Redox Stress Defines the Small Artery Vasculopathy of Hypertension: How Do We Bridge the Bench-to-Bedside Gap?

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Abstract

Although convincing experimental evidence demonstrates the importance of vascular reactive oxygen and nitrogen species (RONS), oxidative stress, and perturbed redox signaling as causative processes in the vasculopathy of hypertension, this has not translated to the clinic. We discuss this bench-to-bedside disparity and the urgency to progress vascular redox pathobiology from experimental models to patients by studying disease-relevant human tissues. It is only through such approaches that the unambiguous role of vascular redox stress will be defined so that mechanism-based therapies in a personalized and precise manner can be developed to prevent, slow, or reverse progression of small-vessel disorders and consequent hypertension.

Considering the high prevalence of hypertension worldwide, the excess heart disease and stroke that it predisposes to, and the fact that it is the strongest modifiable risk factor for cardiovascular disease, it is not surprising that the American Heart Association funded a Strategically Focused Research Network on hypertension and that the Lancet commissioned a call-to-action and a life-course strategy to address the global burden of raised blood pressure (BP).[1] Despite significant advances in understanding the pathophysiology of hypertension and the availability of numerous effective drugs, suboptimal BP control remains the primary predisposing factor for cardiovascular morbidity and mortality. This Hypertension Paradox of more uncontrolled hypertension despite improved therapies, defined by Chobanian,[2] is multifactorial and may relate largely to the still unknown genetic basis and elusive causal molecular mechanisms of hypertension.

Genetics plays some role in human primary hypertension as evidenced in twin studies and monogenetic forms of hypertension where the kidney is a key target. Genome-wide association studies have been disappointing and have failed to identify specific genes that underpin hypertension. Few loci have been validated or translated into therapeutic targets, with multiple genes and their variants collectively accounting for <2.5% of BP variation. In one of the largest recent studies to dissect the genetic architecture of BP, 66 BP-associated loci were identified.[3] What is particularly significant in that study is that the 66 index single nucleotide polymorphisms were enriched with *cis*-regulatory elements, particularly in vascular cells, highlighting a potentially critical role for the vascular system, beyond the kidney, in BP regulation. In an unpublished study, we performed a comprehensive Gene Ontology analysis for the 87 genes reported as being the nearest to the 66 BP-associated single nucleotide polymorphisms[3] searching for genes potentially involved in redox regulation. One of these, *AGT*, encodes angiotensinogen, the precursor of AngII (angiotensin II), a potent inducer of Nox and oxidative stress. Another gene *PNPT1* encodes polyribonucleotide nucleotidyltransferase 1, important in cellular responses to oxidative stress. Other genes, for example, *MTHFR* that encodes methylenetetrahydrofolate reductase and *SH2B3* that regulates cell differentiation, may influence cellular redox states indirectly. Recent studies indicate a high genetic risk of oxidative stress in patients with hypertension, evidenced by increased prevalence of single nucleotide polymorphisms of genes encoding enzymes related to oxidative stress (guanosine triphosphate cyclohydrolase-1 involved in BH4 [tetrahydrobiopterin] synthesis, mSOD [manganese superoxide dismutase], and eNOS [endothelial nitric oxide synthase]).[4] Together genes regulating vascular function and oxidative stress seem to track with hypertension. However, causality has yet to be proven.

The pathophysiology of vascular disorders in hypertension is well established. Although both large and small vessels play a role, small arteries are critically involved because they are key determinants of peripheral resistance, which defines BP. In support of this, experimental and human studies demonstrate that resistance arteries exhibit endothelial dysfunction, remodeling, and subclinical inflammation, processes often preceding emergence of hypertension, and which are reversible with BP lowering.[5,6] As such, hypertension is increasingly being considered a disease of small blood vessels, where vascular injury causes increased BP, which promotes small artery vasculopathy and target organ damage. Microvascular complications are well-recognized consequences of established hypertension, events that are amplified and exacerbated with multimorbidity, such as diabetes mellitus, and which often lead to macrovascular disease, especially with aging.

