Am J Transl Res 2026;18(3):2214-2224
www.ajtr.org /ISSN:1943-8141/AJTR0170803

Original Article

Prolactin-induced platelet activation and endothelial dysfunction in coronary artery disease: insights into PKC and TXA2 pathways

Jingren Li[1,2*], Chong Wang[1*], Shuping Li[1], Yan Wang[2], Lijing Zhang[3], Jian Zhang[1]

[1]Department of Cardiology, Beijing Chest Hospital, Capital Medical University, Beijing 101149, China; [2]Department of Cardiology, Beijing Tuberculosis and Thoracic Tumor Institute, Beijing 101149, China; [3]Department of Cardiology, Dongzhimen Hospital Beijing University of Chinese Medicine, Beijing 100700, China. [*]Equal contributors and co-first authors.

Received December 17, 2025; Accepted February 24, 2026; Epub March 15, 2026; Published March 30, 2026

Abstract: Background: Elevated prolactin level is associated with an increased risk of coronary artery disease (CAD), probably through promoting vascular inflammation and thrombosis. This study investigated whether prolactin exacerbates atherothrombosis by regulating endothelial dysfunction and platelet activation and further explored the underlying mechanisms. Methods: A co-culture model of human umbilical vein endothelial cells (HUVECs) and platelets was employed to simulate the vascular interface. The effects of prolactin, alone or in combination with a protein kinase C (PKC) inhibitor or aspirin (a thromboxane A2 [TXA2] pathway inhibitor) were assessed. Endothelial activation was assessed by measuring proliferation, the expression of adhesion molecules vascular cell adhesion molecule 1 (VCAM-1) and intercellular cell adhesion molecule 1 (ICAM-1), and the production of inflammatory cytokines interleukin (IL)-6 and IL-1β in HUVECs. Platelet function was analyzed by measuring CD61 expression, surface levels of P-selectin and CD40L, and the release of platelet microparticles (PMPs). Results: Prolactin significantly enhanced endothelial proliferation and the expression of adhesion molecules and inflammatory cytokines. Concurrently, it enhanced platelet aggregation and increased the surface expression of activation markers (P-selectin, CD40L) and pro-thrombotic PMPs. These effects were mediated through the PKC pathway, as they were markedly reversed by PKC inhibition. Prolactin partially restored endothelial and platelet activation even in the presence of aspirin, indicating an additional role for the TXA2 pathway. Conclusion: Prolactin coordinately exacerbates endothelial dysfunction and platelet activation through PKC and TXA2 pathway activation. These findings identify a dual-pathway mechanism by which prolactin may promote a pro-thrombotic state, contributing to the pathogenesis of atherothrombosis in CAD.

Keywords: Prolactin, endothelial dysfunction, platelet activation, coronary artery disease TXA2, PKC

Introduction

Coronary artery disease (CAD) remains a leading cause of mortality worldwide [1]. Epidemiologic data indicates that the disease is prevalent in both rural and urban areas, with its incidence demonstrating a progressive annual increase [2]. Atherosclerosis constitutes the fundamental pathologic basis of CAD [3]. During atherosclerosis, vascular endothelial dysfunction and abnormal platelet activation represent two central and interconnected processes that mutually reinforce each other [4]. Dysfunctional endothelial cells adopt a pro-inflammatory and pro-thrombotic phenotype, which not only facilitates the recruitment and adhesion of circulating monocytes but also enhances their interaction with platelets [5]. Subsequently, the activated platelets release inflammatory mediators that further exacerbate endothelial injury, forming a vicious cycle [6]. This cascade promotes atherosclerotic plaque progression and instability, thereby increasing the risk of thrombotic events. Therefore, elucidating the molecular mechanisms underlying this endothelial–platelet crosstalk is of substantial clinical importance.

Beyond traditional metabolic risk factors such as hypertension and dyslipidemia [7], emerging

https://doi.org/10.62347/PIMY8425

evidence suggests that neuroendocrine hormones may play significant roles in CAD [8]. Prolactin is a pleiotropic pituitary hormone primarily involved in lactation [9]. Studies indicate that prolactin is associated with endothelial dysfunction mediated by vascular inflammation [10, 11]. However, its vascular effects of prolactin remain complex and sometimes controversial. On one hand, prolactin has been reported to induce the expression of inflammatory cytokines and adhesion molecules in endothelial cells, promoting leukocyte infiltration and contributing to early atherogenesis [12]. On the other hand, prolactin has been implicated in modulating platelet activation and enhancing platelet adhesion and aggregation [13, 14]. Nevertheless, the precise mechanisms and their connection with endothelial dysfunction remain unclear. These seemingly contradictory effects suggest that prolactin may regulate the functions of both endothelial cells and platelets through distinct downstream signaling pathways, thereby synergistically promoting atherothrombosis.

At the signaling level, protein kinase C (PKC) and thromboxane A2 (TXA2) are two critical mediators of vascular inflammation, vasomotor regulation, and platelet activation [15, 16]. The PKC-δ isoform has been implicated in regulating inflammatory responses and endothelial function in atherosclerosis [17]. Aberrant PKC activation can cause vascular dysfunction, leading to the development of CAD [18]. Furthermore, TXA2 promotes vasoconstriction and platelet aggregation by activation of the thromboxane prostanoid receptor, representing a classic target of antiplatelet agents such as aspirin [19]. Although previous studies have preliminary associated prolactin with endothelial inflammation and platelet activation, direct evidence demonstrating whether and how prolactin coordinately exacerbates endothelial-platelet interactions through the PKC and TXA2 signaling remains lacking. This constitutes a significant gap in current knowledge.

