Tanshinone IIA attenuates LPS-induced HUVECs injury by inhibiting the NF-κB signaling pathway

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Received December 19, 2017; Accepted January 8, 2019; Epub September 15, 2020; Published September 30, 2020

Abstract: Background: Currently, atherosclerosis (AS) is generally considered to be a chronic inflammatory response. Tanshinone IIA (TSIIA) is the most important bioactive lipophilic isolated from danshen, and its pharmacological effects have been generally recognized, including its anti-atherosclerosis effects. However, the mechanism of action of TSIIA in the amelioration of AS has not yet been identified. Methods: First, LPS was used to build a human umbilical vein endothelial cell (HUVECs) inflammatory model. Cultured HUVECs were seeded and preincubated with 2.5 μM TSIIA for 2 h followed by stimulation with 2.0 μg/mL LPS at 37 °C for 12 h. With that, the MTT method was used to determine the effects of LPS and TSIIA on cell cytotoxicity. The protein levels of cytokine TNF-α, IL-1β, IL-6, and IL-8 were determined by ELISA. The total NO production in the supernatants was estimated by using a nitric oxide (NO) assay kit. The adhesive ability of HUVECs to monocytes (THP-1 cells) was analyzed using a cell adhesion assay. RT-qPCR, Western blot and flow cytometry were used to determine the expressions of the different levels of VCAM-1 and ICAM-1 in HUVECs. Western blot was used to analyze the activity of the NF-κB transcription factor. Results: In this study, a significant inhibition of HUVECs viability exposed to 2.0 μg/mL LPS and an obvious relief after the use of 2.5 μM TSIIA were emphasized. Furthermore, TSIIA inhibited the expressions of the inflammatory factors (TNF-α, IL-1β, IL-6), chemokine (IL-8) and adhesion molecules (VCAM-1 and ICAM-1) and suppressed the adhesion of monocytes (THP-1 cells) to HUVECs in LPS-stimulated HUVECs. Remarkably, like the NF-κB inhibitor PDTC, TSIIA also attenuates the activity of the NF-κB signaling pathway effectively, as mainly reflected in the decreased expression of phosphorylated p65 and IκBα. Conclusion: The protective effects of TSIIA on LPS-stimulated HUVECs injury, including promoting viability, anti-inflammation, and anti-adhesion, are likely mediated through the suppression of the NF-κB pathway.

Keywords: Tanshinone IIA, atherosclerosis, LPS, viability, inflammation, adhesion, NF-κB pathway

Introduction

Atherosclerosis (AS) is a series of chronic inflammatory changes that accumulate large amounts of cholesteryl esters on blood vessel walls to form atherosclerotic plaques, a thickening of the vessel walls and stenosis of the lumen and is a crucial pathological factor in the pathogenesis and development of cardiovascular diseases [1-3]. Endothelial cells play an important role in maintaining the homeostasis of the cardiovascular system and its dysfunction, especially the inflammatory response, which has been proven to be the primary cause of AS [4, 5]. Lipopolysaccharide (LPS), as a major component of gram-negative bacteria cell walls, has been applied to many cellular inflammatory models, including endothelial cell inflammatory model [6, 7]. Endothelial cells exposed to LPS cause an inflammatory response by releasing a plentiful amount of pro-inflammatory cytokines, chemokines, cell adhesion molecules, and nitric oxide (NO) [8]. Furthermore, AS is likely to be initiated by the activation of endothelial cells with the expression of cell adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). These cell adhesion molecules can enable the adhesion of mononuclear leukocytes in turn, such as monocytes, which further leads to a cascade of inflammatory reactions [9]. Previous studies showed that NO
plays a dual role in the pathogenesis of AS. On the one hand, the endothelial nitric oxide synthase (eNOS) expressed by endothelial cells, which is dependent on calcium ions or calmodulin and reduced nicotinamide adenine dinucleotide phosphate (NADPH), produces a small amount of NO and exerts many physiological functions such as the regulation of blood pressure and blood flow, anti-platelet aggregation and the inhibition of leukocyte adhesion. On the other hand, inducible nitric oxide synthase (iNOS) can be induced by some inflammatory mediators, producing a large amount of NO, exacerbating inflammation and damaging the body [10, 11]. Therefore, the destruction of monocyte adhesion to the endothelium to reduce the inflammatory reaction is considered a new therapeutic strategy for atherosclerosis.

