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CLINICAL RESEARCH

Human Second Window Pre-Conditioning and Post-Conditioning by Nitrite Is Influenced by a Common Polymorphism in Mitochondrial Aldehyde Dehydrogenase

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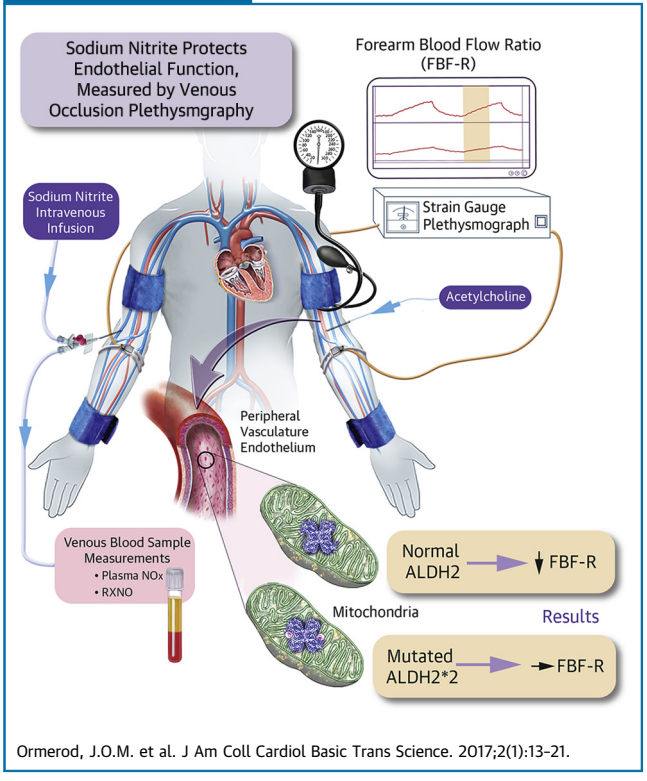
VISUAL ABSTRACT



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HIGHLIGHTS

- **Pre- (and peri-ischemia) conditioning is a potentially powerful protector against ischemia–reperfusion injury, and activation of ALDH2 may be a critical step.**
- **The authors demonstrate second-window pre-conditioning (i.e., with the stimulus 24 h before ischemia) with-low dose sodium nitrite in the vascular endothelium of healthy human volunteers.**
- **They go on to show that nitrite, administered during ischemia, also affords protection to vascular endothelium in participants with the common worldwide variant *ALDH2*2* enzyme, but not in those with wild-type ALDH2, using this particular protocol.**
- **This surprising result shows the challenges of translation in this particular area and the critical importance of dose, location, and timing of the conditioning stimulus.**

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ABBREVIATIONS AND ACRONYMS

Ach = acetylcholine

ALDH2 = mitochondrial aldehyde dehydrogenase

FBF = forearm blood flow

FBF-R = forearm blood flow ratio

GTN = glyceryl trinitrate

IR = ischemia-reperfusion

RIPC = remote ischemic pre-conditioning

SUMMARY

Pre-conditioning is an exciting physiological phenomenon that, despite great efforts, has so far resisted translation to mainstream clinical medicine. Many potential triggers (e.g., ischemia of the organ in question or a remote organ, many different drugs) have been investigated, but recent work has implicated activation of mitochondrial aldehyde dehydrogenase (ALDH2) as central to the process. A genetic polymorphism, known as *ALDH2*2*, is common worldwide (present in up to 40% of Han Chinese people) and produces a functionally different enzyme. The authors used a variety of protocols in the human ischemic forearm model, in participants with both enzyme types, to assess cytoprotection with low-dose sodium nitrite and attempt to further elucidate the role of ALDH2. (J Am Coll Cardiol Basic Trans Science 2017;2:13–21) © 2017 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

There has been great interest in possible beneficial effects of inorganic nitrite (NO_2) in recent years (1-3). Nitrite may have a role in hypoxic vasodilation (4) and has been shown to protect renal tissue (5,6), liver (7), and myocardium (8,9) from ischemia-reperfusion (IR) injury in animal models. Nitrate (NO_3^-), administered orally in beetroot juice, protected endothelium from IR injury in healthy human volunteers (9), an effect attributed to a 2-fold increase in plasma nitrite. More recent work by Ingram et al. (10) in a human forearm model of reperfusion injury demonstrated a protective effect of a 20-min intravenous infusion of sodium nitrite (1.5 μmol/min) administered before the onset of ischemia (pre-conditioning), but administration of the same nitrite infusion during forearm ischemia (post-conditioning) resulted in no reduction in the degree of endothelial dysfunction compared with placebo. This lack of protection from nitrite administered during an ischemic insult contradicts the protective effects observed by Gonzalez et al. (8) in a canine model of myocardial infarction. The NIAMI (Nitrite in Acute Myocardial Infarction) study randomized 280 patients with ST-segment elevation myocardial infarction to receive 70 mmol sodium nitrite or placebo intravenously in the 5 min immediately before reperfusion (11). This post-conditioning protocol demonstrated no difference in infarct size at either 8 days or 6 months as determined by cardiac magnetic resonance imaging.

Mitochondrial aldehyde dehydrogenase (ALDH2) is a member of the 19-strong human aldehyde dehydrogenase family of $NAD(P)^+$-dependent enzymes (12). A common polymorphism in exon 12 (Glu487Lys, or Glu504Lys in the unspliced protein), known as the *ALDH2*2* allele, is present in up to 50% of individuals of East Asian descent (13). Heterozygosity at this allele results in a near inactive enzyme and produces the "Asian Flushing" phenotype, a phenomenon linked to the accumulation of acetaldehyde following alcohol ingestion; mutation of a single subunit destabilizes the cofactor binding site and dimer interface such that heterozygotes are functionally similar to homozygotes with the variant allele (14). Individuals possessing 1 or 2 copies of the *ALDH2*2* allele may be at greater risk of coronary artery disease (15) and myocardial infarction (16). ALDH2 activation by phosphorylation has been postulated to be central to protection conferred against myocardial ischemia reperfusion injury (17). Pre-conditioning was induced by activation of PKCε (which phosphorylates ALDH2) and subsequently by a direct activator of ALDH2, alda-1. In a later study, the volatile anesthetic isoflurane induced cardioprotection in a rat model (18). Protection was associated with activation of ALDH2 and was abolished by an inhibitor of PKCε. On the basis of these data, combined with the observation that ALDH2 also exhibits intrinsic nitrite reductase activity (19), we hypothesized that an interaction between ALDH2 and nitrite might contribute to IR protection in humans.

We hypothesized that nitrite would be protective in the human forearm, either when administered 24 h before ischemia reperfusion ("second-window pre-conditioning") or when administered during ischemia (with the primary effect in the "post-conditioning" window), and that its protective effects would be modified by variations in ALDH2 activity. We used a combination of genetic and pharmacological tools in an established model of IR injury in the human forearm (20), to investigate protection by nitrite and the role of ALDH2.

METHODS

This study was approved by the local research ethics committee. All participants gave written informed consent. All studies were performed in a dedicated