

Disruption of methylarginine metabolism impairs vascular homeostasis

The following commentary was written by Dr Clare Sansom and Professor Neil McDonald. The original article was published in the February 2007 issue of Nature Medicine [1]: Leiper J, Nandi M, Torondel B, Murray-Rust J, Malaki M, O'Hara B, Rossiter S, Anthony S, Madhani M, Selwood D, Smith C, Wojciak-Stothard B, Rudiger A, McDonald NQ & Vallance P. Nature Medicine, 2007, 13(2), 198-203.

Nitric oxide gas (NO) is an important signalling molecule in mammals including humans. It plays a role in regulating the precise functioning of blood vessels and, therefore, in controlling blood pressure. It is synthesised by an enzyme simply known as nitric oxide synthase (NOS), which can be inhibited by methylated forms of the amino acid arginine. These inhibitors are removed from the circulation through metabolism by another enzyme, dimethylarginine dimethylaminohydrolase (DDAH). Their accumulation in blood plasma is known to be associated with increased risk of cardiovascular disease [2] and it has been hypothesised that DDAH dysfunction may be associated with this risk. Now, Neil McDonald from Birkbeck College and Cancer Research UK, London, and a core member of the Institute for Structural Molecular Biology, working with colleagues from both institutes and from University College London, has determined the structure of the human DDAH isoform, DDAH-1, with inhibitors bound [1]. The group also showed that reducing the activity of this enzyme, either by chemical inhibition or by gene knockout, led to accumulation of modified arginines, reduction in nitric oxide signalling, and dysfunction of the vascular system [1].

Methylated arginines are produced when the addition of methyl groups to arginine residues in proteins is followed by the cleavage of those residues out of the proteins by proteases. Several different chemical species can be formed, depending on the number and position of the atoms that are methylated. Two of these, asymmetric dimethylarginine (ADMA) and monomethyl arginine (L-NMMA) are inhibitors of nitric acid synthase and, through reducing the production of this important signalling molecule, affect the function of the cardiovascular system. ADMA, in particular, has been described as an "über marker" (after the German über, meaning more) [2] as increases in ADMA levels correlate with cardiovascular risk factors including raised cholesterol, hypertension and diabetes, and with poor outcome in a variety of cardiac conditions. The principal cause of raised ADMA levels in these conditions is believed to be dysfunction of its metabolising enzyme, DDAH-1 [3], thus highlighting DDAH-1 as a critical regulator of the nitric oxide synthesis pathway and of vascular health.

McDonald and his co-workers used a mouse knockout model to investigate the function of DDAH-1. Mice with only one copy of the *Ddah-1* gene (*Ddah-1*^{+/-} mice) crossed with each other produced normal (*Ddah-1*^{+/+}) and *Ddah-1*^{+/-} offspring in a 1:2 ratio. This indicates that this gene must be essential for normal development, as mice with no *Ddah-1* genes must have been conceived but died before birth. *Ddah-1*^{+/-} mice, however, developed normally. They did have increased levels of ADMA, and their blood vessels showed abnormalities similar to those of humans with cardiac defects linked to elevated ADMA. Similar results were obtained when normal mice were fed diets that included inhibitors of this enzyme.

The mechanism of this enzyme and its inhibition was also probed with X-ray crystallography. The structure of human DDAH-1 was found, as expected, to closely resemble that of the same enzyme from the bacterium *Pseudomonas aeruginosa* [4]. Its fold, unusually, has roughly five-fold symmetry, with the active site at the centre surrounded by five fairly similar motifs (Figure 1).

Two structures were solved, one with the product of the DDAH reaction, citrulline, bound in the active site, and the other with the inhibitor L-257 bound. The positions of three amino acid residues, Cys 273, His 172, and Asp 126, around citrulline indicated that these were the main residues involved in the enzyme's reaction, the so-called "catalytic triad".

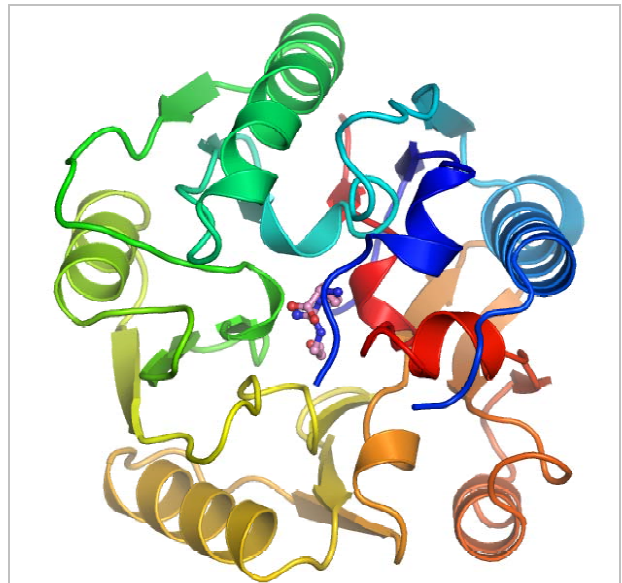


Figure 1: Structure of human DDAH-1 complexed to a small molecule inhibitor

These results clearly show that disrupting the action of DDAH-1, either through chemical inhibition or through gene deletion, leads directly to a build-up of methylated arginines, causing impaired nitrogen oxide metabolism and, thence, to a series of vascular abnormalities that have been associated with more severe cardiac problems (Figure 2).

