As a young medical resident, I encountered a patient suffering from spontaneous coronary vasospasm and was puzzled by these dramatic alterations in vasomotion. This experience piqued my interest in understanding the drivers of vascular reactivity. In a paper published in the JCI, my colleagues and I revealed a role for superoxide production in the vascular dysfunction associated with hypercholesterolemia. Subsequent work by our group and others has unveiled complex associations between ROS generation and vascular disease.

While a second-year internal medicine resident on the Duke coronary care unit in the 1970s, I admitted a young lady who was having repeated episodes of chest pain and profound ST-segment elevation on her electrocardiogram. During several of these episodes, she developed ventricular fibrillation that required cardioversion. An emergency cardiac catherization revealed that her coronary arteries were overtly normal, but her left anterior descending coronary artery spontaneously developed spasm to the point of closure. Thankfully, these episodes eventually resolved. She obviously had variant angina, and I was impressed with the dramatic nature of her illness and the idea that diseases could alter vasomotion in such a striking fashion.

An experimental model and search for mechanisms

After my residency and cardiology fellowship, I accepted a postdoctoral research fellowship under the tutelage of Melvin Marcus, Allyn Armstrong, who were studying the effects of atherosclerosis. Fortunately, around this time, was instrumental in adapting this technique. We were surprised to discover that vessels from cholesterol-fed rabbits produced NO, but it was largely released in the oxidized form of nitrite (NO$_2^-$) (10). Furthermore, cholesterol-fed rabbits treated with superoxide scavenger polyethylene-glycolated superoxide dismutase exhibited improved endothelium-dependent vasodilation (11). Together, our results strongly suggested superoxide involvement in hypercholesterolemia-associated vascular dysfunction and in other molecular events in atherosclerosis.

We next established methods to measure superoxide production by intact vessels. This was challenging, because many commonly used assays to study isolated enzymes or chemical reactions were either insensitive or subject to interference. After exploring several techniques, we settled on chemiluminescence-based methods to study intact vessels, and Dr. Yuichi Ohara, a postdoctoral fellow in our laboratory at the time, was instrumental in adapting this technique. We were surprised to discover that vessels from cholesterol-fed rabbits produced three times more superoxide than did normal vessels (12). Strikingly, removal of the vessel endothelium com-

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Identifying a role of ROS

Our understanding of disease-dependent changes in EDRF production was advanced by the nearly concurrent observations that the EDRF was NO (7) and that the EDRF could be inactivated by superoxide (8). Because NO and superoxide react with one another at a near-diffusion-limited rate (9), we hypothesized that the hypercholesterolemia-associated defect in endothelium-dependent vasodilation was due to oxidative inactivation of NO by superoxide. At the time (late 1980s), apolipoprotein E-null mice, which develop severe hypercholesterolemia, were not available; therefore, we used cholesterol-fed rabbits as a model of hypercholesterolemia and early atherosclerosis.

From ST segments to endothelial pathophysiology: hypercholesterolemia and endothelial superoxide production

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hindsight

Figure 1
Collaboration. David Harrison (left), David Ku (center), and James Bates (right) at a Key-
stone Conference, circa 1990.

Figure 2
Evolving concepts of vascular ROS production. (A) Healthy vascular ECs respond to the presence of vasodilators by producing NO, which diffuses into smooth muscles and promotes vasodilation. Under hypercholes-
terolemia conditions, vascular ECs produce superoxide in addition to NO in response to vaso-
dilators. Superoxide and NO interact within vascular ECs to produce OONO⁻, which is a strong oxidant that contributes to vascular dysfunction in individuals with hypercholesterolemia. (B) Interplay between various sources of ROS in vascular cells. NADPH oxidase–produced ROS can oxi-
dize the eNOS cofactor tetrahydrobiopterin (purple), which un couples these enzymes, re sulting in ROS production. NADPH oxida-
dase–associated ROS also disrupt mito-
ochondrial electron transfer, leading to ROS production. Furthermore, ROS from other sources promote the formation of ROS by xanthine oxidoreductase. This feed-forward nature of ROS production promotes several pathophysiological states.
duced ROS can oxidize tetrahydrobiopterin, an NOS cofactor, and this event uncouples these enzymes, resulting in the production of superoxide rather than NO (23). My colleague, Hua Cai, revealed that hydrogen peroxide inhibits the expression of dihydrofolate reductase, an enzyme involved in maintaining cellular levels of tetrahydrobiopterin, leading to ENOS uncoupling (24). Furthermore, NADPH oxide–associated ROS disrupt mitochondrial electron transfer, leading to ROS production (25). The feed-forward nature of ROS production is recapitulated in several pathophysiological states (20).

It should be noted that all methods for measuring superoxide and related ROS are imperfect. Dr. Sergey Dikalov joined our group and helped us adapt additional methods for ROS detection and quantification, including electron spin resonance, HPLC to monitor oxidation of dihydroethidium, and cytochrome c reduction, as well as hydrogen peroxide–detecting methods. Use of these methods confirmed our initial chemiluminescence-based measurements and proved extremely useful.

Clinical hurdles and therapeutic implications

Several large clinical trials analyzing the use of antioxidant vitamins in cardiovascular disease have failed to show benefit. In fact, high doses of vitamin E seem to be harmful (26). We now understand that ROS act as signaling molecules and are essential for cell growth and survival. Hydrogen peroxide released from the endothelial mitochon-
dria acts as a hyperpolarizing factor to mediate vasodilation (27). Because ROS production can be highly localized and have different roles in various subcellular compartments, treatment with nonspe-
cific antioxidants has proven problematic. An alternative approach has been to develop specific NADPH oxidase (NOX) inhibitors. Recently, Gray and colleagues demonstrated that hyperglycemia activates NOX1 (28), and deletion of Nox1 in mice prevented the diabetes-associated acceleration of atherosclerosis. Excitingly, Gray et al. determined that pharmacological inhibition of NOX1 attenuates lesion formation in hypercholesterolemic mice (28). Mitochondria-targeted antioxidants have proven beneficial in experimental hypertension and type 2 diabetes, perhaps by specifically targeting pathological ROS in mice, without disrupting ROS signaling at other cellular sites (29).

In the years since our initial observations, it has become clear that the etiology of coro-
nary spasm is complicated and involves perturbed endothelial NO production, oxida-
tive injury, inflammation, and enhanced vascular smooth muscle constriction. Other causes of endothelial dysfunction in addition to NO oxidation have been described. Nevertheless, the experience of caring for a patient with coronary spasm left a lasting impression and attracted me to this area of research.

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