All-Trans Retinoic Acid Impairs Platelet Function and Thrombus Formation and Inhibits Protein Kinase CβI/δ Phosphorylation

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Abstract

All-trans retinoic acid (ATRA) is widely used for induction of complete remission in patients with acute promyelocytic leukemia (APL). ATRA also regulates protein kinase C (PKC) activity. Therapeutic use of ATRA reportedly interferes with hemostatic function in APL patients, including effects on coagulation or other vascular cells, although effects of ATRA on platelets remain unclear. This study aims to investigate the effect of therapeutic-relevant doses of ATRA on platelet function. Human platelets were preincubated with ATRA (0–20 μM) for 1 hour at 37°C, followed by analysis of aggregation, granule secretion, receptor expression by flow cytometry, platelet spreading, or clot retraction. Additionally, ATRA (10 mg/kg) was injected intraperitoneally into mice and tail bleeding time and arterial thrombus formation were evaluated. ATRA inhibited platelet aggregation and adenosine triphosphate release induced by collagen (5 μg/mL) or thrombin (0.05 U/mL) in a dose-dependent manner without affecting P-selectin expression or surface levels of glycoprotein (GP) Ibα, GPIIb, or αIIbβ3. ATRA-treated platelets demonstrated reduced spreading on immobilized fibrinogen or collagen and reduced thrombin-induced clot retraction together with reduced phosphorylation of Syk and PLCγ2. In addition, ATRA-treated mice displayed significantly impaired hemostasis and arterial thrombus formation in vivo. Further, in platelets stimulated with either collagen-related peptide or thrombin, ATRA selectively inhibited phosphorylation of PKCβI (Ser661) and PKCδ (Thr505), but not PKCα or PKCβII phosphorylation (Thr638/641). In conclusion, ATRA inhibits platelet function and thrombus formation, possibly involving direct or indirect inhibition of PKCβI/δ, indicating that ATRA might be beneficial for the treatment of thrombotic or cardiovascular diseases.
Introduction

Platelets play critical roles in thrombosis and hemostasis. At site of vascular injury, platelets attach to the subendothelial matrix through binding to exposed von Willebrand factor (vWF) or collagen via surface receptors glycoprotein (GP) Iba or GPVI. Engagement of platelet receptors triggers transduction of intraplatelet signaling, resulting in activation of integrin αIIbβ3, which binds fibrinogen and mediates platelet aggregation. This process is called αIIbβ3 "inside-out" signaling. Ligand binding to αIIbβ3 also initiates αIIbβ3 "outside-in" signaling and subsequent activation of c-Src, Syk, or PLC-γ2, which mediates platelet spreading, clot retraction, and thrombus formation to prevent blood loss. Therefore, abnormal platelet function is closely associated with bleeding or thrombosis under pathological conditions.

As the synthetic and natural forms of vitamin A, retinoids have been shown to regulate cell differentiation, growth, or apoptosis through binding to their cognate nuclear receptors, which include retinoic acid receptor (RAR) and retinoid X receptor (RXR) family members. RARs can bind to all-trans retinoic acid (ATRA) and 9-cis RA, whereas RXRs bind specifically to 9-cis RA. ATRA is an active metabolite of vitamin A under the family retinoid and has been widely used for induction of complete remission in patients with acute promyelo-cytic leukemia (APL) as the only well-established differentiation therapy. In APL, RARα on chromosome 17 is fused with promyelocytic leukemia (PML) protein on chromosome 15, leading to formation of a fusion protein PML-RARα, which exerts negative effect on RA signaling and blocks cell differentiation. Pharmacological concentrations of ATRA are capable to bind PML-RARα and overcome its inhibitory effects, resulting in promotion of terminal differentiation of leukemic promyelocytes. ATRA has been reported to have effects on the hemostatic system of APL patients as demonstrated by decrease or normalization of clotting and fibrinolytic variables as well as reduced proteolysis of the vWF. In addition, ATRA has also been reported to interfere with the hemostatic properties of different cells, such as promyelocytic blast cells, normal human endothelial cells, and monocytes. Furthermore, a previous study has demonstrated that rapid cytoskeletal events (platelet spreading) and actin-dependent morphological changes (extended cell body formation) were all significantly inhibited in ATRA-treated human platelets. However, whether ATRA affects platelet function remains unclear.

