Estrogen-mediated protection against HIV Tat protein-induced inflammatory pathways in human vascular endothelial cells

Yong Woo Lee\textsuperscript{a,}\textsuperscript{,*}, Sung Yong Eum\textsuperscript{a}, Avindra Nath\textsuperscript{b}, Michal Toborek\textsuperscript{a}

\textsuperscript{a}Department of Surgery/Division of Neurosurgery, University of Kentucky College of Medicine, 800 Rose Street, Lexington, KY 40536, USA
\textsuperscript{b}Department of Neurology, Johns Hopkins University, Baltimore, MD 21287, USA

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Abstract

**Objective:** It has been proposed that human immunodeficiency virus (HIV) infection-induced inflammatory environment may contribute to the pathogenesis of cardiovascular diseases. Recent studies have also demonstrated the potential role of estrogen as therapeutic agents in the prevention or treatment of cardiovascular diseases. In the present study, we assessed the hypothesis that estrogen may attenuate the HIV Tat protein-induced inflammatory pathways in human vascular endothelium. **Methods:** Expression of inflammatory mediators in human endothelial cells was analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA). Electrophoretic mobility shift assay (EMSA) also was performed to investigate the DNA-binding activities of several transcription factors, which are known to regulate expression of these inflammatory genes. **Results:** Acute exposure of human endothelial cells to Tat markedly induced the mRNA and protein expression of IL-1\textsubscript{h}, MCP-1, VCAM-1, and E-selectin. Tat also stimulated the adherence of inflammatory cells to endothelial cell monolayers. Significant and dose-dependent increases in NF-\textkappa-B DNA-binding activity were observed in human endothelial cells treated with Tat. However, Tat did not affect DNA-binding activities of AP-1, CREB, and STAT1. Pretreatment with 17\beta-estradiol dramatically blocked the activation of NF-\textkappa-B in human endothelial cells exposed to Tat. In addition, 17\beta-estradiol selectively inhibited the Tat-induced expression of IL-1\textsubscript{h}. **Conclusion:** Our results suggest that estrogen may protect against Tat-induced inflammatory reactions in human vascular endothelium via blocking the NF-\textkappa-B-mediated molecular signaling pathways. These data may contribute to understanding the pathogenesis of cardiovascular complications and development of therapeutic strategies for HIV-infected patients.

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**Keywords:** Atherosclerosis; Cytokines; Estrogens; Gene expression; Infection/inflammation

1. Introduction

Human immunodeficiency virus (HIV) infection, the cause of acquired immunodeficiency syndrome (AIDS), has been reported one of the most common causes of death in the United States [1]. It causes severe impairment of immune function, opportunistic infections, neurological disorders, and cancer [2,3]. In addition, growing body of evidences indicates that HIV infection and its treatment can contribute to the pathogenesis of cardiovascular diseases [4–6]. In fact, accelerated coronary atherosclerosis and arteriosclerosis were detected in the patients infected with HIV [7]. It was also demonstrated that HIV protease inhibitors, key components of highly active antiretroviral therapy (HAART) in HIV-infected patients, promote the formation of atherogenic lipoproteins and endothelial dysfunction [8].

Tat is a nuclear regulatory protein which plays a crucial role in viral gene expression and replication [9]. Previous studies have demonstrated that Tat, actively released from HIV-infected cells, may be an important mediator of HIV-mediated inflammatory responses in a variety of cell types [10,11]. It was also reported that Tat-mediated inflammation in brain can be critically involved in neuronal injury and dysfunction during HIV infection [12]. Additionally, recent
evidence demonstrates that Tat induces oxidative and inflammatory pathways in brain microvascular endothelium [13,14].

