

## **Prasugrel metabolites inhibit neutrophil functions**

Elisabetta Liverani, Mario C. Rico, Analia E. Garcia, Laurie E. Kilpatrick and Satya P.

Kunapuli

Journal of Pharmacology and Experimental Therapeutics

### **Supplemental data**

**Supplemental Figure 1: Formation of neutrophil/platelet aggregates was investigated using flow cytometry.** Top left panel: scatter plot indicating the neutrophil population (gate). Top right panel, plot for CD41 and CD16 in unstimulated identifies the quadrant for double positive events. Bottom right and left blot after fMLP (10 $\mu$ M) and PAF (10nM) stimulation respectively.

**Supplemental Figure 2: Prasugrel metabolites did not alter CD11a surface expression, but they could still inhibit neutrophil/platelet aggregate formation.** Neutrophils were labeled with FITC-Conjugated anti-human CD11a (Clone HI111; eBioscience, San Diego, CA). **(A)** Representative histogram of unlabelled neutrophils (negative control - gray line) compared with unstimulated cells labeled with CD11a (unstimulated – black line) and fMLP (10 $\mu$ M)-stimulated cells (fMLP-stimulated – red line) (left panel). GMFI values of fMLP and PAF (10nM) are shown (right panel; Mean  $\pm$  SEM, n = 3). **(B)** Representative histogram of fMLP-activated cells previously pre-incubated with either negative control (green line) or prasugrel metabolite (2 min – 5  $\mu$ M; purple line) (left

panel). GMFI values of fMLP and PAF activated cells (right panel; Mean  $\pm$  SEM, n = 3). (C) Both neutrophils and platelets were pre-incubated with prasugrel metabolites (5  $\mu$ M, 2 minutes) before stimulation with fMLP (10  $\mu$ M, white bar) or PAF (10 nM, black bar) (n= 3, mean  $\pm$  S.E.M., \*\*p  $\leq$  0.01 PRAS versus Ctr).

**Supplemental Figure 3: P2Y<sub>12</sub> expression in a variety of human cells.**

Western blotting image is showing P2Y<sub>12</sub> expression in a variety of human cells such as neutrophils (PMN), peripheral blood mononuclear cells (PBMC), Monocytes (Mono) and platelets as a positive control. Goat anti-human antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

**Supplemental Figure 4: Hematology cell counts following prasugrel treatment in LPS-induced systemic inflammation.** Graphs show murine blood cell counts of neutrophils (A), lymphocytes (B), platelets (C) and red blood cells (D). Values are expressed as 10<sup>3</sup>/ $\mu$ L, mean  $\pm$  SEM, (n = 2).