In the present study, through incubation of human platelets with different concentrations of ATRA, we aim to investigate the role of ATRA in platelet function.

Materials and Methods

Reagents

ATRA was purchased from Sigma-Aldrich (St. Louis, Missouri, United States) and dissolved in 0.5% v/v (final concentration) dimethyl sulfoxide. Collagen and thrombin (≥ 10 NIH units/vial) were from Chrono-log Corporation (Havertown, Pennsylvania, United States). Collagen-related peptide (CRP) was prepared as previously described. Fluorescein isothiocyanate (FITC)-conjugated mouse antihuman CD41a was from BD Biosciences (San Jose, California, United States). Phycoerythrin (PE)-conjugated antihuman/mouse CD62p (P-selectin) and antihuman GPVI purified antibody were purchased from eBioscience (San Diego, California, United States). FITC-conjugated anti-CD42b antibody was from Abcam (Cambridge, Massachusetts, United States). FITC-conjugated goat antimouse immunoglobulin G (IgG) was purchased from ZSGB-BIO (Beijing, China). β-actin antibody and antirabbit IgG (horseradish peroxidase-linked) antibody were purchased from Cell Signaling Technology (Danvers, Massachusetts, United States). Tirofiban was purchased from Grand Pharma (China) CO. LTD.

Animals

All experimental procedures involving animals were complied with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines and approved by the Ethic Committee of Xuzhou Medical University. C57BL/6 mice, aged 8 to 10 weeks and weighted 24 to 28 g, were purchased from SLAC Laboratory Animal Co., Ltd. (Shanghai, China). All mice were housed in specific pathogen-free grade environment with 12-hour light/dark cycle and free access to food and water.

Platelet Preparation

All experimental procedures involving collection of human and mouse blood were approved by the Ethic Committee of Xuzhou Medical University. Informed consent was obtained from all participants. Platelets were prepared from human and mouse blood as described previously. For human platelets, venous blood was collected into a tube anticoagulated with trisodium citrate, glucose, and citric acid (ACD) and centrifuged for 20 minutes at 120 × g at room temperature to obtain platelet-rich plasma (PRP). Platelet pellets were then collected by centrifugation of PRP at 1,350 × g for 15 minutes, followed by washing three times in citrate-glucose-saline (CGS) buffer and resuspended in Tyrode’s buffer. Mouse platelets were isolated from ACD anticoagulated blood, washed using CGS buffer, and resuspended in Tyrode’s buffer.

Treatment of Platelets with ATRA

Isolated human platelets were incubated with different concentrations of ATRA (0, 1, 10, and 20 μM) at 37°C for 1 hour followed by relevant analysis.

Platelet Aggregation and Adenosine Triphosphate Release

Platelet aggregation was performed in the presence of fibrinogen (0.5 mg/mL). After ATRA treatment, platelet aggregation in response to collagen (5 μg/mL) and thrombin (0.05 U/mL) was evaluated in a Lumi-Aggregometer Model 700 (Chrono-log Corporation) at 37°C with stirring (1,000 revolutions per minute). Platelet aggregation was quantified as the percentage of maximum platelet aggregation in the absence of drug. The release of adenosine triphosphate (ATP) was monitored in parallel with platelet aggregation after addition of luciferin/luciferase reagent (Chrono-log Corporation) to the platelet suspension according to the manufacturer’s
instructions. ATP release was quantified relative to the vehicle (0 μM ATRA) treatment.

