Abstract: **P555**

**Pannexin1 promotes hemostasis and thrombosis by warranting platelet function**

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**Background:** Platelets accumulate at sites of vessel injury to prime hemostasis. This event may occur excessively in atherosclerotic lesions, leading to acute ischemic events. Previous studies report that ATP release through Pannexin1 (Panx1) membrane channels contributes to human platelet aggregation in vitro, which was supported by the association between a Panx1-400A>C gain-of-function genetic polymorphism and collagen-induced platelet reactivity in a small cohort of healthy subjects.

**Aim:** Here, we investigate the effects of genetic or pharmacological reduction of Panx1 channel function on platelet aggregation in vitro and in vivo.

**Methods:** Aggregation responses of WT or Panx1-/- platelets to collagen or arachidonic acid (AA) were measured by turbidimetry. Alternatively, platelets were pre-incubated during 7 min with the Panx1 inhibitor Brilliant Blue FCF (BB-FCF). To study the effects of Panx1 deficiency on hemostasis and thrombosis, we used mice with ubiquitous (Panx1-/-) or specific deletion of Panx1 in platelets (Pf4-CreTgPanx1fl/fl). In vivo bleeding time was assessed on tails, FeCl3-induced arterial thrombosis on mesenteric arteries and venous thromboembolism after injection of a collagen/epinephrine mixture in the jugular vein. Vasomotor function was measured by wire myography on mesenteric arteries.

**Results:** Panx1 channel function blockade with 1 mM of the food dye BB-FCF or Panx1 deletion specifically reduced in vitro platelet aggregation induced by 1 µg/mL collagen as compared to control conditions. The aggregation response induced by AA (75 µM) was not affected. Panx1 deficiency also delayed hemostasis; bleeding time after tail transection was increased in Panx1-/- mice compared with WT controls (788±76 vs 308±62 s, respectively. n=8). Vasoconstriction induced by phenylephrine (10 mM), KCl (10 mM), U446619 (10 nM) or endothelin-1 (10 nM) was decreased also in Panx1-/- mice, partly explaining the decreased hemostatic response. However, bleeding time was also increased in Pf4-CreTgPanx1fl/fl mice compared with Panx1fl/fl mice (536±121 vs 246±37 s, respectively. n=7-9). Time to circulatory arrest after FeCl3-induced vessel wall injury in mesenteric arteries was slightly increased in Pf4-Cre+Panx1fl/fl mice as compared to controls (37.4±6.5 vs 28.4±5.2 min, respectively. n=7-9). Finally, respiratory arrest after induction of venous thromboembolism was delayed in both Panx1-/- (233±14 vs 190±9 s. n=10) or Pf4-Cre+Panx1fl/fl (349±70 vs 241±11 s. n=6-7) mice as compared to their controls.

**Conclusion:** Panx1 contributes to platelet aggregation in thrombus formation during hemostasis, arterial and venous thrombosis. As Panx1 channel inhibitors include a commonly-used food dye and FDA-approved drugs like probenecid and mefloquine, this finding may have important clinical and therapeutic implications.