Asymmetric dimethylarginine-induced oxidative damage leads to cerebrovascular dysfunction

Mushfiquddin Khan*, Inderjit Singh, Jeseong Won

Asymmetric dimethylarginine (ADMA) and its enantiomer, symmetric dimethylarginine (SDMA), are naturally-occurring methylated metabolites of the L-arginine amino acid moiety of proteins followed by proteolysis (Grosse et al., 2020). These metabolites were first identified in human urine in 1970. At present, several other L-arginine metabolic products are known to occur naturally and invoke their distinct biological effects in health and disease. It is well established that both these metabolites, ADMA and SDMA, compete with L-arginine as a substrate of nitric oxide synthases (NOS). ADMA and SDMA inhibit and uncouple NOS, leading to the formation of superoxide (O_2^-) rather than nitric oxide (NO). Thus, these metabolites are linked to a dysregulated NO metabolome in neuronal/inflammatory cells, and this dysregulation is implicated in the neurodegeneration that follows brain trauma. Under pathological conditions, ADMA/SDMA is secreted and therefore found in excess in circulation and picked up by endothelial cells. In endothelial cells, ADMA uncouples endothelial NOS (eNOS). Uncoupled NOS enzymes produce superoxide, and normal NOS form NO in the same compartment, resulting in the formation of increasing amounts of peroxynitrite ($ONOO^-$). As a consequence, the bioavailability of NO is decreased. This reduced level of NO and excessive accumulation of $ONOO^-$ causes a reduction in cerebral blood flow (CBF) and thus hypoxia/hypoperfusion. This "ADMA-induced uncoupling" of eNOS is maintained by sustained and prolonged production of $ONOO^-$ in a vicious cycle (**Figure 1**). This cycle leads to secondary injury to the neurovascular unit and, thus, cerebrovascular dysfunction and functional deficits (Choi et al., 2020). Because the role of high levels of ADMA has been established in cerebrovascular pathologies, including Alzheimer's disease and stroke (Choi et al., 2020; Grosse et al., 2020; Selley, 2003), the discussion in this perspective is limited to ADMA/SDMA-induced aberrant activity of eNOS and its consequences on cellular functions and functional deficits.

Ischemia/reperfusion injury increases eNOS activity and expression. eNOS, a Ca^{2+}-dependent constitutive 135 kDa protein, produces NO, which modulates endothelial integrity and invokes anti-inflammatory properties as well as anti-apoptotic activity (Li and Förstermann, 2000). NO also exerts its beneficial effects by maintaining CBF, inhibiting platelet aggregation and reducing leukocyte adhesion (Cirino et al., 2003). eNOS-deficient transgenic mice show larger infarct volumes than controls after middle cerebral artery occlusion (Huang et al., 1996). These observations indicate that eNOS action has many beneficial effects. However, in the presence of an excessive amount of ADMA, eNOS is uncoupled. This uncoupled eNOS is incapable of transferring electrons to L-arginine and begins to use oxygen as a substrate for O_2^- production. As a consequence, uncoupled eNOS produces O_2^-. However, all eNOS enzymes do not become uncoupled, and those remaining eNOS enzymes produce NO, leading to a condition favoring increased production of $ONOO^-$. There are several sources of O_2^- and ROS within vascular cells, including mitochondria, NADPH oxidase, cyclooxygenase/lipoxygenase, xanthine oxidase and NOS. eNOS produces excessive O_2^- only when uncoupled. A specific role for uncoupled eNOS-derived O_2^- has been documented in atherosclerosis progression. O_2^- produced by eNOS has immediate access to eNOS-derived NO, being present within the same compartment at the same time, to form $ONOO^-$. The involvement of $ONOO^-$ in cerebrovascular diseases has been reviewed by Pacher et al. (2007). The role of $ONOO^-$ in reperfusion injury following middle cerebral artery occlusion has been documented showing neuroprotection after treatment with $ONOO^-$ decomposition catalyst. Other than the role of uncoupling NOS, especially eNOS, the off-target role of ADMA, if any, is less clear. Nevertheless, ADMA is recognized as an independent risk factor in endothelial dysfunction.