We therefore hypothesized that pathologically elevated levels of prolactin may concurrently activate the PKC and TXA2 signaling pathways in both endothelial cells and platelets. This dual activation induces a pro-inflammatory and pro-adhesive phenotypic shift in endothelial cells while simultaneously enhancing platelet activation and aggregation capacity. Collectively, these effects establish a pro-thrombotic microenvironment, possibly accelerating CAD progression. To test this hypothesis, a co-culture system of human umbilical vein endothelial cells (HUVECs) and platelets was employed to model the vascular endothelial-platelet interface. By exogenously adding prolactin, combined with a specific PKC inhibitor or a TXA2 pathway inhibitor (aspirin), this study systematically evaluated the effects of prolactin on endothelial cell viability, activation status, inflammatory cytokine secretion, as well as on platelet aggregation, the expression of activation markers, and platelet microparticle release. This study aimed to elucidate the specific mechanisms, particularly those involving PKC and TXA2, by which prolactin coordinately drives endothelial dysfunction and platelet hyperactivity. Our findings are expected to provide the direct experimental evidence that prolactin functions as a dual-targeting modulator of the endothelial-platelet axis, thereby revealing a novel integrative mechanism of atherothrombosis in CAD.

Materials and methods

Cell culture and treatment

HUVECs was purchased from Xiamen Yimo Biotechnology Co., Ltd. Cells were cultured in IM-H205-1 medium supplemented with L-glutamine and 10% fetal bovine serum (FBS) in T75 flasks, and maintained at 37°C in a humidified incubator with 5% CO_2 (CI-150C, Jet BIOFIL, China).

Human platelets were isolated from peripheral venous blood obtained from healthy adult volunteers according to the established method [20]. All donors provided written informed consent prior to participation, and the study protocol was approved by the Ethics Committee of Beijing Chest Hospital, Capital Medical University.

A co-culture system of HUVECs and platelets was constructed to simulate the vascular endothelial-platelet interface. To investigate the effects of prolactin on the PKC pathway, the co-culture system was assigned to the following experimental groups: PV (co-culture of platelets and HUVECs), PVA (co-culture system treated with 10 μM adenosine diphosphate, ADP), PVAP (co-culture system treated with 10 μM ADP and 100 mU/mL prolactin), PVAPP (co-culture system treated with 10 μM ADP, 100 mU/mL prolactin, and 1 μM PKC inhibitor), and PVAH (co-

culture system treated with 10 μM ADP and 5000 mU/mL prolactin). To investigate involvement of the TXA2 pathway, the following treatment groups were established: PTVα (co-culture system treated with 10 μM TXA2 agonist and 10 nM adrenaline α2A agonist), PTVαA (co-culture system treated with 10 μM TXA2 agonist, 10 nM adrenaline α2A agonist, and 100 μM aspirin), and PTVαAP (co-culture system treated with 10 μM TXA2 agonist, 10 nM adrenaline α2A agonist, 100 μM aspirin, and 100 mU/mL prolactin).

CCK-8 assay

HUVECs were seeded into 96-well plates (1×10^3 cells/well) and allowed to adhere overnight. Following the application of the designated treatments, CCK-8 reagent (Dojindo, Japan) was added to each well and incubated under standard culture conditions for 2 hours. Subsequently, the absorbance was measured at 450 nm using a microplate reader (Bio-Rad, USA).

Flow cytometry

Platelet aggregation and platelet-derived particles were analyzed using flow cytometry with a commercial reagent kit according to the manufacturer's instructions. Briefly, after treatment, platelet suspensions were collected, washed with PBS, and centrifuged. The samples were then resuspended in loading buffer and analyzed by flow cytometry. For size calibration, standard microspheres were used according to the manufacturer's protocol.

Immunofluorescence

The fluorescence intensities of CD62P (P-selectin) and CD40L were measured using a fluorescence microscope (BZ-H4XD, Keyence, Japan). For immunofluorescence staining, cells were fixed with 4% paraformaldehyde for 15 min at room temperature. After washing with PBS to remove residual fixative, cells were permeabilized with 0.1% Triton X-100 in PBS for 10 min. Following another wash, cells were blocked with 5% normal goat serum for 1 h at room temperature and incubated overnight at 4°C with primary antibodies overnight. After thorough washing, cells were incubated with fluorescence-conjugated secondary antibodies (1:200 dilution) for 1 h in the dark. Nuclei were counterstained with DAPI (1 μg/mL) for 5 min. Following a final wash, images were acquired using a fluorescence microscope (BZ-H4XD, Keyence, Japan), and the fluorescence intensity of P-selectin and CD40L was analyzed using Image J software.

Enzyme-linked immunosorbent assay (ELISA)

Following designated treatments, the levels of interleukin-6 (IL-6) and IL-1β in HUVEC culture supernatants were measured using commercial human IL-6 (CB10373-Hu) and human IL-1β (CB10347-Hu) ELISA kits (Shanghai K & E Biotech, China), according to the manufacturers' instructions.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from cells using a commercial RNA isolation kit. 1 μg of total RNA was reverse-transcribed into complementary DNA (cDNA) using PrimeScript™ RT Master Mix (Takara, Dalian, China). Quantitative PCR was performed using Power SYBR Green PCR Master Mix (Takara, Dalian, China) on a QuantStudio system. Primer sequences for amplification were as follows: VCAM-1 (Forward 5'-TCT GTG AAT CCA TCC ACA AAG C-3', Reverse 5'-CAT GTC AAC ATG ACT GAG TCT C-3'); ICAM-1 (Forward 5'-GCT TAT ACA CAA GAA CCA GAC C-3', Reverse 5'-TCG AGT GAC AGT CAC TGA TTC-3'); GAPDH (Forward 5'-GGA AAT CCC ATC ACC ATC TTC-3', Reverse 5'-AGG TTT TTC TAG ACG GCA GG-3'). Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method, with GAPDH serving as the endogenous reference gene.

Western blot analysis

Total cellular protein was extracted using RIPA lysis buffer. Equal amounts of protein (30 μg per lane) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred onto a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked with 5% skim milk for 1 h at room temperature. After blocking, the membrane was incubated with primary antibodies overnight at 4°C and then secondary antibody for 1 h. After further washing, protein bands were visualized using an enhanced chemiluminescence (ECL) substrate and imaged with a chemiluminescence detection system. Band intensities were quantified using ImageJ software.

