LIPID TARGETS IN PREVENTION OF CLOTTING: TRANSLATING *IN VITRO* CONCEPTS TO *IN VIVO* APPLICATION

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Bioactive lipids have been shown to play both pro- and anti-clotting regulatory roles in platelet function resulting in modulation of hemostasis and thrombosis. While much is known about COX-1 regulation and the role of its free fatty acid metabolites in regulation of the platelet, less is known about how 12-LOX and its fatty acid eicosanoids mediate these essential functions. Nearly 33% of deaths annually are associated with cardiovascular disease and platelet activation is essential to arteriothrombotic clots leading to myocardial infarction and stroke. Therefore a greater understanding of the role of 12-LOX in this process is needed and may represent a novel target for prevention of thrombosis. Our group has developed a highly selective 12-LOX inhibitor to target 12-LOX in the platelet and determine its potential role in platelet activation and thrombotic risk. Here, we show for the first time the *in vivo* utility of inhibiting 12-LOX. In human platelets run through a microfluidics system at arterial shear, treatment with the 12-LOX inhibitor ML355 was shown to be more effective at decreasing platelet adhesion to collagen compared to aspirin. *In vivo*, platelet accumulation at the site of injury in a number of thrombotic models in the mouse was prevented in the presence of ML355. Importantly, bleeding, a common side effect of platelet inhibition, was not affected, supporting 12-LOX as an important enzyme in regulation of hemostasis and thrombosis *in vivo* (Adili et al. Arterioscler Thromb Vasc Biol 2017,). These observations, coupled to the earlier observation by our group that inhibition or ablation of 12-LOX was effective in preventing immune-meditated thrombosis in human platelets and mouse models (Yeung et al. Blood 2014), raised the question of whether inhibition of 12-LOX might be a viable treatment of immune-mediated thrombocytopenia and thrombosis (ITTs). To address this question, transgenic mice expressing human immune receptor FcgRIIa but not ALOX12, were retro-orbitally injected with a fluorescent antibody for the platelet receptor □-GPIX to induce ITT-like symptoms. Blood was collected at several time points to assess platelet count and the mice were sacrificed after 4 hours to determine the degree of thrombosis in vascular beds such as the lungs. While induction of ITT resulted in over 80% platelet loss within an hour and significant thrombosis in the lungs within 4 hours, animals lacking 12-LOX showed protection from both of these pathologies. Hence, targeting 12-LOX with ML355 demonstrates that 12-LOX is a viable antiplatelet target for arteriothrombotic events while exhibiting limited bleeding.