Peroxisome proliferator-activated receptor γ alleviates human umbilical vein endothelial cell injury in deep vein thrombosis by blocking endoplasmic reticulum stress

YUNXIN ZHANG[1], YONGSHUAI GE[2], LIYUAN TAO[3], MINGYUAN LIU[4], WEI JIA[1], XUAN TIAN[1], PENG JIANG[1], ZHIYUAN CHENG[1], JINYONG LI[1] and JIANLONG LIU[1]

[1]Department of Vascular Surgery, Beijing Jishuitan Hospital, Beijing 100035; [2]Research Center for Medical Artificial Intelligence, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, Guangdong 518055; [3]Research Center of Clinical Epidemiology, Peking University Third Hospital, Beijing 100191; [4]Department of Vascular Surgery, Beijing Friendship Hospital, Beijing 100050, P.R. China

Received March 31, 2023; Accepted February 15, 2024

DOI: 10.3892/etm.2024.12674

Abstract. The present study aimed to explore the role of peroxisome proliferator-activated receptor γ (PPARγ) in the development of deep vein thrombosis (DVT), as well as to discover the potential regulatory mechanism of PPARγ. Human umbilical vein endothelial cells (HUVECs) were treated with modified glycated human serum albumin (M-HSA) to mimic DVT. PPARγ expression and activity were detected using western blot analysis and the corresponding activity detection kit, respectively. Cell Counting Kit-8 and the terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling assays were employed to detect cell viability and apoptosis, respectively. The levels of thrombosis-related factors and inflammatory cytokines were detected by ELISA. The levels of oxidative stress-related factors were determined by the corresponding commercial kits. In addition, tunicamycin (TM), the agonist of endoplasmic reticulum stress (ERS), was applied to investigate the potential mechanism. The results indicated that M-HSA caused reduced expression and activity of PPARγ in HUVECs; these effects were reversed by PPARγ overexpression, which significantly inhibited M-HSA-induced cell viability loss, cell apoptosis, inflammation and oxidative stress in HUVECs. In addition, ERS was activated following M-HSA stimulation in HUVECs, but was suppressed by PPARγ overexpression. Furthermore, TM partly abolished the protective role of PPARγ overexpression against cell viability loss, cell apoptosis, inflammation and oxidative stress in M-HSA-induced HUVECs. In summary, PPARγ antagonized M-HSA-induced HUVEC injury by suppressing the activation of ERS, which provides a novel strategy for the treatment of DVT.

Correspondence to: Dr Jianlong Liu, Department of Vascular Surgery, Beijing Jishuitan Hospital, 31 Xinjiekou East Street, Xicheng, Beijing 100035, P.R. China
E-mail: liujianlongjll@163.com

Key words: peroxisome proliferator-activated receptor γ, human umbilical vein endothelial cells, deep vein thrombosis, endoplasmic reticulum stress

Introduction

Venous thromboembolism, including deep vein thrombosis (DVT), and its severe form pulmonary embolism (PE), is the third most frequent complication of cardiovascular diseases, affecting ~400,000 people annually (1,2). Currently, compression ultrasound is the first line imaging modality for the diagnosis of DVT (3). The treatment of DVT consists of surgical invention, such as thrombectomy or catheter-based thrombolysis and drug prevention including anticoagulation by heparin, thrombin and vitamin K antagonists (4,5). However, due to the low specificity (40-50%) of the diagnosis and the limited efficacy of these treatments, patients at the acute stage of DVT may develop PE, or even post-thrombotic syndrome, which is the most common long-term complication in patients with DVT, seriously threatening their survival and quality of life (6-8). Therefore, the progress of novel therapeutic strategies can be achieved by improving the understanding of the pathophysiology of DVT.

Previous evidence confirmed that oxidative stress and inflammation are common pathological processes responsible for vascular endothelial cell damage, which is one of the most important causes leading to DVT (9-11). Advanced glycation end products (AGEs) are heterogeneous molecules produced by the non-enzymatic glycation of proteins and lipids under hyperglycemic or oxidative stress conditions (12). These molecules can bind with their receptor [receptor of advanced glycation end products (RAGE)] to evoke an inflammatory response and induce oxidative stress, as well as thrombogenic reactions, playing a central role in the development of vascular complications (11,13-15). The previous study conducted by the authors demonstrated the involvement of AGEs/RAGE in the development of human umbilical vein endothelial cell (HUVEC) injury. The RAGE inhibitor downregulated

endothenin-1 (ET-1) levels, which is considered to be the hallmark of endothelial injury, thereby mitigating HUVEC injury (16). Therefore, blockade of AGEs/RAGE is an effective approach for the prevention of DVT.

