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Nitroglycerin-mediated *S*-nitrosylation of Proteins: A Field Comes Full Cycle

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Nitroglycerin; tolerance; *S*-nitrosylation; guanylate cyclase

Nitroglycerin (glyceryl trinitrate, GTN) has been an important part of the management of patients with angina or heart failure for over 135 years. GTN works through a combined action on the venous circulation and coronary vasculature to reduce preload and improve myocardial blood flow.[1] Its attributes include a potent vasodilatory action on diseased coronary vessels as well as anti-ischemic effects elicited in the microcirculation.[1,2] Dilation of conduit vessels by GTN is mediated in large part through nitric oxide (NO) binding to heme within, and activation of, soluble guanylate cyclase (sGC) in vascular smooth muscle, thereby leading to induction of the second messenger, cyclic GMP. The microvascular action of GTN involves additional effects on red blood cells (RBCs) to improve rheology and oxygen delivery.[2] GTN is an exceptionally potent vasodilator compared to other organic nitrates (isosorbide di- or mono-nitrates), but loses efficacy over time. Tachyphylaxis to GTN is initially specific to GTN (mechanism-based tolerance), but is ultimately associated with diminished responsiveness to other nitro(so)vasodilators (cross-tolerance) and even other classes of drugs (as a result of fluid retention and perhaps cellular injury).[1,3,4] Tolerance and cross-tolerance have generally been thought of in terms of an NO deficiency, resulting in attenuated sGC activity.[5,6] Sayed and co-workers had found recently that *S*-nitrosylation of sGC (the addition of an NO group to a cysteine thiol) by endothelium-derived NO inhibits sGC activity,[7] and they now report that exposure to GTN can result in the *S*-nitrosylation and desensitization of sGC, thereby providing a mechanism for cross-tolerance.[8] In other words, they suggest that aberrant or misdirected NO bioactivity, rather than NO deficiency per se, may contribute to cross-tolerance. These findings are consistent with an emerging paradigm in NO biology in which NO-based signaling is elicited in substantial part by *S*-nitrosothiols (SNOs) and accordingly, dysregulated protein *S*-nitrosylation contributes to cellular dysfunction and disease.[9,10] These new results also help elucidate the long-recognized importance of *S*-nitrosothiols in GTN biotransformation and metabolism.

Classic studies by Murad, Ignarro and Furchgott originally identified the activity of GTN with that of the endothelium-derived vasodilator, NO.[11] Both GTN and NO activated sGC in situ. It is now understood, however, that NO bioactivity cannot be readily differentiated from that of endogenous SNOs, which mediate vasorelaxation and whose role in regulation of vascular resistance has been established by stringent genetic criteria.[12,13] SNO-based activity is transduced by sGC/cGMP and by *S*-nitrosylation of proteins. It is therefore of interest that a

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large part of the acetylcholine-mediated relaxation in the classic Furchgott bioassay (rabbit thoracic aorta) is in fact preserved after inhibition of sGC,[14,15] and probably attributable to *S*-nitrosylation of the charybdotoxin-sensitive potassium channel and perhaps of calcium ATPase.[12,14] The case for *S*-nitrosothiols is perhaps even stronger in the microcirculation. Harrison and colleagues noted long ago that coronary microvessels are far more responsive to low mass nitrosothiols such as *S*-nitrosocysteine than to NO itself.[16] *S*-nitrosothiols are also impervious to the NO-scavenging chemistry of hemoglobin (Hb), which is of particular importance in small vessels where the effective concentration of Hb is highest. Interestingly, vasodilation by GTN is markedly less efficacious in small versus large coronary vessels and is greatly potentiated in microvessels by the addition of cysteine,[16-18] which reacts with GTN to produce *S*-nitrosocysteine.[19] Thus, the role of cGMP in the action of GTN in the microcirculation (especially during low flow states), and more generally in the control of microcirculatory blood flow, is poorly understood. In view of this, and of atypical features of the hamster cheek pouch preparation used by Sayed et al[8] (which is not representative of vascular beds that contribute principally to the effects of nitro(so)vasodilators), their findings will need to be confirmed in more relevant vascular systems.

The observations of Sayed et al[8] nonetheless shed new light on shared biochemical and physiological properties of GTN and *S*-nitrosocysteine with respect to cross-tolerance. These results are reminiscent of early work by Ignarro[19] on the participation of *S*-nitrosothiols, particularly *S*-nitrosocysteine, in GTN biotransformation, and of work by Needleman,[20] who suggested that oxidation of protein thiols may constitute a mechanism of GTN tolerance. Recent experiments by Kaul and colleagues[2] further suggest that the principal function of these *S*-nitrosothiols may be in the microcirculation where they subserve RBC-mediated control of blood flow. Notably, GTN augments the *S*-nitrosylation of hemoglobin in tandem with the increases in oxygen delivery mediated by RBCs.[2] *S*-nitrosohemoglobin is in equilibrium with low-mass SNOs, which convey NO bioactivity from RBCs,[13,21,22] consistent with the accumulating evidence that SNOs play central roles in hypoxic vasodilation, a mechanism that is pivotal in relief from ischemia (Figure).

