Xanthine Oxidoreductase Mediates Nitrite-Induced Platelet Inhibition

Nitrite reduction by Xanthine Oxidoreductase modulates platelet reactivity and in part mediates platelet inhibition by inorganic nitrate in mice

Tipparat Parakaw , Rayomand Khambata , and Amrita Ahluwalia*

* corresponding author
a.ahluwalia@qmul.ac.uk

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"Xanthine oxidoreductase (XOR) is a potential missing link in platelet hyperactivity and cardiovascular diseases. Bioconversion of NO_2^- to NO by XOR modulates platelet reactivity as we observed rises in plasma NO_2^- levels after Xdh allele deletion in mice. This higher plasma NO_2^- in Xdh heterozygous mice was associated with greater platelet reactivity demonstrated in a tail bleeding, thrombus formation, and platelet aggregation study. There is also a potential of nitrate on platelet inhibition in wild-type mice, which developed smaller thrombus formation."

Background

Inorganic nitrate (NO_3^-) is a stable form of nitric oxide (NO) found in green leafy vegetables and beetroots. NO_3^- has been proposed as a potential therapy to improve cardiac function and decrease platelet-hyperactivity (Velmurugan et al. 2013, Velmurugan et al. 2016). These effects are based upon the capacity for NO_3^- to be converted to nitrite (NO_2^-) and further NO in the body. Whilst the conversion of NO_3^- to NO_2^- is governed by the host commensal bacteria, the conversion of NO_2^- to NO is facilitated by mammalian NO_2^- reductases (Lundberg, Weitzberg, and Gladwin 2008) (Figure 1A). One particular prominent NO_2^- reductase is xanthine oxidoreductase (XOR), however, whether this pathway plays a role in the effects of NO_2^- upon platelet activity is unknown. Thus, we assessed the role of XOR in mediating NO_2^- induced platelet inhibition using *Xdh* genetically deleted mice to assess plasma NO_2^- concentrations and platelet reactivity. In addition, the impact of *Xdh* deletion upon two-week NO_3^- supplementation in drinking water (15mM) on platelet functions was also determined.

Method

Wild type (WT), *Xdh* heterozygous (Het), and *Xdh* knock out (KO) mice were used to assess plasma NO_2^- by chemiluminescence and platelet reactivity. *In vivo* platelet function was assessed using tail bleeding (N=15) and a ferric chloride model of thrombosis using intravital microscopy (N=7), while platelet aggregation responses to collagen or protease-activated receptor-4 activating peptide (PAR4-AP) was measured using impedance aggregometry *ex vivo* (N=15). To explore the possibility that deletion of the gene impacted upon the effects of dietary NO_3^-, mice were treated with KNO_3 in drinking water (15 mM) or equimolar salt (15mM KCl) as a control for 2 weeks prior to assessing plasma NO_2^- concentrations, and thrombus formation (N=7). Because *Xdh* KO mice did not survive beyond 4 weeks, we were unable to use *Xdh* KO mice in all experiments.

Result

After *Xdh* allele deletion, rises in plasma NO_2^- levels were observed suggesting lack of conversion of NO_2^- to NO (Figure 1B). *Xdh* Het and KO mice expressed shorter tail bleeding times compared to WT mice. *Xdh* Het mice developed greater thrombosis responses to ferric chloride and increased platelet aggregation induced by collagen compared to WT mice (Figure 1C). Moreover, NO_3^- administration had the potential to increase NO_2^- levels in both WT and Het mice. However, this associates with decreased thrombosis responses in WT mice only.

