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Farnesoid X Receptor and its ligands inhibit the function of platelets

Article

Supplemental Material

Supplementary figures

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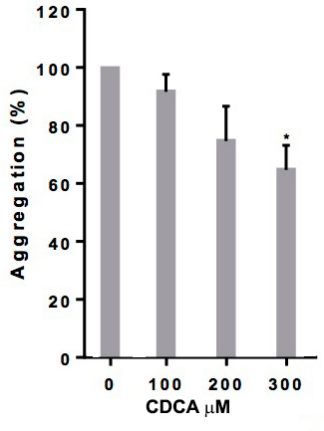

Figure I – FXR ligands inhibit platelet aggregation induced by collagen. Washed platelets were incubated with increasing concentrations of CDCA (100 - 300 μM) or vehicle (containing DMSO (0.1% (v/v)) prior to stimulation for 180 s with Collagen (0.5 μg mL^{-1}) and aggregation measured at 37°C under constant stirring conditions. Numerical data represent the percentage compared with control, mean ± SD (n=4). t-test *$p \leq 0.05$, ** $p \leq 0.01$.

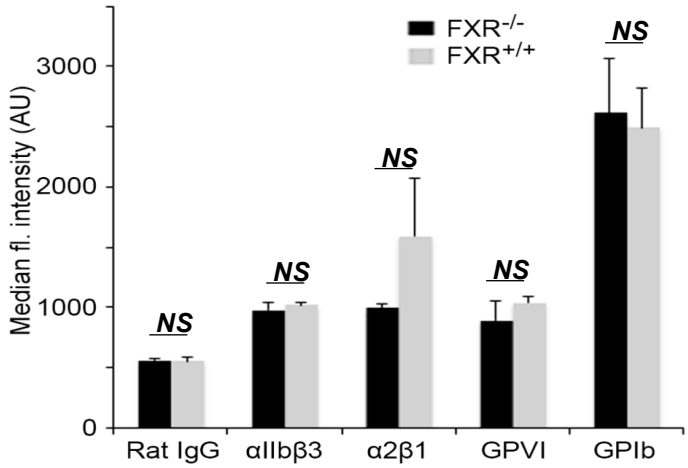

Figure II – Characterization of FXR-deficient platelets. The expression levels of αIIbβ3, α2β1,

GPVI and GPIb were analyzed on $FXR^{-/-}$and $FXR^{+/+}$ platelets by flow cytometry (A). Data represent mean (of median fluorescence intensity) ± SD (n=4). t-test $P > 0.05$ (non-significant - *NS*).

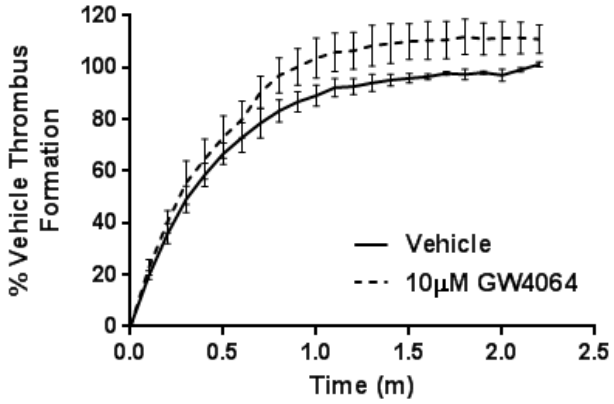

Figure III –GW4064 does not inhibit the adhesion of platelets to collagen under arterial flow conditions. Human whole blood was loaded with DiOC6 and incubated with vehicle (containing DMSO (0.1% (v/v)) (black) or 10 µM GW4064 (dashed line) in the presence of integrilin (10 µM), to prevent platelet aggregation, for 10 minutes before perfusion through collagen coated (100 µg/mL) Vena8Biochips at a shear rate of 20 dyn/cm^2 for 2.5 minutes. Following confocal microscopy using an A1R system (Nikon Instruments, UK) platelet adhesion was determined by comparing fluorescence intensity in the vehicle and treated samples. Data represent mean ± SD (n=3). 2-way ANOVA with Bonferroni post test $p > 0.05$ (non-significant).

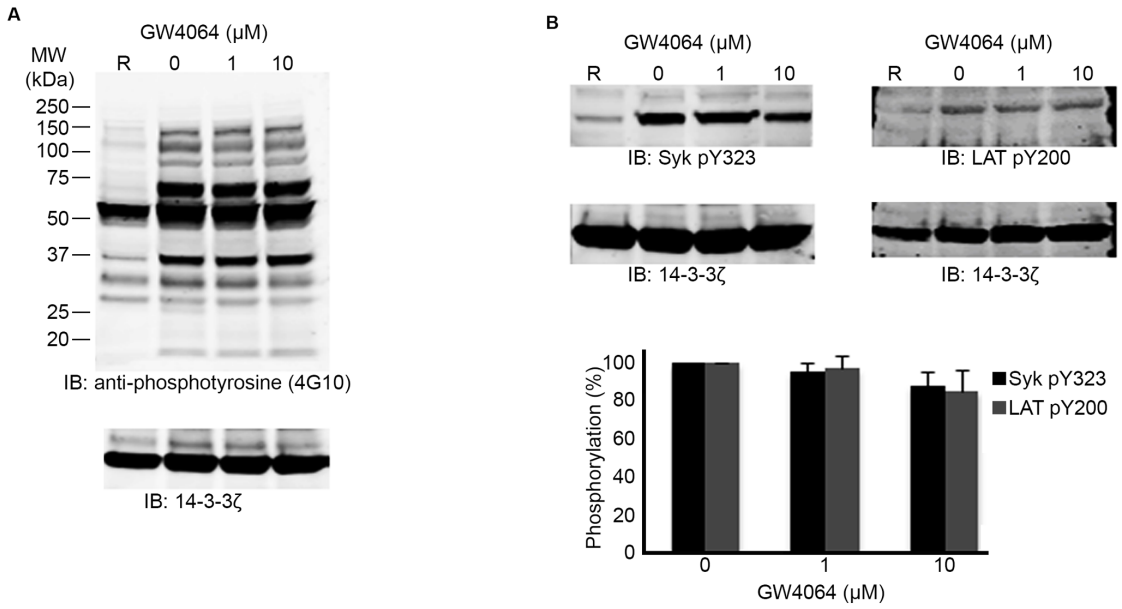

Figure IV – FXR agonists do not alter platelet protein tyrosine phosphorylation levels. (A) Washed human platelets were stimulated with CRP-XL (1μg/mL) in the absence or presence of GW4064. Whole-cell protein tyrosine phosphorylation levels were assessed by immunoblot analysis. Data are representative of 4 experiments. R represents untreated resting platelets. (B) Platelet lysates were subjected to immunoblot analysis using phospho-specific antibodies for the tyrosine kinase Syk (Y323) and the adapter protein LAT (Y200). Fluorescence images were visualised using a fluorimager and analysed using Imagequant software. $P>0.05$, n=4, T-test (non-significant).