Hormone replacement therapy (HRT) may have adverse effects on breast cancer, stroke, and coronary heart disease [15,16]. However, it should be noted that a growing body of evidence indicates the beneficial health effects of estrogens including a protection against neurodegenerative diseases and a prevention of osteoporosis [12,17,18]. Additionally, a number of epidemiological and clinical studies have demonstrated the protective effects of estrogens on cardiovascular diseases [19–23]. Indeed, estrogen replacement therapy attenuates the development of cardiovascular diseases and reduces the cardiovascular disease-related mortality among postmenopausal women [24,25]. The potential mechanisms of action for estrogen-mediated protection against cardiovascular diseases also have been described. For example, exogenous estrogens markedly attenuate hypercholesterolemia and atherosclerosis in rabbits fed a cholesterol-rich diet through the regulation of lipoprotein metabolism [26]. In addition, the lipid peroxidation of LDL was inhibited by 17\(\beta\)-estradiol in cultured bovine aortic endothelial cells [27].

Although previous studies have established the potential protective role of estrogens in the development of cardiovascular diseases, the cellular and molecular mechanisms underlying this process are not fully understood. Specifically, there are no reports to indicate the effects of estrogen on Tat-induced inflammatory reactions in peripheral vascular endothelium. In the present study, we examined whether Tat can induce inflammatory vascular environment through selective transcription factor-mediated upregulation of inflammatory mediators. In addition, we investigated the role of 17\(\beta\)-estradiol in Tat-mediated inflammatory pathways in human vascular endothelial cells.

2. Methods

2.1. Cell cultures

Human umbilical vein endothelial cells (HUVEC) were isolated and cultured as described previously [28]. The human monocyctic leukemia cells (THP-1) were purchased from ATCC (Manassas, VA) and used to study cell adhesion assay. The investigation conforms to the principles outlined in the Declaration of Helsinki [29]. Each experiment was carried out four times.

2.2. Preparation of HIV Tat protein

HIV Tat protein is a transactivating regulatory protein composed of two exons with 86–104 amino acids. Previous studies have shown that the functions of transactivation, neurotoxicity and immune activation are all present within the 72 amino acids formed by the first exon [30,31]. Therefore, Tat\(_{1-72}\) was employed in the present study.

Recombinant Tat\(_{1-72}\) was produced and purified as described earlier [30]. Briefly, the Tat gene encoding the first 72 amino acids of HIV-1BRU (obtained from Dr. Richard Graynor, through the AIDS repository at the National Institutes of Health) was inserted into an *Escherichia coli* vector Pin Point Xa-2 (Promega, Madison, WI). Biotinylated Tat\(_{1-72}\) was purified on a column of soft release avidin resin, cleaved from the fusion protein using factor

<table>
<thead>
<tr>
<th>Genes(^a)</th>
<th>Sequences of the primer pairs (5' to 3')</th>
<th>Product size</th>
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<tr>
<td>IL-1(\beta)</td>
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<tr>
<td>Reverse GAGGCCCAAGGCCACAGG</td>
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<tr>
<td>MCP-1(^b)</td>
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<tr>
<td>Reverse GTGGTCCATGGAATCCTGAA</td>
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<tr>
<td>VCAM-1</td>
<td>Forward ATGACATGCTTGAGCCAGG</td>
<td>260 bp</td>
</tr>
<tr>
<td>Reverse GTGTCCTCCTTTGACACT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-selectin</td>
<td>Forward CTCTGACAGAAAGAAGCCAAG</td>
<td>255 bp</td>
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<tr>
<td>Reverse ACTTGAGTCCACTGAAGCCA</td>
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<tr>
<td>(\beta)-Actin</td>
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<td>Reverse TGTAACGCAACTAAGTCATA</td>
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</table>

\(^a\) IL-1\(\beta\), interleukin-1\(\beta\); MCP-1, monocyte chemoattractant protein-1; VCAM-1, vascular cell adhesion molecule-1.

\(^b\) Primer pairs purchased from R&D Systems, Minneapolis, MN.
2.3. Cell viability assay

Cell viability was determined with the standard 3-[4, 5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) conversion assay. The cell viability of HUVEC was not affected by treatment with Tat at ≤ 100 nM, which was used in the present study (Fig. 1).

