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Downsides to the nitrate–nitrite–nitric oxide pathway in physiology and therapeutics?

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In their recent Review article (The nitrate–nitrite–nitric oxide pathway in physiology and therapeutics. *Nature Rev. Drug Discov.* **7**, 156–167 (2008))[1], Lundberg and colleagues describe the concept of the nitrate–nitrite–nitric oxide (NO) pathway in physiology and therapeutics. However, there are some issues that merit consideration with regard to its physiological and pharmacological significance.

First, the authors are proponents of nitrite as an NO donor under severe hypoxic conditions, and according to REF. 1, this mechanism, as mediated by deoxyhaemoglobin, should operate maximally at 50% haemoglobin saturation (P_{50}). However, although this state may exist momentarily in metabolically active tissues such as the heart, it must be appreciated that the pulmonary venous haemoglobin saturation is usually around 75% ($pO_2 \approx 40$ mm Hg).

Second, it is also not certain that hypoxic conditions are a prerequisite for nitrite bioactivation to NO. Although it is thought that xanthine oxidoreductase (XOR) performs its surrogate "nitrite reductase" role in hypoxic conditions[2], Gladwin and colleagues[3], who reported pO_2 values of around 20 mm Hg during exercise, were surprised that nitrite infusion caused vasodilation in the absence of "hypoxia and low pH", a finding that challenged the XOR mechanism. That nitrite is bioactivated to NO under aerobic conditions is perhaps illustrated by research related to steroid hormone synthesis, indicating that nitrate, and to a greater extent nitrite ions, inhibit steroidogenesis *in vitro* and *in vivo*[4,5] via NO, as the NO scavenger carboxy-2-phenyl-4,4,5,5,-tetramethylimidazoline-1-oxyl 3-oxide (cPTIO) reversed the inhibition. It is most likely that NO inhibits steroidogenesis by binding haem in steroidogenic cytochrome P450 enzymes. All these *in vitro* experiments with Leydig cells were done using buffers with the prevailing 5–6 mg per litre dissolved oxygen, a level that allows aquatic animals to survive in water[6]. Therefore, the requirement for hypoxia to bioactivate nitrite to NO is questionable.

Third, if the nitrate–nitrite–NO pathway operates, then the production of NO, the vasodilator, should indeed be perpetual, with L-arginine-NO synthase producing it when oxygen is abundant, and the former pathway activated under hypoxic conditions. However, this raises the question of why there is a need for therapeutic NO donors in patients with angina. Cardiac ischaemia should create a P_{50} state, and with ample blood nitrite (perhaps some arising from the damaged cardiac tissue), the pathway should generate enough NO to alleviate angina. Could it be that there is no blood nitrite for the purpose, because all of it has been metabolized? Indeed, there is controversy regarding blood nitrite concentrations, which have been reported to range from undetectable to 26 μM[7].

Finally, Lundberg and colleagues also alluded to the potential downsides to the therapeutic side of the pathway, including carcinogenesis (from nitrosoamines) and methaemoglobinaemia[1]. As mentioned above, endocrine disruption by way of steroid deficiency is another potential drawback[8]. The administration of nitrite and nitrate ions at levels allowable by the European Union in drinking water (50 mg per litre) significantly reduced the circulating levels of corticosterone and testosterone in rats[5]. In addition, the adrenal glands of treated animals contained lipid droplets similar to congenital adrenal hyperplasia due to some steroidogenic enzyme deficiencies[9]. This possibility of endocrine disruption due to steroid hormone deficiency should not be neglected when considering the therapeutic potential of targeting the nitrate–nitrite–NO pathway.

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