SYMPOSIA

Free Radical Mechanisms of Cellular Injury Symposium: Introduction

AISAR ATRAKCHI* AND LINDA CLERCH†

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Free radical generation, including reactive nitrogen and reactive oxygen species, is known to participate in cell physiology in both a positive and negative manner. Moreover, alterations in their concentrations are implicated in a number of human diseases. Two of the talks in this mini symposium discussed research on nitroxides and the third talk addressed the clinical application of controlling free radical concentrations. Dr. David Wink eloquently discussed the chemistry of nitric oxide (NO) in the attempt to understand the mechanisms of NO reactivity during pathophysiological events. Such understanding may open the door to therapeutic applications. He specifically reviewed the indirect effects that are mediated by reactive nitrogen oxide species and talked about nitrosative and oxidative stress. Dr. James Mitchell presented data (*in vitro* as well as *in vivo*), and chemical explanations showing how non-toxic levels of nitroxides can actually act as protectors against oxidative damage. Dr. Mitchell's research also involves testing the feasibility of using nitroxides and other paramagnetic probes for *in vivo* electron paramagnetic resonance imaging. The latter allows the study of probe uptake, oxygen content in tissues, and tissue redox reactivity. Dr. Christopher Wilcox discussed the clinical potential of controlling free radical reactions. He used the spontaneously hypertensive rat (SHR) as a model of oxidative stress. The data showed oxidative stress may adversely affect normal epithelial-vascular signaling between the macula densa and afferent arteriole of the kidneys in SHR, thereby inducing pre-glomerular vasoconstriction and salt sensitivity and hypertension. Long-term data using a superoxide dismutase agonist support the possibility of developing this category of compounds as a novel therapeutic approach to lower renal vascular resistance and blood pressure, as well as restoring normal blood vessel physiology.

Oxidative Stress: Cause or Consequence of Hypertension

CHRISTOPHER S. WILCOX AND WILLIAM J. WELCH

Division of Nephrology and Hypertension, Georgetown University Medical Center, Washington, DC 20007

Studies in the blood vessel of animal models of hypertension have shown that oxidative stress can impair the normal physiologic transmission through vasodilator pathways linking the endothelium to the adjacent vascular smooth muscle cells. Reactive oxygen species (ROS) found in the wall of the aorta and major conduit vessels in animals with genetic or renovascular hypertension constitute an endothelium-derived constrictor factor that diminishes the half-life of the endothelium-derived nitric oxide (NO) and can irreversibly inactivate prostacyclin synthase. Furthermore, oxygen radicals can directly oxidize free or esterified arachidonate to form isoprostanes that can engage vasoconstrictor and prothrombotic mechanisms via the TP receptor. These vasoconstrictive, prothrombotic and atherosclerotic pathways linked to ROS are well established in large vessels, where they may contribute to the atherosclerosis. However, their importance in the regulation of peripheral vascular resistance and specifically in the resistance of the preglomerular afferent arterioles in the kidney remains elusive. This has been the focus for our studies.

The afferent arterioles of the kidney contain more than 95% of the rennin secreted by the kidney. They are also the

major resistance vessels that govern the hydraulic pressures in the downstream glomerular and peritubular capillaries that are the sites for the filtration and reabsorption of fluid. An increase in preglomerular vascular resistance is a characteristic feature of hypertension. It contributes to salt sensitivity and to a diminished efficacy in eliminating a salt load. The afferent arteriole of the kidney has unique regulatory mechanisms based on its close association with the specialized epithelial cells of the macula densa segment. This segment forms the junction between the thick ascending limb of the loop of Henle and the early distal convoluted tubule. Reabsorption of solute by macula densa cells leads to the elaboration of a vasoconstrictor stimulus that is dependent on adenosine (A). This acts on A receptors to contract the afferent arteriole. There is a dense expression of the type 1, or neuronal nitric oxide synthase (NOS) isoform in macula densa cells. Functional studies with inhibitors of NOS have shown that this pathway serves as a vasodilator pathway from the macula densa to the afferent arteriole. It is activated by solute transport and blocks vasoconstriction of the preglomerular vessel. Immunocytochemical studies have shown that nitrotyrosine is densely expressed over the extraglomerular mesangial cells and interstitial cells between the macula densa and the afferent arteriole. This has prompted the hypothesis that reactive oxygen species at this site form a "superoxide curtain" that isolates NO generated in the macula densa from targets in the afferent arteriole. This could provide for physiologic regulation of the afferent arteriole. It may be a pathogenic force in hypertension.

Studies of the kidney of the spontaneously hypertensive rat (SHR), compared to its normotensive control, show increased immunoreactive expression of nitrotyrosine and increased excretion of isoprostanes implying oxidative stress. The abundance of mRNA or protein for nNOS in macula densa cells of the SHR is increased, yet the function of the L-arginine-NO pathway between the macula densa and afferent arteriole is lost as indicated by studies with NOS inhibitors. To investigate the potential role of increased ROS generation in the interstitium as a means of abrogating NO signaling between macula densa and afferent arteriole, the stable, membrane-permeable nitroxide, tempol, was utilized. Tempol is superoxide dismutase mimetic. Microperfusion of tempol into the efferent arteriole supplying the peritubular capillaries surrounding the test nephron enhances NO signaling between the macula densa and the afferent arteriole, as shown by increased sensitivity of the afferent arteriole to a NO donor microperfused into the macula densa. Moreover, during tempol administration, the normal functioning of the macula densa-afferent arteriolar L-arginine NO pathway is restored in the SHR. Finally, studies of tempol administration over a two-week period show that it moderates oxidative stress in the SHR, as indexed by a reduction in the rate of excretion of isoprostanes, and moderates the increases in blood pressure and renovascular resistance.

These studies provide support for the hypothesis that the SHR is a model of oxidative stress. They show further that oxidative stress may be a pathophysiologic mechanism that can disrupt normal epithelial-vascular signaling between macula densa and afferent arteriole in the kidney of hypertensive models thereby promoting preglomerular vasoconstriction and salt sensitivity. Finally, the longer term studies with a membrane-permeable superoxide dismutase mimetic suggest novel therapeutic strategies based on abrogation of oxidative stress that may not only lower renovascular resistance and blood pressure, but also restore normal physiologic regulation of the circulation and blood vessels through the L-arginine NO pathway.

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Protection Against Oxidative Stress by Nitroxides

JAMES B. MITCHELL,* MURALI C. KRISHNA,* PERIANNAN KUPPUSAMY,*† JOHN A. COOK,* AND ANGELO RUSSO*

Radiation Biology Branch, Division of Clinical Sciences, National Cancer Institute, Bethesda, Maryland 20892 and †Division of Cardiology, Johns Hopkins University, Baltimore, Maryland 21205

Oxidative stress is implicated in the pathogenesis of a variety of human diseases, as well as evoking fundamental genetic responses. The final common pathway in the mechanism of action of ionizing radiation, many chemotherapeutic agents, and immunologic regulation is through oxidizing radical species. Stable nitroxide free radicals have been employed to probe various biophysical and biochemical processes involving oxidative stress.