

Farnesoid X Receptor and its ligands inhibit the function of platelets

Article

Supplemental Material

Supplementary figures

Moraes, L. A., Unsworth, A. J., Vaiyapuri, S., Ali, M. S., Sasikumar, P., Sage, T., Flora, G. D., Bye, A. P., Kriek, N., Dorchies, E., Molendi-Coste, O., Dombrowicz, D., Staels, B., Bishop-Bailey, D. and Gibbins, J. M. (2016) Farnesoid X Receptor and its ligands inhibit the function of platelets. *Arteriosclerosis Thrombosis and Vascular Biology*, 36 (12). pp. 2324-2333. ISSN 1524-4636 doi: <https://doi.org/10.1161/ATVBAHA.116.308093> Available at <http://centaur.reading.ac.uk/67335/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

To link to this article DOI: <http://dx.doi.org/10.1161/ATVBAHA.116.308093>

Publisher: Lippincott, Williams & Wilkins

including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the [End User Agreement](#).

www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading

Reading's research outputs online

Supplemental Figures

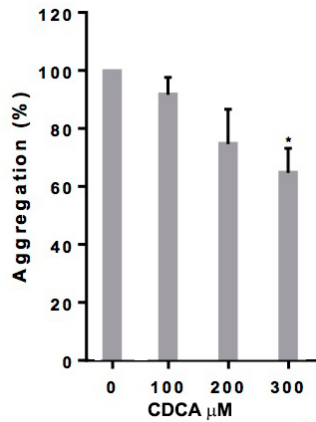


Figure I – FXR ligands inhibit platelet aggregation induced by collagen. Washed platelets were incubated with increasing concentrations of CDCA (100 - 300 μM) or vehicle (containing DMSO (0.1% (v/v))) prior to stimulation for 180 s with Collagen (0.5 $\mu\text{g mL}^{-1}$) and aggregation measured at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

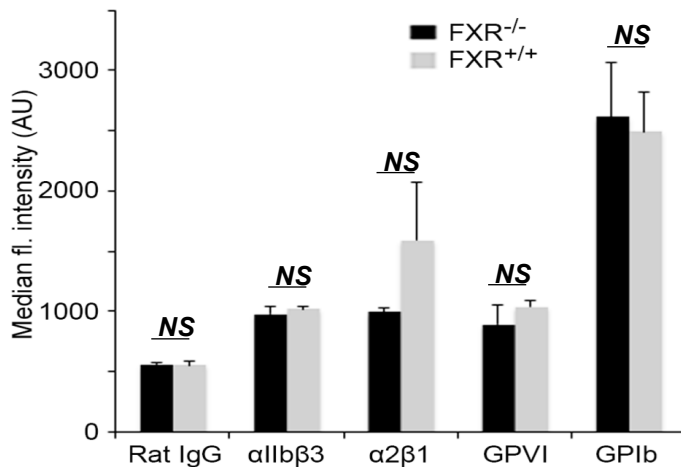


Figure II – Characterization of FXR-deficient platelets. The expression levels of $\alpha\text{IIb}\beta\text{3}$, $\alpha\text{2}\beta\text{1}$,

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

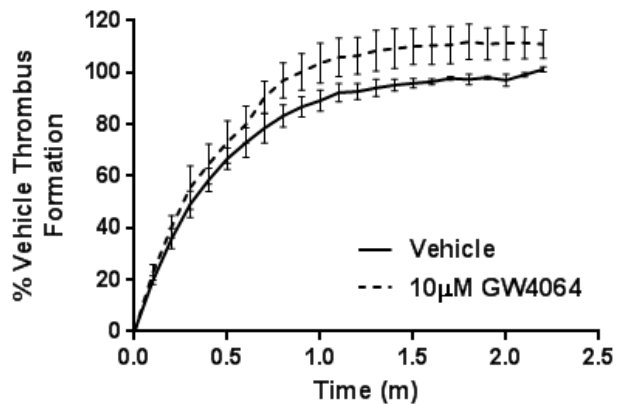


Figure III –GW4064 does not inhibit the adhesion of platelets to collagen under arterial flow conditions. Human whole blood was loaded with DiOC6 and incubated with vehicle (containing DMSO (0.1% (v/v))) (black) or 10 μ M GW4064 (dashed line) in the presence of integrilin (10 μ M), to prevent platelet aggregation, for 10 minutes before perfusion through collagen coated (100 μ g/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 \pm SD (n=3). 2-way ANOVA with Bonferroni post test $p > 0.05$ (non-significant).

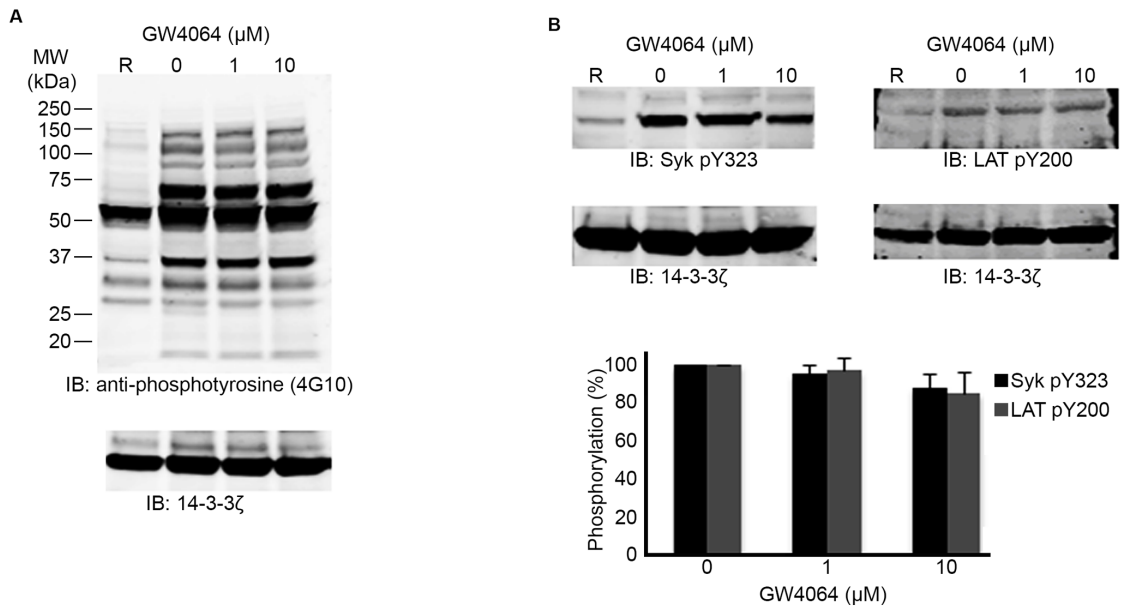


Figure IV – FXR agonists do not alter platelet protein tyrosine phosphorylation levels. (A) Washed human platelets were stimulated with CRP-XL (1 $\mu\text{g}/\text{mL}$) in the absence or presence of GW4064. Whole-cell protein tyrosine phosphorylation levels were assessed by immunoblot analysis. Data 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).