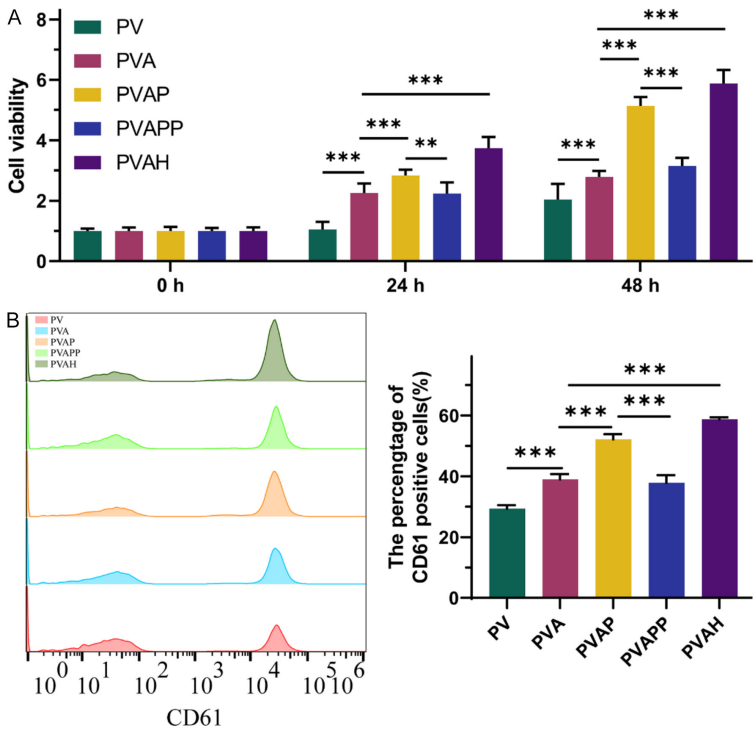

Figure 1. Effects of prolactin and PKC inhibition on endothelial proliferation and platelet aggregation via the protein kinase C (PKC) pathway. The co-culture system of human umbilical vein endothelial cells (HUVECs) and platelets was constructed and subjected to the specified treatments: PV (co-culture of platelets and HUVECs), PVA (PV + 10 μM adenosine diphosphate, ADP), PVAP (PVA + 100 mU/mL prolactin), PVAPP (PVAP + 1 μM PKC inhibitor), and PVAH (PVA + 5000 mU/mL prolactin). A. The proliferation of HUVECs was assessed by CCK-8 at 0 h, 24 h, and 48 h. B. The platelet aggregation marker CD61 was analyzed using flow cytometry and the results were quantified. Data are presented as mean ± standard deviation (SD) (N = 3). $^{**}P < 0.01$, $^{***}P < 0.001$.

Statistical analysis

Statistical analyses were conducted using GraphPad Prism 9 software. Data from three independent experiments were presented as mean ± standard deviation (SD). For comparisons among multiple groups, one-way analysis of variance (ANOVA) was applied, followed by Tukey's post-hoc test. A two-sided P value < 0.05 was considered significant. The specific levels of significance are indicated in the figures as $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

Results

Prolactin promoted endothelial proliferation and platelet aggregation through the PKC pathway

To investigate the role of prolactin in endothelial-platelet interactions, we first examined its effects on endothelial proliferation and platelet aggregation in a HUVEC-platelet co-culture system with modulation of PKC signaling. As shown in **Figure 1A**, stimulation of platelet aggregation agonist ADP (PVA group) significantly promoted cell proliferation compared to the basal control (PV group). The addition of prolactin (PVAP group) further potentiated this effect. Importantly, this prolactin-induced enhancement was effectively reversed by a specific PKC inhibitor (PVAPP group), suggesting PKC-dependent signaling. Moreover, a high concentration of prolactin (PVAH group) elicited a more pronounced increase in cell proliferation than that observed in the PAVP group.

Flow cytometry analysis of platelet aggregation marker, integrin CD61, showed that prolactin significantly augmented ADP-induced platelet aggregation (**Figure 1B**). This pro-aggregatory effect of prolactin was also abolished by PKC inhibition. Consistently, higher prolactin concentrations further increased CD61 expression, indicating enhanced platelet aggregation. Collectively, these findings demonstrate that prolactin simultaneously promotes endothelial activation and platelet aggregation in a PKC-dependent manner.

These results support the existence of a positive feedback loop in which prolactin-driven endothelial proliferation facilitates platelet recruitment and activation, while activated platelets release mediators that further exacerbate endothelial proliferation and dysfunction.

Prolactin upregulated platelet activation markers CD40L and P-selectin through the PKC pathway

The expression of key platelet activation markers in the co-culture system was further assessed using immunofluorescence. CD40 ligand (CD40L) is a potent pro-inflammatory and im-

mune-modulatory molecule expressed on activated platelet. P-selectin is a critical adhesion molecule that facilitates platelet adhesion to endothelial cells and leukocytes, driving inflammation and thrombosis. Immunofluorescence signals for both CD40L and P-selectin exhibited a characteristic punctate, aggregate-like distributions, identifying their primary cellular source as the activated platelet clusters adhering to the HUVEC monolayer (**Figure 2**). Quantitative analysis revealed that ADP stimulation elevated the levels of both markers compared to the baseline control. Prolactin treatment significantly potentiated this increase in platelet CD40L (**Figure 2A**) and P-selectin (**Figure 2B**). The upregulation of both markers by prolactin was effectively abolished by PKC inhibition. Furthermore, the induction exhibited a clear dose-dependent relationship, with higher prolactin concentrations inducing higher levels of CD40L and P-selectin on platelets.