Peroxisome proliferator-activated receptor γ (PPARγ), a member of the PPAR family of highly conserved nuclear hormone receptors, is widely known for its important role in regulating adipocyte differentiation, blood pressure, lipid metabolism and insulin sensitivity (17). A previous study has reported the eliminating effect of the activation of PPARγ on hypercoagulability, one of the major factors contributing to DVT, by inhibiting thrombin-induced platelet aggregation, which indicates a potential role of PPARγ during the process of DVT (18). It was also reported that the activation of PPARγ may prevent thrombosis by downregulating the expression of pro-inflammatory cell adhesion molecules and by enhancing endothelial nitric oxide production. Of note, PPARγ has been revealed to be associated with the AGE/RAGE axis to exert its protective role against vascular inflammation and oxidative stress (19). Chrysin, a natural flavonoid, which acts as a PPARγ agonist, has shown the ability to reduce serum AGE levels, inhibit AGEs-RAGE-mediated oxidative stress and inflammation and attenuate endothelial dysfunction (20). Previous studies have confirmed the protection of PPARγ on endothelial cells (21-23); however, whether PPARγ participates into AGE-RAGE-triggered endothelial dysfunction, including oxidative stress and inflammation, during DVT, remains poorly elucidated. Furthermore, AGE-RAGE signaling is a pivotal inducer of endoplasmic reticulum stress (ERS), which is closely associated with inflammation and oxidative stress (24). PPARγ has also been shown to be a critical mediator of ERS, as the inhibition of it was demonstrated to relieve ERS and reduce the production of pro-inflammatory cytokines and reactive oxygen species (ROS), thereby alleviating ischemia-reperfusion injury (25).

The present study not only investigated the role of PPARγ on AGEs-RAGE-triggered HUVEC injury, but also explored the potential mechanism of action of PPARγ. The findings of the present study contributed to the understanding of the pathophysiology of DVT and offered novel strategies for the prevention of DVT.

Materials and methods

Cell culture and induction. HUVECs (cat. no. iCell-h110) were purchased from iCell Bioscience, Inc. and were incubated in Endothelial Cell Culture Medium (Cellverse Bioscience Technology Co., Ltd.) containing 100 U/ml penicillin and 100 μg/ml streptomycin in the presence of 5% CO_2 at 37˚C. To mimic AGE-induced DVT, HUVECs were treated with modified glycated human serum albumin (M-HSA) for 24 h (26) and M-HSA was prepared by co-incubation of HSA (15 mg/ml; Sigma-Aldrich; Merck KGaA) and 3-deoxyglucosone (3-DG, 1 mM; Sigma-Aldrich; Merck KGaA) for 2 weeks as described in a previous study conducted by the authors (16). Tunicamycin (TM; Sigma-Aldrich; Merck KGaA), the agonist of ERS, was used for pre-treatment (5 μg/ml) for 6 h prior to M-HSA induction in HUVECs.

Cell transfection. The sequences of PPARγ were cloned into the pcDNA 3.1 vector (Invitrogen; Thermo Fisher Scientific, Inc.) to establish a PPARγ overexpression vector (oe-PPARγ). The pcDNA 3.1 vector was used as a negative control (oe-NC). HUVECs were transfected with oe-PPARγ (15 nM) or oe-NC (15 nM) using Lipofectamine™ 3000 reagent (Thermo Fisher Scientific, Inc.) at 37˚C strictly in line with the manufacturer's guidelines upon reaching 80% confluence. At 48 h post-transfection, the transfected HUVECs were harvested for subsequent experiments.

PPARγ activity assay. PPARγ activity was evaluated as previously reported (27). In brief, nuclear extracts were initially obtained from cultured HUVECs using a Nuclear Extraction Kit (cat. no. ab113474; Abcam). Subsequently, PPARγ activity was determined using a PPARγ Transcription Factor Assay Kit (cat. no. ab133101; Abcam) by measuring the absorbance at 450 nm.

Cell viability assay. Cell viability was determined using the Cell Counting Kit-8 (CCK-8) assay (16). In brief, HUVECs were cultured in 96-well plates ($3.0x10^3$ cells/well) and incubated for 24, 48 and 72 h, respectively. At different time points, 10 μl CCK-8 solution (cat. no. KGA9305-500; Nanjing KeyGen Biotech Co., Ltd.) was added into each well and HUVECs were cultured in the incubator for an additional 2 h at 37˚C. Finally, the absorbance at 450 nm of each well was detected using a microplate reader.

Terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling (TUNEL) assay. Cell apoptosis was assessed using the TUNEL assay (28). HUVECs ($5x10^4$ cells/well) were cultured in 6-well plates with cell culture silicon slides. Following treatment, the cells were fixed with 4% paraformaldehyde for 30 min, permeabilized with 0.2% Triton X-100 for 5 min and blocked with 3% BSA (Wuhan Servicebio Technology Co., Ltd.) for 30 min at room temperature. Subsequently, the cells were incubated with a TUNEL reaction mixture (Roche Diagnostics) at 37˚C for 1 h and the cell nuclei were stained with 1 mg/ml 4',6-diamidino-2-phenylindole (Invitrogen, Thermo Fisher Scientific, Inc.) for 10 min at 37˚C in the dark. Anti-fluorescence quenching liquid was used for sealing. The apoptotic cells were observed in five random fields using an inverted fluorescence microscope (Olympus IX71; Olympus Corporation).

ELISA. The culture medium was harvested and centrifugated at 12,000 x g, 4˚C for 10 min and the supernatant was then collected. ELISA kits for 6-keto prostaglandin-F1 α (6-K-PGF1α; E-EL-0054, Elabscience), ET-1 (ml025101), TNF-α (ml077385), IL-1β (ml058059) and IL-6 (ml028583; all from Shanghai Enzyme-linked Biotechnology Co., Ltd.) were applied to evaluate the corresponding protein levels in the culture supernatant in accordance with the manufacturer's instructions (16).

