Evaluation of a new range of platelet agonists for the diagnosis of inherited or acquired platelet dysfunctions

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Introduction

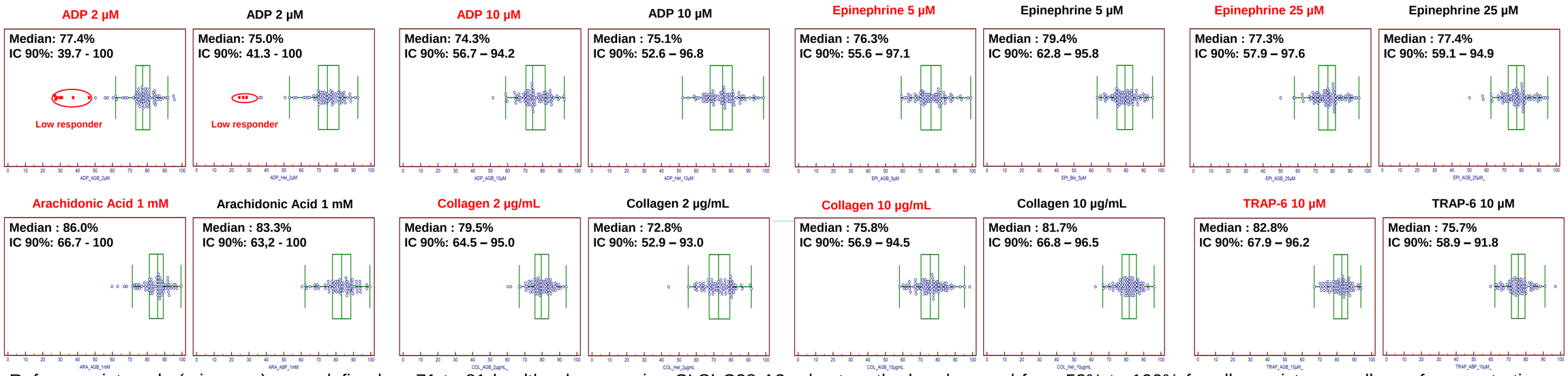
Diagnosis of platelet dysfunctions (inherited or acquired) are performed by light transmission aggregometry (LTA) using specific agonists targeting the major receptors: thrombin receptor (PAR-1), ADP receptors ($P2Y_1$ & $P2Y_{12}$), collagen receptors ($\alpha_2\beta_1$ & GPVI), epinephrine receptor (α2A) and signaling pathways (arachidonic acid cascade and Thromboxane A_2 receptor). The aim of this study was to evaluate the performances of a new range of agonists (Arachidonic Acid, ADP, Collagen, Epinephrine and TRAP-6). Results obtained in healthy donors and patients with inherited or acquired platelet dysfunctions were compared to those obtained with reference agonists (CE marked).

Material and Methods

LTA (aggregometer TA-8V, SD-Medical) was performed using citrated platelet-rich plasma (cPRP). Platelet aggregation was induced by different agonists (Agro-Bio a Stago brand) at different concentrations (low and high) for ADP (2 and 10 µM), Collagen (2 and 10 µg/mL), Epinephrine (5 and 25 µM),TRAP-6 (10 and 50 µM) and only one concentration for Arachidonic acid (1 mM). Statistical analyzes (outliers research, reference intervals, Band & Altman, correlation tests) were carried out using the MedCalc® software.

Results

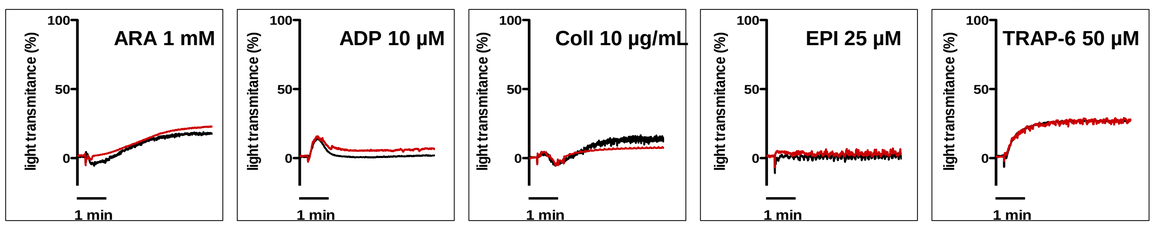
Reference intervals determination on 71 to 81 healthy donors (Agro-Bio vs Reference)



