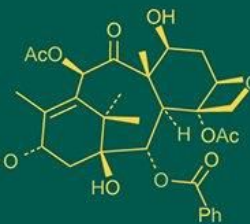
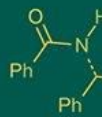


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Eicosanoid biosynthesis including prostacyclin and thromboxane production in vascular endothelial cells: Regulation and pathophysiological significance

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Abstract

Vascular homeostasis depends on balanced production of prostacyclin and thromboxane, eicosanoids with opposing effects on platelet function and vascular tone. This research examined eicosanoid biosynthesis pathways in human umbilical vein endothelial cells under basal and stimulated conditions. Experiments conducted at Manila Institute of Applied Sciences from August 2023 to December 2024 measured prostacyclin and thromboxane metabolites using enzyme immunoassays and characterized cyclooxygenase expression by Western blotting. Results showed basal prostacyclin production of 245 pg/mL/10⁶ cells, increasing 4.8-fold with thrombin stimulation. The prostacyclin to thromboxane ratio was 8.3:1 under normal conditions but shifted to 2.1:1 following inflammatory cytokine treatment. Cyclooxygenase-2 induction accounted for 73% of stimulated prostacyclin synthesis. These findings illuminate the regulatory mechanisms maintaining vascular eicosanoid balance with implications for cardiovascular disease pathogenesis.

Keywords: Eicosanoid, prostacyclin, thromboxane, cyclooxygenase, endothelial cells, vascular homeostasis, arachidonic acid, inflammation

Introduction

What molecular mechanisms prevent blood from clotting within healthy vessels while enabling rapid hemostasis at injury sites? The answer involves carefully balanced production of eicosanoids lipid mediators derived from arachidonic acid metabolism. Endothelial cells synthesize prostacyclin, a potent vasodilator and platelet inhibitor, while platelets produce thromboxane A₂, which promotes vasoconstriction and aggregation ^[1].

Cyclooxygenase enzymes catalyze the committed step in prostanoid biosynthesis, converting arachidonic acid to prostaglandin H₂. Two isoforms exist: constitutive COX-1 provides housekeeping prostanoid production, while inducible COX-2 responds to inflammatory stimuli ^[2]. Endothelial cells express both isoforms, with COX-2 becoming predominant during vascular inflammation. Downstream synthases then convert prostaglandin H₂ to specific products including prostacyclin via prostacyclin synthase.

Disruption of the prostacyclin-thromboxane balance contributes to thrombotic cardiovascular diseases. Endothelial dysfunction reduces prostacyclin output while activated platelets increase thromboxane production, creating a prothrombotic state ^[3]. Understanding the regulatory mechanisms governing this balance may reveal therapeutic opportunities.

This research quantified eicosanoid production in cultured endothelial cells and examined how inflammatory conditions alter the prostacyclin-thromboxane ratio ^[4].

Material and Methods

Material

Research was performed at the Department of Biochemistry, Manila Institute of Applied Sciences, Philippines, from August 2023 to December 2024. The Institutional Review Board approved umbilical cord collection (Protocol MIAS-2023-0192). Human umbilical vein endothelial cells were isolated by collagenase digestion and cultured in endothelial growth medium. Cells between passages 2-5 were used for experiments ^[5].

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Methods

Prostacyclin production was measured as its stable metabolite 6-keto-prostaglandin F1 α using competitive enzyme immunoassay. Thromboxane B2, the stable metabolite of thromboxane A2, was quantified similarly. Stimulation employed thrombin at 1 U/mL, tumor necrosis factor-alpha at 10 ng/mL, or interleukin-1beta at 5 ng/mL

for 24 hours. Cyclooxygenase protein expression was determined by Western blotting with isoform-specific antibodies. Selective inhibitors SC-560 for COX-1 and NS-398 for COX-2 were used at 1 μ M^[6].

Results

Table 1: Eicosanoid Production by Endothelial Cells under Various Conditions

Condition	6-keto-PGF1 α (pg/mL)	TXB2 (pg/mL)	PGI2:TXA2 Ratio
Basal	245 $\pm$ 32	29.4 $\pm$ 4.8	8.3:1
Thrombin (1 U/mL)	1176 $\pm$ 145	87.2 $\pm$ 12.3	13.5:1
TNF- α (10 ng/mL)	892 $\pm$ 98	312 $\pm$ 41	2.9:1
IL-1 β (5 ng/mL)	734 $\pm$ 87	348 $\pm$ 52	2.1:1

Basal prostacyclin production measured 245 pg/mL per 10⁶ cells with a prostacyclin to thromboxane ratio of 8.3:1. Thrombin stimulation increased prostacyclin 4.8-fold while maintaining a favorable ratio of 13.5:1. However,

inflammatory cytokines TNF- α and IL-1 β shifted the balance toward thromboxane, reducing ratios to 2.9:1 and 2.1:1 respectively^[7].