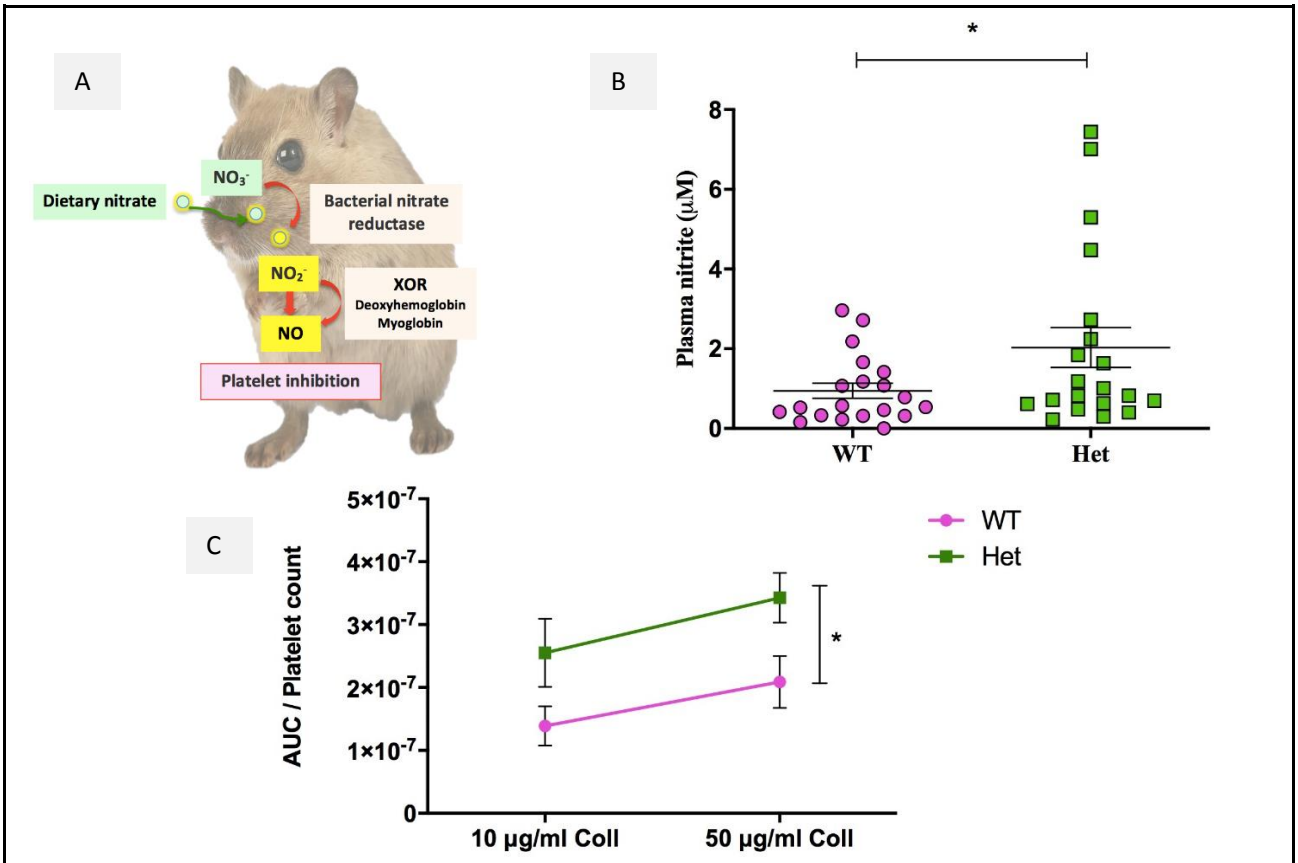

Figure 1 Role of XOR, plasma nitrite, and platelet aggregation in the *Xdh*$^{+/-}$ colony. XOR is responsible for bioconversion of NO_2^- to NO, which potentially modulates platelet activation (A). In this study, plasma collected from *Xdh* WT and Het mice was measured for nitrite by chemiluminescence (B). Platelet aggregation by collagen were recorded by impedance aggregometry and normalized to platelet count (C). Data are mean ± SEM. For plasma nitrite n=20 and platelet aggregation n=15. Statistical significance was determined using an unpaired T-test (A), and Two-way ANOVA followed by Bonferroni's multiple posttests (B) represented by *$P < 0.05$.

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Discussion and conclusion

Here we show for the first time that with each allele deletion there is a rise in nitrite levels in plasma. We speculate that this rise in nitrite levels may relate to a loss of nitrite processing from XOR to NO. Longer tail bleeding time, smaller thrombus formation and lower platelet aggregation observed in WT mice suggesting a role for XOR in mediating NO production and platelet activity. In addition, there is a potential of nitrate on platelet inhibition mediated by XOR.

About the authors

Ms Parakaw is a PhD student in Prof Ahluwalia laboratory at the William Harvey Research Institute, Barts and London Medical School, Queen Mary University of London, London, UK. This research is conducted under the supervision of Prof Ahluwalia, professor of pharmacology and Mr Khambata, a lecturer in vascular pharmacology at the William Harvey Research Institute. Prof Ahluwalia is the major contributor to the idea and project design and Ms Parakaw contributes to all laboratory experiments.

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References

Lundberg, J. O., E. Weitzberg, and M. T. Gladwin. 2008. "The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics." *Nat Rev Drug Discov* no. 7 (2):156-67.

Velmurugan, S., J. M. Gan, K. S. Rathod, R. S. Khambata, S. M. Ghosh, A. Hartley, S. Van Eijl, V. Sagi-Kiss, T. A. Chowdhury, M. Curtis, G. G. Kuhnle, W. G. Wade, and A. Ahluwalia. 2016. "Dietary nitrate improves vascular function in patients with hypercholesterolemia: a randomized, double-blind, placebo-controlled study." *Am J Clin Nutr* no. 103 (1):25-38.

Velmurugan, S., V. Kapil, S. M. Ghosh, S. Davies, A. McKnight, Z. Aboud, R. S. Khambata, A. J. Webb, A. Poole, and A. Ahluwalia. 2013. "Antiplatelet effects of dietary nitrate in healthy volunteers: involvement of cGMP and influence of sex." *Free Radic Biol Med* no. 65:1521-32.

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Office of Science and Technology, Royal Thai Embassy, Brussels, Belgium.
Tel: +32 (02) 675 0797
E-mail: info@thaiscience.eu

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