Prolactin induced pro-adhesive endothelial phenotype and promoted platelet microparticle release through the PKC pathway

A hallmark of endothelial dysfunction is the increased expression of adhesion molecules, which are essential for the adhesion and transmigration of monocytes into the vascular wall, a critical event in atherothrombosis. RT-qPCR analysis showed that ADP treatment significantly increased the mRNA levels of vascular cell adhesion molecule 1 (VCAM-1) and intercellular cell adhesion molecule 1 (ICAM-1) in HUVECs. Prolactin treatment significantly enhanced this effect (**Figure 3A**), whereas PKC inhibition markedly reversed this prolactin-induced effect. A dose-dependent relationship was observed, with higher concentrations of prolactin inducing greater expression of both adhesion molecules.

Furthermore, the activated platelets release platelet microparticles (PMPs), subcellular vesicles that are pro-thrombotic and pro-inflammatory, contributing to disease progression. Prolactin treatment significantly increased ADP-induced PMP release, and this effect was abolished by PKC inhibition (**Figure 3B**). These results indicate that prolactin promotes a pro-adhesive endothelial phenotype and enhances the release of bioactive platelet-derived particles through PKC-dependent signaling.

Prolactin upregulated inflammatory cytokine production and PKC upstream signaling molecule PLCβ in HUVECs

The effects of prolactin and PKC inhibition on the levels of pro-atherogenic inflammatory cytokine and PKC upstream signaling molecule PLCβ were next explored in the HUVEC-platelet co-culture system. As shown in **Figure 4A**, ADP treatment significantly increased the production of IL-6 and IL-1β. Prolactin treatment further enhanced their production in a dose-dependent manner, while PKC inhibition reversed the effects of prolactin. Furthermore, PKC upstream signaling molecule phospholipase C beta (PLCβ), an enzyme that generates second messengers for PKC activation, were then analyzed. Prolactin treatment significantly increased PLCβ expression, which was reversed by PKC inhibition (**Figure 4B**), suggesting prolactin may enhance a positive feedback loop sustaining PKC pathway activity.

Prolactin partially restored endothelial proliferation and platelet aggregation through the TXA2 pathway under aspirin treatment

To investigate whether prolactin modulates platelet-endothelial interactions through the TXA2 pathway, the HUVEC-platelet co-culture system was treated with TXA2 agonist and aspirin (a TAX2 inhibitor) in the absence or presence of prolactin. As expected, aspirin treatment (PTVαA group) significantly reduced both HUVEC proliferation and platelet integrin CD61 expression compared with the TXA2-activated control (PTVα group). However, the addition of prolactin (PTVαAP group) partially but significantly reversed the suppressive effects of aspirin on both endothelial proliferation and platelet CD61 expression (**Figure 5A**, **5B**).

Prolactin restored platelet P-Selectin expression through the TXA2 pathway under aspirin treatment

Whether prolactin affects platelet activation through the TXA2 pathway was further investigated. Consistent with its known antiplatelet effects, aspirin effectively reduced the expression of platelet adhesion molecule P-selectin (**Figure 6**). Importantly, treatment with prolactin significantly increased P-selectin levels even in the presence of aspirin.

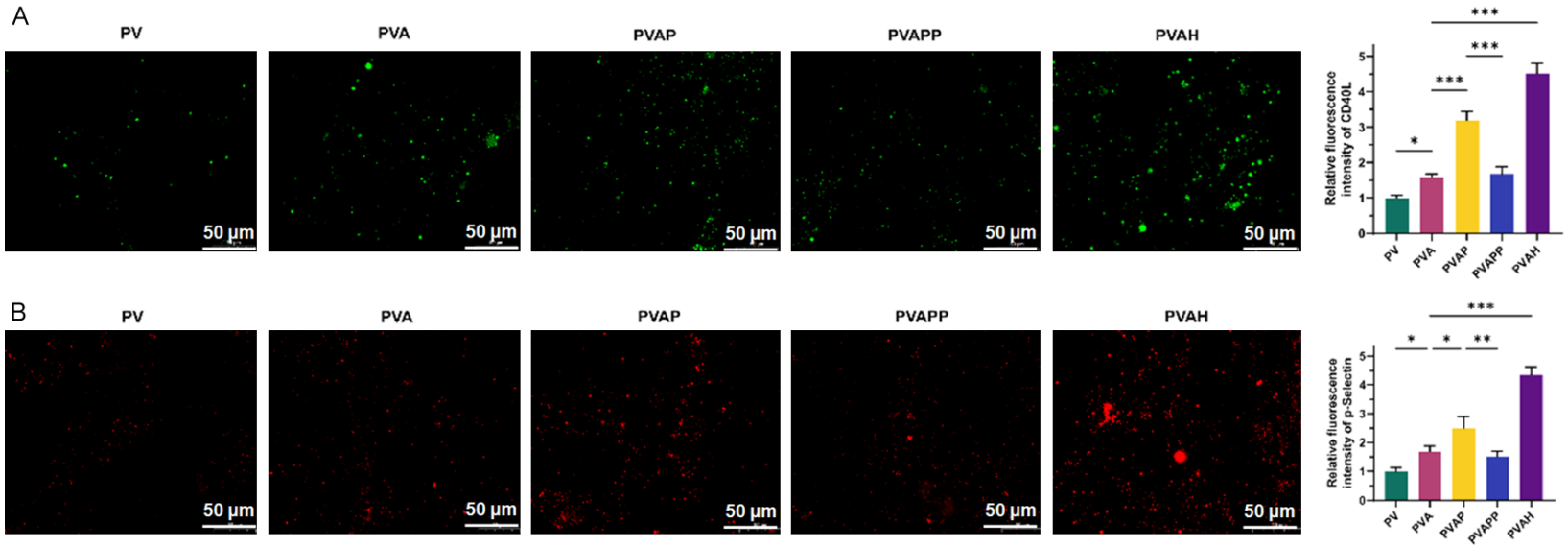

Figure 2. Effects of prolactin and PKC inhibition on the expression of platelet activation marker CD40 Ligand (CD40L) and P-selectin by the PKC pathway. The co-culture system of HUVECs and platelets was constructed and subjected to the specified treatments: PV (co-culture of platelets and HUVECs), PVA (PV + 10 μM ADP), PVAP (PVA + 100 mU/mL prolactin), PVAPP (PVAP + 1 μM PKC inhibitor), and PVAH (PVA + 5000 mU/mL prolactin). The expression of (A) CD40L and (B) P-selectin on platelets was assessed by immunofluorescence assay. Scale bar = 50 μm. Data are presented as mean ± SD (N = 3). **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

