Organic nitrates have served as mainstays of therapy for the treatment of coronary artery disease for more than a century. Their use, however, has been complicated by unwanted side effects, including hypotension and the development of nitrate tolerance. Nitrate tolerance is a term used to define tachyphylaxis to nitrates with long-term use. The mechanisms of nitrate tolerance have been debated for some time and include depletion of thiols, an increase in venous blood volume limiting vasodilator responsiveness, and increased generation of reactive oxygen species.

Recent observations provide evidence for the reactive oxygen species hypothesis: nitrate tolerance is associated with an increase in the vascular production of superoxide anion, and the sources of this superoxide are membrane-bound NAD(P)H oxidase and endothelial nitric oxide synthase itself. The superoxide anion produced by these enzyme systems inactivates the nitric oxide derived from the metabolism of the organic nitrate by forming peroxynitrite; evidence for this process has been found by observing an increase in 3-nitrotyrosine in the urine of nitrate-tolerant subjects.

An additional feature of nitrate tolerance is the recognition that cross-tolerance to endogenous endothelium-dependent vasodilators also occurs. The mechanism by which an organic nitrate suppresses the bioactivity of endothelial nitric oxide involves several determinants. Vascular superoxide anion can react with and inactivate endogenous nitric oxide just as it can nitrate-derived nitric oxide. In addition, organic nitrates increase superoxide production by endothelial nitric oxide synthase via a protein kinase C–dependent mechanism.

The “uncoupling” of endothelial nitric oxide synthase activity, ie, the conversion of the oxidoreductase’s enzymatic activity from the oxidation of L-arginine to the reduction of molecular oxygen, by long-term organic nitrate exposure is, therefore, common to both tolerance to organic nitrates and cross-tolerance to endogenous nitric oxide. Complementing this increase in superoxide anion generation is a decrease in Cu/Zn superoxide dismutase, further enhancing the steady-state flux of vascular superoxide anion in nitrate-tolerant individuals.

Central to the increase in superoxide production by endothelial nitric oxide synthase is a decrease either in the substrate L-arginine or in a critical cofactor required for nitric oxide generation by the enzyme, tetrahydrobiopterin. When tetrahydrobiopterin levels fall below the concentration required to saturate the enzyme (ie, \( \approx 2 \, \text{µmol/L} \)), superoxide forms by heme-catalyzed reduction of molecular oxygen.

In 1973, Needleman and colleagues first recognized the redox-dependence of the action of organic nitrates. Several studies followed and indicated that nitrate tolerance is, in part, dependent on the thiol redox state of vascular smooth muscle or platelets and that exogenous thiols could ameliorate nitrate tolerance. The reported benefits of improving the thiol redox state include enhancing the reductive denitrification of organic nitrates; improving the activity of guanylyl cyclase by protecting critical thiols from oxidation, especially its active-site vicinal dithiols; and protecting nitric oxide from oxidative inactivation by reactive oxygen species, both by scavenging oxygen-derived free radicals and by forming the more stable S-nitrosothiol derivatives, which are themselves less likely to induce tolerance.

More recently, other rational pharmacological approaches to the prevention of nitrate tolerance have met with similar success, including the use of ascorbate, hydralazine, and \( \alpha \)-tocopherol as antioxidants; angiotensin-converting enzyme inhibitors to attenuate the angiotensin II–dependent activation of NAD(P)H oxidase; tetrahydrobiopterin to restore pools of this reductive cofactor for nitric oxide synthase and to prevent uncoupling of enzyme activity; and L-arginine to prevent uncoupling and to enhance nitric oxide production by providing more substrate to endothelial nitric oxide synthase.

In the present edition of Circulation, Gori and colleagues report that another simple pharmacological intervention, folic acid, can prevent nitroglycerin-induced nitrate tolerance and cross-tolerance to endothelial nitric oxide. Building on prior published work in vitro and in human subjects with and without hyperhomocyst(e)inemia, these investigators posited that folic acid should enhance the bioavailability of tetrahydrobiopterin in the setting of the oxidant stress accompanying long-term nitroglycerin administration. The results of their study support this hypothesis, but do so without a clearly established mechanism. In the setting of hyperhomocyst(e)inemia, the benefits of folic acid are clear-cut: folate is required for the remethylation of homocysteine to methionine, which, in turn, reduces the concentration of homocysteine available to support oxidative stress. However, in the
absence of hyperhomocyst(e)inemia, the mechanism of folate’s action is not obvious.

Folate is a vitamin essential for methylation reactions. Dietary folate must be reduced to tetrahydrofolate to enter the methylation cycle, and this reduction is accomplished by dihydrofolate reductase using NADPH as a cofactor. NADPH is also a required cofactor for nitric oxide synthase activity and for the synthesis of tetrahydrobiopterin from dihydrobiopterin. It is directly involved in the catalytic formation of nitric oxide, principally by mediating reductive activation of the ferrous heme-O₂ moiety of the enzyme; this mechanism likely also accounts for the benefits of ascorbate in potentiating the action of endothelial nitric oxide synthase. In support of this mechanism, recent evidence suggests that there is a peridrin binding domain in nitric oxide synthases that is similar to the folate-binding site of dihydrofolate reductase, and this may serve as a site through which 5-methyltetrahydrofolate acts to facilitate tetrahydrobiopterin’s radical-mediated electron transfer to heme.

Whatever the precise molecular mechanism, the observation that this simple intervention improves endothelial function and ameliorates nitrate tolerance has great clinical implications. These data can be generalized to advocate for the broad use of folic acid for patients with atherothrombotic vascular disease (provided they do not have vitamin B12 deficiency), regardless of their homocyst(e)ine status. By preventing the uncoupling of endothelial nitric oxide synthase and its generation of superoxide anion, this straightforward treatment will enhance endothelial and vascular function. Clearly, these short-term improvements in vascular function will need to be assessed in longer term studies that also have clear clinical endpoints. Nevertheless, these intriguing and encouraging results support the need for further studies of this simple treatment strategy, both to prevent nitrate tolerance and thereby broaden the use of these age-old agents in the treatment of cardiovascular disease and to improve endothelial function and restore vascular health in patients at risk for atherothrombotic disease.

References


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Folate and Nitrate-Induced Endothelial Dysfunction: A Simple Treatment for a Complex Pathobiology
Joseph Loscalzo

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