Assessment of oxidative stress. The levels of ROS, malondialdehyde (MDA) and superoxide dismutase (SOD) were detected to evaluate the degree of oxidative stress (16). For ROS measurement, HUVECs were stained with 20 μM 2',7'-dichlorodihydrofluorescein diacetate strictly in line with the instructions of the Fluorometric Intracellular ROS

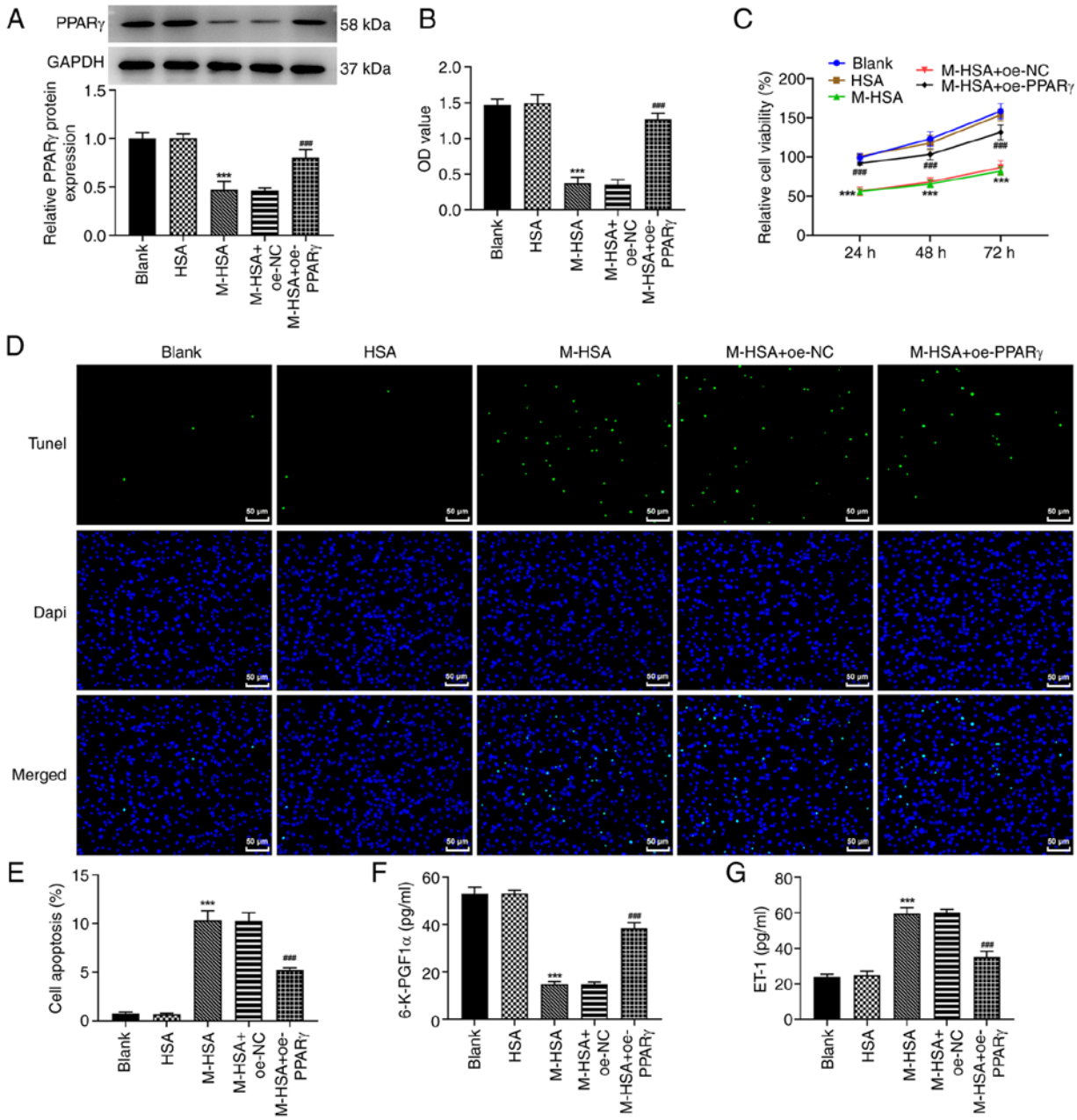

Figure 1. PPARγ restores cell viability loss, apoptosis and levels of 6-K-PGF1α and ET-1 in M-HSA-stimulated HUVECs. HUVECs were stimulated by M-HSA for 24 h to mimic advanced glycation end products-induced vein thrombosis. Meanwhile, HUVECs were transfected with oe-PPARγ or oe-NC for 48 h. (A) The protein expression level of PPARγ was detected using western blot. (B) The PPARγ activity was assessed at the absorbance of 450 nm. (C) Cell viability was evaluated using Cell Counting Kit-8 assay at indicated time points (24, 48 and 72 h). (D and E) Cell apoptosis was determined using TUNEL assay. The concentrations of (F) 6-K-PGF1α and (G) ET-1 were measured by ELISA. ***P<0.001 vs. HSA and $^{###}$P<0.001 vs. M-HSA + oe-NC. PPARγ, proliferator-activated receptor γ; 6-K-PGF1a, 6-keto prostaglandin-F1α; ET-1, endothenin-1; M-HSA, modified glycated human serum albumin; HUVECs, human umbilical vein endothelial cells; oe, overexpressing; NC, negative control.