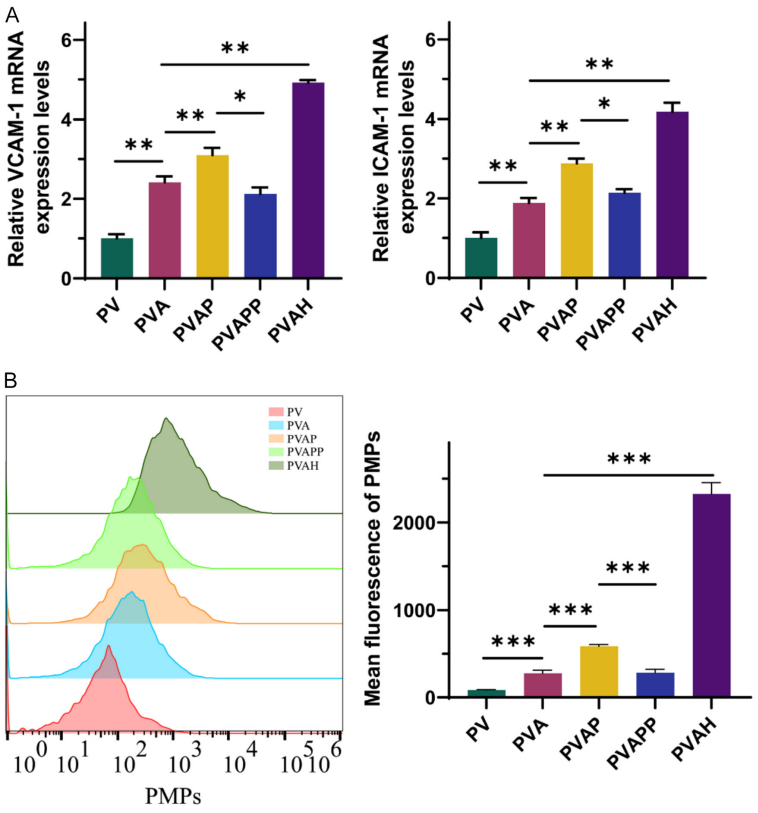

Figure 3. Effects of prolactin and PKC inhibition on the expression of vascular cell adhesion molecule 1 (VCAM-1), intercellular cell adhesion molecule 1 (ICAM-1), and platelet microparticles (PMPs). The co-culture system of HUVECs and platelets was constructed and subjected to the specified treatments: PV (co-culture of platelets and HUVECs), PVA (PV + 10 μM ADP), PVAP (PVA + 100 mU/mL prolactin), PVAPP (PVAP + 1 μM PKC inhibitor), and PVAH (PVA + 5000 mU/mL prolactin). A. The mRNA levels of VCAM-1 and ICAM-1 in HUVECs were quantified by RT-qPCR. B. The expression of PMPs were analyzed by flow cytometry and mean fluorescence intensity was quantified. Data are presented as mean ± SD (N = 3). **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Discussion

This study elucidates a novel and coordinated mechanism by which prolactin exacerbates endothelial-platelet interactions, a process pivotal to atherothrombosis [21, 22]. Our findings indicate that prolactin concurrently activates both the PKC and TXA2 pathways, thereby promoting a synergistic pro-thrombotic response. This dual-pathway activation simultaneously induces a pro-inflammatory and pro-adhesive phenotype in endothelial cells while heightening platelet aggregation and the release of bioactive microparticles, ultimately contributing to thrombus formation *in vivo* [23]. Mechanistically, prolactin does not merely stimulate endothelial proliferation but drives a dysfunctional phenotype characterized by increased expression of adhesion molecules and inflammatory mediators. Concurrently, prolactin lowers the activation threshold of platelets and enhances their responsiveness, creating a feed-forward loop that amplifies vascular inflammation and thrombotic potential.

Prolactin-driven enhancement of endothelial proliferation and platelet aggregation by the PKC pathway establishes a self-perpetuating cycle central to atherosclerotic progression. During atherosclerosis, endothelial proliferation is associated with an activated and dysfunctional state, leading to upregulated adhesion molecules like VCAM-1 and ICAM-1, which transforms the endothelial lining into a receptive interface for monocyte and platelet adhesion [23, 24]. Recruited monocytes subsequently migrate into the subendothelial space, driving plaque formation. Simultaneously, prolactin-enhanced platelet activation promotes the release inflammatory factors and PMPs, which in turn aggravate endothelial dysfunction and inflammation, thereby accelerating plaque progression and destabilization [25]. These PMPs are bioactive vesicles rich in pro-inflammatory and pro-coagulant molecules that can directly activate endothelial cells, recruit leukocytes, and provide a catalytic surface for thrombin generation. Taken together, prolactin-activated endothelium activation promotes platelet recruitment, while activated platelets release factors that further aggravate endothelial dysfunction. This vicious cycle accelerates plaque progression, inflammation, and ultimately leads to plaque instability and rupture.

