The effect of peroxynitrite decomposition catalyst MnTBAP on aldehyde dehydrogenase-2 nitration by organic nitrates: role in nitrate tolerance

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Bioconversion of glyceryl trinitrate (GTN) into nitric oxide (NO) by aldehyde dehydrogenase-2 (ALDH-2) is a crucial mechanism which drives vasodilatory and antiplatelet effect of organic nitrates in vitro and in vivo. Oxidative stress generated by overproduction of free radical species including superoxide anions and NO-derived peroxynitrite, has been suggested to play a pivotal role in the development of nitrate tolerance, though the mechanism still remains unclear. Here we investigated on the possible role of peroxynitrite in attenuating the bioconversion of organic nitrate esters into NO via nitration of ALDH-2. Human washed platelets were made nitrate tolerant by incubation for 6 h with GTN. This was expressed by attenuation of platelet aggregation induced by thrombin (60 U/ml). This effect was accompanied by reduction of cGMP levels in platelets undergoing thrombin-induced aggregation and by attenuation of nitrite formation, suggesting a reduced generation of NO. In addition, a prominent nitration of platelet ALDH-2 was found in GTN-tolerant platelets compared to control cells. GTN tolerance in platelets was antagonized by co-incubation of platelets with Mn(III)tetrakis(4-Benzoic acid) porphyrin (MnTBAP), a selective peroxynitrite decomposition catalyst. Furthermore, MnTBAP reduced platelet ALDH-2 nitration subsequent to GTN tolerance, an effect accompanied by enhanced cGMP and nitrite formation. A similar effect was found in vivo. Indeed, chronic administration of isosorbide-5-mononitrate (IS-5-MN) in Wistar rats for 7 consecutive days induced tolerance in the hypotensive response which follows bolus injection of GTN. This effect was antagonized by co-administration of MnTBAP (10 mg/Kg/daily), thus confirming that overproduction of peroxynitrite represents a crucial mechanism in the development of nitrate tolerance.
Thus, oxidative stress via nitration of ALDH-2, the enzyme which makes NO from organic nitrates, represents a key event in GTN tolerance, an effect counteracted both in vitro and in vivo by novel peroxynitrite decomposition catalyst.