosis in rats was reduced by PTX probably via decreasing of platelet-activating factor and reactive oxygen species levels [19]. It was suggested that the cardioprotective effects of PTX against ischemia and reperfusion injury may be due to reductions in the activation of NF κ B and the production of TNF- α content [20]. An early marker of atherosclerosis, a cross-sectional area of intima media thickness, was

decreased after PTX administration in type I diabetic patients [21]. These studies have shown PTX administration has a versatile role in inhibition of inflammatory response in CVD.

According to these studies, PTX has an inhibitory effect on ICAM-1, VCAM-1, MCP-1, and IL-18 expression and could suppress other inflammatory steps that may be involved in atherosclerosis. Furthermore, a recent study has shown the inhibitory effect of PTX on CD40/CD40 ligands, inflammatory elements involved in atherosclerosis progression, system in patients with atherosclerosis [16]. Our results supported the decreasing effect of PTX on ICAM-1 and VCAM-1 serum levels.

ACKNOWLEDGEMENTS

This study was a part of a thesis of R. Rasooli, and it has been supported financially by Mashhad University of Medical Sciences (Mashhad, Iran).

REFERENCES

- Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med.* 2005 Apr; 352(16):1685-95.
- Gerdes N. The Role of the Pro-Inflammatory Cytokine Interleukin-18, Its Processing Enzyme Caspase-1, and Potential Alternative IL-18-Activating Pathways in Atherosclerosis. Köln: University of Köln; 2005.
- Mohammadpour AH, Shamsara J, Nazemi S, Ghadirzadeh S, Shahsavand S, Ramezani M. Evaluation of RANKL/OPG Serum Concentration Ratio as a New Biomarker for Coronary Artery Calcification: A Pilot Study. *Thrombosis.* 2012; 0212:306263.
- Daniels SR. Cardiovascular disease risk factors and atherosclerosis in children and adolescents. *Curr Atheroscler Rep.* 2001 Nov; 3(6): 479-85.
- Wung BS, Hsu MC, Wu CC, Hsieh CW. Resveratrol suppresses IL-6-induced ICAM-1 gene expression in endothelial cells: effects on the inhibition of STAT3 phosphorylation. *Life Sci.* 2005 Dec; 78(4): 389-97.
- Nahrendorf M, Jaffer FA, Kelly KA, Sosnovik DE, Aikawa E, Libby P, et al. Noninvasive vascular cell

- adhesion molecule-1 imaging identifies inflammatory activation of cells in atherosclerosis. *Circulation* 2006 Oct; 114(14):1504-11.
7. Martinovic I, Abegunewardene N, Seul M, Vosseler M, Horstick G, Buerke M, et al. Elevated monocyte chemoattractant protein-1 serum levels in patients at risk for coronary artery disease. *Circ J.* 2005 Dec; 69(12): 1484-9.
 8. Dinarello CA. Interleukin-18 and the pathogenesis of inflammatory diseases. *Semin Nephrol.* 2007 Jan; 27(1): 98-114.
 9. Ustunsoy H, Sivrikoz MC, Tarakcioglu M, Bakir K, Guldur E, Celkan MA. The effects of pentoxifylline on the myocardial inflammation and ischemia-reperfusion injury during cardiopulmonary bypass. *J Card Surg.* 2006 Jan-Feb; 21(1):57-61.
 10. Sima AV, Stancu CS, Simionescu M. Vascular endothelium in atherosclerosis. *Cell Tissue Res.* 2009 Jan; 335(1):191-203.
 11. de Campos T, Deree J, Martins JO, Loomis WH, Shenvi E, Putnam JG, et al. Pentoxifylline attenuates pulmonary inflammation and neutrophil activation in experimental acute pancreatitis. *Pancreas.* 2008 Jul; 37(1):42-9.
 12. Neuner P, Klosner G, Pourmojib M, Knobler R, Schwarz T. Pentoxifylline *in vivo* and *in vitro* down-regulates the expression of the intercellular adhesion molecule-1 in monocytes. *Immunology.* 1997 Mar; 90(3):435-9.
 13. Boldt J, Heesen M, Padberg W, Martin K, Hempelmann G. The influence of volume therapy and pentoxifylline infusion on circulating adhesion molecules in trauma patients. *Anaesthesia.* 1996 Jun; 51(6):529-35.
 14. Hyun YM, Chung HL, McGrath JL, Waugh RE, Kim M. Activated integrin VLA-4 localizes to the lamellipodia and mediates T cell migration on VCAM-1. *J Immunol.* 2009 Jul; 183(1):359-69.
 15. Pei Z, Kai-zheng G, Hong-guang SUN, Xiao-lei LV, Shun-di W, Xiao-ning SUN, et al. Long-term effects of pentoxifylline in patients with congestive heart failure. *Chinese Heart J.* 2008; 51:51-4.
 16. Fernandes JL, de Oliveira RT, Mamoni RL, Coelho OR, Nicolau JC, Blotta MH, et al. Pentoxifylline reduces pro-inflammatory and increases anti-inflammatory activity in patients with coronary artery disease- a randomized placebo-controlled study. *Atherosclerosis.* 2008 Jan; 196(1):434-42.
 17. El-Bassossy HM, El-Moselhy MA, Mahmoud MF. Pentoxifylline alleviates vascular impairment in insulin resistance via TNF-alpha inhibition. *Naunyn Schmiedebergs Arch Pharmacol.* 2011 Sep; 384(3):277-85.
 18. Maiti R, Agrawal NK, Dash D, Pandey BL. Effect of Pentoxifylline on inflammatory burden, oxidative stress and platelet aggregability in hypertensive type 2 diabetes mellitus patients. *Vascul Pharmacol.* 2007 Aug-Sep; 47(2-3):118-24.
 19. Prasad K, Lee P. Suppression of hypercholesterolemic atherosclerosis by pentoxifylline and its mechanism. *Atherosclerosis.* 2007 Jun; 192(2): 313-22.
 20. Zhang R, Bharadwaj U, Li M, Chen C, Yao Q. Effects of pentoxifylline on differentiation, maturation, and function of human CD14+ monocyte-derived dendritic cells. *J Immunother.* 2007 Jan; 30(1):89-95.
 21. Atabek ME, Kurtoglu S, Selver B, Baykara M. Effectiveness of pentoxifylline on the cross-sectional area of intima media thickness and functions of the common carotid artery in adolescents with type 1 diabetes. *J Pediatr Endocrinol Metab.* 2011; 24(11-12): 945-51.
 22. Shamsara J, Mohammadpour AH, Behravan J, Falsoleiman H, Ramezani M. Pentoxifylline decreases soluble CD40 ligand concentration and CD40 gene expression in coronary artery disease patients. *Immunopharmacol Immunotoxicol.* 2012 Jun; 34(3): 523-9.