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ORAL ABSTRACTS

301.VASCULATURE, ENDOTHELIUM, THROMBOSIS AND PLATELETS: BASIC AND TRANSLATIONAL

Apoptosis Signal-Regulating Kinase 1 (Ask1) Inhibition Prevents *In Vivo* Thrombosis without Compromising Hemostasis

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Cardiovascular diseases are currently among the leading causes of death in mankind. Although significant progress has been made in prevention and treatment, the currently available pharmacological interventions have a significant rate of severe bleeding side effects due to their effect on hemostasis. Recently, we have shown that apoptosis signal-regulating kinase 1 (ASK1) plays an important role in platelet activation and thrombus formation. Here, we show that two chemically distinct ASK1 inhibitors, dose-dependently inhibited thrombin-induced phosphorylation of ASK1 and its downstream effectors MKK3/4/6, p38 MAPK, and cPLA2 in human platelets, as detected by western blotting using phospho-specific antibodies. ASK1 inhibitors also dose-dependently inhibited platelet a granule release and $\alpha_{IIb}\beta_3$ activation which are the readouts for inside-out signaling events as analyzed by flow cytometry. Moreover, pretreatment of human platelets with ASK1 inhibitors completely impaired clot retraction and platelet spreading on immobilized fibrinogen two events regulated by outside-in signaling. To investigate the *in-vivo* effect of Ask1 inhibitor on thrombus formation, we intraperitoneally injected C57BL/6 mice with Ask1 inhibitor (1mg/kg), and the blood was withdrawn at 1-, 4-, and 24-hour time points and analyzed for *in vitro* thrombus formation on collagen matrix under arterial flow. Ask1 inhibitor significantly decreased thrombus growth ($P<0.01$) which was sustained for at least 24 hours. Platelet adhesion to collagen was not affected as determined by surface coverage.In a 10% $FeCl_3$ -induced carotid artery injury model of thrombosis, we found that Ask1 inhibitor (100μg/kg)showed a significantly extended time of vessel occlusion ($P<0.01$) . Moreover, Ask1 inhibitor (100μg/kg) treated C57BL/6 mice were protected from collagen/epinephrine-induced pulmonary thromboembolism ($P<0.001$). Furthermore, in a well-established ischemic stroke model of transient middle cerebral artery occlusion, C57BL/6 mice treated with Ask1 inhibitor (1mg/kg) showed increased survival and improved neurological deficits than vehicle treated mice. The extent of the brain damage was assessed by staining the brain sections with 2% 2,3,5-triphenyltetrazoliun chloride, which showed significant reduction in infarct size and edema ($P<0.0001$). Interestingly, mice treated with Ask1 inhibitor (1mg/kg), a 10-fold more than the amount needed to inhibit thrombosis, had no effect on hemostasis as observed by both the tail bleeding assay and laser-induced cremaster injury model. Whereas clopidogrel (5-30mg/kg) showed significantly increased ($P<0.0001$) tail bleeding time. Next, we performed cerebral bleeding assay in which brain injury was induced by inserting a 26-gauge needle through the skull in the cerebrum 2mm laterally to the bregma and 4mm deep into the cerebrum and rotated 360 degrees. After 20 minutes the extent of bleeding in the brain was assessed by imaging. Treatment of C57BL/6 mice with clopidogrel (30mg/kg) showed significantly enhanced bleeding ($P<0.05$) compared to vehicle treated mice. However, Ask1 inhibitor treated mice had same extent of bleeding as that of vehicle treated mice. Additionally, a liver bleeding assay was performed by excising a calibrated piece (~4-5mg) of inferior edge of the right lobe of the liver of the mouse under anesthesia. After 20 minutes the bleeding was assessed by counting red blood cells in the peritoneal lavage. As observed in brain bleeding assay, treatment of mice with clopidogrel (30mg/kg), showed significantly increased liver bleeding ($P<0.05$) compared to vehicle treated mice whereas Ask1 inhibitor treated mice had same extent of liver bleeding as that of vehicle treated mice. Taken together, our results provide evidence that ASK1 inhibitors are novel therapeutic agents to combat thrombotic disorders without any side effects of bleeding, which is inherent to current anti-thrombotic drugs.

Disclosures No relevant conflicts of interest to declare.

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