Organic nitrates are still quite frequently used for the treatment of acute and chronic angina, and acute and chronic congestive heart failure. When given acutely, their effectiveness is undisputable; however, their long-term efficacy is substantially limited due to the development of tolerance (1) and the induction of endothelial dysfunction (2), a parameter that has prognostic significance in patients with coronary artery disease, hypertension, and heart failure (3).

In this issue of the Journal, Thomas et al. (4) showed that therapy with isosorbide mononitrate (ISMN) leads to endothelial dysfunction, increases oxidative stress, and that this condition is improved by acute administration of vitamin C. One might conclude that this was predictable since this has been shown for nitroglycerin (GTN) and all nitrates are the same. In fact, all nitrates are not all the same and, therefore, the paper by Thomas et al. (4) has substantial importance.

Organic nitrates need to undergo intracellular metabolism in order to produce vasodilatation, a process often referred to as bioactivation. During the past 3 to 4 years, substantial insight into this bioactivation process has been gained and we have also learned a substantial amount about the mechanisms underlying nitrate tolerance. In the early 1990s, we found that treatment of animals with GTN patches caused tolerance and endothelial dysfunction (the phenomenon of cross-tolerance) and that this phenomenon was linked to the capacity of GTN to stimulate the production of reactive oxygen species (ROS) within the vascular tissue (5).

As potential superoxide sources, the vascular nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (6) and an uncoupled nitric oxide (NO) synthase (7) were identified. At that time we believed that this concept could be extended to all other nitrates. There was no reason to believe that different organic nitrates such as GTN, pentaerythrityl tetranitrate (PETN), isosorbide dinitrate (ISDN), and ISMN were bioactivated by different enzymes. This view, however, has changed substantially within the last couple of years.

A breakthrough in this field came with the discovery that the mitochondrial aldehyde dehydrogenase (ALDH-2) was involved in this bioactivation process and could metabolize GTN leading to NO or a related compound (Fig. 1) (8). In cooperation with Chen et al. (8), Sydow et al. (9) demonstrated that oxidative stress within the mitochondria caused inactivation of ALDH-2, and that this is a key mechanism underlying impaired GTN biotransformation during prolonged treatment of cultured cells, animals (9), and patients (U. Hink, unpublished observation, 2006) with GTN. This observation linked the oxidative stress concept of tolerance with the impaired biotransformation concept of nitrate tolerance.

Interestingly, ALDH-2 is only responsible for bioactivation of nitrates with the highest vasodilator potency such as GTN and PETN as well as for the PETN-derived trinitrate PETriN; ALDH-2 does not seem to be involved in bioactivation of dinitrates and mononitrates (Fig. 1). Recent studies by our group suggest that the propensity for ALDH-2 to bioactivate an organic nitrate is related to the number of nitrate moieties within the compound (10).

It is also important to note that, in ALDH-2 knockout animals, the shift of the GTN dose–response curve is identical as compared with the shift observed in vessels from wild-type animals treated with an ALDH-2 inhibitor. Importantly, inhibition of this enzyme by knockout or inhibitors, however, does not completely prevent the GTN and PETN vasodilating capacity suggesting that other enzymes are almost certainly involved in GTN bioactivation. A likely candidate is 1 or more cytochrome P450 subtypes in the endoplasmic reticulum. These alternate enzymes seem to be involved in the metabolism of higher concentrations—a so-called low-affinity pathway for GTN biotransformation (11). Thus, it is likely that GTN and PETN undergo biotransformation via completely different enzymes than ISDN and ISMN (Fig. 1). Of note, incubation of isolated mitochondria with GTN markedly increases mitochondrial superoxide production while incubation with ISMN does not (10). This observation is important since both tolerance and cross-tolerance to endothelium-dependent vasodilators have been recently linked to the capacity of GTN to stimulate mitochondrial superoxide formation (12).

Based on this background, the study by Thomas et al. (4) should be of substantial interest to cardiologists because mononitrates are still frequently used worldwide to treat patients with stable angina symptoms. These investigators treated healthy volunteers with isosorbide-5-mononitrate (IS-5-MN) once daily or with a placebo. One week of ISMN treatment caused a marked blunting of the increase in forearm blood flow in response to acetylcholine. In

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addition, the vasoconstriction caused by the NO synthase inhibitor N\textsuperscript{G}-monomethyl-L-arginine was substantial reduced. These 2 findings suggested that both baseline and stimulated bioactivity of endogenous endothelial NO was altered by ISMN therapy. The exciting point was that administration of vitamin C completely normalized vasodilation induced by acetylcholine demonstrating a crucial role of superoxide anions or other related ROS in this vascular abnormality caused by ISMN therapy. Taken together, this is the first study to show that mononitrates cause endothelial dysfunction and the first study to show that treatment with mononitrates stimulates oxidative stress within vascular tissue.

What is the source of superoxide in the case of ISMN-induced tolerance? Surprisingly, there have been no studies of experimental animals using mononitrates to address this question. It is unlikely the mitochondria because, as mentioned above, ISMN is not bioactivated by the mitochondria. Likely candidates for superoxide-producing enzymes contributing to endothelial dysfunction in response to ISMN include xanthine oxidase, the vascular NADPH oxidase, and also an uncoupled NO synthase.

Thus, the results of Thomas et al. (4) will stimulate additional research on this topic, and it is likely that the enzyme involved in mononitrate bioactivation, still a mystery, will be identified.

The study by Thomas et al. (4) might be criticized because it was performed in control subjects rather than patients, which may make it difficult to translate these data into the clinical situation. In our opinion, this is not a problem at all. Usually, in patients with coronary artery disease, vascular oxidative stress already exists. Thus, treating these patients with NO (via mononitrates) very likely will lead to formation of the NO/superoxide reaction product peroxynitrite (ONOO\textsuperscript{-}) (13), which in turn can impair endothelial function by uncoupling endothelial NO synthase (7), by causing tyrosine nitration of prostacyclin synthase leading to a reduction of PGI\textsubscript{2} formation (13) and by inhibiting the NO signaling within smooth muscle cells. Thus, the demonstration that endothelial dysfunction can be induced in control subjects, where NO generally exceeds superoxide production, illustrates to us how potently mononitrates diminish endothelial function.

The results of this study might also explain the recent meta-analysis showing how treatment of post-infarction patients with mono- and dinitrates actually worsened their outcome (14).

These results also again challenge the concept that patients with coronary artery disease and stable angina should routinely be treated with nitrates to improve symptomatology and raise the question of whether organic nitrates should be used at all. The guidelines of the American Heart Association/American College of Cardiology for treatment of stable coronary artery disease have already taken this question into account and recommended short-acting nitrates or a calcium antagonist such as amloidipine rather than a mono- or dinitrate, when patients remain symptomatic even after treatment with a beta-blocker, aspirin, and a statin (15).
REFERENCES