**Platelet α-Granule Release**
Platelet α-granule release was assessed through measuring the surface expression of the α-granule GP, P-selectin by flow cytometry as described previously. After ATRA treatment, human platelets were stimulated with collagen (5 and 10 μg/mL), thrombin (0.05 and 0.1 U/mL), or CRP (2 μg/mL) in the presence of PE-conjugated anti-P-selectin antibody followed by analyzing platelet P-selectin expression by flow cytometry. P-selectin expression was defined as the percentage of platelets in platelet-specific gate with positive staining of anti-P-selectin antibody.

**Expression of Platelet Receptors**
After treatment with ATRA, FITC-conjugated anti-CD42b antibody (GPIIb), FITC-conjugated mouse antihuman CD41a antibody (αIIb), or antihuman GPVI antibody (detected by FITC-conjugated goat antimouse IgG) were added and incubated for 30 minutes at room temperature followed by flow cytometry analysis of the expression of platelet receptors as described previously.

**Platelet Spreading**
Human platelets were placed on glass coverslips which were precoated with fibrinogen (10 μg/mL) or collagen (10 μg/mL) (4°C overnight) at 37°C for 90 minutes followed by washing with phosphate-buffered saline. Then, platelets were fixed, permeabilized, stained with Alexa Fluor-546-labeled phalloidin, and observed under a fluorescence microscopy (Nikon-80i) using an ×100 oil objective. The surface coverage was quantified using Image J software.

**Clot Retraction**
Human platelets-mediated clot retraction was initiated by addition of thrombin (1 U/mL) in the presence of 2 mM Ca2+ and 0.5 mg/mL fibrinogen at 37°C as described previously. Images were captured every 30 minutes.

**Tail Bleeding Assay**
Mice received intraperitoneal injection of ATRA (10 mg/kg). After 30 minutes, tail bleeding time was measured as described previously.

**FeCl3-Induced Arterial Thrombosis**
After treated with ATRA (20 μM) or vehicle at 37°C for 30 minutes, mouse platelets (106) were labeled with calcein and infused into ATRA-treated mice or wild-type mice, respectively, via tail vein injection. After 30 minutes, 10% W/v (final concentration) FeCl3 was used to cause damages to the mesenteric arteries and thrombus formation was monitored by a fluorescence microscopy (Olympus BX53).

**Western Blotting**
Washed human platelets were treated with CRP (5 μg/mL) or thrombin (under the condition of clot retraction) (1 U/mL) in the presence of different concentrations of ATRA or vehicle for 15 minutes. Levels of total and phosphorylated Syk (anti-Tyr-525 and pan-Syk, Bioworld Technology), PLCγ2 (anti-Tyr-1217 and pan-PLCγ2; Bioworld Technology), protein kinase C (PKC) α/βII (anti-Thr638/641, Cell Signaling Technology, and pan-PKCα, Affinity Biosciences), PKCβI (anti-Ser661 and pan-PKCβI, Affinity Biosciences), or PKCδ (anti-Thr505, Cell Signaling Technology, and pan-PKCδ, Affinity Biosciences) were assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis/Western blot. The density of protein band was quantified using Image J software and the phosphorylation level was presented as a ratio to the total level.

**Statistical Analysis**
Data are represented as mean ± standard deviation or mean ± standard error and analyzed using GraphPad Prism software. One-way analysis of variance (ANOVA) was conducted for comparison of difference among different groups. Two-way ANOVA with Bonferroni posttests was performed for comparison among different groups over time. A p-value of < 0.05 indicates statistical significance.

