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

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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.

We have demonstrated that nitroxides at non-toxic concentrations are effective as *in vitro* and *in vivo* antioxidants when oxidation is induced by superoxide, hydrogen peroxide, organic hydroperoxides, ionizing radiation, or specific DNA-damaging anticancer agents. The protection of oxidative damage in biological systems (both *in vitro* and *in vivo*) by non-toxic levels of nitroxides has several plausible chemical explanations: 1) SOD-mimicking action; 2) oxidation of reduced metals that have potential to generate site specific -OH radicals; 3) termination of free radical chain reactions induced by alkyl, alkoxyl, alkylperoxyl radical species, and detoxifying drug-derived radicals; and 4) detoxification of hypervalent toxic metal species such as ferryl and cupryl ions. Examples of the protection of nitroxides against oxidative stress at the cellular and animal level, proposed chemical mechanisms underlying the protective action(s), and the potential use of nitroxides in clinical settings is presented.

Additionally, the application and feasibility of nitroxides and other paramagnetic probes for *in vivo* Electron Paramagnetic Resonance imaging (EPRI) to study probe uptake, oxygen concentration in tissues, and tissue redox reactivity is discussed. The development of "functional imaging" approaches, in addition to providing the physical architecture of a structure, will provide physiological/metabolic information about the structure. EPRI, a magnetic resonance technique similar to Magnetic Resonance imaging, probes the magnetic properties of species containing unpaired electrons (free radicals, transition metals, etc.). With the availability of exogenous, non-toxic, biologically compatible free radical probes, EPRI has the potential to provide, in a non-invasive manner, valuable physiologic information in three dimensions. For example, nitroxides are redox sensitive probes, which are useful to non-invasively delineate tissue heterogeneity such as that occurring between normal and malignant tissue with respect to distribution, redox status, and oxygen concentration. Measurements using spin label oximetry showed significant hypoxia in tumors compared to normal tissue. These results suggest that tumor hypoxia results in more rapid reduction of nitroxides than in normal tissue, which in turn may explain the selective radioprotection of normal tissue by nitroxides.

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Cytotoxicity Related to Oxidative and Nitrosative Stress by Nitric Oxide

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The role of nitric oxide (NO) in toxicology and pathophysiology has received considerable attention since its discovery as an endogenous mediator of different physiological functions. As additional biological aspects of NO have been discovered, speculation has emerged that this diatomic radical plays quintessential roles in various pathophysiological mechanisms. This has lead to considerable effort to understand the underlying mechanisms involving NO during pathophysiological events, to possibly point to therapeutic strategies.

The chemistry is the most important determinant of NO in biological systems. However, deciphering the chemistry