Kit (cat. no. MAK143; Sigma-Aldrich; Merck KGaA). The fluorescence intensity was detected by a microplate fluorometer (Molecular Devices, LLC). For MDA and SOD measurements, the cell supernatant was measured with Lipid Peroxidation MDA Assay Kit (cat. no. S0131S) and Total Superoxide Dismutase Assay Kit (cat. no. S0101S) (both from Beyotime Institute of Biotechnology) in accordance with the manufacturer's guidelines, respectively.

Western blot analysis. Total protein was extracted from cells using a radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology), followed by the determination of protein concentrations using an Enhanced BCA protein assay kit (Beyotime Institute of Biotechnology). The same amount (30 μg/lane) of protein was separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes. Following blocking with 5% non-fat milk at room temperature for 2 h, the membranes were incubated with primary antibodies against PPARγ (1:1,000; cat. no. ab178860; Abcam), C/EBP homologous protein (CHOP; 1:1,000; cat. no. 2895; Cell Signaling Technology, Inc.), glucose-regulated protein 78

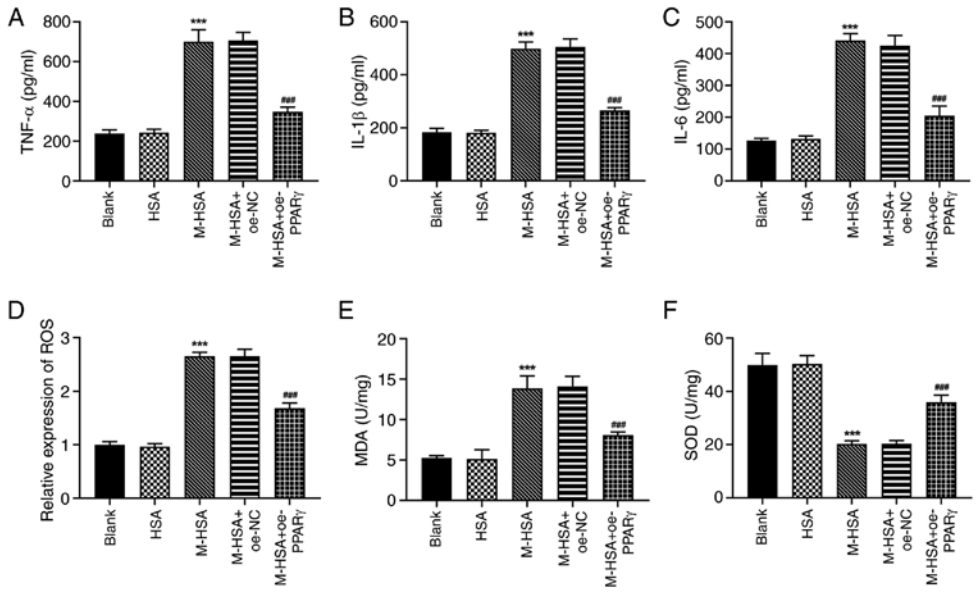

Figure 2. PPARγ reduces inflammation and oxidative stress in M-HSA-stimulated HUVECs. The production of (A) TNF-α, (B) IL-1β and (C) IL-6 in HUVECs was measured by ELISA. (D) The level of ROS was detected using CFH-DA probe. The levels of (E) MDA and (F) SOD in HUVECs were measured using their corresponding commercial kits. ***P<0.001 vs. has and ###P<0.001 vs. M-HSA + oe-NC. PPARγ, proliferator-activated receptor γ; M-HSA, modified glycated human serum albumin; HUVECs, human umbilical vein endothelial cells; ROS, reactive oxygen species; MDA, malondialdehyde; SOD, superoxide dismutase; oe, overexpressing; NC, negative control.

