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COMMENTARY

Arginine and nitrate tolerance

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Organic nitrates remain in widespread use as NO donors for the treatment of coronary artery disease and chronic heart failure, where dilator effects on capacitance veins and conduit arteries contribute to their therapeutic efficacy. Tolerance to the dilator effects of nitrates remains a persisting therapeutic problem, often occurring within 24 h of continuing use, and leading to the emergence of break-through symptoms. To date the only effective strategy to prevent tolerance is to ensure nitrate free intervals during treatment, but this deprives patients of continuous treatment with NO donors (Glasser, 1999).

Over the past 20 years the mechanism of nitrate tolerance has been investigated in animal and human blood vessels and a complex picture has emerged. Local mechanisms have been implicated and nitrate tolerance has been induced in isolated vessels using high concentrations of nitrates for short incubation periods (1–2 h). Organic nitrates require metabolism to release NO, and certain studies indicate that tolerance is secondary to reduce metabolism as a result of thiol depletion (Needleman & Johnson, 1973). However, there is also evidence for cross-tolerance between nitrates, other NO donors and endogenous NO-dependent relaxation, suggesting that there are additional mechanisms independent of the biotransformation of organic nitrates (Münzel *et al*., 2000). These include changes in the smooth muscle response to NO through reduced responsiveness of soluble guanylate cyclase to NO (Moncada *et al*., 1991) or increased phosphodiesterase activity (Bohyn *et al*., 1991). Recent data implicates the endothelium in the development of nitrate tolerance, because in some models endothelium-denuded vessels are partly resistant to the development of tolerance. This effect of the endothelium has been ascribed to the generation of superoxide radicals (Münzel *et al*., 2000). Nitrate tolerance can also be induced *in vivo* by administration of nitrates systemically over several days, where activation of the sympathetic and renin-angiotensin systems and intravascular volume expansion have been implicated as mechanisms of resistance to the dilator actions of nitrates (Cheesman & Benjamin, 1994).

In the current edition of the journal, Abou-Mohammed *et al*. presented intriguing data demonstrating that nitrate tolerance in isolated blood vessels can be prevented by L-arginine. Using a standard protocol for inducing nitrate tolerance *in vitro* (incubating vessels for 2 h with a supramaximal relaxant concentration of glyceryltrinitrate; GTN), the resulting 40 fold shift in the EC_{50} to GTN was largely prevented by the presence of L-arginine in the organ bath. Abou-Mohammed *et al*. hypothesise that GTN causes vasorelaxation in part through stimulation of eNOS, that prolonged exposure to GTN causes L-arginine depletion through activation of eNOS, and this in turn contributes to the development of tolerance to GTN. In support of this hypothesis, GTN reduced tissue arginine in rat aorta, and caused an approximate 40% increase in arginine uptake and apparent eNOS activity in cultured endothelial cells.

However, there are a number of problems with this hypothesis. Firstly, the finding that GTN stimulates eNOS is surprising, given the well-recognized effects of NO and NO-donors to inhibit NOS isoforms through reaction with the haem moiety (Buga *et al*., 1993). Recently Münzel *et al*. (2000) have demonstrated that induction of nitrate tolerance *in vivo* reduced NO generation by eNOS in rat aortic rings, a finding that is inconsistent with the data presented by Abou-Mohammed *et al*. It is possible that the increase in citrulline production measured in endothelial cells exposed to GTN merely reflects increased uptake of L-arginine, and that eNOS activity *per se* is unaffected. Conclusions drawn about eNOS activity using the photometric assay are likely to be confounded by NO generated directly from GTN.

Secondly, given that L-arginine affords a large degree of protection against GTN tolerance (and implies a major role for reduced eNOS activity), the small reduction in relaxation to acetylcholine in GTN-tolerant vessels in puzzling. If their hypothesis is correct, one would expect reduced eNOS activity to have at least as great an effect on an endothelium-dependent relaxant as on the GTN response, and that incubation with L-arginine would be expected to cause a major increase in the response to acetylcholine (which was not demonstrated in this or a previous study (Münzel *et al*., 2000)). The role of the endothelium in the action of L-arginine to prevent tolerance to GTN would be clear if studies were performed on endothelium-denuded vessels. Based on their hypothesis, L-arginine would have no protective effect in this setting.

This begs the question of how arginine might be working in these studies. The lack of a significant effect of D-arginine suggests that L-arginine is acting through an enzymatic process, rather than as a chemical anti-oxidant (Rehman *et al*., 1997). It is possible that L-arginine prevents superoxide generation by eNOS, but this would imply cross tolerance with other NO donors as have been shown previously (Münzel *et al*., 2000), but which was not evident in the present study. L-arginine might increase the biotransformation of GTN to NO, but there is no biological precedent for this.

Irrespective of these mechanistic uncertainties, these observations add L-arginine to the growing list of agents that have been shown to reduce nitrate tolerance in animal (vitamin C, ACE inhibitors, glutathione) and human (ACE inhibitors, beta adrenergic blockers, hydrallazine, N-acetylcysteine, dipyridamole) studies. It remains to be determined whether exogenous arginine might also be effective in the prevention of GTN tolerance *in vivo* where, in contrast to the organ bath, there is no shortage of L-arginine.

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