Letter to the Editor
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Vascular tolerance to nitroglycerin in ascorbate deficiency: results are in favour of an important role of oxidative stress in nitrate tolerance

In their recent article ‘Vascular tolerance to nitroglycerin (GTN) in ascorbate deficiency’ Wölkart et al. demonstrated an impaired vasodilator potency of GTN in ascorbate-deficient guinea pigs.1 The authors also conclude from the lack of an effect of polyethylene-glycolated superoxide dismutase (PEG-SOD) and catalase (PEG-catalase), that the impairment of GTN vasodilator potency induced by ascorbate deficiency is not mediated by induction of oxidative stress (their Figure 3). Although these data are interesting, especially in the context of the previous literature suggesting a role of oxidative stress in the development of nitrate tolerance, we believe that several points deserve further discussion.

Recent research emphasizes the role of mitochondria in the bioactivation of organic nitrates and in their clinical processes that mediate their clinical implications, including release of a nitric oxide (NO)-containing vasodilator, induction of ischaemic preconditioning, and tolerance.2–4 A number of studies, using antioxidants of different type and different affinity, show that the release of reactive oxygen species (ROS), which appears to be instantaneous upon exposure to GTN, is a key mediator of at least the latter two processes.2,5 Of note, administration of PEG-SOD and PEG-catalase, for structural characteristics of these molecules, would be unable to modify mitochondrial ROS production, which supports an alternate explanation to the findings of Wölkart et al., i.e. that depletion of ascorbate might have impaired the redox balance of mitochondria, causing, among other changes, partial oxidative inhibition of the nitrate bioconverting enzyme aldehyde dehydrogenase-2 (ALDH-2). Because PEG-SOD and PEG-catalase would not interfere with this effect of ascorbate depletion, the absence of any effects of these antioxidants would not be unexpected. In support of this interpretation, and consistent with a low penetration depth of these antioxidants, it was repeatedly shown that extracellular superoxide dismutase overexpression (which is mimicked by PEG-SOD) does not affect smooth muscle dependent relaxation [e.g. in response to sodium nitroprusside or dethylation NONOate] while improving oxidatively impaired endothelial function.6–8 Also the concentration of PEG-SOD (100 U/mL) used by Wölkart et al. may be not enough for competitive interference with the decrease in NO bioavailability. Use of lipophilic antioxidants (since GTN is also highly lipophilic) in future studies would solve this issue.

Depletion of ascorbate necessarily modifies the redox-balance of the cytoplasm and intracellular organelles,14,15 and results in oxidative stress.16–18 Measurement of the redox state of thiols (e.g. glutathione:glutathione disulfide) would have clarified this issue, particularly because activity of the ALDH-2 is highly sensitive to thiol oxidation.2,19,20 Data presented in Figure 4 of the paper indeed show a difference in the effects of daidzin and chloralhydrate between control and ascorbate-deprived animals. The absolute shift could be twice as high in ascorbate-deficient vessels. This may suggest that ascorbate-deficient tissue is more sensitive to ALDH-2 inhibition, which in turn could reflect a decreased expression and/or activity of ALDH-2. The interpretation of these data appears very complex, and direct assessment of the activity of ALDH-2 would have solved this issue (unfortunately the expression cannot be assessed since the sequence of guinea pig ALDH-2 is yet unknown).

Interestingly, a recent paper from the same laboratory proposes the involvement of ALDH-1 as the second GTN bioactivation pathway (and probably the backup system in case of ALDH-2 inefficiency).21 The authors show that ALDH-1 is at least 10-fold less efficient in bioactivating GTN (Figure 5 in Ref.21) suggesting that the thiols in ALDH-1 are less activated as compared with those in ALDH-2 and nucleophilicity of the ALDH-1 is presumably 10-fold less as compared with ALDH-2-thiols. This would again suggest that shifts in the redox balance would gradually impair these GTN bioactivating pathways.

The interaction of ascorbate and nitrate therapy is extremely complex, and even more so when one tries to dissect it from any redox effect of the two drugs. Evidence that nitrate tolerance clearly continues to develop in ascorbate-deprived animals suggests that there have to be ascorbate-independent mechanisms of nitrate bioactivation and function. In humans, while some studies have shown an effect of ascorbate on nitrate tolerance, others failed to do so.22 This question is further complicated by the scarce absorption of orally administered ascorbate.23 In sum, while we believe that the data of Wölkart et al. are interesting and deserve attention, we also believe that, in the absence of measures of mitochondrial or cellular oxidative stress and of ALDH-2 activity, and in the absence of observations on the effects of more potent antioxidants, they do not lead to the firm conclusion that ROS production is not a key factor in nitrate pharmacology.

References

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