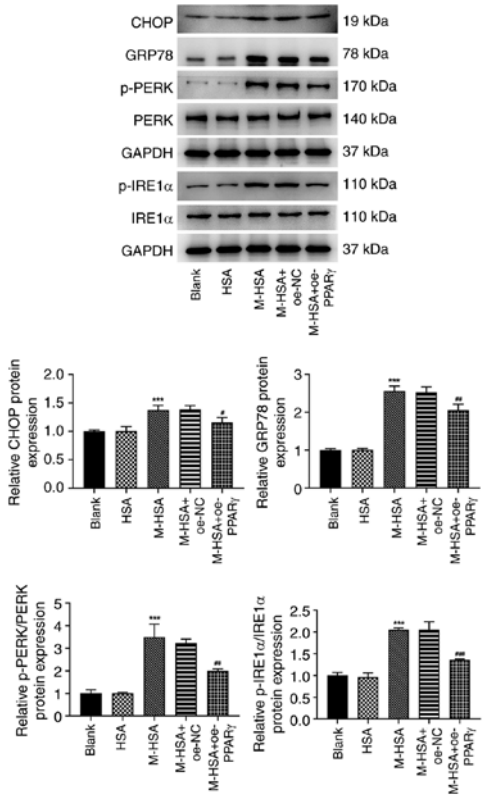

Figure 3. PPARγ weakens the activation of endoplasmic reticulum stress in M-HSA-stimulated HUVECs. The protein expression levels of CHOP, GRP78, p-PERK, PERK, IRE1α and p-IRE1α were detected using western blot analysis. ***P<0.001 vs. HSA; #P<0.05, ##P<0.01 and ###P<0.001 vs. M-HSA + oe-NC. PPARγ, proliferator-activated receptor γ; M-HSA, modified glycated human serum albumin; HUVECs, human umbilical vein endothelial cells; CHOP, C/EBP homologous protein; GRP78, glucose-regulated protein 78; p, phosphorylated; PERK, protein kinase (PKR)-like ER kinase; IRE1α, p-inositol requiring enzyme 1α; oe, overexpressing; NC, negative control.

(GRP78; 1:1,000; cat. no. ab21685; Abcam), phosphorylated (p)-protein kinase (PKR)-like ER kinase (p-PERK; 1:200; cat. no. orb504147; Biorbyt), PERK (1:500; cat. no. orb1294328; Biorbyt), p-inositol requiring enzyme 1α (p-IRE1α; 1:1,000; cat. no. ab243665; Abcam), IRE1α (1:1,000; cat. no. ab37073; Abcam) and GAPDH (1:2,500; cat. no. ab9485; Abcam) at 4˚C overnight. On the following day, the membranes were washed with Tris-buffered saline containing 0.1% Tween-20, and subsequently incubated with horseradish peroxidase-conjugated goat anti-rabbit (1:5,000; cat. no. ab6721; Abcam) or goat anti-mouse (1:5,000; cat. no. ab6789; Abcam) secondary antibodies at room temperature for 2 h. The signals were visualized using Amersham ECL Prime Western Blotting Detection Reagent (Amersham; Cytiva) and were semi-quantified by ImageJ software (Version 1.52; National Institutes of Health).

Statistical analysis. All data were expressed as mean ± standard deviation from at least three independent experiments. Data analysis was conducted using GraphPad Prism 8 (GraphPad Software; Dotmatics). One-way ANOVA analysis followed by the Tukey's post-hoc test was used to compare the differences among groups. P<0.05 was considered to indicate a statistically significant difference.

Results

PPARγ restores cell viability loss, apoptosis and the levels of 6-K-PGF1α and ET-1 in M-HSA-stimulated HUVECs. To investigate the role of PPARγ in AGE-induced VT, HUVECs were stimulated by M-HSA to mimic AGE-induced DVT and the expression levels of PPARγ were detected. As demonstrated in Fig. 1A, the protein expression levels of PPARγ were significantly reduced following M-HSA stimulation in HUVECs. Therefore, a gain-of function experiment was