2.4. Reverse transcriptase-polymerase chain reaction (RT-PCR)

2.4.1. Semiquantitative RT-PCR

Total RNA was extracted by the use of TRI reagent and reverse transcribed at 42 °C for 60 min in 20 μl of Reverse Transcription System (Promega) with 0.5 μg of oligo(dT)$_{15}$ primer. The sequences of the primer pairs used for PCR amplification of the studied genes are shown in Table 1. The PCR mixture consisted of a Taq PCR Master Mix Kit (Qiagen), 2 μl of a product of the reverse transcriptase reaction, and 20 pmol of primer pairs in a total volume of 50 μl. For each individual gene, a linear range of PCR amplification was established and the induction of the target gene was studied within the range. The following thermocycling profiles were used to determine the induction of the genes encoding the studied inflammatory mediators:

- IL-1β: 94 °C for 30 s, 55 °C for 45 s, and 72 °C for 45 s, repeated 35 times;
- MCP-1: 94 °C for 4 min before the first cycle, 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 45 s, repeated 22 times followed by a final extension at 72 °C for 10 min;
- VCAM-1: 94 °C for 4 min, 55 °C for 1 min, and 72 °C for 1 min, repeated 22 times;
- E-selectin: 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, repeated 24 times.

Amplification of the β-actin, a housekeeping gene, was determined by using the same number of cycles and thermocycling profiles as for the target gene and performed in separate tubes. PCR products were separated by 2% agarose gel electrophoresis, stained with SYBR® Green I.

**Fig. 2.** HIV Tat protein upregulates the mRNA expression of inflammatory mediators in human vascular endothelial cells. HUVEC were either untreated or treated with the indicated concentrations of Tat for 4 h. The mRNA levels of IL-1β (A), MCP-1 (B), VCAM-1 (C), and E-selectin (D) were measured by semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR), respectively. β-Actin (a housekeeping gene) was used to indicate that the same amount of RNA was used per sample. The amplified PCR products were analyzed by 2% agarose gel electrophoresis and visualized by using phosphorimaging technology. M, molecular weight markers (100-bp DNA ladder).
(Molecular Probes) and visualized by using phosphorimaging technology (FLA-5000, Fuji, Stamford, CT).

2.4.2. Real-time RT-PCR

Total RNA was isolated using RNeasy Mini Kit (Qiagen) and reverse transcribed at 25 °C for 15 min, 42 °C for 45 min and 99 °C for 5 min in 20 μl of Reverse Transcription System (Promega) with 0.5 μg of random hexamers. For quantitative PCR, amplifications of individual gene were performed on ABI PRISM® 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) using TaqMan® Universal PCR Master Mix, gene-specific TaqMan PCR probes and primers, and a standard thermal cycler protocol (50 °C for 2 min before the first cycle, 95 °C for 15 s and 60 °C for 1 min, repeated 45 times). For specific probes and primers of PCR amplifications, TaqMan® Pre-Developed Assay Reagents for human IL-1β, MCP-1 and β-actin, and Assay-on-Demand™ Products for human VCAM-1 and E-selectin were obtained from Applied Biosystems. The threshold cycle (CT), which indicates the fractional cycle number at which the amount of amplified target gene reaches a fixed threshold, from each well was determined using ABI Prism 7000 SDS software. Relative quantification, which represents the change in gene expression from real-time quantitative PCR experiments between Tat-treated group and untreated control group, was calculated by the comparative CT method as described earlier [35,36]. The data were analyzed using the equation $2^{-\Delta\Delta CT}$, where $\Delta\Delta CT=[CT$ of target gene − CT of housekeeping gene]treated group − [CT of target gene − CT of housekeeping gene]untreated control group. For the treated samples, evaluation of $2^{-\Delta\Delta CT}$ represents the fold change in gene expression, normalized to a housekeeping gene (β-actin) and relative to the untreated control.