**Results**

**ATRA Inhibits Platelet Aggregation and Dense-Granule Secretion**
To investigate the effect of ATRA on platelet aggregation, we incubated human platelets with different concentrations of ATRA (0, 1, 10, and 20 μM) followed by measuring collagen- or thrombin-induced platelet aggregation. As seen in Fig. 1A, since dimethyl sulfoxide has been shown to have an impact on platelet function, weak response was observed in vehicle-treated platelets. Compared with vehicle treatment (0 μM ATRA), ATRA significantly impaired platelet aggregation in response to collagen (5 μg/mL) or thrombin (0.05 U/mL) in a dose-dependent manner. To further evaluate whether ATRA affects platelet dense-granule secretion, we also measured ATP release in collagen or thrombin-stimulated platelets and showed ATRA significantly inhibited ATP release from collagen- or thrombin-treated platelets in a dose-dependent manner (Fig. 1B). Interestingly, ATRA did not affect platelet α-granule secretion after collagen or thrombin stimulation even at a higher dose as demonstrated by no significant changes of P-selectin expression after ATRA treatment (Fig. 1C). Taken together, these data show that ATRA inhibits platelet aggregation and dense-granule secretion.

**ATRA Does Not Affect the Surface Expression of Platelet Receptors**
Platelet surface receptors GPIIbα, GPVI, and αIIbβ3 were demonstrated to play an important role in the regulation of platelet aggregation through engagement of their specific ligand, such as vWF (GPIIbα), collagen (GPVI), and fibrinogen (αIIbβ3). Given impaired platelet aggregation in response to agonist stimulation, surface expression of platelet receptors GPIIbα, GPVI, and αIIbβ3 after ATRA treatment was measured by flow cytometry. As shown in Fig. 2A, ATRA treatment did not affect the surface expression of GPIIbα (Fig. 2A), GPVI (Fig. 2B), and αIIbβ3 (Fig. 2C) after being treated with different ATRA concentrations.
concentrations of ATRA even at a higher dose as indicated by no significant changes of the expression of these platelet receptors compared with vehicle treatment.

**Impaired Platelet Spreading and Clot Retraction after ATRA Treatment**

To assess whether ATRA affects platelet spreading, after being treated with different concentrations of ATRA, human platelets were allowed to spread on immobilized fibrinogen or collagen and found that platelet spreading was significantly impaired in ATRA-treated platelets in a dose-dependent manner as the surface coverage area of spread platelets on fibrinogen (Fig. 3A) or collagen (Fig. 3B) after ATRA treatment was significantly smaller than platelets after vehicle treatment, consistent with a previous study showing platelet spreading was inhibited in ATRA-treated human platelets. In addition, we also evaluate the effect of ATRA on platelet-mediated clot retraction, a process regulated by αIIbβ3 outside-in signaling. Consistent with platelet spreading, clot retraction in ATRA-treated platelets was significantly inhibited in a dose-dependent manner as demonstrated by the significantly higher clot volume of ATRA-treated platelets than vehicle-treated platelets (Fig. 3C). These data suggest that ATRA inhibits platelet αIIbβ3 outside-in signaling transduction.

**Reduced Phosphorylation of Syk and PLCγ2 in ATRA-Treated Platelets**

Since ATRA inhibits platelet aggregation, spreading, and clot retraction, we next assessed the effect of ATRA on platelet intracellular signaling transduction through measuring the phosphorylation level of Syk and PLCγ2, which have been shown to be important in the regulation of platelet function and αIIbβ3 signaling transduction. Our data showed that the phosphorylation of Syk and PLCγ2 in CRP (5 μg/mL)-stimulated platelets was significantly impaired after ATRA treatment in a dose-dependent manner (Fig. 4A). To investigate whether the impaired Syk phosphorylation is due to reduced GPVI signaling or integrin αIIbβ3 outside-in signaling, platelets were treated with tirofiban to block integrin αIIbβ3 followed by analysis of Syk phosphorylation and found that there was no difference of Syk phosphorylation in platelets treated with ATRA compared with that in platelets treated with vehicle in the presence of tirofiban (Supplementary Fig. S1, available in the online version), indicating that the impaired Syk phosphorylation is due to impaired integrin αIIbβ3 outside-in signaling. Since thrombin-mediated clot retraction was inhibited in ATRA-treated platelets, the phosphorylation level of Syk and PLCγ2 which have been demonstrated to regulate clot retraction was also measured in thrombin (1 U/mL)-treated platelets under the conditions of clot retraction and found reduced phosphorylation of Syk and PLCγ2 after ATRA treatment (Fig. 4B). Taken together, these data show that ATRA inhibits αIIbβ3 outside-in signaling transduction possibly through inhibition of the phosphorylation of Syk and PLCγ2.