The fundamental question though is, is the vasculopathy a primary cause or a secondary consequence of elevated BP? Disentangling this relationship is complex, but what is clear is that primary events beget secondary events that beget tertiary events and hence the circuitous interaction between vascular dysfunction and elevated BP is an amplifying system, that becomes

pathological when compensatory processes are decompensated. Interrupting this feedforward process would prevent or slow progression of small-vessel disorders and hence ameliorate development of hypertension and its complications, such as stroke and cardiac disease.

The increasing recognition that small-vessel disorders are central to chronic pathologies including hypertension, together with the relative paucity of mechanistic insights into how small vessels cause these pathologies, prompted the National Institutes of Health to strategize Small blood vessels: Big health problems as a top scientific priority. To advance research in this area, a National Institutes of Health white paper emphasized the importance of a greater understanding of specific phenotypes of small vessels in pathophysiological conditions, including hypertension, with the goal of transforming diagnostic and therapeutic strategies to improve vascular health.[7]

Vascular Phenotype in Hypertension

Impaired vasorelaxation, vasoconstriction, eutrophic remodeling, reduced distensibility and rarefaction, processes associated with endothelial cell dysfunction, vascular smooth muscle cell hyper-reactivity, fibrosis, extracellular matrix remodeling, perivascular inflammatory cell activation, and immune cell responses characterize small arteries in hypertension and typify the vascular phenotype or vasculopathy of hypertension.[5,6] These phenomena are dynamic, occurring at different phases during development of hypertension and are defined by complex interactions between vascular cells and circulating elements, including vasoactive agents (AngII, ET-1 [endothelin-1], aldosterone, and dopamine), growth factors (EGF [epidermal growth factor], IGF-1 [insulin growth factor 1], and PDGF [platelet-derived growth factor]), sex hormones, microRNAs, exosomes, and endothelial progenitor cells. Common to many of these processes is RONS generation and activation of redox signaling pathways.[5,6,8–10]

Oxidative Stress Causes Hypertensive-Associated Vasculopathy: Experimental Evidence

The vascular redox state is tightly controlled by activation of Nox-driven ROS (reactive oxygen species) generation, mitochondrial dysfunction, uncoupled eNOS, Nrf2-regulated, and antioxidant systems.[8–10] Physiological redox signaling is characterized by tightly controlled production and degradation of RONS (superoxide anion [O_2^-], hydrogen peroxide [H_2O_2], nitric oxide [NO], and peroxynitrite [$ONOO^-$]) and reversible post-translational oxidoreductive modification of proteins that influence signaling through PLC-PKC (phospholipase 3-protein kinase C), c-Src, Rho kinase, ion channels, SHP1/2 (Src homology region 2 domain-containing phosphatase-1/2), MAP (mitogen-activated protein) kinases, JAK/STAT (Janus kinase/signal transducer and activator of transcription kinase), and MMPs (matrix metalloproteinase)/TIMPs (tissue inhibitor of metalloproteinase).[11] ROS are localized spatially and kinetically in subcellular compartments and microdomains and regulate vascular function. Perturbations in these systems and a shift to irreversible oxidative modifications cause cell injury and vascular dysfunction.[6,9,10,12] Molecular, cellular, transgenic, and genetic models of experimental hypertension demonstrate unambiguously a causal role for oxidative stress (shift in the oxidative:reductive potential to an oxidized state because of increased ROS production and reduced antioxidant defences) in the pathophysiology of hypertensive vasculopathy.[8–10]Robust

approaches to reduce Nox activity, normalize excess ROS, and reduce oxidative stress reverse vascular remodeling, ameliorate endothelial dysfunction, and improve reactivity, processes associated with BP lowering.[5,6,8–10] This redox stress phenomenon is apparent in almost every model of experimental hypertension studied, and accordingly the presumption has been that it should also hold true in human hypertension. However, this is not the case.