2.5. Enzyme-linked immunosorbent assay (ELISA)

The protein levels of human IL-1β and MCP-1 were determined using Human IL-1β Immunoassay and Human MCP-1 Immunoassay kits (R&D Systems, Minneapolis, MN) according to the protocol of manufacturer, respectively.

Cell surface expressions of adhesion molecules such as VCAM-1 and E-selectin were quantified by ELISA Development kits (R&D Systems) according to the manufacturer’s procedure with modifications. Briefly, HUVEC monolayers were incubated with either anti-human VCAM-1 or E-selectin monoclonal antibody (2.5 μg/ml) for 1 h at 37 °C. The cells were then incubated with biotinylated goat anti-mouse IgG antibody (1:1000 dilution) for 1 h at 37 °C. After washing the wells thoroughly, the working dilution of streptavidin-HRP were added to each well and incubated for 20 min at room temperature. The cells were incubated with...
HRP Substrate Solution for 20 min at room temperature with subsequent addition of Stop Solution. After color development, absorbance from each well was measured by a microtiter plate reader at 450–570 nm.

2.6. Cell adhesion assay

Adhesion studies were performed with the human monocytic leukemia cell line, THP-1, as previously described [37] with modifications. Briefly, HUVEC were grown to confluence on 24-well plates and exposed to Tat for 8 h. Prior to the cell–cell adhesion assay, the HUVEC monolayers were washed twice with HBSS and then washed with M199 medium containing 10% FBS. Calcein acetoxymethyl ester (calcein AM; Calbiochem, La Jolla, CA) was employed to label THP-1 cells. The fluorescence labeling of THP-1 cells was measured by fluorescent microplate reader using excitation of 490 nm and emission of 517 nm. Data are means ± S.D. of four determinations. *Statistically significant compared with the control group (P < 0.05).

2.7. Electrophoretic mobility shift assay (EMSA)

Nuclear extracts from HUVEC were prepared and analyzed by EMSA as described earlier [28,38]. Individual double-stranded oligonucleotides containing the consensus sequences of the binding sites for transcription factors AP-1 (5'-CGGTCATGAGCTAGGAA-3'), CREB (5'-AGAGATTGCTACGGAGCTAG-3'), NF-κB (5'-AGTGGGAGGATTTCC CAGGC-3'), or STAT1 (5'-ATTGCCAGGGCGGGCGAGGC-3') were end labeled with [γ-32P]-ATP using bacteriophage T4 polynucleotide kinase. Resultant protein–DNA complexes were resolved on native 5% polyacrylamide gels using 0.25 × TBE buffer (50 mM Tris–Cl, 45 mM boric acid, 0.5 mM EDTA, pH 8.4). The intensity of the bands corresponding to specific NF-κB-DNA binding was determined using UN-SCAN-IT gel™ image analysis software (Silk Scientific, Orem, UT).

Fig. 4. HIV Tat protein upregulates the adhesion of leukocytes to human vascular endothelial cell monolayers. HUVEC were either untreated or treated with the indicated concentrations of Tat for 8 h. To perform the adhesion assay, the calcein AM-labeled human monocytic leukemia cells (THP-1) were incubated with endothelial cell monolayers for 20 min at 37 °C. The adherence of calcein AM-labeled THP-1 cells was measured by fluorescence microplate reader using excitation of 490 nm and emission of 517 nm. Data are means ± S.D. of four determinations. *Statistically significant compared with the control group (P < 0.05).

Fig. 5. HIV Tat protein enhances the NF-κB DNA-binding activity in human vascular endothelial cells. HUVEC were either untreated or treated with the indicated concentrations of Tat for 2 h. Nuclear extracts were prepared and analyzed by electrophoretic mobility shift assay (EMSA). Competition study was performed by the addition of excess unlabeled NF-κB DNA-binding consensus sequence using nuclear extracts from cells treated with 100 nM Tat (A). Supershift analysis of NF-κB DNA-binding activity. Nuclear extracts were prepared from cells treated with 100 nM Tat for 2 h and incubated with anti-p50 antibody or anti-p65 antibody, respectively, for 25 min before the addition of 32P-labeled probe. SS, supershift (B).
2.8. Statistical analysis

Routine statistical analysis of data was completed using SigmaStat 2.03 (SPSS, Chicago, IL). One-way ANOVA was used to compare mean responses among the treatments. For each endpoint, the treatment means were compared using Bonferroni least significant difference procedure. Statistical probability of $P<0.05$ was considered significant.

3. Results

3.1. HIV Tat protein upregulates the mRNA and protein expression of inflammatory mediators in HUVEC

The effects of Tat on the mRNA and protein expression of a variety of inflammatory mediators were investigated in human vascular endothelial cells. Semiquantitative RT-PCR showed that increasing concentrations of Tat dramatically induced the gene expression of pro-inflammatory cytokine IL-1β (Fig. 2A). A significant and dose-dependent induction of chemokine MCP-1 gene was also observed in HUVEC treated with Tat (Fig. 2B). Additionally, Tat markedly and dose-dependently upregulates gene expression of adhesion molecules, such as VCAM-1 and E-selectin, compared with control cell cultures (Fig. 2C and D). Expression of β-actin (housekeeping gene), however, was not affected by treatment with Tat. In order to determine whether Tat-induced increases in mRNA levels can translate to elevated protein expression, a series of ELISA was employed. Consistent with the data on gene expression, treatment with Tat resulted in a significant and dose-dependent upregulation of protein expression of IL-1β, MCP-1, VCAM-1, and E-selectin (Fig. 3A–D). These results suggest that Tat can induce pro-inflammatory environment in human vascular endothelial cells through the upregulation of inflammatory mediators.

3.2. HIV Tat protein upregulates cell adhesion to endothelial cell monolayers

The adherence of human acute monocytic leukemia cells, THP-1, to HUVEC monolayers was assessed to verify the functional integrity of the inflammatory mediators upregulated by human vascular endothelial cells following Tat activation. As shown in Fig. 4, Tat markedly and in a dose-dependent manner stimulated the adherence of THP-1 cells to endothelial cell monolayers.

3.3. HIV Tat protein specifically activates NF-κB DNA-binding activity in HUVEC

To investigate the molecular regulatory mechanisms of Tat-mediated upregulation of inflammatory mediators, a series of electrophoretic mobility shift assay (EMSA) was performed.
performed. Fig. 5 represents the effects of Tat exposure on the DNA-binding activity of transcription factor NF-κB in HUVEC. A low level of NF-κB DNA-binding activity was detected in nuclear extracts isolated from control cell cultures (Fig. 5A, lane 2). In contrast, exposure of HUVEC to increasing concentrations of Tat markedly enhanced NF-κB DNA-binding activity in a dose-dependent manner (Fig. 5A, lanes 3–5). The specificity of the NF-κB binding was determined by competition experiments with molar excess of unlabelled oligonucleotide containing the consensus NF-κB binding site and supershift analysis with specific antibodies against NF-κB p50 and p65. As shown in Fig. 5A (lane 6), molar excess of competitor oligonucleotide completely diminished the bands that corresponded to NF-κB DNA-binding. In addition, incubation of nuclear extracts with anti-NF-κB p50 antibody produced a marked supershift of both upper and lower bands (Fig. 5B, lane 3). In contrast, when the samples were incubated with anti-NF-κB p65 antibody, only the upper band disappeared (Fig. 5B, lane 4). These results indicate that the lower band can be NF-κB p50/p50 homodimer and the upper band is most likely NF-κB p50/p65 heterodimer, respectively.

It has been proposed that several other transcription factors, such as AP-1, CREB, or STAT1, can play an important role in the molecular mechanisms of inflammatory pathways in response to a variety of stimuli. Therefore, EMSA studies were also carried out to determine the effects of Tat on DNA-binding activities of these transcription factors. As illustrated in Fig. 6, treatment of HUVEC with Tat did not activate AP-1, CREB and STAT1. In contrast, a
significant increase in DNA-binding activity of these transcription factors was observed in the parallel studies with TNF-α or IL-4 (positive control).

