

Stability evaluation of a new synthetic Collagen Related Peptide (CRP-A) agonist compared to the equine fibrillar collagen type I-III

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INTRODUCTION

Diagnosis of thrombopathies (inherited or acquired) are performed through platelet function tests (PFTs) using specific agonists targeting the major platelet receptors (PAR-1, P2Y₁, P2Y₁₂, α2A, GPVI, TxA₂). While naturally occurring collagen can target multiple Gp receptors, CRP-A almost exclusively targets GPVI.

AIM

The present study compares the stability of synthetic CRP-A to equine collagen at room temperature (RT), +5±3 °C or after several freeze/thaw cycles. The agonists activities were evaluated in functional tests such as flow cytometry (FCM) or light transmission aggregometry (LTA).

METHOD

The stability of CRP-A (PplusMedical, UK) and collagen (Stago, France) reagents, as well as their respective controls, were evaluated under various storage conditions using LTA on citrated platelet-rich plasma (PRP) and FCM on citrated fresh whole blood. Platelets were isolated by FCM using CD42a (Gp IX) counterstaining. Mean fluorescence intensity (MFI) of platelet glycoproteins GPIIb/IIIa (CD41/CD61-fibrinogen (Fg) binding), CD63 (dense granules), and CD62P (P-selectin) was measured on resting and activated platelets. For each marker, the ratio of MFI activated platelets (S) to MFI resting platelets (B) was calculated for all test conditions and corresponding controls in order to assess reagent stability. At each stability time point, three pooled samples from healthy volunteers were analyzed. Agonist concentrations were predefined for each technique to achieve either 100% response (CRP-A: 1 and 0.1 µg/mL; collagen: 2 µg/mL for LTA, 25 µg/mL for FCM) or ~50% response (CRP-A: 0.05 µg/mL for LTA, 0.25 µg/mL for FCM; collagen: 0.31 µg/mL for LTA, 13 µg/mL for FCM).

LTA

Acceptance Criteria for CRP-A and Collagen Agonists (Maximum % Aggregation):

- Tests performed 1 to < 4 hours post-collection
- PRP platelet count between 150 – 600 G/L
- Control agonist should induce maximum aggregation > 50% → interpretable results

FCM

Acceptance Criteria for Agonist:

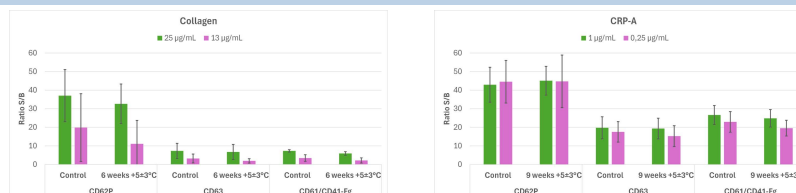
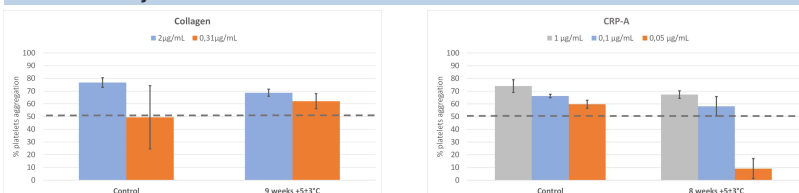
- S/B ratio > 1.0 for all three markers (CD62P, CD63, CD61/CD41) → interpretable results
- For CD61/CD41-fibrinogen marker, S/B ratios were too low to allow reliable relative difference assessment

