Thrombosis Research (2008) **122**, 125–133

THROMBOSIS RESEARCH

intl.elsevierhealth.com/journals/thre

REGULAR ARTICLE

C-reactive protein increases plasminogen activator inhibitor-1 expression in human endothelial cells

Changyi Chen*, Bicheng Nan, Peter Lin, Qizhi Yao

The Molecular Surgeon Research Center, Division of Vascular Surgery and Endovascular Therapy, Michael E. DeBakey Department of Surgery, Baylor College of Medicine, One Baylor Plaza, NAB 2010, Houston, TX 77030, United States

Received 5 May 2007; received in revised form 25 July 2007; accepted 19 September 2007
Available online 22 October 2007

KEYWORDS
C-reactive protein;
PAI-1;
Endothelial cells;
Thrombosis;
CD32;
Curcumin

Abstract

C-reactive protein (CRP) is an inflammatory marker which predicts cardiovascular disease. However, it is not fully understood whether CRP has direct effects on endothelial functions and gene expression. The purpose of current study was to determine the effects and molecular mechanisms of CRP on the expression of plasminogen activator inhibitor-1 (PAI-1) in human endothelial cells. Human coronary artery endothelial cells (HCAEC) were treated with CRP at clinically relevant concentrations for different durations. PAI-1 mRNA, protein and enzyme activities were studied. The effects of CRP on MAPK p38 phosphorylation was also studied by Bio-Plex luminex immunoassay. In addition, other types of human endothelial cells isolated from umbilical vein, skin, and lung microvessels were tested. CRP significantly increased PAI-1 mRNA levels in a time- and concentration-dependent manner. The protein level and enzyme activity of PAI-1 in the supernatant of CRP-treated HCAEC cultures were significantly increased. Anti-CD32 antibody effectively blocked CRP-induced PAI-1 mRNA expression. In addition, CRP significantly increased CD32 mRNA levels and enhanced phosphorylation of MAPK p38. Furthermore, antioxidant curcumin dramatically inhibited CRP-induced PAI-1 mRNA expression. The effect of CRP on PAI-1 expression was also confirmed in other types of human endothelial cells. In conclusion, CRP significantly increased the expression of PAI-1 in HCAEC and other human endothelial cells. CRP also increased its receptor CD32

Abbreviations: CRP, C-reactive protein; PAI-1, plasminogen activator inhibitor-1; HCAEC, human coronary artery endothelial cell; MAPK, mitogen-activated protein kinase; HMVEC-L, human lung microvascular endothelial cell; HUVEC, human umbilical vein endothelial cell; HMEC, human dermal microvascular endothelial cell; EBM-2, endothelial cell basal medium-2; ELISA, Enzyme-linked immunosorbent assay.

* Corresponding author. Tel.: +1 713 798 4401; fax: +1 713 798 6633.
E-mail address: jchen@bcm.tmc.edu (C. Chen).

0049-3848/$ - see front matter
doi:10.1016/j.thromres.2007.09.006

expression which may further enhance its action. CRP-induced PAI-1 expression may be mediated by oxidative stress and p38 signal pathway as antioxidant effectively blocks the effect of CRP on HCAEC.

Introduction

C-reactive protein (CRP) is a prototypical acute-phase reactant protein. Serum CRP is less than 1 μg/ml in healthy people, and however, it may increase as much as 1000-fold within 24 h in response to acute stresses such as inflammation and tissue injury [1]. CRP has been widely used as a biomarker of the presence and severity of inflammatory diseases including cardiovascular disorders [2]. The elevated CRP levels have been demonstrated in myocardial infarction, coronary artery disease, stroke, peripheral arterial disease, metabolic syndrome and type-II diabetes [3–6]. In addition, CRP may have direct biological functions in the vascular system such as altering gene expression and functions of endothelial cells and smooth muscle cells [7–9]. Some of these effects may be mediated through the interaction between CRP and their potential receptors including CD16 and CD32 molecules [10]. However, these studies are very preliminary, and the roles and molecular mechanisms of CRP in endothelial functions such as mediating thrombosis are largely unknown.

