HDAC inhibitor CS014 inhibits platelet activity, small and large vessel thrombosis while maintaining haemostasis in a dose-dependent manner

M. Holinstat¹, L. Stanger¹, S. Lambert¹, B. Dahlof², N. Bergh²
¹University of Michigan, Ann Arbor, United States of America
²University of Gothenburg, Gothenburg, Sweden

Funding Acknowledgements: Type of funding sources: Private company. Main funding source(s): Cereno Scientific

Background: It is a significant challenge to develop new targets for prevention of platelet activity and clotting and clot formation leading to occlusive thrombosis remains a significant cause of cardiovascular morbidity and mortality. Using the HDAC inhibitor, CS014, we can show an in vivo benefit in reduced clotting without increased bleeding. Understanding the benefits of epigenetic modulation with CS014 would validate its utility in prevention of life-threatening clot formation in vivo.

Purpose: Develop a first-in-class HDAC inhibitor, CS014, that prevents clotting without a risk for bleeding. Compared to valproic acid (VPA), CS014 exhibits a better clot prevention profile.

Methods: Thrombosis and bleeding in mouse models were assessed with VPA and CS014 at multiple doses by labelling platelets and fibrin and measuring accumulation at the site of injury using intravital microscopy. HDAC inhibitor were given IP for 5 days at different doses. Thrombosis in the vessel was assessed in the 1) laser-induced cremaster arteriole thrombosis assay for assessment of clotting in the small vessel, 2) carotid artery FeCl3 thrombosis assay for assessment of clotting in the large vessel, and 3) saphenous vein rebleeding laser-induced assay for assessment of clotting and haemostasis in the vein. Bleeding was assessed in the tail vein bleeding assay.

Results: CS014 treatment significantly reduced clot formation and fibrin formation at the site of injury in the laser-induced cremaster assay. The observed inhibition of clot formation and fibrin accumulation at the site of injury was significantly greater than the inhibition observed with VPA at lower doses. FeCl3-induced injury of the carotid artery resulted in full occlusion of the vessel within 12-15 minutes. Treatment with CS014 or VPA were able to prevent full occlusion of the carotid artery, supporting their benefit in arterial injury conditions. In the saphenous vein rebleeding assay, fibrin and platelet accumulation at the site of injury wound was significantly inhibited, suggesting that CS014 and VPA function in both arterial and venous systems to attenuate clot and thrombosis. The tail vein bleeding assay confirmed that while the thrombus formation and stability was decreased based on the cremaster and carotid artery assays, no change was observed in bleeding time under these conditions.

Conclusions: We have shown for the first time that CS014, a first-in-class HDAC inhibitor, results in better inhibition of mouse thrombosis and decreased time to clot resolution without bleeding. This discovery represents a new class of treatment for prevention of platelet activation and thrombosis with potential for protection from myocardial infarction and stroke. Additionally, the decreased fibrin formation in the blood should limit the risk of thromboembolism. CS014 has proven that HDAC inhibition offers a new alternative for protection in the blood from thrombotic risk without an added risk of bleeding.
CS014 doesn't cause bleeding risk