**ATRA Impairs Hemostasis and Arterial Thrombosis In Vivo**

To investigate the effect of ATRA on mouse platelet function in vivo, ATRA (10 mg/kg) was intraperitoneally injected into mice followed by analysis of platelet aggregation and ATP release as well as tail bleeding time. As seen in Fig. 5, ATRA-treated mice displayed significantly reduced CRP- or thrombin-induced platelet aggregation (Fig. 5A) and ATP release (Fig. 5B) compared with vehicle-treated mice. Regarding the effect of ATRA on in vivo hemostasis and thrombosis, mice receiving injection of ATRA showed a significantly prolonged tail bleeding time (265.80 ± 46.93 seconds) compared with mice receiving vehicle (96.00 ± 23.59 seconds) (p < 0.05) (Fig. 5C).
Meanwhile, the arterial thrombus formation which was induced by FeCl₃ was also significantly delayed in ATRA-treated mice receiving infusion of ATRA-treated platelets (29.67 ± 3.51 minutes) compared with that in mice receiving vehicle-treated platelets (16.50 ± 1.36 minutes) (p < 0.01) (►Fig. 5D). Taken together, these data show that ATRA impairs hemostasis and arterial thrombosis in vivo.

ATRA Inhibits the Phosphorylation of PKCβI and PKCδ

A previous study has demonstrated a direct interaction of ATRA with PKC, and PKC has been shown to play an important role in the regulation of platelet aggregation, granule secretion, and spreading. To evaluate whether ATRA affects platelet function through regulation of PKC, we measured the phosphorylation level of PKCα, PKCβI/II, and PKCδ in CRP (5 μg/mL)- or thrombin (1 U/mL)-stimulated platelets in the presence of different doses of ATRA, and found that the phosphorylation level of PKCβI (Ser661) and PKCδ (Thr505) was significantly reduced in CRP- (►Fig. 6A) and thrombin-treated (►Fig. 6B) platelets in the presence of ATRA in a dose-dependent manner. However, ATRA did not affect the phosphorylation of PKCα or PKCβII (Thr638/641). Consistently, ATRA administration into mice also decreased the phosphorylation of PKCβI and PKCδ in CRP-stimulated platelets (►Fig. 6C).

Discussion

As an active metabolite of vitamin A under the family retinoid, ATRA has been demonstrated to be widely applied for induction of complete remission in APL patients as the only well-established differentiation therapy. However, therapeutic use of ATRA is reported to interfere with the hemostatic function in APL patients, including effects on coagulation or other vascular cells, such as promyelocytic blast cells, normal human endothelial cells, and normal human monocytes, although effects of ATRA on platelets remain poorly understood. In the present study, through incubating human platelets with different doses of ATRA, we investigated the effect of ATRA on platelet function and showed ATRA inhibits platelet aggregation, spreading, clot retraction, as well as hemostasis and arterial thrombosis in vivo.

ATRA has been previously shown to downregulate tumor necrosis factor-induced expression of tissue factor in vascular endothelial cells, increase tissue plasminogen activator activity in rats, as well as thrombomodulin expression in human U937 monoblast-like cells and human MEG01 megakaryocyte-like cells. In addition, ATRA has also been demonstrated to reduce the cancer procoagulant activity in APL cells and improve the coagulopathy in APL patients as demonstrated by decreased markers of clotting activation and...
brin degradation, increased protein C, as well as reduced proteolysis of vWF after ATRA treatment, which was paralleled the improvement of clinical signs of the coagulopathy in APL patients. Furthermore, ATRA has been reported to interfere with the hemostatic function of promyelocytic blast cells, normal human endothelial cells, and normal human mono-
cytes to exert beneficial antithrombotic effects. Consistent with the antithrombotic role of ATRA, in the present study, we showed that ATRA inhibits platelet function as demonstrated by reduced platelet aggregation, spreading, clot retraction, as well as impaired in vivo hemostasis and arterial thrombus formation after ATRA treatment, suggesting ATRA might also

