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EDITORIAL

Superoxide dismutase: Master and Commander?

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Inhaled nitric oxide (NO) has long been known to be an effective pulmonary vasodilator in the normal pulmonary vasculature. In the classical NO signalling pathway, it activates soluble guanylate cyclase in the cytoplasm of pulmonary artery smooth muscle cells, leading to the production of cyclic guanosine monophosphate (cGMP), which then activates protein kinase G (PKG). PKG, acting through several downstream targets, causes vasodilatation. In addition, NO acts through induction of post-translational changes, such as *S*-nitrosylation of proteins with reactive thiols. NO levels are decreased in idiopathic pulmonary arterial hypertension (IPAH) [1, 2], in patients with PAH secondary to anorectic agents [3] and in infants with persistent pulmonary hypertension of the newborn (PPHN) [4]. In part, NO is decreased because of the reduced endothelial NO synthase (eNOS) that has been reported in PAH and in PPHN [5, 6]. More recently, an additional mechanism has been described. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide synthase and IPAH patients have increased levels of ADMA in their blood and lungs [7]. ADMA is metabolised by dimethylarginine dimethylaminohydrolase (DDAH) 1 or DDAH2, two enzymes encoded by different genes. Although solid evidence supporting a role for DDAH2 in the degradation of lung ADMA is not available, DDAH1 gene deletion in vascular endothelial cells causes accumulation of ADMA in the lung tissue of mice [8]. Similarly, monocrotaline injection causes a decrease in lung DDAH1 expression and DDAH activity, leading to the accumulation of ADMA in rats, which may then contribute to the development of pulmonary hypertension [9]. The increased ADMA levels provide another mechanism to explain the decreased NO levels in PAH. It is also worth noting that in IPAH patients, higher ADMA plasma levels correlate with worse pulmonary haemodynamics and decreased survival [7].

Given the reduced level of NO in PAH, it is not surprising that efforts have been made to increase endogenous NO production. Endothelial NO synthetase generates NO through the oxidation of L-arginine, using tetrahydrobiopterin (BH_4), oxygen, nicotinamide adenine dinucleotide phosphate (NADPH) and calcium/calmodulin as cofactors. Decreased availability of L-arginine or BH_4 can lead to uncoupling of NOS and production of superoxide anion (O_2^-). O_2^- production can also be increased in the lung through other mechanisms, such as NAD(P)H oxidase activity or mitochondrial metabolism. O_2^- in turn, can combine with NO to form peroxynitrite or, through the action of one of three classes of superoxide dismutase (SOD), generate hydrogen peroxide (H_2O_2). Supplementation of L-arginine, either by intravenous infusion for 30 min [10] or orally for a week [11], produces modest decreases in pulmonary artery pressure (16 and 9%, respectively) and resistance (28 and 16%, respectively).

Production of BH_4 requires the activity of GTP-cyclohydrolase 1 (GTP-CH1). The hyperphenylalaninemic mouse (hph-1) has a 90% reduction in GPT-CH1 activity, decreased NO levels and develops pulmonary hypertension, even under normoxic circumstances [12]. These mice have a marked increase in O_2^- production in the lungs [13]. When hph-1 mice are crossed with mice that overexpress GTP-CH1 to increase BH_4 synthesis, the progeny have increased NOS activity and decreased O_2^- in the lungs. They do not develop normoxic pulmonary hypertension [13]. In an elegant paper in this issue of the *European Respiratory Journal* (*ERJ*), FRANCIS *et al.* [14] examine the role of BH_4 in altering the severity of hypoxic pulmonary vasoconstriction (HPV) in isolated rat and mouse lungs [14]. They show that the addition of BH_4 to the perfusate of the rat lungs increased nitrate/nitrite levels and reduced HPV. Similar effects of BH_4 on HPV were observed in wild-type mouse lungs. This would be predictable based on increased NO production. However, there are two additional parts of the BH_4 inhibition of HPV. One is the finding that an SOD mimetic (MnTMPyP) increased the vasodilator effect of BH_4. This, and a reduction in HPV achieved by the antioxidant NH_4, which is not a NOS co-factor, indicate that O_2^-, or radicals downstream, such as peroxynitrite or hydroxyl radical, were contributing to the vasoconstriction. The other component that was identified was a dilatory effect of H_2O_2, based on the observation that catalase diminished the BH_4 inhibition of HPV. This effect of H_2O_2 would be concordant with its proposed pulmonary vasodilator effect [15–17]. The vasodilatation may occur as a result of stimulation of guanylate cyclase, associated with the reduction of H_2O_2 by catalase or changes in NAD(P)H redox status [18]. It is also known that proteins containing deprotonated cysteine residues can be oxidised by H_2O_2. Several phosphatases are inactivated by this mechanism, prolonging phosphorylation in signalling pathways.