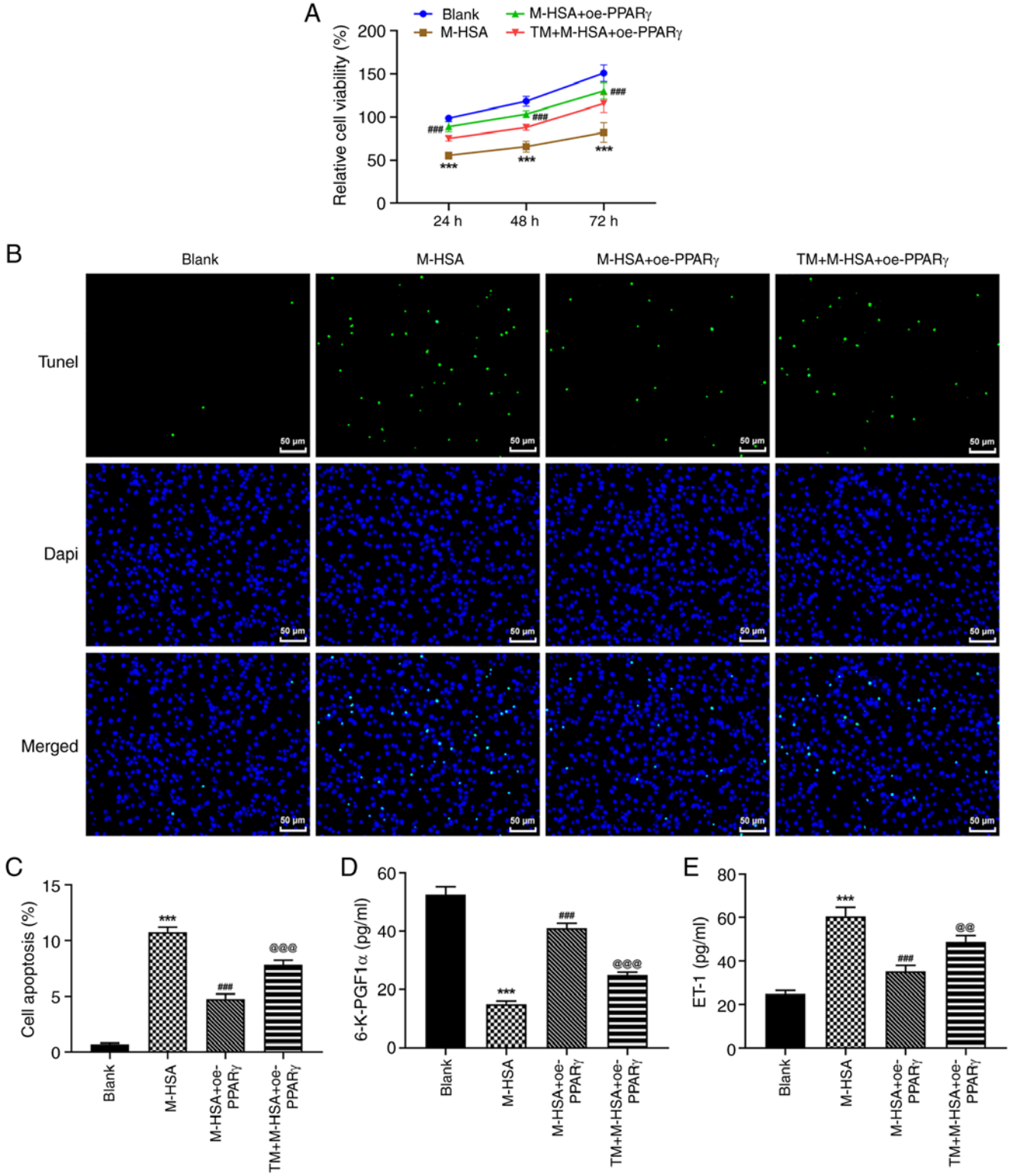

Figure 4. TM partly diminishes the effects of PPARγ on M-HSA-induced endothelial injury in HUVECs. HUVECs were stimulated by M-HSA for 24 h to mimic advanced glycation end products-induced vein thrombosis. Meanwhile, HUVECs were transfected with oe-PPARγ for 48 h, with or without additional treatment of TM, an agonist of endoplasmic reticulum stress. (A) Cell viability was evaluated using Cell Counting Kit-8 assay at indicated time points (24, 48 and 72 h). (B and C) Cell apoptosis was determined using TUNEL assay. The concentrations of (D) 6-K-PGF1α and (E) ET-1 were measured by ELISA. ***P<0.001 vs. Blank; $^{###}$P<0.001 vs. M-HSA; $^{@@}$P<0.01 and $^{@@@}$P<0.001 vs. M-HSA + oe-PPARγ. TM, tunicamycin; PPARγ, proliferator-activated receptor γ; M-HSA, modified glycated human serum albumin; HUVECs, human umbilical vein endothelial cells; oe, overexpressing; 6-K-PGF1a, 6-keto prostaglandin-F1α; ET-1, endothenin-1.

conducted to upregulate PPARγ (Fig. S1). The expression levels of PPARγ in the M-HSA + oe-PPARγ group were significantly higher than those in the M-HSA + oe-NC group (Fig. 1A). In addition, PPARγ activity was also weakened by M-HSA stimulation while it was increased following PPARγ overexpression (Fig. 1B). Subsequently,