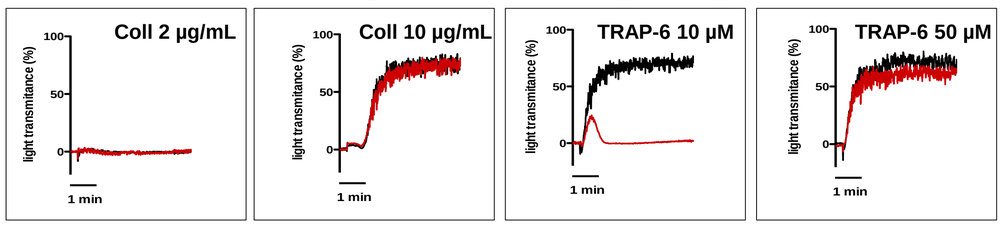
Reference intervals (min, max) were defined on 71 to 81 healthy donors using CLSI C28-A3 robust method and ranged from 53% to 100% for all agonists regardless of concentration, except for 2 µM ADP where we found 7 low responder (Agro-Bio reagent) and 3 low responder (Reference reagent) of 75 included healthy donors. However, a good correlation can be observed between results (median) obtained with Agro-Bio reagents as compared to those obtained with reference reagents.

Diagnosis of inherited platelet dysfunctions (representative aggregation curves: Agro-Bio vs Reference)

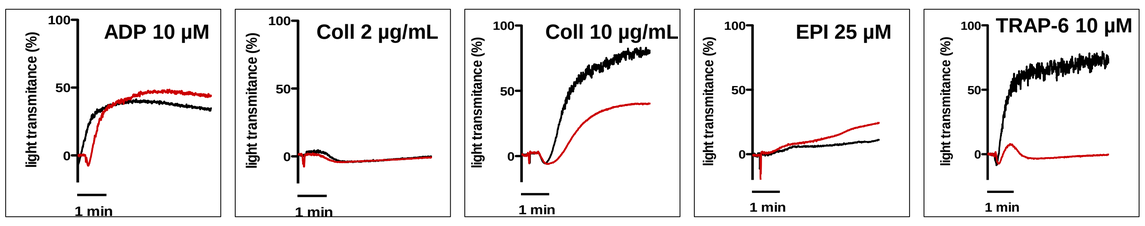
Glanzmann-like disease induced by Mab anti-GPIIb-IIIa (Abciximab)



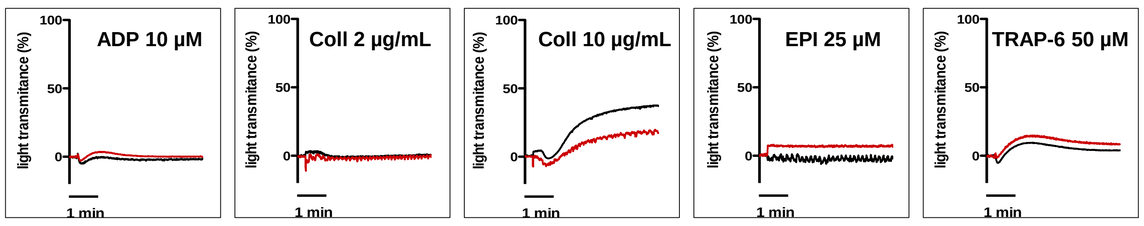
Storage Pool Disease (δ-SPD)