3.4. Estrogen selectively inhibits HIV Tat protein-induced mRNA and protein expression of IL-1β in HUVEC

To determine the effects of estrogen on Tat-mediated upregulation of inflammatory mediators, HUVEC were pretreated with 2 nM 17β-estradiol for 24 h, followed by exposure to 100 nM Tat, and expression levels of inflammatory mediators were analyzed by real-time RT-PCR and ELISA. In agreement with the data shown in Figs. 2A and 3A, Tat significantly increased the mRNA and protein expression of IL-1β. Pretreatment with 17β-estradiol, however, significantly attenuated the Tat-mediated upregulation of mRNA and protein expression of IL-1β (Fig. 7). In contrast, Fig. 8 shows the effects of 17β-estradiol on the induction of other inflammatory mediators, such as chemokine and adhesion molecules, in the Tat-treated HUVEC. As indicated, a significant and marked induction of MCP-1, VCAM-1 and E-selectin was observed in the cells exposed to Tat. On the other hand, 17β-estradiol did not affect the Tat-mediated induction of these inflammatory mediators in HUVEC.

3.5. Estrogen attenuates HIV Tat protein-induced DNA-binding activity of NF-κB in HUVEC

In order to elucidate the possible molecular mechanisms of estrogen-mediated protective effects against Tat-induced inflammatory pathways in human vascular endothelial cells, we examined the effects of 17β-estradiol on the NF-κB DNA-binding activity in Tat-treated HUVEC. Consistent with the data shown in Fig. 5, exposure to Tat at the concentration of 100 nM markedly increased in NF-κB DNA-binding activity by approximately 2.9-fold as compared to control values. In contrast, NF-κB binding was significantly decreased by 45% in nuclear extracts prepared from HUVEC treated with Tat in the presence of 17β-estradiol (Fig. 9).

4. Discussion

The present study demonstrated that stimulation of cultured human vascular endothelial cells with HIV Tat protein specifically activates transcription factor NF-κB and leads to the upregulation of inflammatory mediators, including IL-1β, MCP-1, VCAM-1 and E-selectin. Additionally, we suggested the potential protective effects of estrogen on the inflammatory vascular environment produced by Tat.

It is now widely believed that atherosclerosis is an inflammatory disease of the vessel wall and inflammatory reactions in endothelial cells are primarily regulated through the production of inflammatory mediators [39]. In fact, enhanced expression of chemokines and adhesion molecules in the vascular endothelium facilitate recruiting and adhering inflammatory cells into the vessel wall, and thus stimulate transendothelial migration, which can be considered an early atherogenic process [40,41]. These studies strongly support the idea that the inflammatory vascular environment is critical for the initiation and development of atherosclerosis. In the present study, we first examined whether HIV Tat protein can stimulate an inflammatory response in human vascular endothelial cells. Figs. 2 and 3 showed a significant and dose-dependent increase in the mRNA and protein expression of IL-1β, MCP-1, VCAM-1, and E-selectin in Tat-treated HUVEC, indicating that Tat may produce the inflammatory vascular environment through the overexpression of inflammatory mediators. Additionally, Tat markedly stimulated the adherence of inflammatory cells to HUVEC monolayers (Fig. 4). Thus, it is possible that Tat-induced pro-inflammatory environment in the vascular endothelium...
can contribute to the accelerated development of atherosclerosis observed in HIV patients.

