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THEMED SECTION: ENDOTHELIUM IN PHARMACOLOGY

COMMENTARY

Understanding organic nitrates – a vein hope?

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The organic nitrate drugs, such as glyceryl trinitrate (GTN; nitroglycerin), are clinically effective in angina because of their dilator profile in veins and arteries. The exact mechanism of intracellular delivery of nitric oxide (NO), or another NO-containing species, from these compounds is not understood. However, mitochondrial aldehyde dehydrogenase (mtALDH) has recently been identified as an organic nitrate bioactivation enzyme. Nitrate tolerance, the loss of effect of organic nitrates over time, is caused by reduced bioactivation and/or generation of NO-scavenging oxygen-free radicals. In a recent issue of the *British Journal of Pharmacology*, Wenzl *et al.* show that guinea-pigs, deficient in ascorbate, also have impaired responsiveness to GTN, but nitrate tolerance was not due to ascorbate deficiency that exhibited divergent changes in mtALDH activity. Thus, the complex function of mtALDH appears to be the key to activation of GTN, the active NO species formed and the induction of tolerance that can limit clinical effectiveness of organic nitrate drugs.
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The paper by Wenzl *et al.* is available from http://www3.interscience.wiley.com/cgi-bin/fulltext/122221718/PDFSTART

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Abbreviations: GDN, glyceryl dinitrate; GTN, glyceryl trinitrate; mtALDH, mitochondrial aldehyde dehydrogenase; ODQ, -[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one

Clinical efficacy of the organic nitrates

The organic nitrates, such as glyceryl trinitrate (GTN; nitroglycerin), have been used to provide relief from the symptoms of angina since the birth of modern pharmacology, yet we still do not fully understand how they work. Their clinical effectiveness in the treatment of angina and heart failure is due to their profile of dilator activity in large veins and arteries via a, as yet unidentified, method of delivering nitric oxide (NO) via enzymatic transformation. Recently, it has been demonstrated that their major bioactivation pathway appears to be through the mitochondrial enzyme, aldehyde dehydrogenase (mtALDH or ALDH2) (Chen *et al.*, 2002; Daiber *et al.*, 2009; Mayer and Beretta, 2008). However, there remain several unanswered questions including the mechanism of reactivation of mtALDH following GTN bioactivation, the intermediate form of NO produced and the mechanism of nitrate tolerance. Although it was recognized that GTN acted through NO before the physiological role of NO in the vascular endothelium was discovered, advances in NO pharmacology have largely passed by the organic nitrates. More recently developed classes of drugs, such as other NO donors, the NO adducts, phosphodiesterase inhibitors and the antioxidants have powerful actions yet do not bring the same clinical benefits as the organic nitrates (Miller and Megson, 2007).

Nitrate tolerance

A limitation of the organic nitrates is the loss of effect with continuous use due to the development of nitrate tolerance. Despite extensive investigation, the underlying cause of nitrate tolerance has yet to be established. Impaired bioactivation of nitrates is the most likely explanation, although other mechanisms such as the enhanced generation of

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NO-scavenging oxygen-free radicals (Munzel *et al.*, 1995) and, perhaps, endothelial dysfunction (see Daiber *et al.*, 2009) may also be involved. A clearer understanding of nitrate tolerance could lead to the development of new drugs free from this shortcoming. However, it is debatable how important this is for clinical practice as with the exception of a few variants of angina, significant tolerance can be avoided with asymmetric dosing.

GTN and ascorbate deficiency

In a recent issue of the *British Journal of Pharmacology*, Wenzl *et al.* (2009) use a guinea-pig model involving 3 days of GTN injections to induce nitrate tolerance, which was shown to cause inactivation of mtALDH. This group has presented the novel finding that GTN lowers plasma levels of ascorbate, although not tissue ascorbate. Their current research is founded on the hypothesis that ascorbate deficiency could underlie tolerance to organic nitrates (Wolkart *et al.*, 2008). Wenzl *et al.* (2009) showed that guinea-pigs that were deficient in ascorbate did indeed have impaired responsiveness to GTN. However, a detailed analysis of the pattern of GTN metabolites revealed fundamental differences between nitrate tolerance and ascorbate deficiency. Experimental nitrate tolerance was accompanied by a loss of the selective formation of 1,2-glyceryl dinitrate (1,2-GDN; the isomer associated with bioactivation of GTN); however, in ascorbate deficiency the normal 1,2-GDN : 1,3-GDN ratio was maintained. The authors conclude that while mtALDH is involved in GTN impairment of both models, there is a difference in the underlying mechanisms. Physiological intracellular reducing agents such as ascorbate appear to be important in the activation and/or tolerance of the organic nitrates, but we still lack a full picture of their role, and a number of questions remain, such as why does antioxidant treatment not always prevent/reverse nitrate tolerance, and how does the redox susceptibility of the mtALDH pathway fit into the proposed venous selectivity of the organic nitrates?

Does GTN act independently of NO?

Glyceryl trinitrate and the other organic nitrates activate soluble guanylate cyclase, and their vasodilator action is completely prevented by the selective soluble guanylate cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ). All the active members of the organic nitrate class incorporate a moiety capable of generating NO within their molecule, and chemically plausible pathways have been established whereby the organic nitrate molecule can be reduced to free NO. Yet even high concentrations of reducing agents, above that contained within cells, are unable to release NO from GTN in the absence of biological tissue (Miller and Megson, 2007). It remains a puzzle that, despite application of several extremely sensitive techniques (electron paramagnetic resonance with iron-diethyldithiocarbamate, cell-permeable NO fluorescent dyes and NO microelectrodes placed adjacent to the endothelium), no study has yet directly measured NO release from physiological concentrations of GTN in functioning vascular tissue (Kleschyov *et al.*, 2003; Nunez *et al.*, 2005; Miller *et al.*, 2008). This may merely be indicative of a specific intracellular site of nitrate activation that releases only minute amounts of NO that are locally active and rapidly consumed by the haem group of the target enzyme, guanylate cyclase. However, an alternative interpretation is that an NO-containing species, not NO itself, is the direct product emanating from organic nitrate biotransformation (Kleschyov *et al.*, 2003). Some recent evidence suggest that this pathway may only be relevant to high-potency organic nitrates such as GTN, and not other nitrates (including isosorbide mono-/di-nitrate), highlighting the observation that not all nitrates are the same. For example, pentraerithrityl tetranitrate does not develop tolerance and may have antioxidant properties (Daiber *et al.*, 2004), and the recently developed organic nitrate, LA419, may also act independently of NO in reducing left ventricular hypertrophy (Ruiz-Hurtado *et al.*, 2007).

Conclusions

Clearly there is some way to go before we fully establish the bioactivation pathway of the organic nitrates, the intermediate species and the underlying cause of nitrate tolerance. Through the use of detailed investigations, such as that by Wenzl *et al.* (2009), we will get closer to the answers to persistent questions, such as why do certain organic nitrates act differently and in what way does this explain their clinical efficacy? These questions become even more pertinent with the design of hybrid drugs that contain nitrate groups in order to maximize their actions or minimize side effects through NO-mediated pathways (Miller and Megson, 2007). In its long life, GTN has been transformed from a dangerous explosive to a healing medicine. After more than a century of research we have accumulated extensive knowledge of the action of organic nitrates on the cardiovascular system, yet there remains a great deal more that we still need to NO.

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Conflict of interest

None.

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