CalDAG-GEFI deficiency

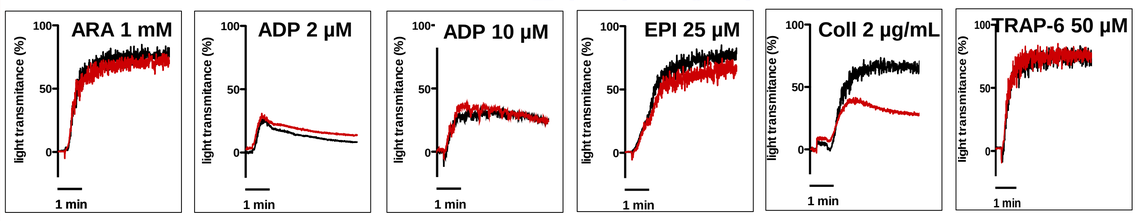


Kindlin-3 deficiency

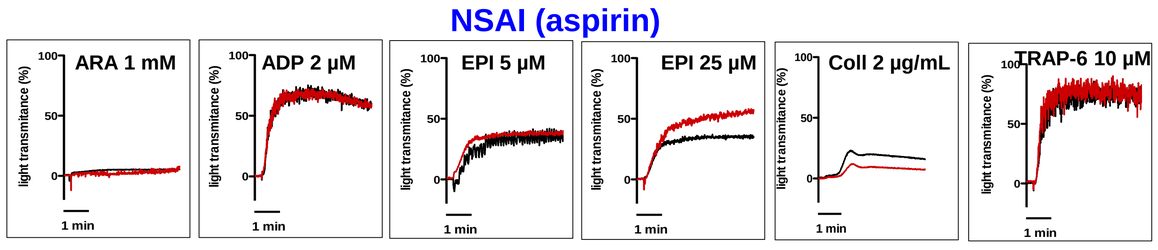


Diagnosis of acquired thrombopathies (representative aggregation curves: reference vs Agro-Bio)

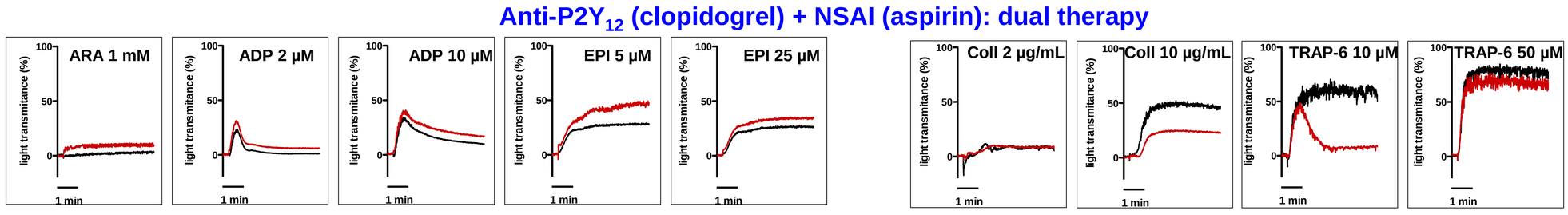
Anti-$P2Y_{12}$ (clopidogrel)



NSAI (aspirin)



Anti-$P2Y_{12}$ (clopidogrel) + NSAI (aspirin): dual therapy



The performances of these agonists were evaluated in the diagnosis of inherited platelet dysfunctions [Glanzmann-like disease induced with MAb anti-GPIIbIIIa (Abciximab, n=5), δ-storage pool disease (n=2), CalDAG-GEFI deficiency (n=2), Kindlin-3 deficiency (n=1)] and in the diagnosis of acquired thrombopathies like drug-induced platelet disorders [Clopidogrel (n=6), Aspirin (n=14) or dual therapies (n=18)] and showed good matching in LTA, Bland & Altman or Correlation curves (data not shown) for all agonists between Agro-bio and reference reagents (CE marked). Nevertheless, we observed a slight decrease in aggregation induced by collagen at 2 µg/mL and in lesser extent by TRAP-6 for CalDAG-GEFI and Kindlin-3 deficiency, Aspirin, dual therapy treated patient as compared to the reference reagent, suggesting a better sensitivity for the diagnostic of thrombopathies involving collagen or thrombin receptors.

Conclusion

Taken together, all these results and statistical analysis have shown a same effectiveness between this new range of agonists as compared to the existing reference (CE marked) agonists in the diagnosis of inherited or acquired platelet dysfunctions.