One of the great enigmas in the study of GTN has been the inability to detect NO as a byproduct.[23] The likely solution is found in the recent discovery that GTN is bioactivated within mitochondria by the enzyme aldehyde dehydrogenase (MtALDH or ALDH 2).[24,25] The main product is nitrite. However, whereas the cytosolic isoform of aldehyde dehydrogenase also generates nitrite, only the mitochondrial enzyme subserves vasodilation.[4,24,25] Cytosolic nitrite is thus effectively inert. Rather, either nitrite within mitochondria or some other action of MtALDH[26] generates vasodilatory NO bioactivity that is exported to dilate blood vessels, and that bioactivity is not conveyed by NO itself. It is therefore of interest that this activity is precisely replicated by *S*-nitrosoglutathione (GSNO) (which may undergo further biotransformation to *S*-nitrosocysteine).[24] Furthermore, excessive amounts of GTN or SNO may oxidize the active site thiols of MtALDH, providing a mechanism for tolerance.[4,27-29] Increased amounts of *S*-nitrosothiols will also *S*-nitrosylate and/or oxidize mitochondrial respiratory proteins (complex 1),[30] leading to an oxidant leak[4] that can accentuate cross-tolerance. Increased *S*-nitrosylation (nitrosative stress) thus begets oxidation (oxidative stress), a formula underlying tolerance and cross-tolerance.

There are about 750 million individuals worldwide with the Asian variant of MtALDH. These individuals do not respond appropriately to GTN.[31,32] Notably, isosorbide dinitrate is not metabolized by MtALDH,[24] and may represent an appropriate first-line agent for these patients. The proper dosing of GTN during intravenous administration is not known, and it would seem appropriate to restudy this drug in light of the new information on mechanisms of biotransformation, tolerance and cross-tolerance. Monitoring of MtALDH activity may allow for therapeutic benefits of GTN without induction of tolerance.

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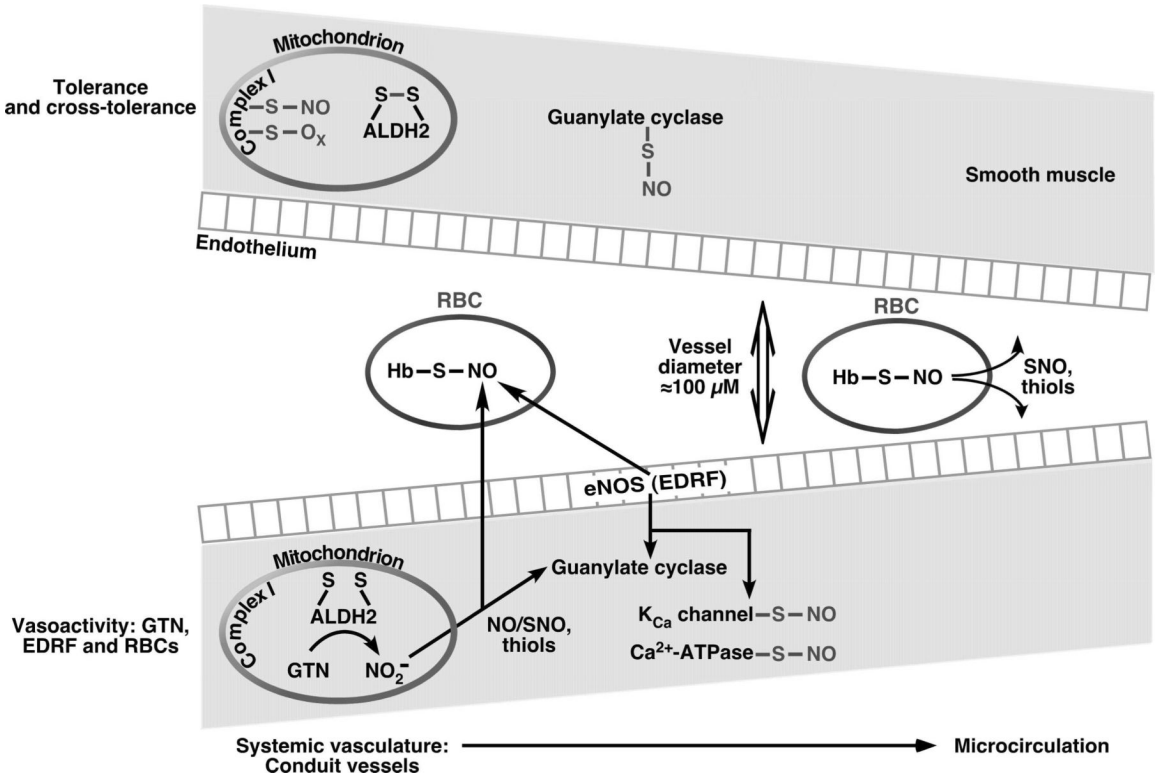

Figure.

The vasoactivity of nitroglycerin derives from its biotransformation (veins > arteries) by mitochondrial ALDH[33,34] and through a microcirculatory effect that is mediated by RBCs[2]. *S*-nitrosothiols likely contribute substantively to the actions of GTN. The action spectrum of GTN thus overlaps that of NO bioactivity derived from the endothelium (shear- or G-protein coupled receptor-mediated) as well as RBCs (hypoxia regulated), which is conveyed at least in part through protein *S*-nitrosylation, as well as activation of guanylate cyclase. Prolonged use of GTN results in the aberrant *S*-nitrosylation and/or oxidation of MtALDH, mitochondrial complex 1, guanylate cyclase (as shown in present paper) and likely other proteins, which constitute the basis of tolerance and cross-tolerance (see text).