Fig. 3  Platelet spreading and clot retraction. After all-trans retinoic acid (ATRA) treatment, washed human platelets were placed on fibrinogen- (A) or collagen (B)-coated glass coverslips and allowed to spread at 37°C for 90 minutes followed by staining with Alexa Fluor-546-labeled phalloidin (mean ± standard deviation [SD], n = 3). ATRA-treated platelets were supplemented with 2 mM Ca2+ and 0.5 mg/mL fibrinogen and clot retraction was initiated after addition of thrombin (1 U/mL). Images were captured every 30 minutes (mean ± SD, n = 3) (C). For A and B, data were analyzed by one-way analysis of variance (ANOVA). Compared with 0, *p < 0.05; **p < 0.01; ***p < 0.001. For C, data were analyzed by two-way ANOVA. Compared with 1, 10, or 20, ***p < 0.001.
Fig. 4  Phosphorylation level of Syk and PLC\(\gamma_2\). After all-trans retinoic acid (ATRA) treatment, human platelets were treated with 5 \(\mu\)g/mL collagen-related peptide (CRP) (A) or 1 U/mL thrombin (under the conditions of clot retraction) (B) for 15 minutes followed by analysis of the phosphorylation level of Syk and PLC\(\gamma_2\) by Western blot. The protein expression was quantified using Image J software and represented as a ratio of phosphorylation to the total level (mean \pm standard deviation [SD], \(n = 3\)). Data were analyzed by one-way analysis of variance (ANOVA). Compared with 0, \(*p < 0.05; **p < 0.01; ***p < 0.001.\)

Fig. 5  Effect of all-trans retinoic acid (ATRA) administration on mouse platelet function. Mice received intraperitoneal injection of ATRA (10 mg/kg) followed by analysis of platelet aggregation (A), adenosine triphosphate (ATP) release (B), and tail bleeding time (mean, \(n = 5\)) (C). After treated with ATRA (20 \(\mu\)M) or vehicle, mouse platelets were labeled with calcein and infused into ATRA-treated or vehicle-treated mice, respectively, via tail vein injection followed by challenge with 10% FeCl\(_3\) to induce arterial thrombus formation which was monitored by a fluorescence microscopy (Olympus BX53) and the vessel occlusion time was then measured (mean, \(n = 6\)) (D). For A and B, data were presented as mean \pm standard error (SE) (\(n = 3\)). Data were analyzed by Student’s t-test. \(*p < 0.05; **p < 0.01.\)
exert antithrombotic effects through inhibition of platelet function.

The platelet surface GP receptors, such as αIIBβ3, GPIbα, and GPVI, play an important role in the regulation of platelet function. In response to vascular injury, platelet receptors GPIbα and GPVI adhere to the damaged blood vessel wall through recognition of exposed vWF and collagen, respectively, leading to transduction of intraplatelet signaling.

**Fig. 6** Phosphorylation of protein kinase C (PKC) α/βI, PKCβI, and PKCδ. All-trans retinoic acid (ATRA)-treated human platelets were stimulated with 5 μg/mL collagen-related peptide (CRP) (A) or 1 U/mL thrombin (under the conditions of clot retraction) (B) for 15 minutes and the phosphorylation level of PKCα/βI, PKCβI, and PKCδ was measured by Western blot. In addition, platelets were isolated from mice treated with ATRA (10 mg/kg) and treated with 5 μg/mL CRP followed by analysis of the phosphorylation of PKCβI and PKCδ (C). The protein expression was quantified using Image J software and represented as a ratio of phosphorylation to the total level (mean ± standard deviation [SD], n = 3). Data were analyzed by one-way analysis of variance (ANOVA). Compared with 0, **p < 0.01; ***p < 0.001.
pathway and subsequent activation of αⅡbβ₃, which binds to fibrinogen or vWF and mediates platelet aggregation and thrombus formation.²⁻³⁻⁴ Considering the inhibition of platelet aggregation and thrombosis by ATRA, as well as importance of platelet receptors αⅡbβ₃, GPⅠbα, and GPⅣ in platelet function, we evaluated whether ATRA affects the surface expression of these receptors and found no changes of the surface expression of these receptors in ATRA-treated platelets, suggesting ATRA does not affect the surface expression of platelet GP receptors.