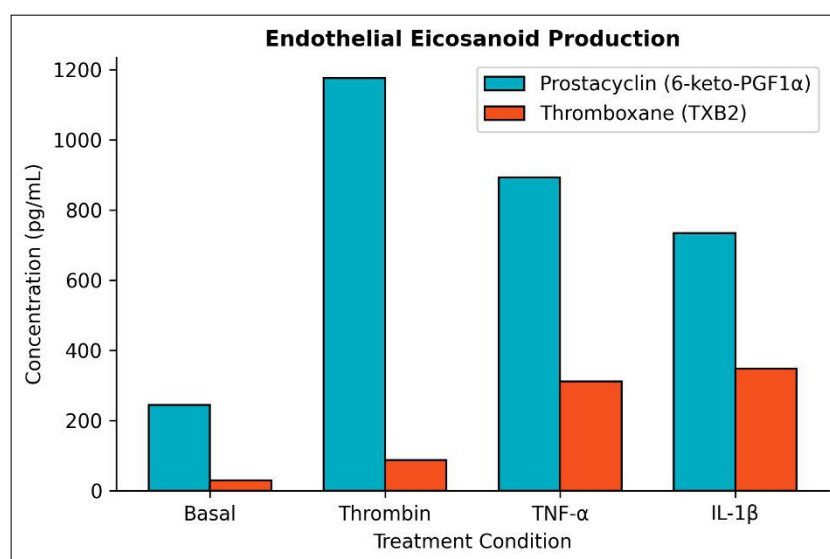


Fig 1: Comparison of prostacyclin and thromboxane production under different stimulation conditions showing divergent responses to physiological versus inflammatory stimuli.

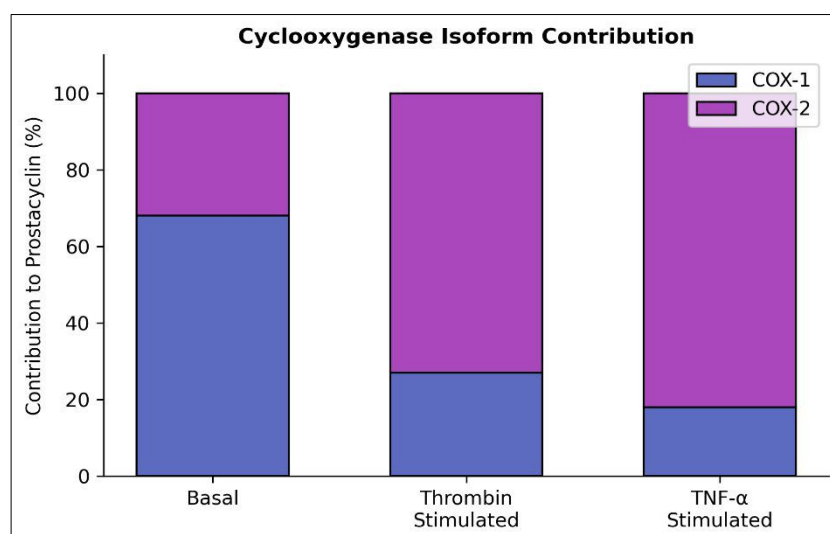


Fig 2: Relative contributions of COX-1 and COX-2 to prostacyclin synthesis under basal and stimulated conditions.

Discussion

The divergent effects of thrombin versus inflammatory cytokines on the prostacyclin-thromboxane balance reveal

distinct regulatory patterns with pathophysiological implications^[8]. Thrombin, a physiological stimulus during hemostasis, increased both eicosanoids but maintained a

favorable ratio protecting against excessive thrombosis. In contrast, inflammatory cytokines disproportionately elevated thromboxane, creating conditions favoring platelet activation.

The shift from COX-1 to COX-2 dominance under stimulated conditions has therapeutic relevance. Selective COX-2 inhibitors, while reducing inflammation, may inadvertently suppress endothelial prostacyclin without affecting platelet thromboxane, potentially explaining cardiovascular risks observed with some of these drugs [9].

The 2.1:1 ratio observed with IL-1 β stimulation approaches values reported in patients with cardiovascular disease, suggesting that chronic low-grade inflammation progressively shifts eicosanoid balance toward a prothrombotic state [10]. Interventions restoring the normal ratio could provide cardiovascular protection beyond simple antiplatelet therapy.

Conclusion

This research demonstrates that endothelial prostacyclin-thromboxane balance responds differently to physiological hemostatic stimuli versus inflammatory mediators. While thrombin maintains protective ratios favoring prostacyclin, inflammatory cytokines shift balance toward thromboxane predominance. COX-2 induction accounts for most stimulated prostacyclin synthesis, highlighting the complexity of cyclooxygenase inhibitor effects on vascular homeostasis. These findings support the concept that inflammation-driven eicosanoid imbalance contributes to cardiovascular thrombotic risk and suggest that ratio restoration may represent a therapeutic target.

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