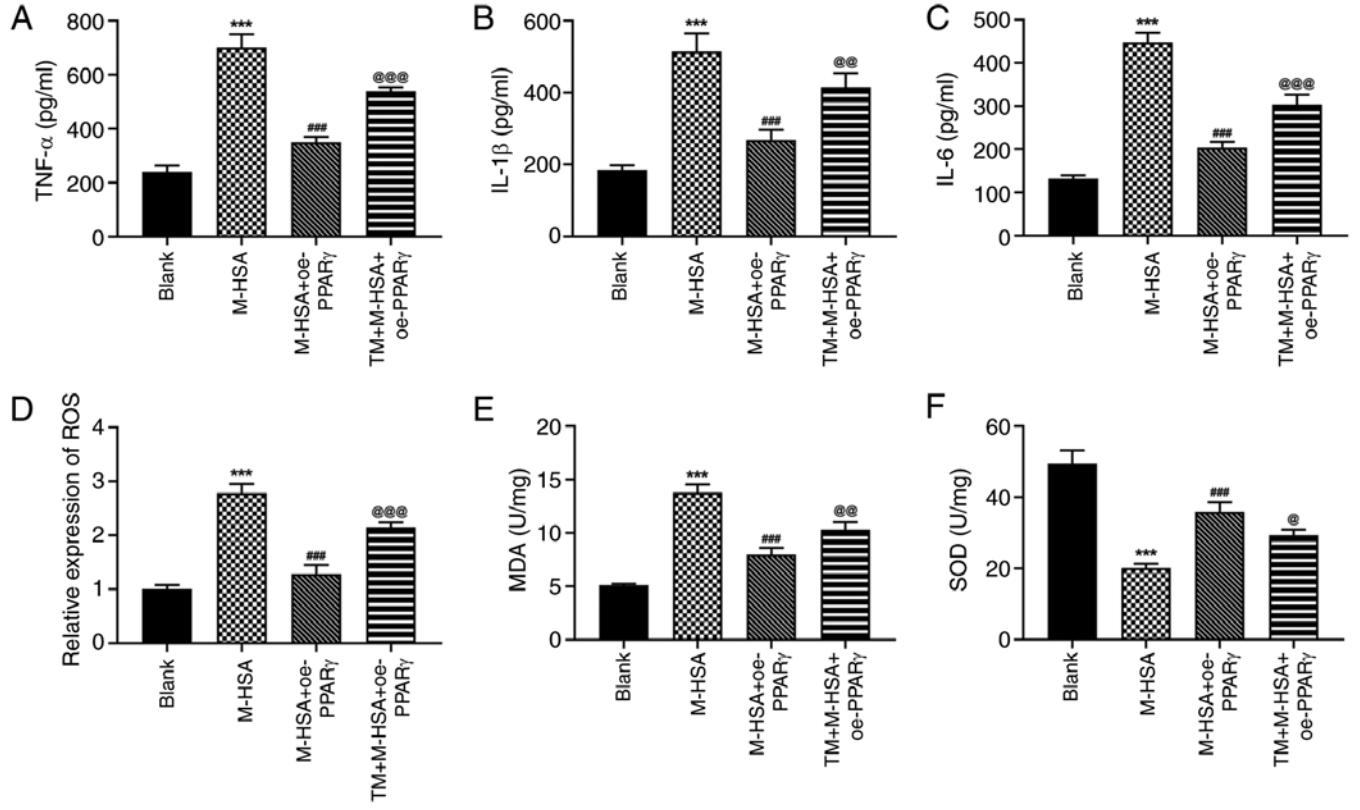

Figure 5. TM partly diminishes the effects of PPARγ on M-HSA-induced inflammation and oxidative stress in HUVECs. The production of (A) TNF-α, (B) IL-1β and (C) IL-6 in HUVECs was measured by ELISA. (D) The level of ROS was detected using CFH-DA probe. The levels of (E) MDA and (F) SOD in HUVECs were measured using their corresponding commercial kits. ***P<0.001 vs. Blank; $^{###}$P<0.001 vs. M-HSA; $^{@}$P<0.05, $^{@@}$P<0.01 and $^{@@@}$P<0.001 vs. M-HSA + oe-PPARγ. TM, tunicamycin; PPARγ, proliferator-activated receptor γ; M-HSA, modified glycated human serum albumin; HUVECs, human umbilical vein endothelial cells; ROS, reactive oxygen species; MDA, malondialdehyde; SOD, superoxide dismutase; oe, overexpressing.

the data from the CCK-8 and TUNEL assays indicated that M-HSA led to a significant reduction in cell viability and an apparent elevation in TUNEL-positive cells, whereas these changes were inhibited when PPARγ was overexpressed (Fig. 1C-E), suggesting that PPARγ had the ability to alleviate M-HSA-induced cell viability loss and apoptosis in HUVECs. In addition, the downregulated 6-K-PGF1α levels and upregulated ET-1 levels in HUVECs, which were caused following M-HSA induction, were also partly abolished by PPARγ overexpression (Fig. 1F and G). These data suggested that PPARγ overexpression attenuated M-HSA-induced endothelial injury in HUVECs by improving cell viability, inhibiting cell apoptosis, upregulating 6-K-PGF1α levels and downregulating ET-1 levels.

PPARγ reduces the induction of inflammation and oxidative stress in M-HSA-stimulated HUVECs. Since oxidative stress and inflammation are common pathological processes responsible for vascular endothelial cell damage, the regulatory role of PPARγ was also investigated on inflammation and oxidative stress in M-HSA-stimulated HUVECs. As expected, M-HSA resulted in excessive production of TNF-α, IL-1β and IL-6 in HUVECs, while PPARγ overexpression was capable to suppress the overproduction of these markers (Fig. 2A-C). Furthermore, elevated levels of ROS and MDA and a reduced level of SOD were observed in HUVECs following M-HSA stimulation; these effects were partly reversed by PPARγ overexpression (Fig. 2D-F). The aforementioned data indicated a protective role of PPARγ against M-HSA-stimulated inflammation and oxidative stress in HUVECs.

PPARγ weakens the activation of ERS in M-HSA-stimulated HUVECs. Subsequent studies investigated the potential regulatory mechanism by which ERS is induced by a variety of physiological and pathological factors including oxidative stress. The protein expression levels of CHOP, GRP78, p-PERK and p-IRE1α were significantly increased following M-HSA stimulation, suggesting that M-HSA triggered the activation of ERS in HUVECs (Fig. 3). However, this activation was weakened by PPARγ overexpression, as demonstrated by the restoration of the protein expression changes following PPARγ overexpression in M-HSA-stimulated HUVECs.

TM partly diminishes the effects of PPARγ on M-HSA-induced endothelial injury in HUVECs. To verify the critical role of ERS during the regulation of PPARγ in M-HSA-induced HUVECs, the agonist of ERS, TM, was used and the aforementioned cellular experiments were re-conducted. It was observed that the inhibitory effects of PPARγ on M-HSA-induced cell viability loss and cell apoptosis in HUVECs were weakened by TM (Fig. 4A-C). Moreover, additional treatment of TM caused a decrease in 6-K-PGF1α levels and an increase in ET-1 levels compared with the corresponding levels noted in the M-HSA + oe-PPARγ group (Fig. 4D and E). In addition, the protective effects of PPARγ against M-HSA-induced inflammation and oxidative stress were also weakened by TM

in HUVECs (Fig. 5A-F). Therefore, these data suggested that the protective role of PPARγ against M-HSA-induced HUVEC injury could be diminished by TM, implying that PPARγ may exert its functions by inhibiting the activation of ERS.

