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| **Targeting the platelet internal membrane reveals a novel approach for improved anti-platelet therapies.** |
| **Background**  The class II PI3K, PI3KC2α, is a broadly expressed lipid kinase with emerging biological roles. We have recently shown that PI3KC2α is important in platelet structure and function using a mouse genetic approach - PI3KC2α-deficient mice have impaired thrombotic function that appears due to a dysregulated open canalicular system (OCS) structure (Mountford, Nat Comm 2015). However, without pharmacological inhibitors of PI3KC2α, it remains unknown if a similar mechanism occurs in human platelets and whether targeting this is a viable anti-thrombotic approach.  **Aim**  To determine the role of PI3KC2α in regulating human platelet membrane structure and function, and assess the viability of targeting PI3KC2α as an anti-thrombotic strategy.  **Methods**  A rational drug design approach was used to develop an inhibitor of PI3KC2α, MIPS-19416. MIPS-19416 was used to examine the impact of PI3KC2α inhibition on the structure of the platelet OCS using both TEM and FIB-SEM. Platelet function was examined in a series of standard in vitro assays, alongside ex vivo thrombosis in human blood, and in vivo thrombosis in mice.    **Results**  PI3KC2α inhibition with MIPS-19416 in human platelets fully recapitulated the structural and functional effects of PI3KC2α-deficiency in mouse platelets. Here, MIPS-19416 increased the volume of the OCS in human platelets. These membrane changes did not impact in vitro platelet activation or aggregation. However, thrombus formation was significantly reduced with MIPS-19416 in two distinct in vivo mouse models, and two ex vivo human whole blood flow models, including one where thrombosis occurs largely independently of canonical platelet activation.  **Conclusion**  PI3KC2α is involved in regulating platelet membrane structure and function in both mouse and human. Inhibition of PI3KC2α has an anti-thrombotic effect that occurs specifically under shear stress and largely independently of platelet activation, suggesting that targeting the platelet membrane via PI3KC2α may provide potential as a novel anti-thrombotic drug target. |