Thrombosis factors play a crucial role in the pathogenesis of cardiovascular disease. For instance, plasminogen activator inhibitor-1 (PAI-1) contributes to fibrinolysis by inhibiting tissue plasminogen activator [11]. It is well known that PAI-1 is an acute-phase protein, which was shown originally by Kluft et al. [12] and in more detail by Juhan-Vague et al. [13]. PAI-1 is synthesized in the liver, endothelial cells, vascular smooth muscle cells, and macrophages. PAI-1 expression is highly regulated by many factors including cytokines, oxidative stress, and cellular signaling molecules such as mitogen-activated protein kinases (MAPKs). The elevated PAI-1 is closely associated with enhanced thrombosis by impairing fibrinolysis [14–18], which has been recognized as an important risk factor for atherosclerotic vascular disease [15]. However, it is not fully understood whether CRP could affect PAI-1 expression in different endothelial cells though a unique molecular mechanism.

The main objective of current study was to determine the effect of CRP on PAI-1 expression in human endothelial cells. The molecule mechanisms including the interaction with CRP receptor CD32, effect of antioxidant, and involvement of MAPKs were investigated. Current study provides a better understanding of biological functions of CRP in the vascular system and may suggest new strategies to control CRP-mediated endothelial dysfunction.

Materials and methods

Chemicals and reagents

Recombinant CRP was purchased from Calbiochem (La Jolla, CA, USA). Purity was confirmed by SDS-PAGE showing single band. We determined the endotoxin level as 0.0005 EU/μg for this CRP preparation by *Limulus* amebocyte lysate assay. TRI reagent, monoclonal mouse anti-human β-actin and monoclonal rabbit anti-goat IgG were purchased from Sigma (St. Loius, MO). iQ SYBR Green Supermix kit and iScrip cDNA Synthesis kit were obtained from Bio-Rad Laboratories (Hercules, CA). Goat polyclonal anti-human PAI-1 antibody was obtained from Santa Cruz (Santa Cruz, CA). Spectrolyse PAI-1 kit and IMUBIND tissue PAI-1 ELISA test Kit were obtained from American Diagnostica Inc. (Greenwich, CT). Sheep anti-mouse Ig and ECL plus Western Blotting Detection System were purchased from Amersham (Piscataway, NJ). Mouse anti-human monoclonal CD32 and CD16 were purchased from BD Pharmingen (San Diego, CA).

Cell culture

Human endothelial cells including human coronary artery endothelial cells (HCAEC), human lung microvascular endothelial cells (HMVEC-L), and human umbilical vein endothelial cells (HUVEC) were purchased from Clonetics (Walkersville, MD) at passage 3. Immortalized human dermal microvascular endothelial cells (HMEC) were generously provided by Dr. Wright S. Caughman, Department of Dermatology, Emory University (Atlanta, GA). HCAEC, HUVEC, HMEC and HMVEC-L were cultured in the endothelial cell basal medium-2 (EBM-2) contained with 10% fetal bovine serum and EGM-2 SingleQuots (Invitrogen, Carsbad, CA). All cells were maintained at 37 °C in a 5% CO_2 humidified milieu. Cells were cultured to 90% confluence and in the starvation medium including EBM-2 supplemented with 1% fetal bovine serum, 0.1% gentamicin sulfate and amphotericin-B, heparin and ascorbic acid for 24 h. Cells were treated by CRP (5, 10 or 25 μg/ml) in the fresh starvation medium at 37 °C for 3, 6, 12, 24 or 48 h. Control cells received the fresh medium without CRP. All experiments were performed in triplet.

RNA extraction and quantitative real time PCR

The cells were washed with cold PBS and total RNA was extracted by TRI reagent following manufacturer's protocol. RNA from each well was resuspended in 20 μl of RNase-free water and the concentration was determined by absorbance at 260-nm wavelength. cDNA was generated by reverse transcription from mRNA using the iScript cDNA Synthesis Kit (Bio-Rad) following the manufacturer's instructions. Specific primers, including PAI-1, CD32 and CD16 (Table 1), were designed with Beacon designer software (Bio-Rad) and synthesized by Sigma-Genosys (Woodlands,