Discussion

DVT is recognized as a multifactorial disease originating from complicated interactions between environmental and genetic predisposing factors (29). In the present study, the role of PPARγ in DVT was identified and the regulatory functions and molecular mechanism of PPARγ were elucidated with regard to HUVEC-mediated injury. The present study used M-HSA to stimulate HUVECs so as to mimic AGE-induced DVT. It was determined that PPARγ was significantly decreased following the stimulation of M-HSA in HUVECs. Simultaneously, the protective role of PPARγ in AGEs-induced DVT was verified by its inhibitory effects on cell apoptosis, endothelial injury, inflammation and oxidative stress in M-HSA-stimulated HUVECs, illustrating a potential therapeutic approach against DVT.

The functional capability of the vessel wall endothelium is essential to maintain vascular function and a non-thrombotic state. Endothelial dysfunction, which occurs due to the imbalance between proinflammatory and anti-inflammatory mediators, oxidative and antioxidant factors, procoagulant and anticoagulant substances and relaxing and contracting factors, plays a prominent role in the development of DVT by arousing the prothrombotic response (10,30-32). PPARγ is widely expressed in muscle, liver, heart and adipose tissue, as well as in vascular endothelial and smooth muscle cells (33). Evidence has shown that *Panax notoginseng* saponins-induced activation of PPAR-γ inhibits thrombin-induced platelet aggregation *in vitro* and effectively improves hypercoagulability *in vivo* (18). The PPARγ agonist rosiglitazone effectively inhibited inflammation and oxidative stress in injured HUVECs (34). Notoginsenoside Fc, a novel triterpenoid derived from *P. notoginseng*, can prevent endothelial cell injury via the PPARγ pathway (35). As expected, the present study demonstrated that PPARγ participated into AGEs-RAGE-triggered oxidative stress and inflammation during DVT and served as a protective mediator against the formation of endothelial cell injury by inhibiting inflammation and oxidative stress.

ERS, also known as the unfolded protein response, plays an important role in preventing cells against toxic stimuli or cellular stress-caused deposition of misfolded proteins (36). Under ERS conditions, GRP78 chaperone binds to misfolded proteins to trigger an adaptive mechanism via the activation of subsequent signaling pathways, including PERK, activating transcription factor (ATF) 6α and IRE1α. Once the unfolded or misfolded proteins are excessive, activated PERK will phosphorylate eukaryotic initiation factor 2 and further activate ATF4, which promotes the expression of CHOP and triggers cell apoptosis (37,38). It has been revealed that AGEs directly induce ERS in human aortic endothelial cells, playing an important role in endothelial cell apoptosis (39). As AGE-triggered HUVEC injury simulates the cellular environment of DVT, it is suggested that ERS may be involved in the development of DVT. In the present study, an activation of ERS was found following M-HSA stimulation, as determined by the upregulation of the protein expression levels of CHOP, GRP78, p-PERK and p-IRE1α. Simultaneously, PPARγ greatly suppressed the activation of ERS, which was consistent with previous studies exploring the regulation of PPARγ on ERS (25,40). Nevertheless, whether ERS is the cause or the effect of the regulation of PPARγ during the development of DVT remains unknown; therefore, the present study addressed this question. Surprisingly, when TM was employed to promote ERS, the protective function of PPARγ against inflammation, oxidative stress and apoptosis in M-HSA-stimulated HUVECs was weakened, demonstrating that ERS is essential for contributing to HUVEC injury. In addition, PPARγ may exert its protective role by inhibiting ERS.

However, the present study contains certain limitations. First, only the regulatory role of PPARγ in M-HSA-stimulated HUVECs was investigated, which was an *in vitro* cell model of DVT. *In vivo* or clinical studies are required to verify the findings of the present study. In addition, more in-depth and comprehensive research is required to elucidate the molecular mechanism of DVT, so as to discover novel therapeutic strategies for the clinical treatment of this disease.

In summary, the present study demonstrated that PPARγ antagonized M-HSA-induced HUVEC injuries by inhibiting cell apoptosis and balancing thrombosis-related factors, inflammatory cytokines and oxidative stress-related factors via suppressing the activation of ERS. Therefore, these findings highlight the protective role of PPARγ during the development of DVT by alleviating endothelial injury and imply a promising strategy for the treatment of DVT.

Acknowledgements

Not applicable.

Funding

The present study was supported by Beijing Jishuitan Hospital Nova Program (grant no. XKXX202110) and Guangdong Basic and Applied Basic Research Foundation (grant no. 2019A1515011262).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JLiu conceived and designed the study. YZ, YG, LT, ML, WJ, XT, PJ, ZC and JLi performed the experiments and collected the data. YZ, YG, LT and ML analyzed and interpreted the data. YZ and JLiu wrote and revised the manuscript. YZ and JLiu confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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