RESULTS

LTA

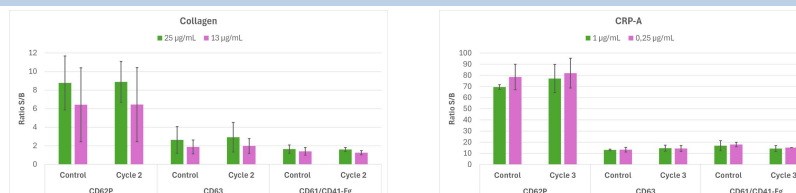
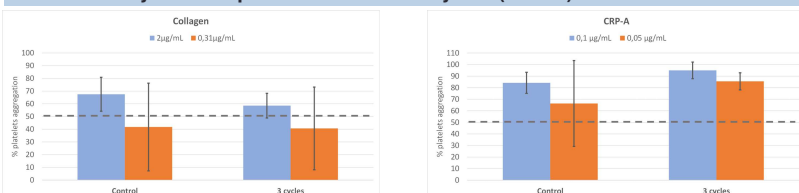
FCM

In-use stability at +5±3°C



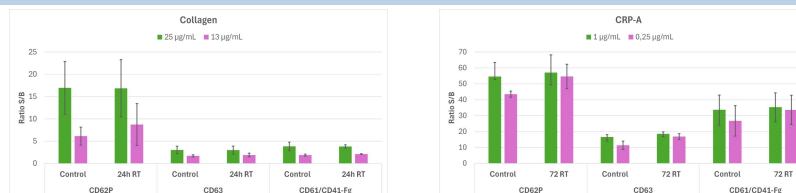
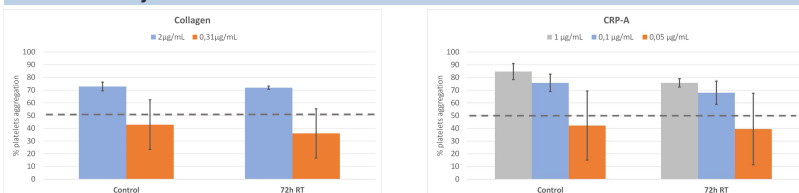
Both CRP-A and collagen exhibited acceptable stability when stored at +5±3 °C. Collagen remained stable for up to 6–8 weeks, whereas CRP-A maintained stability for up to 8–9 weeks, depending on the analytical method used (FCM or LTA).

In-use stability under repeated freeze–thaw cycles (–20 °C)



Freeze–thaw cycles showed that collagen tolerated up to 2–3 cycles, depending on the analytical method, while CRP-A remained stable for 3 cycles with both methods.

In-use stability at RT



At RT, collagen stability was confirmed for up to 24 hours and 72 hours based on FCM and LTA data, respectively. In contrast, CRP-A remained stable for up to 72 hours according to FCM and LTA data, respectively.

DISCUSSION

Given the distinct activation pathways triggered by collagen and CRP-A—where collagen engages multiple adhesion receptors, including GPIIb/IIIa and GPVI, while CRP-A selectively targets GPVI—the platelet activation response was evaluated using two complementary functional assays, LTA and FCM.

LTA provides an overall assessment of platelet aggregation, whereas FCM enables a more detailed characterization of platelet activation and intracellular signaling events, including fibrinogen binding to GPIIb/IIIa and dense granule secretion. These fundamental methodological differences likely contribute to the discrepancies observed between LTA and FCM measurements.

Moreover, the use of whole blood in FCM, in contrast to PRP in LTA, may further influence the observed responses. This distinction may also partly explain the greater variability observed with CRP-A in LTA compared with collagen.

CONCLUSION

The results indicate that the synthetic CRP-A is an effective platelet agonist with very good potency and stability which can be an excellent alternative to collagen from animal origin with clinical utility for identifying platelet disorders or monitoring anti-platelet therapy. Moreover, CRP-A is more suitable for FCM handling, as it does not require a 37 °C incubation step, in contrast to collagen.

	Collagen		CRP-A	
	FCM	LTA	FCM	LTA
Stability				
Stability @ +5±3°C	6 weeks	8 weeks	9 weeks	8 weeks
Stability after freeze/thaw	2 cycles	3 cycles	3 cycles	3 cycles
Stability @ RT	24h	72h	72h	72h