Soluble guanylate cyclase activators as inhibitors of platelet function

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Purpose: PGI₂ and NO synergize with P2Y₁₂ receptor blockade to inhibit platelet aggregation. Recently novel allosteric activators of soluble guanylyl cyclase (sGC) have been identified that can stimulate the formation of cyclic nucleotides through both heme-dependent (e.g. riociguat and BAY 41-2272) and heme-independent (e.g. cinaciguat and BAY 60-2770) pathways. Therefore, we hypothesize that in the presence of P2Y₁₂ receptor blockade these sGC modulators could produce enhanced platelet inhibition.

Materials and methods: Blood was taken from healthy humans, aged 18-40. To assess platelet reactivity, platelet rich plasma (PRP) was obtained by centrifugation of whole blood and 96-well plate aggregometry (1) was used to measure platelet aggregation in response to TRAP-6 amide (thrombin receptor agonist peptide) 10 and 25 µM and collagen 3 and 10 µg/ml in the presence of sGC modulators and/or the P2Y₁₂ blocker, prasugrel active metabolite (PAM 3 µM), and/or vehicle. Further studies examined the aggregation of platelets in response to TRAP-6 in whole blood, following treatment with/without sGC modulators and/or PAM, using as an end point determinant fluorescence-activated cell sorting (FACS) (2). Finally, adhesion of platelets under shear to collagen-coated surfaces was assessed by visualizing the perfusion through IBIDI chambers of mepacrine-labelled platelets in whole blood treated with sGC modulators and/or PAM and/or vehicle.

Results: sGC activators were more potent than sGC stimulators as inhibitors of platelet function in both platelet rich plasma and whole blood. In combination with a P2Y₁₂ blocker, a powerful synergistic effect was observed in whole blood experiments (e.g. aggregation induced by TRAP-6: PAM alone, 76 ± 4%; PAM + BAY 60-2770, 22 ± 6%; n=7). This pattern was also replicated in studies examining platelet adhesion under shear, where less platelet adherence was seen when a sGC activator was included in addition to PAM: geometric mean fluorescence index (MFI) for whole blood plus vehicle 60 ± 5; PAM alone, 48 ± 3, PAM + BAY 60-2770, 39 ± 3; n=3.

Conclusion: Both allosteric activators have the potential to be anti-platelet agents when used in combination with P2Y₁₂ receptor blockers. We suggest that providing the sGC activators in low dose combinations with P2Y₁₂ receptor blockers could provide a focused and powerful anti-platelet effect with relatively lesser effects at other sGC sites such as the vascular smooth muscle. Thus in combination with a P2Y₁₂ receptor blocker, sGC activators could produce a strong anti-platelet effect with reduced incidence of headache and hypotension.

References:

