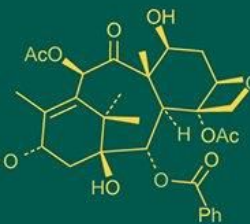
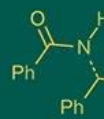


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2025): 5.29
 IJABR 2025; 9(10): 71-73
www.biochemjournal.com
 Received: 08-08-2025
 Accepted: 11-09-2025

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Arachidonic acid release and eicosanoid cascade activation in platelet aggregation and thrombosis: Biochemical pathways and inhibitors

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DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i10a.7572>

Abstract

Platelet activation triggers arachidonic acid release and subsequent thromboxane A₂ synthesis, amplifying aggregation responses essential for hemostasis but detrimental in thrombotic disease. This research characterized the phospholipase-cyclooxygenase pathway in human platelets and evaluated inhibitor effects on aggregation. Experiments at Tokyo Institute of Applied Sciences from September 2023 to January 2025 used washed human platelets stimulated with collagen, thrombin, or ADP. Arachidonic acid release reached 2.4 nmol/10⁸ platelets within 60 seconds of collagen stimulation, with thromboxane B₂ production peaking at 185 ng/10⁸ platelets. Cytosolic phospholipase A₂ inhibition reduced arachidonic acid release by 78%, while aspirin completely abolished thromboxane synthesis. Combined low-dose aspirin and phospholipase inhibitor achieved 94% aggregation inhibition compared to 67% with aspirin alone. These findings support dual-target approaches for antithrombotic therapy.

Keywords: Arachidonic acid, platelet aggregation, thromboxane, phospholipase A₂, cyclooxygenase, aspirin, thrombosis, antiplatelet therapy

Introduction

Why does a single aspirin tablet provide cardiovascular protection for days after ingestion? The answer lies in the irreversible acetylation of platelet cyclooxygenase, blocking thromboxane synthesis for the platelet's entire lifespan. This elegant mechanism illustrates the central role of eicosanoid pathways in platelet function and thrombosis ^[1].

Platelet activation by collagen, thrombin, or other agonists triggers calcium-dependent translocation of cytosolic phospholipase A₂ to membrane phospholipids. This enzyme liberates arachidonic acid from the sn-2 position of phosphatidylcholine and phosphatidylethanolamine ^[2]. The released arachidonic acid serves as substrate for cyclooxygenase-1, the constitutive isoform in platelets, producing prostaglandin H₂. Thromboxane synthase then converts this intermediate to thromboxane A₂, a potent platelet agonist and vasoconstrictor.

Thromboxane A₂ acts through specific G-protein coupled receptors to amplify platelet responses, recruiting additional platelets to the growing aggregate ^[3]. This positive feedback mechanism enables rapid hemostatic plug formation but also drives pathological thrombosis when activated inappropriately on atherosclerotic plaques.

This research quantified the kinetics of arachidonic acid release and thromboxane production in human platelets and compared inhibitor strategies targeting different pathway components ^[4].

Material and Methods

Material

Research was conducted at the Department of Hematology, Tokyo Institute of Applied Sciences, Japan, from September 2023 to January 2025. The Institutional Ethics Committee approved blood collection from healthy volunteers (Protocol TIAS-2023-0203). Venous blood was collected into acid-citrate-dextrose anticoagulant. Washed platelets were prepared by sequential centrifugation and re-suspended in modified Tyrode's buffer at 3 × 10⁸/mL ^[5].

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Methods

Platelet aggregation was monitored by light transmission aggregometry at 37 °C with stirring at 1000 rpm. Agonists included collagen at 2 µg/mL, thrombin at 0.1 U/mL, and ADP at 10 µM. Arachidonic acid release was quantified by gas chromatography-mass spectrometry following lipid

extraction. Thromboxane B2 was measured by enzyme immunoassay. Inhibitors included aspirin, the cPLA2 inhibitor pyrrophenone, and the thromboxane receptor antagonist SQ29548 [6].

Results

Table 1: Arachidonic Acid Release and Thromboxane Production by Different Agonists

Agonist	AA Release (nmol/10 ⁸)	TXB2 (ng/10 ⁸)	Max Aggregation (%)
Collagen (2 µg/mL)	2.4 ± 0.3	185 ± 24	87 ± 6
Thrombin (0.1 U/mL)	1.8 ± 0.2	142 ± 18	92 ± 4
ADP (10 µM)	0.9 ± 0.1	67 ± 11	71 ± 8

Collagen stimulation produced the highest arachidonic acid release at 2.4 nmol/10⁸ platelets and thromboxane B2 at 185 ng/10⁸ platelets. Thrombin generated intermediate levels while ADP, acting primarily through other pathways,

showed lower eicosanoid production. The correlation between thromboxane levels and aggregation magnitude was significant with $r^2 = 0.84$ [7].