Evidence indicates that the expression of inflammatory mediators is regulated at the transcriptional level through activation of redox-responsive transcription factors, such as AP-1, NF-κB, CREB and STAT1 [28,32,42,43]. Therefore, we determined the DNA-binding activities of these transcription factors to elucidate the molecular signaling mechanisms of Tat-mediated upregulation of inflammatory mediators in HUVEC. As shown in Fig. 5, Tat selectively increased NF-κB DNA-binding activity among all transcription factors analyzed in the present study. These data are in agreement with the recent studies, which indicate the Tat-induced activation of NF-κB in other cell types [12,13]. On the other hand, our previous study demonstrated that treatment of porcine brain microvascular endothelial cells with Tat resulted in activation of AP-1 [13]. It was also shown that HIV Tat protein activates c-Jun N-terminal kinase (JNK) and AP-1 in human histiocytic lymphoma U937 cells [44]. In contrast to these reports, the present study did not show any apparent effect on AP-1 DNA-binding activity in HUVEC treated with Tat (Fig. 6). This discrepancy may be due to the different cell types. However, it should be noted that the present results showing the distinct effects of Tat on NF-κB and AP-1 activation indicated that these transcription factors may be regulated via different molecular signaling mechanisms in human peripheral endothelial cells.

It has been suggested that the protective effects of estrogen involve its anti-inflammatory properties. For instance, it was demonstrated that pretreatment with 17β-estradiol markedly inhibited the lipopolysaccharide (LPS)-stimulated inflammatory reactions [45]. Recent study also showed that 17β-estradiol significantly attenuated Tat-mediated pro-inflammatory and pro-inflammatory effects [12]. Additionally, previous studies have demonstrated that estrogen improves endothelial dysfunction through upregulation of eNOS [46–48], suggesting that eNOS can be involved in estrogen-mediated protection against Tat-induced endothelial dysfunction. However, the specific effects of estrogens on the Tat-mediated inflammatory reactions in human vascular endothelium have not been elucidated. Therefore, we determined whether estrogen treatment has anti-inflammatory effects in Tat-stimulated HUVEC. Interestingly, pretreatment of HUVEC with 17β-estradiol selectively attenuated Tat-induced overexpression of IL-1β (Fig. 7). In fact, IL-1β is generally thought of as prototypical pro-inflammatory cytokine. A number of previous studies have demonstrated that this cytokine is the critical mediator of inflammatory events in the initiation and progression of atherosclerosis [49]. Our findings demonstrating the significant inhibition of Tat-induced upregulation of IL-1β by 17β-estradiol provide the strong evidence to suggest the potential protective role of estrogens in Tat-mediated inflammatory pathways in human vascular endothelium. Future studies, however, are needed to elucidate the exact mechanisms of the estrogen’s selective effects on inflammatory mediators induced by Tat in human vascular endothelial cells.

Recent studies have indicated that the protective effects of estrogen on the inflammatory responses in vascular endothelium are associated with inhibition of NF-κB [50,51]. In the present study, we examined the effects of 17β-estradiol on the Tat-mediated activation of NF-κB to further investigate the molecular mechanisms by which estrogen regulates the overexpression of IL-1β in Tat-treated HUVEC. In agreement with the previous studies, NF-κB DNA-binding activity was significantly attenuated in nuclear extracts prepared from Tat-treated HUVEC in the presence of 17β-estradiol (Fig. 9). Although it is widely believed that inflammatory genes are regulated by redox-responsive transcription factors, specific mechanisms and cooperation among different transcription factors can be involved in induction of these genes. Our data indicated that exposure to estrogen can inhibit Tat-induced NF-κB DNA-binding activity and IL-1β overexpression. Thus, the effects of estrogen on Tat-induced inflammatory reactions appear to be highly selective.

In conclusion, we presented for the first time new evidence that Tat can induce the mRNA and protein expression of pro-inflammatory cytokine IL-1β in human peripheral vascular endothelial cells that can contribute to understanding of the pathogenesis of cardiovascular complications in HIV-infected patients. More importantly, our findings demonstrating that the estrogen-mediated inhibition of Tat-induced upregulation of IL-1β is, at least in part, through the NF-κB-dependent mechanism can open up new areas of investigation toward the development of therapeutic strategies for the prevention of atherosclerosis specifically targeted against inflammatory pathways.

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References