One important point to note is that, although O_2^- and H_2O_2 both cause oxidation, O_2^- causes vasoconstriction in this BH_4 model, while H_2O_2 causes vasodilatation. Consequently it is their specific molecular interactions or the microlocation within the cell that determines the outcome. The variety of SODs (Cu Zn, Mn, extracellular) and peroxidases (gluthathione peroxidase, catalase, peroxiredoxin, thioredoxin) may determine where and how oxidation occurs and what signals are

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relayed [19]. For instance, O_2^- causes oxidation of many proteins but not protein-tyrosine phosphatase 1B (PTP1B), which is oxidised and inhibited by H_2O_2 [20]. Given that BH_4 stimulates the production of NO, O_2^-, H_2O_2 and a variety of radicals derived from their interaction, the role of the SODs, controlling the watershed between O_2^- and H_2O_2 is critically important. Inhibition of, or decreased expression of, SOD leading to higher O_2^- levels increases oxidative damage [21], while in the setting of HPV, the use of an SOD mimetic (decreasing O_2^- and increasing H_2O_2) abolishes HPV in the isolated rat lung [22]. Similarly, in a model of persistent PPHN in the lamb, ATP causes constriction of pulmonary artery rings [23] but in the presence of an SOD mimetic, ATP causes dose-dependent relaxation. It is not clear how much the O_2^- effect impaired ATP-induced vasodilatation and how much H_2O_2 contributed to it. In the paper in this issue of the *ERJ* [14], the SOD mimetic did not alter HPV; however, catalase increased HPV in hph-1 mice and heterozygotes, and tended to increase it in the wild-type mice. This suggests a vasodilator role for H_2O_2 in the setting of HPV, a concept proposed in 1986 [24] and supported by recent observations in bovine pulmonary arteries [25].

The activity of SOD becomes even more interesting when its role in PAH is considered. Mitochondrial SOD is decreased in the pulmonary artery smooth muscle cells of IPAH patients and fawn-hooded rats [26]. The latter spontaneously develop pulmonary hypertension. The use of an SOD mimetic in rats exposed to chronic hypoxia prevents pulmonary hypertension and reduces right ventricular hypertrophy, suggesting that the observed decrease in SOD in IPAH patients may play a role in the aetiology [27]. These findings are supported by observations made in PPHN lambs, which have decreased SOD. Intralobar pulmonary arteries taken from such lambs, ventilated with 100% O_2, show markedly increased contraction to norepinephrine [28]. A single dose of recombinant human SOD (rhSOD), given intratracheally at the time of delivery, reduces the norepinephrine contraction to the levels seen in control lambs. The one dose of rhSOD also increased the activity of GTP-CH1, the rate-limiting enzyme in the synthesis of BH_4, and levels of BH_4 in the lungs.

FRANCIS *et al.* [14] suggest consideration of BH_4 administration in the treatment of pulmonary hypertension. A large number of studies indicate that BH_4 improves systemic vascular endothelial function in patients with hypertension, hypercholesterolaemia, heart failure, coronary artery disease, old age and in smokers [29]. From the discussion here, it seems that BH_4 is a Jekyll and Hyde intervention, with O_2^- playing the part of the evil Mr Hyde and NO and H_2O_2 acting in concert as the benevolent Dr Jekyll. However, the most important role is that of the stage director, superoxide dismutase, who determines whether good or ill will predominate. Administration of BH_4 in conjunction with SOD may be the best therapy to test in the long-term treatment of pulmonary hypertension.

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STATEMENT OF INTEREST

None declared.

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