Ascorbic acid: what do we really NO?

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THE HUMAN CUTANEOUS CIRCULATION has emerged as a potential representative vascular bed for investigating mechanisms of microvascular dysfunction in clinical populations. Microvascular dysfunction, including a loss of nitric oxide-dependent vasodilation, is one of the earliest pathological changes associated with cardiovascular disease and is clearly evident in human skin. Several putative mechanisms of cutaneous microvascular dysfunction have been characterized in primary aged (3), essential hypertensive (6), hyperlipidemic (2), diabetic (7), and postural orthostatic tachycardia syndrome (8) patient populations.

In this issue of the Journal of Applied Physiology, DuPont et al. (1) report their findings that oxidant stress mechanisms and a relative deficit in nitric oxide synthase substrate availability (L-arginine) both contribute to cutaneous microvascular dysfunction in a chronic kidney disease (CKD) patient population. These novel observations in the cutaneous circulation extend what is currently understood about the mechanisms mediating microvascular dysfunction in this patient population. CKD patients are more likely to die of cardiovascular disease than end stage renal disease (1), but it is clear that the two processes are mechanistically linked, possibly through increased concentrations of endogenous NOS inhibitors and systemic microvascular dysfunction. Therefore, in this clinical population, identifying the mechanisms underlying systemic microvascular dysfunction is particularly important for monitoring the progression of the disease and for identifying and testing the efficacy of intervention strategies and may even be indicative of events preceding CVD.

This study used a high concentration of ascorbic acid given locally through intradermal microdialysis to explore the contribution of oxidant stress mechanisms to attenuated nitric oxide-dependent cutaneous vasodilation in CKD patients (1). Acute administration of ascorbic acid is a commonly used pharmacological tool to explore these mechanisms in humans because it can be given in high concentrations as it is rapidly cleared through the kidneys. While the use of this drug is a first step to establish the involvement of oxidant stress mechanisms, the limitations of this drug for making mechanistic determinations for how various oxidants are altering NO metabolism through synthesis or degradation must be acknowledged (Fig. 1). Ascorbic acid is commonly used as it readily quenches oxidant species including superoxide generated from enzymatic and/or mitochondrial sources. However, ascorbic acid can also reduce oxidant stress mechanisms and increase functional NO synthesis by 1) promoting the degradation of the endogenous NOS inhibitor asymmetrical dimethyl L-arginine (ADMA; 9), 2) stabilizing and increasing the synthesis of NOS cofactors (tetrahydrobiopterin, BH4; 10), and 3) by inhibiting the arginase pathway (5) with competes for the common NOS substrate L-arginine. Dupont et al. have established the involvement of oxidant stress mechanisms and further explored relative NOS substrate utilization in their study. These initial observations establish a basis for future studies to examine the precise enzymatic and/or mitochondrial sources of the increased oxidant stress in CKD-associated microvascular dysfunction. In addition, because an increase in endogenous NOS inhibitors (ADMA) is associated with this condition, other mechanisms that may explain the L-arginine paradox, including the potential involvement of upregulated arginase, are necessary to explore.

Local skin heating has been increasingly used to examine mechanisms affecting NO metabolism in clinical populations exhibiting microvascular dysfunction. Local heating vasodilation is mediated by two independent mechanisms, including an initial axon reflex with a small NO contribution followed by a secondary rise to a plateau that is primarily dependent on the

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Fig. 1. A schematic representation of the mechanisms by which the antioxidant ascorbic acid can influence nitric oxide synthesis. 1. Ascorbic acid quenches superoxide produced through a variety of enzymatic sources. 2. The ascorbic acid-induced decrease in superoxide promotes the breakdown of the endogenous nitric oxide synthase (NOS) inhibitor asymmetrical dimethyl L-arginine (ADMA) through dimethylaminohydrolase (DDAH). 3. Ascorbic acid inhibits the degradation of the essential NOS cofactor tetrahydrobiopterin (BH4) and promotes the synthesis of BH4 from BH2 via the salvage pathway. 4. Ascorbic acid inhibits the S-nitrosylation activation of arginase which competes with NOS for the common substrate L-arginine (L-arg).
production of functional NO (~70% in young health humans; 4). Interestingly, Dupont et al. found that their localized interventions into the NOS pathway differentially affected the phases of the local heating response. Specifically, the initial peak was increased with ascorbic acid administration but not by L-arginine; however, both of these interventions similarly increased the plateau response. These data are difficult to interpret from a mechanistic standpoint because of the many non-specific actions of ascorbic acid, but they do confirm that both substrate deficits for NO synthesis and other oxidant stress mechanisms are contributing to microvascular dysfunction in this population. For further clarification of how increased oxidant stress and NOS substrate deficiency contribute to cutaneous microvascular dysfunction in this population, additional physiological and pharmacological stimuli should be used to examine the full range of vasoreactivity.

Dupont et al. have added to the growing body of evidence illustrating that microvascular dysfunction is a systemic disease process and that the mechanisms can be explored in the cutaneous circulation. This new evidence also highlights that there are likely common mechanisms underlying microvascular dysfunction in the various populations that have been systematically examined. Additional experiments with specific inhibitors of the enzymatic sources of oxidant species and physiological and pharmacological stimuli to evaluate a range of vasoreactivity are necessary to fully elucidate these mechanisms.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author.

AUTHOR CONTRIBUTIONS

Author contributions: L.A.H. conception and design of research; L.A.H. performed experiments; L.A.H. analyzed data; L.A.H. interpreted results of experiments; L.A.H. prepared figures; L.A.H. drafted manuscript; L.A.H. edited and revised manuscript; L.A.H. approved final version of manuscript.

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