A large number of inflammatory signaling pathways has been shown to be involved in the occurrence and development of AS. The nuclear factor-κB (NF-κB) signaling pathway, which can be activated by a number of inflammatory stimuli, such as exogenous LPS or endogenous tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β, is the most important signaling pathway in AS [4, 12]. The activated NF-κB signaling pathway dominates the development of AS by manipulating the function of some genes. For example, the activated NF-κB signaling pathway has been demonstrated to be directly responsible for promoting leukocyte adhesion to the endothelium and for the increased expression of adhesion molecules in TNF-α-stimulated endothelial cells [13]. Similarly, a lot of animal experiments have shown that the inhibition of the NF-κB pathway results in reduced lesion size and reduced inflammatory cell infiltration and thus alleviates disease [14]. Hence, the use of drugs that inhibit the NF-κB signaling pathway is a top priority in the treatment of AS.

Danshen (Salvia miltiorrhiza), an herbal supplement, has been widely used in cardiovascular disease for hundreds of years in China [15]. Tanshinone IIA (TSIIA) is the most important bioactive lipophilic isolated from the root of danshen (Figure 1). Its pharmacological effects have been generally recognized, including anti-oxidation [16], anti-inflammation [17], anti-cholesterol [18], myocardial regeneration [19], and even anti-obesity [20], and so on. In particular, its characteristics of anti-inflammatory, anti-cholesterol, and anti-aggregation make it an important research object in the treatment of AS in recent years. The significant inhibitory effects of TSIIA on AS both at the cellular and animal levels have been highlighted in multiple studies. However, the mechanism of action of TSIIA in the amelioration of injury to endothelial cells has not yet been unified.

In this study, we demonstrate that TSIIA can effectively improve viability, inhibit inflammatory response and the activity of viscous molecules (ICAM-1 and VCAM-1) of LPS-induced endothelial cells in large part by suppressing the NF-κB signaling pathway, which will lay the theoretical foundation for the clinical treatment of AS and related diseases.

Materials and methods

Chemicals and reagents

Tanshinone IIA (TSIIA, ≥ 97% (HPLC), Code: T4952) and lipopolysaccharides (From Escherichia coli 055: B5; Code: L6529) were purchased from Sigma Aldrich (St. Louis, MO, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation and cytotoxicity detection kit (Code: C0009), NF-κB inhibitor/antioxidant (pyrrolidine dithiocarbamate, PDTC, Code: S1809) and 2’, 7’-Bis (2-carboxyethyl)-5 (6)-carboxyfluorescein acetoxy-methyl ester (BCECF-AM, 5 mM in DMSO, Code: S1006) were purchased from Beyotime (Shanghai, China). Total protein extraction kit (Code: W034), the total protein assay kit (BCA method, Code: A045-3), nitric oxide (NO) assay
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Tanshinone IIA kit (Code: A012-1) and enzyme-linked immuno-sorbent assay (ELISA) kits of Human TNF-α (Code: H007), IL-1β (Code: H002), IL-6 (Code: H007) and IL-8 (Code: H008) were purchased from Nanjing Jiancheng Bioengineering Institute (Jiangsu, China). TRizol reagent (Code: 15596-018) and SYBR™ Green PCR Master Mix (Code: 4364344) were purchased from Thermo Fisher Scientific (USA). Antibodies against CD106 (VCAM-1-FITC, Code: 551146) and CD54 (ICAM-1-FITC, Code: 561899) were purchased from BD (New Jersey, USA). Antibodies against eNOS (Code: ab5589), iNOS (Code: ab3523), VCAM-1 (Code: ab134047), ICAM-1 (Code: ab53013), p-IKBα (Code: ab92700), NF-κB/p-p65 (Code: ab86299), GAPDH (Code: ab9485) and Goat Anti-Rabbit IgG H&L (HRP) (Code: ab6721) were purchased from Abcam (Cambridge, UK). Other chemicals and reagents were from Sinopharm Chemical Reagent Co., Ltd and Sigma.

