The correlation between soluble endothelial protein c receptor (sEPCR) and tumor necrosis factor-alpha (TNF-α) levels in vivo

Soluble endotelial protein c reseptörü ile tümör nekrozis faktör arasındaki in vivo ilişki

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To the Editor,

In addition to the anticoagulant property of activated protein C (APC), it has been shown that APC also has profibrinolytic, anti-inflammatory and anti-apoptotic roles. At the endothelial cell surface, endothelial protein C receptor (EPCR) augments APC formation by representing the thrombin-thrombomodulin (TM) complex to protein C. The influence of EPCR on APC generation has been shown by cell culture and animal model studies in vitro. It has been demonstrated that a recombinant human APC (rhAPC) upregulates the synthesis of cyclooxygenase (COX)-2 expression via EPCR binding and protease-activated receptor-1 (PAR-1) signaling mechanism [1].

The results of this study have shown in vitro effects of TNF-alpha (TNF-α), EPCR and PAR-1 on APC response. Three different antibodies raised against the inhibition of EPCR binding with APC, an antibody for the detection of normal human EPCR and anti-human PAR-1 antibody were used in human umbilical vein endothelial cells (HUVEC) cell lines. When the stimulated endothelial cells were treated with anti-human EPCR blocker, the expression level of COX-2 decreased, and the same was true for the anti-human PAR-1 antibody that blocks the expression of COX-2 protein. Additionally, TNF-α ??levels also increased the expression level of this protein with a synergistic effect with rhAPC [1]. This study has shown the effect of EPCR-APC binding on protein expression, especially on the very important one, COX-2, that regulates prostaglandin synthesis and TNF-α-APC response relationship. The in vitro effects of TNF-α on EPCR have been reported in human endothelial cells [2]. The effect of TNF-α on TM and EPCR expression has been evaluated by measuring APC formation. It has been suggested that APC and TNF-α induce microparticle-associated EPCR formation in HUVEC and monocytes [3]. However, there is no study that elicits the possible effects of TNF-α on APC formation via EPCR. The three inflammatory cytokines --interleukin-1,, TNF-α and endotoxin -- have been claimed to reduce TM, EPCR and protein S levels [4]. A recent study [5] showed that there was no association between the interleukin-6 (IL-6) and TNF-α gene promotor polymorphisms in a Turkish pediatric stroke group when compared to healthy controls.

A rare 23 bp insertion mutation leading to a truncated protein generation has been reported to decrease the membrane expression of EPCR. Most recently, two studies revealed that plasma soluble (s)EPCR levels are genetically controlled by a set of haplotypes, including 6936 A-G (Ser219Gly) or the A3 haplotype and several single nucleotide polymorphisms (SNPs)

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[6,7]. It has been suggested that membrane-bound EPCR is cleaved by metalloproteinases and it increases the level of sEPCR levels in the plasma. Increased levels of sEPCR cause a shift in the role of EPCR, which functions as a modulator and enhancer of APC generation by inhibiting the anticoagulant activity of APC [8].

In this study, the possible effects of plasma sEPCR and TNF-α levels were investigated in a group of 104 healthy controls together with the TNF-α promoter-308 G-A polymorphism and their associations. The mean age of the controls was 28.58±7.8 and the median age was 25. The plasma levels of the parameters were determined by enzyme linked immunosorbent assay (ELISA) according to the manufacturer's instructions using the commercially available Asserachrom® sEPCR ELISA kit (Diagnostica Stago, Asnieres-France) and TNF-α kits (Biosource International, USA). To determine TNF-α–308 G-A polymorphism, a previously described technique was performed [5].

For statistical analysis, SPSS statistical package version 11 was used. Pearson'scorrelation test was used to determine any correlation between the levels of parameters in healthy controls. Correlation was significant at the 0.01 level (2-tailed) between TNF-α and sEPCR levels. The distributions of the parameters assumed to be related were also compared by using Wilcoxon signed rank test and the analysis gave positive ranks (P=0.015). The relationship between TNF-α –308 G-A polymorphism and sEPCR levels was comparedusing chi-square test, and odds ratios (OR) were calculated with 95% confidence interval (CI). Two groups were generated according to GG and GA genotypes of TNF-α gene polymorphism. Among 84 individuals with TNF-α GG genotype, 12 were found to have sEPCR levels higher than 100 ng/ml, and in the 20 TNF-α GA group, 3 individuals were found to have high levels of sEPCR. These groups were compared with respect to their sEPCR levels. The OR was 1.05 (CI: 0.27-4.07) and P value was 0.77. The same approach was used for the TNF-α levels and the promoter gene polymorphism.

Our study is the first study to evaluate the in vivo effects of TNF-α and sEPCR levels and to determine associations with TNF-α gene polymorphism. Here, we report a correlation between TNF-α and sEPCR levels among healthy controls, whereas we were unable to find an association between the TNF-α promoter gene variant and either plasma parameter according to the performed risk analysis. The significant correlation between TNF-α and sEPCR levels may be attributable to the low levels of the parameters among controls. We think that this correlation should be analyzed in patients with thrombosis, as a correct approach to determine the influences.

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