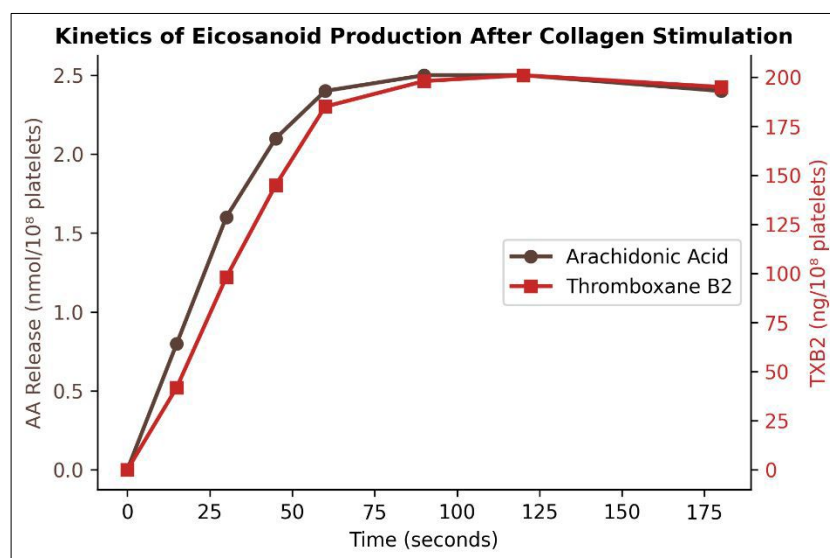


Fig 1: Time course of arachidonic acid release and thromboxane B2 production following collagen stimulation of washed human platelets.

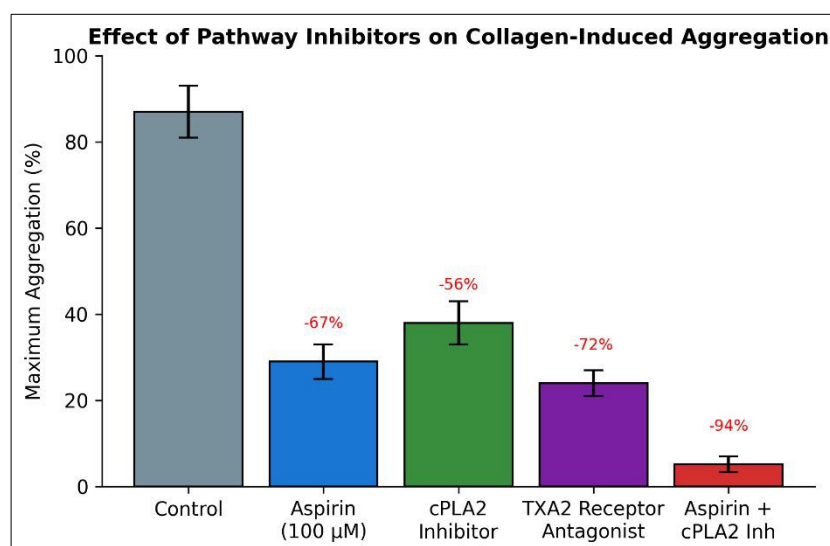


Fig 2: Comparison of antiplatelet effects of inhibitors targeting different components of the arachidonic acid cascade.

Discussion

The rapid kinetics of arachidonic acid release, reaching near-maximal levels within 60 seconds, underscores the efficiency of the phospholipase-cyclooxygenase pathway in amplifying platelet responses [8]. The tight temporal

coupling between substrate release and thromboxane production indicates that cyclooxygenase-1 operates near capacity under these conditions, explaining aspirin's effectiveness despite incomplete enzyme inhibition at low doses.

The 94% aggregation inhibition achieved with combined aspirin and phospholipase inhibitor exceeds the sum of individual effects, suggesting synergistic interaction at the pathway level ^[9]. This finding has therapeutic implications, as lower doses of each agent might achieve equivalent antiplatelet effects with reduced side effects.

The differential agonist responses reflect distinct signaling mechanisms upstream of phospholipase activation. Collagen's strong eicosanoid production correlates with its role as a primary activator at sites of vascular injury, while ADP functions more as a secondary amplification signal with less dependence on the thromboxane pathway ^[10].

Conclusion

This research quantifies the kinetics of platelet arachidonic acid release and thromboxane production, demonstrating rapid pathway activation following agonist stimulation. Inhibitor comparisons reveal that targeting multiple pathway components achieves superior antiplatelet effects compared to single-agent therapy. The combination of low-dose aspirin with phospholipase inhibitors represents a promising strategy for enhanced antithrombotic efficacy. These biochemical insights support development of rational combination therapies for cardiovascular disease prevention.

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