Cell culture

The HUVEC cell line (Code: BNCC337616) and the F12K culture medium were purchased from ATCC (Manassas, VA, USA). The cells were cultured in a 90% F12K culture medium, 10% fetal bovine serum (FBS), 100 units/mL of penicillin and 100 mg/mL of streptomycin. THP-1 cell line was purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China) and cultured in 90% Gibco™ RPMI-1640 medium, 10% FBS, 0.05 mM β-Mer (Sigma-Aldrich) and 1% penicillin/streptomycin solution. Both the HUVECs and THP-1 cells were maintained at 37°C with a humidified 95% air, 5% CO₂ atmosphere and passaged about 3 times before doing the experiments. In all cell experiments, the culture medium was replaced by a serum-free medium for 12 h before medication.

Toxicity assay of LPS and TSIIA to HUVECs by MTT

In order to select the optimal concentration of LPS and TSIIA for the HUVECs cells, we first performed a toxicity assay of LPS and TSIIA to HUVECs, respectively. Following culture in 96-well plates, the HUVEC cells were plated at a density of 1.0 × 10⁴ cells/well. The cells were added to 50 μL of LPS suspended in sterilized water at different concentrations (0, 0.1, 0.5, 1.0, 1.5, 2.0 μg/mL) or 50 μL of TSIIA suspended in 0.9% normal saline at different concentra-

Cytokine assays by ELISA

Cultured HUVECs were seeded in 96-well plates and pre-incubated with 2.5 μM TSIIA for 2 h, and the supernatants and cell aggregates were collected after 12 h of stimulation with 2.0 μg/mL LPS at 37°C and stored at -20°C or -80°C until assay. The protein levels of cytokine TNF-α, IL-1β, IL-6, and IL-8 in supernatants were determined by an ELISA kit according to manufacturer's instructions. The absorbance was measured with a microplate reader (Bio-Rad, USA) at 450 nm. Finally, the value of various cytokines was calculated by a standard curve. Stored cell aggregates were used for subsequent RNA and protein extraction.

Measurement of nitric oxide (NO) production

Cultured HUVECs were seeded in 96-well plates once more and pre-incubated with 2.5 μM TSIIA for 2 h. The supernatants were collected after 12 h of stimulation with 2.0 μg/mL LPS at 37°C, and we measured the level of NO in each sample of supernatants immediately. Total NO production was estimated using a nitric oxide (NO) assay kit according to the manufacturer's instructions. The absorbance at 550 nm was measured using a microplate reader (Bio-Rad, USA). Finally, the concentration of NO in each sample of the supernatants was calculated by comparing the absorptions with a standard curve. The remaining cell aggregates were stored at -80°C and used for collecting and evaluating the proteins later.

Adhesive ability of HUVECs to THP-1 cells by cell adhesion assay

According to the previous experimental operation, cultured HUVECs were seeded in 96-well plates and pre-incubated with 2.5 μM TSIIA for 2 h followed by stimulation with 2.0 μg/mL LPS at
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Table 1. Primer sequences

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequences (5’→3’)</th>
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<tr>
<td>Human VCAM-1 forward primer</td>
<td>TACAACCGTCTTGGTCAGCCC</td>
</tr>
<tr>
<td>Human VCAM-1 reverse primer</td>
<td>CGCATCCTCACTGGGCTT</td>
</tr>
<tr>
<td>Human ICAM-1 forward primer</td>
<td>GTGACCACTACAGCTTTACC</td>
</tr>
<tr>
<td>Human ICAM-1 reverse primer</td>
<td>CTGCTACCACTGGATGAA</td>
</tr>
<tr>
<td>Human GAPDH forward primer</td>
<td>AGAGGGTGGGGGTGGTATTG</td>
</tr>
<tr>
<td>Human GAPDH reverse primer</td>
<td>AGGGGCCATCCACAGTCTTC</td>
</tr>
</tbody>
</table>

37°C for 12 h. The THP-1 cells that grow well were labeled with 10 μM BCECF-AM for 30 min in RPMI-1640 culture medium and added to the treated HUVECs and incubated for 1.5 h. Then, the unbound THP-1 cells were removed with 3 washes with soft phosphate-buffered saline (PBS). Subsequently, the adsorbed THP-1 cells were counted at least three times by a fluorescence microscope (Olympus, Tokyo, Japan), and the average of each group was incorporated into the final result.