Oxidative Stress and Small Artery Disease in Human Hypertension: Still to Be Confirmed

Despite the populist belief and ongoing hype in the lay-press and scientific journals about the injurious effects of free radicals and the health value of antioxidants, major clinical trials failed to demonstrate expected cardiovascular benefit, and there is still no direct proof that vascular Nox activity is altered or that intravascular RONS generation is actually increased in patients with hypertension or cardiovascular disease. In fact, to date, no disease has convincingly been successfully treated by antioxidants. Besides the discussion and ongoing debate related to appropriateness of choice and dosing of antioxidants used in cardiovascular clinical trials, there are many potential reasons why the redox stress theory in human hypertension has not yet been proven. Among these is the paucity of sensitive and specific methods in the clinical setting to accurately quantify RONS concentrations, to evaluate oxidative/reductive stress, and to measure oxidative modification of proteins.[7] There is a relative lack of understanding of fundamental mechanisms that regulate Nox activity and RONS generation in the human cardiovascular system with challenges in studying human tissue in a disease-specific manner. More specifically, at the molecular level (1) Nox isoforms are localized in various organelles (plasmalemma, nucleus, endoplasmic reticulum, and mitochondria) in a vascular cell-specific manner (endothelial cells, vascular smooth muscle cells, fibroblasts, adipocytes, and macrophages), (2) RONS that are short-lived and unstable are compartmentalized in specific subcellular microdomains (caveolae/lipid rafts, endosomes), (3) proteins are differentially oxidized through numerous post-translational processes (carbonylation, s-sulfenylation, s-nitrosylation, s-glutathionylation, and disulphide formation), (4) oxidative modification is both reversible and irreversible, and (5) redox-sensitive signaling occurs alongside, as well as intertwined with, other signaling pathways that regulate vascular function.

Development of innovative approaches to quantify intracellular RONS in a compartment-targeted manner, elaboration of oxidative proteomics to identify redox modifications, and innovation of in silico tools to model redox signaling and oxidative changes in humans will advance the understanding of human redox biology.[12]However, unless we study clinically appropriate human tissue, the bench-to-bedside gap in defining the role of redox stress in the small artery vasculopathy of human hypertension will widen. Furthermore, without tissue that is germane to human disease, moving forward in the era of precision medicine will be hampered.

New Frontiers in Vascular Redox Biology of Human Hypertension: Accessing Inaccessible Hypertension-Relevant Tissue

Advancing the field of redox biology in cardiovascular medicine and identifying druggable vascular targets demand new approaches where disease-relevant tissue from deeply phenotyped individuals is studied. Although cancer research has benefitted by relatively easy access to tumors, which has facilitated progress in pharmacogenomics, functional genomics/proteomics, and precision medicine in oncology, this is more challenging in cardiovascular medicine where access to patient cardiac and vascular tissue is limited. Recognizing this, there has been much effort in identifying surrogate readouts or biomarkers of vascular disorders in body fluids. Although this approach may have some value, it is almost certain that disease-applicable tissues hold more clinically useful molecular/cellular information than what could be obtained from biomarkers. Relevant to vascular molecular phenotyping, redox biology, and pharmacogenomics in hypertension, we think that it is timely and necessary to focus directly on vascular tissue and hypertension-relevant cells from humans, especially because experimental models do not fully recapitulate clinical hypertension. In our view, several approaches, using tissue from clinically characterized patients with hypertension, could be used, including (1) the gluteal biopsy technique to isolate small arteries and vascular cells from subcutaneous tissue,[11] (2) the endovascular guidewire technique to isolate endothelial cells,[13] (3) genomic profiling of human vascular cells,[14] and (4) hypertensive patient–derived induced pluripotent stem cells.[15] These procedures that may seem onerous from the perspective of clinical application have enormous potential to unravel molecular and redox mechanisms of hypertensive vasculopathy and are clinically attractive as strategies for testing the functional genomic/proteomic/-omic approach to precision medicine of cardiovascular diseases.

Coupling of these approaches with new tools to accurately measure RONS will help decode the significance of free radical biology in vascular cells and will provide scientific mechanistic insights into how redox stress and oxidative damage cause endothelial dysfunction and vascular injury in clinical hypertension. It is only through such advancements that pseudoscientific health claims of antioxidants can be truly addressed and that the bench-to-bedside gap in the oxidative stress theory of human hypertension can be closed.

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Disclosures

None.

Footnotes

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