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ALTERED INHIBITION OF PLATELET AGGREGATION IN HYPERCHOLESTEROLEMIA IN MICE AND HUMANS

Division of BioTherapeutics, Leiden Academic Centre for Drug Research, Leiden University,
Gorleaus Research Building, Einsteinweg 55, 2333CC, Leiden, The Netherlands

Martin Houde^{1,2*}, Émilie Carrier^{1*}, Michel Grandbois¹, Ghassan Bkaily³, Timothy D. Warner⁴,
Suzanne J.A. Korpelaar⁵, Miranda van Eck², Pedro D'Orléans-Juste¹

¹ Department of Pharmacology and Physiology, Université de Sherbrooke, Sherbrooke, Québec, Canada

² Division of BioTherapeutics, Leiden Academic Centre for Drug Research, Universiteit Leiden, Leiden, The Netherlands

³ Department of Anatomy and Cell Biology, Université de Sherbrooke

⁴ The William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom

⁵ Experimental Cardiology, Utrecht Medical Centre, Utrecht, The Netherlands

* Contributed equally to this work

✉ m.houde@lacdr.leidenuniv.nl

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Background

Bradykinin (BK) induces endothelium-dependent inhibition of platelet aggregation (Labonté et al, 2001)

Liver X receptor (LXR) activation inhibits aggregation directly in platelets (Spyridon et al, 2011).

Platelet activity is increased in atherosclerotic patients, but it is unknown if this is because inhibitory pathways are inactive.

Aim

Evaluate the efficiency of platelet inhibitory mechanisms in hypercholesterolemia.

Set-up & Methods

Conclusions

Hypercholesterolemia and atherosclerosis alter the platelet inhibitory mechanisms, tipping the platelet activity balance to aggregation.

Results

1. PLATELET REACTIVITY INCREASES AGE-DEPENDENTLY IN HYPERCHOLESTEROLEMIC MICE

In vitro platelet aggregation measured with a microplate reader following injection of growing concentrations of either ADP (A, C) or U46619 (B, D) in the PRP of WT or ApoE^{-/-} mice aged 8-10 weeks or 18-20 weeks.

**₁: p < 0.01, *₁: p < 0.05 vs 8-10 week old mice, n = 4-8.

2. LOSS OF BRADYKININ-INDUCED INHIBITION OF PLATELET AGGREGATION IN THE HYPERCHOLESTEROLEMIC 18-20 WEEK OLD MICE

(A) Maximum variation (Δ) of MAP (mmHg) of PBS, ET 1, and BK in WT and ApoE^{-/-} mice aged 8-10 (A) and 18-20 weeks (B). Effects of the intravenous administration of vehicle (PBS) and two different doses of ET-1 (0.1 or 1 nmol kg⁻¹) or BK (2.5 or 10 nmol kg⁻¹) on platelet aggregation *ex vivo* these same strains of mice (C: 8-10 weeks old, D: 18-20 weeks old). n = 5-8 (8-10 weeks old), n = 10-16 (18-20 weeks old). ##₁: P < 0.01; #₁: P < 0.05 vs the same dose in WT mice. **₁: P < 0.01; *₁: P < 0.05 vs PBS in the same group of mice

3. NORMAL PGI₂ RELEASE IN VIVO AND PLATELET INHIBITION IN VITRO

Plasma concentrations of 6-keto PGF_{1α} following the intravenous administration of vehicle (PBS), ET-1 (0.1 nmol kg⁻¹) (A) or BK (10 nmol kg⁻¹) (B) in WT and ApoE^{-/-} mice (18-20 weeks old). n = 5-8. (C) Inhibition of ADP-induced platelet aggregation *in vitro*, obtained following addition of iloprost (ILO: 100 nM), a prostacyclin analogue, or sodium nitroprusside (SNP: 40 μM) in the PRP of WT and ApoE^{-/-} mice (18-20 weeks old). (n = 6-7. **₁: P < 0.01; *₁: P < 0.05 vs control (CTL).

6. REDUCED POTENCY OF LXR AGONIST TO INHIBIT PLATELET AGGREGATION IN A HYPERCHOLESTEROLEMIC PATIENT

In vitro platelet aggregation traces of washed platelets from a control subject or a familial hypercholesterolemic patient (FH), after stimulation with a protease activated receptor 4 (PAR-4) agonist or collagen. Platelets were pre-incubated with the LXR agonist T0901317 at increasing concentrations prior to aggregation.

4. PGI₂ INFUSION FAILS TO INHIBIT PLATELET AGGREGATION IN APOE^{-/-} MICE

(A) The average MAP profile of PGI₂ intravenous infusion (1 μg kg⁻¹ min⁻¹, 10 μl min⁻¹, 5 min) in WT and ApoE^{-/-} mice (8-10 and 18-20 weeks old). n = 10-12. (B) *Ex vivo* ADP-induced platelet aggregation in 18-20 week old WT and ApoE^{-/-} mice following PGI₂ infusion or its vehicle (PBS pH 9.0). n = 5-7. **₁: P < 0.01 vs. PBS; *₁: P < 0.05 vs PBS

5. BRADYKININ AND PROSTACYCLIN INCREASE PLATELET cAMP LEVELS IN THE WILD TYPE AND HYPERCHOLESTEROLEMIC MOUSE

Fig. 6. Intra-platelet cAMP concentrations following intravenous administration of vehicle (PBS), bradykinin (BK: 10 nmol kg⁻¹), iloprost (ILO: 10 μg kg⁻¹) or PGI₂ infusion (1 μg kg⁻¹ min⁻¹, 10 μl min⁻¹) in WT and ApoE^{-/-} mice aged 8-10 and 18-20 weeks. **₁: P < 0.01; *₁: P < 0.05 vs vehicle (PBS), injection in the same mouse strain from the same age. n = 5-8.

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Acknowledgments