The central role of the PKC pathway is further underscored by its mediation of these key adhesive and inflammatory events. Our results confirm that prolactin mediates the PKC pathway, leading to increased proliferation of

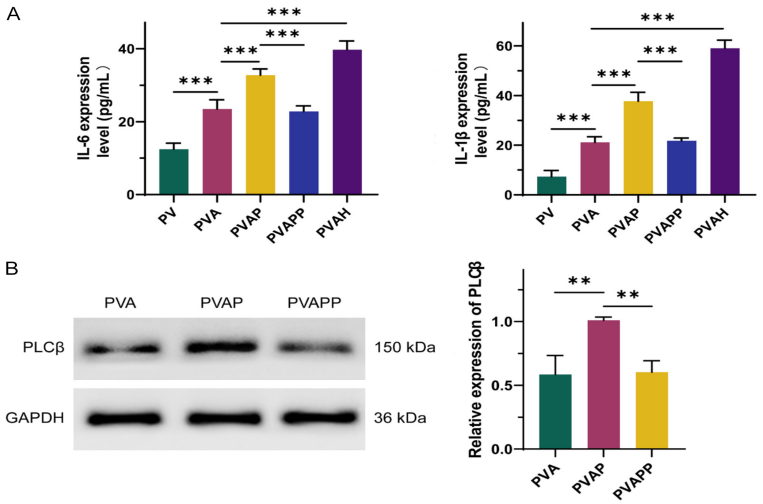

Figure 4. Prolactin upregulated inflammatory cytokine production and PKC upstream signaling molecule phospholipase C beta (PLCβ) in HUVECs. The co-culture system of HUVECs and platelets was constructed and subjected to the specified treatments: PV (co-culture of platelets and HUVECs), PVA (PV + 10 μM ADP), PVAP (PVA + 100 mU/mL prolactin), PVAPP (PVAP + 1 μM PKC inhibitor), and PVAH (PVA + 5000 mU/mL prolactin). A. The levels of IL-6 and IL-1β were confirmed using enzyme-linked immunosorbent assay (ELISA) kits in the culture supernatants of HUVECs. B. Western blot analysis of PLCβ expression in the treated HUVECs. Data are presented as mean ± SD (N = 3). $^{**}P < 0.01$, $^{***}P < 0.001$.

HUVECs and platelet activation, an effect that is dose-dependent and reversible upon PKC inhibition. As a key regulatory factor, PKC critically modulates vascular homeostasis by regulating both platelets and endothelial cells, affecting processes including cell activation and adhesion [26-28]. Some key adhesion molecules play pivotal roles in endothelial-platelet crosstalk. Among these, CD40L and P-selectin are key mediators of thromboinflammatory interactions [29, 30]. The central role of the PKC pathway is further underscored by its mediation of key adhesive events. Prolactin upregulates CD40L and P-selectin on platelets through the PKC pathway. CD40L is a potent pro-inflammatory and immune-modulatory molecule expressed on endothelial cells and activated platelet [31]. P-selectin, upon exposure on activated platelet surface facilitates leukocyte tethering and adhesion to the vessel wall, effectively promoting leukocyte recruitment into developing plaques [32]. This prolactin-mediated coordinated upregulation provides a direct mechanistic explanation for the significantly enhanced endothelial-platelet interaction observed following prolactin stimulation. Furthermore, PKC-dependent increase in inflammatory cytokines (IL-6, IL-1β) and pro-thrombotic PMPs further solidifies therole of prolactin in creating a localized pro-atherogenic and pro-inflammatory microenvironment [24, 25].

Our data further reveal a clinically relevant role of the TXA2 pathway, which influences vascular function, platelet activation, and inflammatory processes [33]. This pathway can regulate vascular constriction and platelet aggregation, triggering various cardiovascular and cerebrovascular diseases [34]. Aspirin can significantly reduce platelet activation levels, which is frequently used in antiplatelet therapy in clinic [35]. Our results indicate that prolactin can partially counteract the effects of aspirin and restore platelet activation levels. Prolactin restored endothelial cell proliferation and the expression of platelet aggregation marker CD61L and platelet adhesion molecule P-selectin even in the presence of aspirin. These findings suggest that prolactin activates complementary signaling routes, potentially through the robust amplification of the PKC pathway.

Despite these findings, several limitations should be acknowledged. First, although the *in vitro* co-culture model is instrumental for mechanistic dissection, it cannot replicate the full systemic, hemodynamic, and neuroendocrine complexity of *in vivo* disease. Critical factors such as shear stress, circulating immune cells, and hormonal feedback regulation are absent. Future studies utilizing animal models of hyperprolactinemia (e.g., in ApoE$^{-/-}$ mice) are necessary to validate these pathophysiological interactions in a living system. Second, while PKC and TXA2 signaling were identified as key pathways, the precise upstream receptors and downstream molecular effectors remain incompletely defined. Investigations into which specific prolactin receptor isoforms mediate these effects, and the identification of specific PKC