Expression of VCAM-1 and ICAM-1 in HUVECs by flow cytometry

The TSIIA and PBS-treated HUVECs were collected, and the supernatants were removed by 2 washes with PBS. The cells were then resuspended in a 200 μL binding buffer. Subsequently, 20 μL antibodies of anti-VCAM-1-FITC or 20 μL antibodies of anti-ICAM-1-FITC was added in cells according to the group, blended gently and incubated 30 min in the dark at 4°C. At last, 300 μL binding buffer was added in each sample, followed by flow cytometry. The expression of VCAM-1 and ICAM-1 in HUVECs were counted by FC500 MCL flow cytometer (Beckman, USA) and expressed as a percentage.

Reverse transcription-quantitative PCR (RT-qPCR)

The VCAM-1 and ICAM-1 mRNA expression levels in HUVECs were measured by reverse transcription-quantitative PCR (RT-qPCR). The stored cell aggregates in the previous ELISA experiment were reused and the total RNA was extracted using TRIzol reagent according to the manufacturer’s instructions and quantified by spectrophotometry. RT-qPCR was carried out using a first strand cDNA synthesis kit and SYBR™ Green PCR Master Mix for QuantStudio™ 6 Flex Real-Time PCR System (Applied Biosystems). The relative amount of each gene was normalized to the housekeeping gene GAPDH. Relative quantity values were analyzed using the 2^−ΔΔCt method, which reflects the difference in threshold for each target gene relative to that of control group. The primer sequences synthesized by Sangon Biotech (Shanghai, China) are listed in Table 1.

Protein extraction and Western blotting analysis

The total proteins of TSIIA and PBS-treated HUVECs in previous experiments were extracted using a total protein extraction kit and quantified by the total protein assay kit (BCA method). With that, whole proteins were electrophoresed under the conditions in 12% polyacrylamide gels. The separated proteins were transferred to polyvinylidene difluoride membranes. Nonspecific binding was blocked with 5% skimmed milk for 2 h at 37°C. And then, the membranes were then incubated with rabbit anti-human eNOS (1:600 dilution), rabbit anti-human iNOS (1:500 dilution), rabbit anti-human VCAM-1 (1:10000 dilution), rabbit anti-human ICAM-1 (1:1000 dilution) monoclonal or polyclonal antibodies (Abcam, Cambridge, UK) at 4°C overnight. Rabbit anti-human GAPDH polyclonal antibody (1:2500 dilution; Abcam, Cambridge, UK) was used as a control. Next, the membranes were incubated with goat anti rabbit secondary antibody IgG (1:5000 dilution; Abcam, Cambridge, UK). Finally, images were obtained from a multifunctional gel imaging system (Bio-Rad), and the gray value of each band was calculated and analyzed.

Western blot for downstream proteins of NFκB

Cultured HUVECs were seeded in 96-well plates once more and preincubated with 2.5 μM TSIIA or 20 μM PDTC for 2 h. Cell aggregates were collected after 12 h of stimulation with 2.0 μg/mL LPS at 37°C. And the total protein in each sample was extracted and quantified. Then, the protein levels of NF-κB/p-p65 and p-IKBα were measured according to the previous procedure. This was followed by these steps: SDS-PAGE, transfer membrane, membrane blocking, primary antibody incubation (Rabbit anti-human p-IKBα antibody, 1:500 dilution; rabbit anti-human NF-κB/p-p65 antibody, 1:5000 dilution; Rabbit anti-human GAPDH antibody, 1:2500 dilution), washing membrane, secondary antibody incubation (Goat anti rabbit sec-
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IL-1β, IL-6 and IL-8 production in LPS-stimulated HUVEC cells effectively (Figure 3).