Pathological role of ADMA: All known naturally-occurring methyl derivatives of L-arginine are biologically active and play roles in cardiovascular and cerebrovascular diseases. Based on high levels of ADMA in hypoxic/ischemic pathology, reducing the levels of ADMA is considered an attractive therapeutic target for functional improvement in stroke and Alzheimer's disease. Using APPSwDI transgenic mice, we showed, for the first time, the pathological role of ADMA in the deterioration of cognitive functions under the conditions of cerebrovascular β-amyloidosis (Choi et al., 2020). In general, high ADMA levels are associated with endothelial dysfunction, hypertension, cardiovascular and cerebrovascular diseases. ADMA is also known as a uremic toxin and causes liver and kidney damage. Elevated ADMA levels also occur in critically ill patients. An increased level of ADMA in parallel with a decreased level of NO in serum from stroke patients was identified as an independent risk factor for ischemic stroke (Ercan et al., 2019). ADMA levels in the perilesional area of traumatic brain injury (TBI) brains are positively correlated with neuroscore performance. However, an as-yet unexplained considerable variation in ADMA levels with time after TBI indicates that the roles of ADMA are less clear in TBI-induced secondary injury. Similar to TBI, the role of ADMA in spinal cord injury is also not clear.

Measurement of ADMA in blood as an index of pathology: ADMA is a metabolite of L-arginine. While L-arginine is the natural substrate for eNOS, its dimethyl derivative ADMA is an endogenous inhibitor of eNOS. Exogenously administered ADMA to normal control animals does not induce any known cognitive deficits, whereas a similar treatment of animals with cerebral amyloid angiopathy potentiates cognitive pathology (Choi et al., 2020). Furthermore, ADMA's effect is more pronounced with age. In normal human subjects, the levels of ADMA (0.45–1.57 μM) and L-arginine (74.5–99.9 μM) vary significantly in plasma (Bode-Böger et al., 2007). The levels of ADMA also vary significantly in plasma of patients from cerebrovascular pathology (Bode-Böger et al., 2007). Some vegetables also contain a significant amount of ADMA and should be taken into account in the measurements. The measurements of ADMA/L-arginine also vary with analytical methods (ELISA or high-performance liquid chromatography); thus, a precise value or definite range has not been adequately characterized. For this reason, the ratio of L-arginine and ADMA represents a better index for ADMA-induced pathologies (Bode-Böger et al., 2007). The significance of the ratio is supported by the antagonizing effect of exogenously administered L-arginine on ADMA-induced pathologies. However, the L-arginine metabolism itself is a complex phenomenon, as recognized by the L-arginine paradox (Bode-Böger et al., 2007). The mechanism of L-arginine and ADMA metabolisms are also regulated by the activity of arginases and dimethylarginine aminodehydrolases (DDAHs), respectively, adding another layer of complexity.

Metabolism: ADMA is secreted from the cells, and thus a large amount of ADMA is found in the blood in a number of cardiovascular and cerebrovascular diseases, including Alzheimer's disease and stroke. ADMA is recognized as a possible marker for the outcome as well as a risk factor of cerebrovascular diseases. Its status as a marker stems from its role as an oxidative stress-induced methylated product of L-arginine. L-arginine methylation is the major post-translational modification catalyzed by protein-arginine methyltransferases (PRMTs). While oxidative stress induces the activity of PRMTs, it inhibits the activity of ADMA-degrading