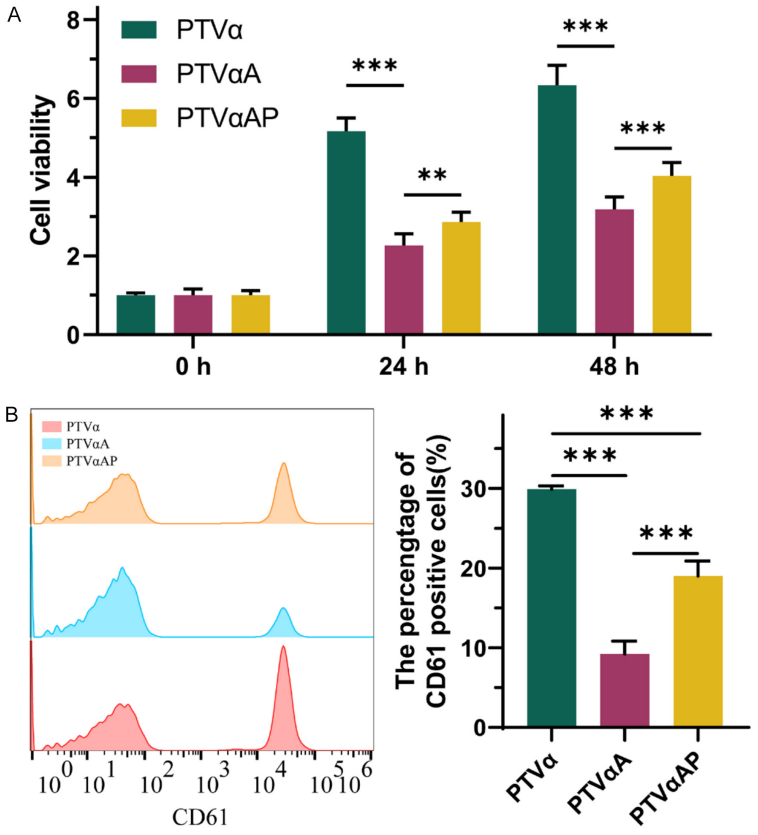

Figure 5. Prolactin modulated HUVEC viability and platelet function through the TXA2 pathway under aspirin treatment. The co-culture system of HUVECs and platelets was constructed and subjected to the specified treatments: PTVα (co-culture of platelets and HUVECs + 10 μM TXA2 agonist + 10 nM adrenaline α2A agonist), PTVαA (PTVα + 100 μM aspirin), and PTVαAP (PTVαA + 100 mU/mL prolactin). A. The proliferation of HUVECs was assessed by CCK-8 at 0 h, 24 h, and 48 h. B. The platelet aggregation marker CD61 was analyzed using flow cytometry and the results were quantified. Data were presented as mean ± SD (N = 3). $^{**}P < 0.01$, $^{***}P < 0.001$.

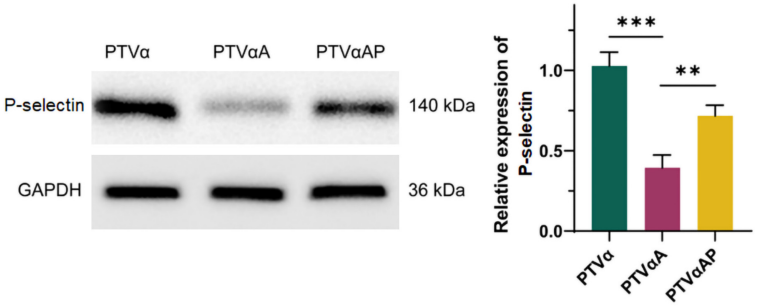

Figure 6. Prolactin partially reversed aspirin-induced suppression of platelet P-Selectin expression via the TXA2 pathway. The co-culture system of HUVECs and platelets was constructed and subjected to the specified treatments: PTVα (co-culture of platelets and HUVECs + 10 μM TXA2 agonist + 10 nM adrenaline α2A agonist), PTVαA (PTVα + 100 μM aspirin), and PTVαAP (PTVαA + 100 mU/mL prolactin). The protein expression of P-selectin was examined using western blot analysis. Data are presented as mean ± SD (N = 3). $^{**}P < 0.01$, $^{***}P < 0.001$.

isoforms would substantially refine our understanding of this signaling network. Finally, the clinical significance of prolactin in CAD progression needs to be assessed through well-designed longitudinal cohort studies. Measuring prolactin levels alongside markers of endothelial function, platelet activity, and plaque imaging in patients with CAD could clarify its use as a prognostic biomarker or a therapeutic target.

Conclusion

Prolactin promotes a pro-thrombotic state by concurrently activating both the PKC and TXA2 pathways. Specifically, prolactin promotes a pro-inflammatory and adhesive phenotype in endothelial cells and enhances platelet activation and aggregation. These coordinated effects may contribute to the dysregulation of vascular homeostasis and elevate atherothrombotic risk, thereby contributing to the pathogenesis of CAD and possibly influencing its clinical management. Our work offers mechanistic insight into the interplay between prolactin and cardiovascular pathology, establishing a basis for future investigations to explore whether targeting this axis has therapeutic relevance in specific patient populations.

Acknowledgements

This study was supported by National Key Research and Development Program Project (No. 2022YFC3500101).

Disclosure of conflict of interest

None.

Address correspondence to: Lijing Zhang, Department of Cardiology, Dongzhimen Hospital Beijing University of Chinese Medicine, No. 5, Haiyun Cang, Dongcheng District, Beijing 100700, China. E-mail: dzmyyccu@163.com; Jian Zhang, Department of Cardiology, Beijing Chest Hospital, Capital Medical University, Zone 1, Courtyard 9, Beiguan Street, Tongzhou District, Beijing 101149, China. E-mail: 15178856085@163.com

References

[1] Wang Y, He L, Du D, Cheng Z and Qin C. A metabolomics-based study on NMDAR-mediated mitochondrial damage through calcium overload and ROS accumulation in myocardial infarction. Front Biosci (Landmark Ed) 2023; 28: 140.

[2] Komilovich EBz. Coronary artery disease. Eur J Mod Med Pract 2023; 3: 81-87.

[3] Health WCotARoC and China Di. Interpretation of the annual report on cardiovascular health and diseases in China 2022. Cardiol Discov 2024; 4: 58-80.

[4] Zhang J. Biomarkers of endothelial activation and dysfunction in cardiovascular diseases. Rev Cardiovasc Med 2022; 23: 73.

[5] Theofilis P, Sagris M, Oikonomou E, Antonopoulos AS, Siasos G, Tsioufis C and Tousoulis D. Inflammatory mechanisms contributing to endothelial dysfunction. Biomedicines 2021; 9: 781.

[6] van der Poll T and Parker RI. Platelet activation and endothelial cell dysfunction. Crit Care Clin 2020; 36: 233-253.

[7] Isath A, Koziol KJ, Martinez MW, Garber CE, Martinez MN, Emery MS, Baggish AL, Naidu SS, Lavie CJ, Arena R and Krittanawong C. Exercise and cardiovascular health: a state-of-the-art review. Prog Cardiovasc Dis 2023; 79: 44-52.

[8] Glezer A, Santana MR, Bronstein MD, Donato J Jr and Jallad RS. The interplay between prolactin and cardiovascular disease. Front. Endocrinol. 2023; 13: 1018090.

[9] Jacob B and Pita MRT, Prolactin, in Encyclopedia of Sexual Psychology and Behavior. 2023, Springer. p. 1-4.

[10] Zhao H, Gong S, Shi Y, Luo C, Qiu H, He J, Sun Y, Huang Y, Wang S and Miao Y. The role of prolactin/vasoinhibins in cardiovascular diseases. Anim Models Exp Med 2023; 6: 81-91.