Tanshinone IIA inhibited iNOS-derived excessive production of NO induced by LPS

Inflammatory cytokines and chemokines, such as TNF-α, IL-8 and so on, have been reported to actively participate in the occurrence and development of inflammation. Therefore, to analyze the potential anti-inflammatory effect of TSIIA in LPS-stimulated HUVECs, levels of TNF-α, IL-1β, IL-6 and IL-8 were determined by ELISA. The results showed that the levels of TNF-α, IL-1β, IL-6 and IL-8 increased significantly when HUVECs were stimulated in 2.0 μg/mL LPS for 12 h. On the other hand, 2.5 μM TSIIA was found to suppress TNF-α, IL-1β, IL-6 and IL-8 production in LPS-stimulated HUVEC cells effectively (Figure 3).

Results

Tanshinone IIA promoted the viability of LPS-induced HUVECs

Before the formal experiment, we explored the experimental conditions. We found that the viability of HUVECs correlated with LPS and Tanshinone IIA in a dose-dependent manner. 2.0 μg/mL LPS will cause significant viability inhibition in 12 h (Figure 2A), and 2.5 μM TSIIA is its maximum safe concentration (Figure 2B).

So, 2.0 μg/mL LPS and 2.5 μM TSIIA were used for final experiment. A significant inhibition of cell viability exposed to 2.0 μg/mL LPS and an obvious relief after the use of 2.5 μM TSIIA were noted (Figure 2C).

Statistical analyses

All values are presented as the means ± SD and were obtained from at least three independent experiments. ANOVA followed by Tukey’s post hoc test were used to compare three and more groups using SPSS 19.0 software. P<0.05 was considered to indicate a statistically significant difference.

Tanshinone IIA inhibited the secretion of inflammatory cytokines induced by LPS

In order to more intuitively show the adhesive ability of HUVECs to THP-1 cells, the adherent THP-1 cells labeled with BCECF-AM dye were
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examined under a fluorescence microscope, digital images were captured, and the visible cells were counted at × 200 magnification. The observations showed that 2.0 μg/mL LPS markedly increased the adhesive ability between THP-1 cells and HUVECs following 12 h of previous incubation with the HUVECs and accompanied by a marked increase of fluorescent THP-1 cells. On the other hand, the pre-treatment of the HUVECs with 2.5 μM TSIIA significantly suppressed the LPS-induced adhesive ability between the THP-1 cells and HUVECs, and with a significant reduction of fluorescent THP-1 cells (Figure 5A, 5B).

Tanshinone IIA decreased the expression of ICAM-1 and VCAM-1 induced by LPS

ICAM-1 and VCAM-1 are universally considered the main adhesion molecules which mediate the adhesive action of monocytes to HUVECs. Therefore, we hypothesized that TSIIA modulates adhesion between HUVECs and THP-1 cells by mediating the expression of ICAM-1 and VCAM-1. We determined the protein expression of VCAM-1 and ICAM-1 in HUVECs by flow cytometry and western blot. The consistent results showed that 2.0 μg/mL LPS alone significantly increased the protein expression of ICAM-1 and VCAM-1. However, the protein levels of ICAM-1 and VCAM-1 were markedly down-regulated following pre-treatment with 2.5 μM TSIIA (Figure 6A, 6B, 6D). In addition, we also examined the mRNA levels of ICAM-1 and VCAM-1 by RT-qPCR. The results showed that the mRNA expression levels of ICAM-1 and VCAM-1 were markedly up-

Figure 3. Tanshinone IIA inhibited the secretion of inflammatory cytokines induced by LPS. Cultured HUVECs were seeded in 96-well plates and pre-incubated with 2.5 μM TSIIA for 2 h followed by stimulation with 2.0 μg/mL LPS at 37 °C for 12 h. A: Expression level detection of TNF-α in supernatants by ELISA. B: Expression level detection of IL-1β in supernatants by ELISA. C: Expression level detection of IL-6 in supernatants by ELISA. D: Expression level detection of IL-8 in supernatants by ELISA. Data are the mean ± S.D. **, P<0.01 vs. the control group; ##, P<0.01 vs. the model group.