Therefore, drug interventions that enhance activity of this enzyme may have a role in the treatment of heart disease. However, inhibitors of this enzyme may also have a therapeutic role, in restoring blood pressure to near normal levels in serious conditions including septic shock. Results presented here, showing that injection of a DDAH-1 inhibitor increased blood pressure in rats in which this was falling due to endotoxaemia, offer an early indication that this may be a useful strategy in clinical practice.

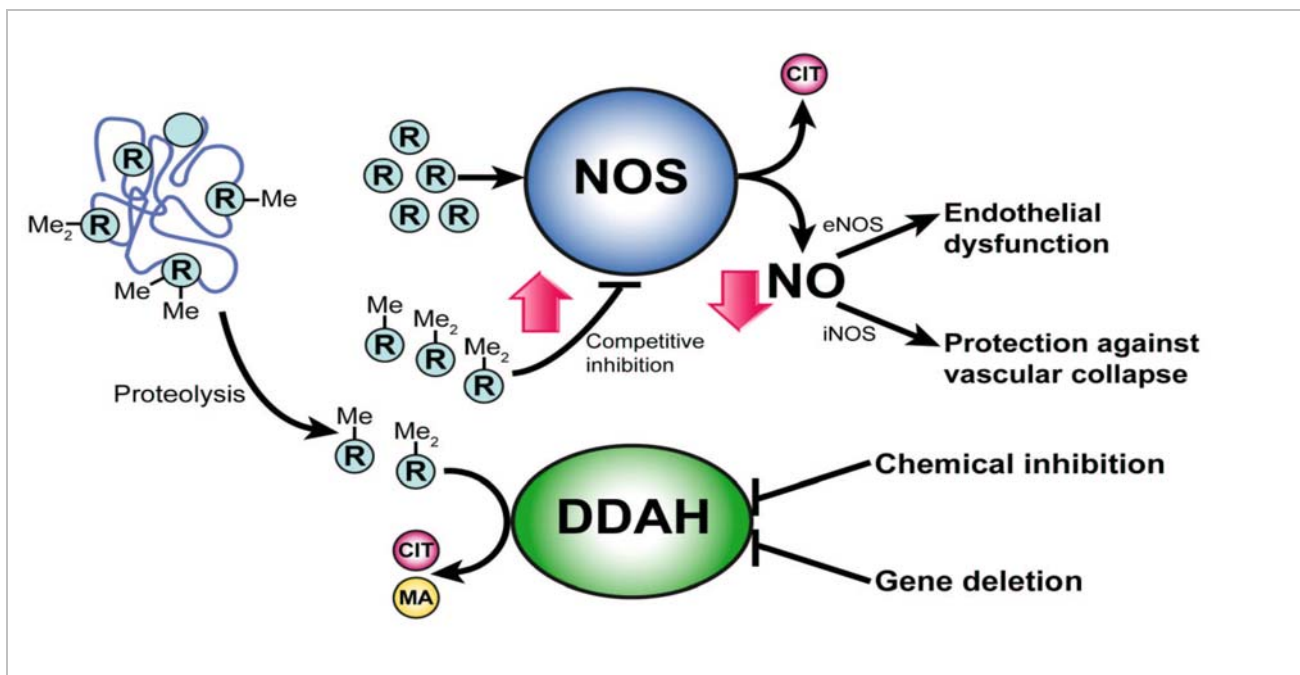


Figure 2: Schematic summarising the main findings of study.

Methylation of arginine residues (R) in proteins and subsequent proteolysis results in the liberation of free methylarginines, including asymmetric dimethylarginine (ADMA; R-Me₂), an inhibitor of nitric oxide synthases (NOS). ADMA is metabolised by dimethylarginine dimethylaminohydrolase (DDAH) to citrulline (CIT) and dimethylamine (MA). ADMA is recognised as a plasma marker of increased cardiovascular risk but it is unclear whether it ever accumulates to sufficient levels to affect NO pathways. Using a combined approach of chemical biology and gene deletion we demonstrate that loss of DDAH function elevates plasma and tissue ADMA levels. This in turn inhibits NO generation, causes endothelial dysfunction and produces a phenotype of pulmonary hypertension. On the other hand, during endotoxemia when inducible NOS (iNOS) is expressed and excess NO causes profound vasodilatation and vascular paresis, inhibition of DDAH restores vascular reactivity and may have therapeutic effects.

References

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