PKC belongs to a family of serine/threonine kinases and are classified into three subfamilies, which are conventional isoforms (α, βI/II, and γ), novel isoforms (δ, ε, η, and θ), and atypical isoforms (ζ and ι/λ). Human platelets express PKCα, PKCβ, PKCδ, and PKCζ, which are demonstrated to play important role in the regulation of platelet aggregation, granule secretion, integrin activation, spreading, as well as procoagulant activity.⁵⁻⁶ A previous study has demonstrated a direct interaction of ATRA with PKC,⁷ showing ATRA binding site in three PKC isozymes (α, βI, and γ) after amino acid alignments and comparison of the crystal structures of several ATRA-utilizing proteins. Using photoaffinity labeling assay, they further showed that other PKC isozymes (α, βI, βII, γ, δ, ε, and ζ) were also photolabeled with PKCβII having the highest affinity for ATRA and PKCζ having the lowest affinity. Consistent with the interaction between ATRA and PKC, in the present study, we showed that ATRA treatment selectively inhibited phosphorylation of PKCβII (Ser661) and PKCζ (Thr505), but not PKCα or PKCβII phosphorylation (Thr63/641) in CRP- or thrombin-stimulated platelets. In addition, we also demonstrated that ATRA significantly reduced dense-granule secretion (ATP release) from collagen- or thrombin-treated platelets, which was consistent with a previous study showing that inhibition of PKCα/β or PKCζ significantly reduced dense-granule release from human platelets after treatment with convulxin or thrombin receptor (pro tease-activated receptor [PAR]) agonists, respectively.⁸⁻⁹ Interestingly, inhibition of both classical and novel PKC isoforms completely abolished dense-granule secretion from convulxin- or PAR agonists-treated platelets.⁹ In the present study, both classical and novel PKC isoforms (PKCβII and PKCζ) phosphorylation was significantly reduced in CRP- or thrombin-stimulated platelets in the presence of ATRA, which might be the reason why dense-granule secretion was both inhibited in collagen- or thrombin-treated platelets. Surprisingly, ATRA treatment did not affect α-granule secretion (P-selectin expression) from collagen- or thrombin-treated platelets, which was in accordance with a previous study showing that PKCζ had minimal effect on platelet P-selectin expression.¹⁰

In conclusion, therapeutic dose of ATRA inhibits platelet function, hemostasis, and arterial thrombus formation in vivo, which might be through direct or indirect inhibition of the phosphorylation of PKCβII/δ, suggesting that it may represent a novel inhibitor of platelet function and thrombus formation.

What is known about this topic?

- All-trans retinoic acid (ATRA) has been widely used for induction of complete remission in patients with acute promyelocytic leukemia (APL).
- ATRA has effects on the hemostatic system of APL patients as demonstrated by decrease or normalization of clotting and fibrinolytic variables.
- ATRA inhibits cytoskeletal events (platelet spreading) and actin-dependent morphological changes (extended cell body formation) in human platelets.

What does this paper add?

- ATRA inhibits platelet aggregation, ATP release, spreading on fibrinogen or collagen, and clot retraction.
- ATRA impairs in vivo hemostasis and arterial thrombus formation.
- ATRA selectively inhibits phosphorylation of PKCζ (Ser661) and PKCδ (Thr505).

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Conflict of Interest

None declared.

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