Figure 4. Tanshinone IIA inhibited iNOS-derived excessive production of NO induced by LPS. Cultured HUVECs were seeded in 96-well plates and pre-incubated with 2.5 μM TSIIA for 2 h followed by stimulation with 2.0 μg/mL LPS at 37 °C for 12 h. A: Determination of total NO production in supernatants of HUVECs using a nitric oxide (NO) assay kit. B: Protein level detection of iNOS and eNOS by western blot. C: Relative expression analysis of iNOS protein. D: Relative expression analysis of eNOS protein. Data are the mean ± S.D. **, P<0.01 vs. the control group; ##, P<0.01 vs. the model group.
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Regulated following stimulation with 2.0 μg/mL LPS. Consistent with the results of analysis for protein levels, pre-treatment with 2.5 μM TSIIA significantly decreased the mRNA levels of ICAM-1 and VCAM-1 (Figure 6C).

Tanshinone IIA and PDTC promoted the viability of LPS-induced HUVECs and decreased the expression of downstream proteins of NFκB

The effects of the NF-κB inhibitor PDTC on LPS-induced HUVECs viability are unclear. As shown in Figure 7A, like TSIIA, PDTC also promoted the viability of LPS-induced HUVECs compared with the model group. NF-κB is a key transcription regulator of inflammation and proliferation, so its important position is self-evident. Therefore, we hypothesized that the anti-inflammatory and repair effects of TSIIA on the endothelial cells are closely related to the NF-κB pathway. In this study, we determined the effects of TSIIA on LPS-induced NF-κB activation. The results of the western blot showed that 2.0 μg/mL LPS activated the NF-κB pathway with a high expression of phosphorylated NF-κB/p65 and IκBα. However, such LPS-induced activation was suppressed by pre-treatment with 2.5 μM TSIIA and accompanied by a marked decrease of p-p65 and p-IκBα expression levels. And PDTC also decreased of p-p65 and p-IκBα expression levels (Figure 7B-D). So, we suspect that TSIIA and the NF-κB inhibitor PDTC had the same function of promoting the viability of LPS-induced HUVECs by suppressing the NF-κB pathway.

Discussion

Atherosclerosis (AS) is a crucial pathological factor in the development of cardiovascular diseases [21]. It is understood that the atherosclerotic mechanisms include lipid metabolism disorders, oxidative stress, increased inflammation, proliferation, enhanced adhesive action to monocytes, as well as NO damage, and so on, which result in endothelial dysfunction and further atherosclerotic plaque formation [22, 23]. Vascular endothelial dysfunction and inflammatory reactions occur when endothelial cells are exposed to physiological stress or exogenous and endogenous chemicals, leading to the earliest pathological changes in the development
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[Graphs and images showing experimental results]
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Figure 6. Tanshinone IIA decreased the expression of ICAM-1 and VCAM-1 induced by LPS. The mRNA levels of ICAM-1 and VCAM-1 were tested by RT-qPCR, and the protein expression of VCAM-1 and ICAM-1 in HUVECs were evaluated by flow cytomtery and Western blot, respectively. A: Percentage detection of ICAM-1 protein level by flow cytometry. B: Percentage detection of VCAM-1 protein level by flow cytometry. C: The mRNA level detection of ICAM-1 and VCAM-1 by RT-qPCR. D: The protein level detection of ICAM-1 and VCAM-1 by Western blot. Data are the mean ± S.D. **, P<0.01 vs. the control group; ***, P<0.01 vs. the model group.

Consistent results show that TSIIA significantly increases the viability of H₂O₂-injured human umbilical vein endothelial cell line by flow cytometric analysis and MTT assay [33]. In addition, TSIIA also attenuates the inflammatory response and apoptosis in the model of spinal cord injury in adult rats, which is accompanied by an obviously decreased production of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) and a low rate of apoptosis [34]. Therefore, the abilities of TSIIA to promote damaging cell viability and suppress inflammatory response are evident.

