THIENOPYRIDINES, BUT NOT ELINOGREL, RESULT IN OFF-TARGET EFFECTS AT THE VESSEL WALL THAT CONTRIBUTE TO BLEEDING

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Supplemental data file

Materials and Methods:

Supplemental Figure 1:

The pharmacokinetic profile of elinogrel following single oral dosing in mice or single i.v. bolus was performed as followed. Multiple groups of 3 mice were administered elinogrel at either 7.5, 20 and 60 mg/kg (oral dosing (A)) or 0.1, 0.5 and 1 mg/kg (i.v. bolus dosing, (B)). At specific time points post administration, groups of 3 mice were bled and elinogrel plasma concentration determined as presented in materials and methods.

Supplemental Figure 2:

A) For dethrombosis experiments in vivo, elinogrel (0, 0.01, 0.05, 0.1, 0.5 and 1 mg/kg, 50 μL) was injected via a butterfly (25G) placed in the tail vein of the mouse when thrombi formed in response to FeCl₃-induced vascular injury reached 50-75% occlusive size. Fluorescence intensity was followed until 40 min post-injury.
B) For dethrombosis experiments in vitro in human blood, rectangular capillaries (Vitrocom, 0.2 mm x 2 mm section) were coated with human type III fibrillar collagen (Sigma) as previously described (Andre et al., 2003b). Blood was collected from the antecubital vein of aspirinated (325 mg/day for 3 days) healthy volunteers who gave written informed consent to the protocol (approved by the local Human Subjects Committee of Portola Pharmaceuticals Inc.) via butterfly 19G on syringes containing 5 μM (final concentration) of the factor Xa inhibitor C921-78. Platelets were labeled in situ with rhodamine 6G (final concentration 1.25 μg/ml) and blood was perfused through the capillary at 1500/sec for a period of 210 seconds. Immediately thereafter, a whole blood solution treated with either saline of 5 μM elinogrel was perfused over the freshly grown thrombotic deposits.

C) In a second set of experiments (stabilization study), the second perfusion consisted in a pure saline solution containing increasing amounts of ADP.

Evaluation of the thrombotic deposits using the real time thrombosis profiler was performed at 8 mm from the proximal end of the capillary. All experiments were performed within one hour of blood collection. Deposition of labeled platelets was visualized using a high-power light emitting diode with a spectral maximum at 530 nm and a spectral half width of 35 nm (Luxeon V, Lumileds Lighting, San Jose, CA) avoiding bleaching that is often associated with UV source. A microscope imaged an area of 360 x 270 μm² on the internal wall of the collagen-coated capillary onto a Sony XCD X-710 digital camera (resulting magnification ca. 13 x). Images were recorded at a frequency of 1 Hz. Thrombus size was plotted as the measurement of the fluorescence intensity divided by total area over the duration (sec) of the experiment.