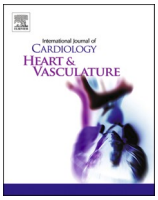
IJC Heart & Vasculature 52 (2024) 101403

Contents lists available at ScienceDirect

IJC Heart & Vasculature

journal homepage: www.sciencedirect.com/journal/ijc-heart-and-vasculature



Protease-activated receptor 2 at the intersection of thrombo-inflammation and beyond

ARTICLE INFO

Keywords
PAR2
Anticoagulants
Neutrophils
Pharmacology
Thrombo-inflammation

In this issue of the *International Journal of Cardiology Heart & Vasculature*, Nakano et al. [1] highlight protease-activated receptor type-2 (PAR2) as a candidate target for the anti-inflammatory actions of rivaroxaban on circulating blood neutrophils.

Coagulation is intimately connected with immunity, [2] and inflammation provides a critical interface of the two systems, conceptually captured by the term "thrombo-inflammation". Thrombin and activated factor X (FXa) promote inflammatory signaling via a unique PAR family. PAR1, the prototypical receptor for thrombin and FXa, is ubiquitously expressed and involved in many developmental and pathophysiological processes. PAR2 is preferentially activated by FXa, but can respond to higher thrombin levels. [3] PAR2, unlike PAR1, is transcriptionally regulated via NFkB and moreover self-regulates in a positive feed-forward manner boosted by cellular redox-stress. [4] Thus, functional expression of PAR2 specifically increases during inflammation and inhibition of thrombin versus FXa is expected to have different cellular consequences.

Nanako et al. [1] provide novel insights into this aspect of thrombo-inflammation specifically in neutrophils of patients with atrial fibrillation (AF). AF is a state of hypercoagulation and inflammation that may be both cause and consequence of the arrhythmia and the underlying atrial cardiomyopathy. [5,6] Stroke prophylaxis is obligatory in patients with AF, and this is increasingly achieved with direct oral anticoagulants (DOAC) that target thrombin (eg. dabigatran) or FXa (eg. rivaroxaban, apixaban, edoxaban). [7] In the present study, 40 DOAC-naïve patients with newly diagnosed AF were enrolled, and blood neutrophils were assessed at baseline and after 4 weeks of DOAC therapy for expression of PAR1 and PAR2. Of note, the authors utilized classical immunoblot for quantification, an approach which notoriously difficult to apply in blood neutrophils. The protocol provided in the data supplement involves a sequential isolation procedure with an acid-precipitation step that yields convincing and clear immunoblot bands. Alone for this methodological feat Nanako et al. are to be highly commended.

PAR1 abundance was largely unaltered after 4-week treatment with any of the DOAC, in keeping with the relatively inert constitutive expression of PAR1 in the presence of thrombin and FXa, at least in vascular cells. [4] Neutrophil PAR2, by contrast, was lower with rivaroxaban treatment, possibly reflecting blockade of FXa-driven feed-forward regulation. However, the mean PAR2/β-actin ratio was decreased from approximately 0.3 before treatment to approximately 0.25 after 4 weeks of treatment (17 %, $P = 0.016$). Although the effect is rather small, it could be biologically and clinically relevant and should be studied in the future. Potential work could include assessment of oxidative burst, neutrophil extracellular trap (NET) extrusion, cytokine production or adhesion to endothelial cells, platelets and other circulating cells in isolated neutrophil and whole blood assays. Particularly NET formation in the AF cohort reported by Nakano et al. [1] would be important to address, because NETs are culprit drivers of AF-related thromboembolism and PAR2 is a potent NETosis trigger.

Although all DOAC significantly and comparably reduced coagulation markers, solely rivaroxaban impacted on PAR2 expression. It appears that rivaroxaban exerts a substance-specific effect at least in endothelial cells, [8] and this is consistent with evidence that different DOAC exert characteristic clinical responses [9,10]. None of the included patients received antiplatelet drugs, so a differential contribution of platelet-neutrophil interactions could well contribute. Subsequent work should systematically assess the mechanisms of distinct regulation of neutrophil PAR expression and function by the individual DOAC, along with potential context-dependent responses in the presence of comorbidities and polypharmacy.

Neutrophils are the first immune cells recruited to combat infection and direct inflammation upon injury. The role of PAR2 in this context is highly dichotomous. A possible reason for this is the capacity of PAR2 to mediate so-called biased signaling, leading to distinct cellular responses

DOI of original article: https://doi.org/10.1016/j.ijcha.2024.101387.

https://doi.org/10.1016/j.ijcha.2024.101403

Available online 6 April 2024

to different proteolytic and synthetic activators. [11] The multifunctional role of this receptor was exemplified by a recent study in a hepatitis mouse model, where PAR2 activation in immune cells aggravated organ injury, while PAR2 activation in damaged liver promoted regeneration. [12] Since many patients receiving DOAC have comorbidities and age-associated frailty that predispose to opportunistic infections, the potential interference with immune defense requires careful consideration. Although PAR2 is generally associated with pro-inflammatory effect, some studies have identified a protective function of PAR2 in maintaining autophagic and mitochondrial function, and resolving inflammation. In a cooperative action with platelets involving 12-lipoxygenase, neutrophils can moreover produce pro-resolving and organ-protective mediators such as maresin-1. Perhaps counter-intuitively, PAR2 has also been shown to *reduce* neutrophil chemotaxis in a mouse model of acute respiratory distress syndrome. [13] This enigmatic nature of PAR2 has made development of clinically useful modulators challenging, although one PAR2-targeting antibody (MEDI0618) has recently passed into phase-1 clinical trials (NCT041985558).

The precise consequences for PAR2 function in AF patients receiving rivaroxaban is difficult to predict. Rivaroxaban suppresses PAR2 expression, neutrophil accumulation and inflammatory markers for example in rodents with angiotensin II-induced AF [14] and human atrial slices with *ex vivo* tachypacing. [15] Although the culprit PAR2-expressing cells were not identified in these studies, these studies corroborate that rivaroxaban negatively regulates PAR2. The findings by Nakano et al. [1] provide clear evidence for additional pleiotropic actions of DOAC on thrombo-inflammation and immune responses that require further validation in experimental models and patient populations.

Sources of Funding

Grants from the Deutsche Forschungsgemeinschaft (FE 1365/4-1 to A.F.), National Institutes of Health (R01HL136389, R01HL163277, R01HL131517, R01HL08959, R01HL160992, and R01HL165704, to D. D.), and the European Union (large-scale network project MAESTRIA No. 965286, to D.D.).

Disclosures

None.

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