Nitric oxide (NO), a short-lived free radical and an internal messenger and an important indicator of endothelial function, is synthesized from L-arginine by nitric oxide synthases (NOS) [35]. Studies show that endothelial nitric oxide synthase (eNOS) is responsible for the low level of NO production involved in endothelium-dependent vasodilatation and maintains endothelial homeostasis [36]. However, inducible nitric oxide synthase (iNOS) can be easily induced by pro-inflammatory substances and then releases a lot of NO [37]. In our results, iNOS expression was increased and eNOS expression was decreased in the LPS-induced model group, but total NO content in the medium was increased significantly. Oppositely, TSIIA prominently increased eNOS enzymatic activity and reduced the increased iNOS expression caused by LPS, but the NO content in the medium was decreased significantly. All of these results suggest that LPS-induced the release amount of total NO increase is due mainly to iNOS and that TSIIA ameliorates this state. Research by other scholars showed that TSIIA can prevent the loss of nigrostriatal dopaminergic neurons by inhibiting NADPH oxidase and iNOS in the MPTP model of Parkinson’s disease [38]. Consequently, based on the data shown in present research and other scholars, we conclude that TSIIA effectively suppresses iNOS-derived NO over-expression and regulates eNOS-derived NO to the normal levels in HUVECs, thereby...
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The adhesion of endothelial cells to monocytes is a crucial step in the inflammatory response which precedes the development of atherosclerosis. Undoubtedly, ICAM-1 and VCAM-1, as the important molecules involved in adhesion, play an important role in the regulation of endothelial cell adhesion and also in the inflammatory response [39]. Accordingly, impeding the adhesion of monocytes to the endothelial cells is of great importance in early AS. In addition, it is remarkable that a previous study linked the unbalanced expression of ICAM-1 and VCAM-1 to an increased risk of the incidence of clinical coronary artery disease [40]. In this study, we found that TSIIA inhibits the adhesion of fluorescent THP-1 cells to LPS-stimulated endothelial cells. Meanwhile, TSIIA suppresses the up-regulation of VCAM-1 and ICAM-1 in mRNA and protein levels induced by LPS. Similar results have demonstrated a novel underlying mechanism for anti-inflammatory effect of TSIIA by modulating TNF-α-induced expression of VCAM-1, ICAM-1 and fractalkine in HUVECs [41]. Thus, TSIIA may prove to be effective in protecting against the development of early AS lesions.

The NF-κB pathway, as a major signaling pathway, is involved in the activation of HUVECs induced by exogenous LPS. Once stimulated with LPS, NF-κB is activated by phosphorylation and regulates the expression of a network of inflammatory mediators [42]. Under normal physiological conditions, NF-κB is sequestered into the cytoplasm by IkB proteins. Upon inflammatory events stimuli, including LPS or TNF-α, IkBα is phosphorylated and degraded, which further enables NF-κB to translocate to the nucleus. And then NF-κB starts playing its powerful transcriptional role in numerous genes, such as the inflammatory factors (TNF-α, IL-1β and IL-6), chemokine (IL-8) and adhesion molecules (VCAM-1 and ICAM-1) [43]. But up to now, the relationship between TSIIA and NF-κB pathway has been unclear. With this confusion in mind, in this study, we detected the effects of TSIIA on LPS-induced NF-κB activation. Our results demonstrated that TSIIA may inhibit LPS-induced pro-inflammatory mediator, adhesion molecule production by suppressing NF-κB activation. The effect is similar to that of the NF-κB inhibitor (PDTC), accompanied by a decrease in phosphorylated NF-κB/p-65 (p-p65) level.

Briefly, the present study shows that TSIIA promotes viability, restrains the expression of inflammatory factors, chemokines, and adhesion molecules, and suppresses the adhesion of monocytes (THP-1 cells) to HUVECs in LPS-stimulated HUVECs. Moreover, the protective effects of TSIIA on LPS-stimulated HUVECs are likely mediated through the suppression of the NF-κB pathway. These findings not only shed new light on the mechanisms of action of TSIIA.
but also suggest that TSIIA may play an important role in the protection against the early development of atherosclerosis.

Disclosure of conflict of interest

None.

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References