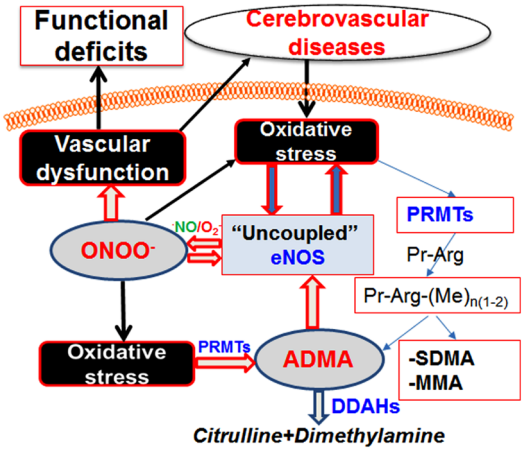

Figure 1 | **Hypothesized role of ADMA in cerebrovascular diseases.**
Asymmetric (ADMA), symmetric (SDMA) dimethylarginine and monomethyl arginine (MMA) are produced by the PRMTs-mediated methylation of the arginine residue of a protein followed by proteolysis. ADMA is the dominant product, and its biological activity is well documented. It plays an injurious role in several diseases associated with brain trauma, cerebrovascular diseases, and endothelial dysfunctions. Under normal conditions, a comparatively small amount of ADMA is produced, which is secreted, in part, in urine, with the remaining degraded by the ADMA-metabolizing enzyme DDAHs. No harmful role of ADMA is reported in normal subjects. In contrast, a large amount of ADMA is produced under the oxidative stress associated with cardiovascular and cerebrovascular pathologies. Under such conditions, ADMA potentiates and exacerbates the disease by inhibiting/uncoupling eNOS. Uncoupled eNOS produces both NO and superoxide in the same compartment and thus forms the most potent and deleterious oxidant $ONOO^-$. $ONOO^-$ oxidizes biomolecules and alters their structure/function. $ONOO^-$ causes blood-brain barrier leakage and perforation, thus inducing/potentiating endothelial dysfunctions. Excessive and sustained oxidative stress and endothelial dysfunctions hamper functional recovery and prolong the disease conditions. ADMA: Asymmetric dimethylarginine; DDAHs: dimethylarginine dimethylaminohydrolases; eNOS: endothelial nitric oxide synthase; MCAO: middle cerebral artery occlusion; MMA: monomethyl arginine; NO: nitric oxide; $ONOO^-$; peroxynitrite; O_2^-: superoxide; Pr-Arg-(Me)n(1-2): protein-L-arginine mono- or dimethylated; Pr-Arg: protein-L-arginine; PRMTs: protein arginine methyltransferases; SDMA: symmetrical dimethylarginine.

enzyme DDAHs (Sydow and Münzel, 2003), resulting in excessive accumulation of ADMA in cells and circulation. The dysregulated activity of the PRMTs/DDAHs pathway plays a significant role in cerebrovascular pathology. Enhancing the DDAH activity by antioxidants has been shown to reduce the ADMA levels, which correlates well with the improved disease conditions. N-acetylcysteine, a precursor of glutathione, has been shown to significantly reduce ADMA levels in renal disease. Other antioxidants, such as vitamin E and pyrrolidine dithiocarbamate, also reduce ADMA levels and improve endothelial dysfunction. In the absence of specific inhibitors of either PRMTs or DDAHs, the use of antioxidants to reduce the levels of ADMA may be an effective approach. However, our studies show that scavenging $ONOO^-$ downstream to ADMA/uncoupled eNOS might be a better strategy to reduce ADMA-induced cerebrovascular pathology and to improve cognitive functions (Choi et al., 2020).

Mechanisms: Decreased NO bioavailability and increased $ONOO^-$ (Pacher et al., 2007; Khan and Singh, 2016, 2019) are associated with the increased levels of ADMA and, more accurately, with a higher ratio of ADMA/L-arginine. Blood-brain barrier leakage and reduced CBF are the major events associated with ADMA/$ONOO^-$. In cell culture models, ADMA induces the production of proinflammatory mediators, including TNF-α, IL-6, NF-κB, ICAM-1, and activates mitogen-activated kinases (Grosse et al., 2020). These results indicate the critical role of ADMA as an inhibitor/uncoupler of eNOS in maintaining the proposed vicious cycle (**Figure 1**).

In conclusion, the vicious ADMA/uncoupled eNOS/$ONOO^-$ cycle, as shown in **Figure 1**, plays a significant role in neurovascular injury and cerebrovascular pathologies. Targeting/disrupting the vicious cycle is a potential approach for treating cerebrovascular diseases.

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