[11] Chasseloup F, Bernard V and Chanson P. Prolactin: structure, receptors, and functions. Rev Endocr Metab Disord 2024; 25: 953-966.

[12] Baba MS, Laway BA, Misgar RA, Wani AI, Bashir MI, Bhat IA, Haq MG and Shah ZA. Metabolic abnormalities, inflammatory markers and endothelial dysfunction in hyperprolactinemia due to prolactinoma before and after normalization of serum prolactin: a prospective case control study. Indian J Endocrinol Metab 2023; 27: 357-364.

[13] García-Rodrigo JF, Ortiz G, Martínez-Díaz OF, Furuzawa-Carballeda J, Ruíz-Herrera X, Macias F, Ledesma-Colunga MG, Martínez de la Escalera G and Clapp C. Prolactin inhibits or stimulates the inflammatory response of joint tissues in a cytokine-dependent manner. Endocrinology 2023; 164: bqad156.

[14] Urban A, Masopust J, Maly R, Hosák L and Kalnická D. Prolactin as a factor for increased platelet aggregation. Neuroendocrinol Lett 2007; 28: 518-523.

[15] Sabe SA, Zhao A, Kononov MA, Sabra M, Li J, Ehsan A, Feng J and Sellke FW. Increased coronary contraction to thromboxane A2 in cardiac surgery patients with poorly controlled hypertension. J Surg Res 2024; 294: 249-256.

[16] Beccacece L, Abondio P, Bini C, Pelotti S and Luiselli D. The link between prostanoids and cardiovascular diseases. Int J Mol Sci 2023; 24: 4193.

[17] Miao LN, Pan D, Shi J, Du JP, Chen PF, Gao J, Yu Y, Shi DZ and Guo M. Role and mechanism of PKC-δ for cardiovascular disease: current status and perspective. Front Cardiovasc Med 2022; 9: 816369.

[18] Jubaidi FF, Zainalabidin S, Taib IS, Abdul Hamid Z, Mohamad Anuar NN, Jalil J, Mohd Nor NA and Budin SB. The role of PKC-MAPK signalling pathways in the development of hyperglycemia-induced cardiovascular complications. Int J Mol Sci 2022; 23: 8582.

[19] Ozen G, Aljesri K, Abdelazeem H, Norel X, Turkyılmaz G, Turkyılmaz S and Topal G. Comparative study on the effect of aspirin, TP receptor antagonist and TxA2 synthase inhibitor on the vascular tone of human saphenous vein and internal mammary artery. Life Sci 2021; 286: 120073.

[20] Sánchez G, Estrada O, Acha G, Cardozo A, Peña F, Ruiz MC, Michelangeli F and Alvarado-Castillo C. The norpurpureine alkaloid from Annona purpurea inhibits human platelet activation in vitro. Cell Mol Biol Lett 2018; 23: 15.

[21] Grismaldo A, Sobrevia L and Morales L. Role of platelet-derived growth factor c on endothelial dysfunction in cardiovascular diseases. Biochim Biophys Acta Gen Subj 2022; 1866: 130188.

[22] Zhou Y, Zhu X, Cui H, Shi J, Yuan G, Shi S and Hu Y. The role of the VEGF family in coronary heart disease. Front cardiovasc med 2021; 8: 738325.

[23] Gao Y and Galis ZS. Exploring the role of endothelial cell resilience in cardiovascular health and disease. Arterioscler Thromb Vasc Biol 2021; 41: 179-185.

[24] Momi S and Gresele P. The role of platelets in atherosclerosis: a historical review. Semin Thromb Hemost 2025; 51: 894-907.
[25] Jing H, Wu X, Xiang M, Wang C, Novakovic VA and Shi J. Microparticle phosphatidylserine mediates coagulation: involvement in tumor progression and metastasis. Cancers 2023; 15: 1957.
[26] Babel RA and Dandekar MP. A review on cellular and molecular mechanisms linked to the development of diabetes complications. Curr Diabetes Rev 2021; 17: 457-473.
[27] Henke P. Endothelial cell-mediated venous thrombosis. Blood 2022; 140: 1459-1460.
[28] Moriya J and Ferrara N. Inhibition of protein kinase C enhances angiogenesis induced by platelet-derived growth factor C in hyperglycemic endothelial cells. Cardiovasc diabetol 2015; 14: 1-10.
[29] Yadav R, Kumari K, Joshi R, Verma K, Paliwal S, Dwivedi J and Sharma S. P-selectin and E-selectin: key macromolecules in thrombus formation and resolution. Int J Biol Macromol 2025; 318: 145259.
[30] Tang T, Cheng X, Truong B, Sun L, Yang X and Wang H. Molecular basis and therapeutic implications of CD40/CD40L immune checkpoint. Pharmacol Ther 2021; 219: 107709.
[31] Lont S, Mohr F, Hecker M and Wagner A. Role of CD40 ligand-mediated endothelial cell-monocyte interaction at atherosclerosis predilection sites. Biochem Pharmacol 2022; 206: 115298.
[32] Obeagu E and Obeagu G. P-selectin and platelet activation in hiv: implications for antiviral therapy. Elite J Sci Res Rev 2024; 2: 17-41.
[33] Badimon L, Vilahur G, Rocca B and Patrono C. The key contribution of platelet and vascular arachidonic acid metabolism to the pathophysiology of atherothrombosis. Cardiovasc Res. 2021; 117: 2001-2015.
[34] Allen MF, Hutchinson JL, Keith M, Mallah S, Corey RA, Trory JS, Jing C, Fang H, Wei L and Bennett SH. Difluorinated thromboxane A2 reveals crosstalk between platelet activatory and inhibitory pathways by targeting both the TP and IP receptors. Br J Pharmacol 2024; 181: 3685-3699.
[35] Santos-Gallego CG and Badimon J. Overview of aspirin and platelet biology. Am J cardiol 2021; 144: S2-S9.