Editorial

Lesions and Lipids and Radicals—O My!

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Radical is a word with many meanings. It is extreme, excellent, or exorbitant. It is a root word, a root number, or just a root. For most of us, radical describes a rather significant departure from the norm. In fact, you might classify as radical the publication of a basic science article (see Al-Ruzzeh and colleagues, page 127)[1] with an accompanying editorial in the *Texas Heart Institute Journal.* In the lexicon of the cell biologist, reference to a radical is quite specific. It is an atom or molecule with at least 1 unpaired electron, capable of easy, enzyme-free modification of neighboring molecular bonds. This radical and its contribution to vascular disease are the focus of discussion in these 2 articles.

Because of their promiscuous nature, small molecules that are radicals or that are easily converted to radicals are loosely referred to as reactive species, subcategorized by their primary atom, oxygen or nitrogen (Table I). Reactive species are capable of oxidative modification or destruction of all cellular constituents, including lipids, proteins, and DNA. One can easily envision hydroxyl radicals or hypochlorous acid (common bleach) deforming DNA, corroding connective tissue, and participating in the explosive arterial injury that occurs during postischemic reperfusion. The presence of reactive species or oxidants in sufficient quantity to produce oxidized macromolecules is termed "oxidant stress." Radical injury—or oxidant stress—has been linked to the mechanism of aging, cancer, hypertension, ischemia–reperfusion injury, and atherosclerosis.

A rational link between aging, cancer, degenerative diseases, and reactive species spawned a logical hypothesis: that fortifying our innate protective mechanisms with large quantities of willing victims of radical attack will impede the advance of these illnesses. Like little Dorothy from Kansas, this hypothesis was swept away from the dreary landscape of scientific rigor to the colorful land of Oz where vitamin E, vitamin C, and antioxidants of varied origin and combination are proffered to stem the tide of aging and atherosclerosis, stop skin from wrinkling, and even cure the common cold. Fantastic claims attract true believers from the depleted ranks of pyramidology and picketers of Area 51. Fortunately, skepticism dominates the minds of conservative physicians who have before them a plethora of clinical trials unable to offer proof of principle.[2]

The antioxidant debate rages with almost religious fervor in areas extending well beyond the scope of vascular disease. Meanwhile, HMG-CoA reductase inhibitors or statins have added new fuel to the fire of this debate. Their positive influence on a wide range of clinical conditions that are linked to oxidant stress is now accompanied by evidence of their antioxidant properties.[3-6] Statin therapy reduces reactive oxygen species (ROS) generation and improves endothelial function as early as a few hours after the 1st dose. This timing implies effects unrelated to low-density lipoprotein (LDL) cholesterol concentrations. In today's issue of the *Texas Heart Institute Journal*, Al-Ruzzeh and colleagues[1] put cerivastatin to task, examining its ability to protect human endothelial cells in culture from hydrogen peroxide (H_2O_2) toxicity. Cell cultures were incubated with graded concentrations of H_2O_2 in combination with either cerivastatin or no protection, and the proportion of surviving cells was tabulated with disappointing results. The now discredited cerivastatin seemed to offer no protection from H_2O_2 at all. Taken at face value, these results challenge the newly forged reputation of the statins as antioxidants. Read face value as, "Pay no attention to the man behind the curtain." Measuring protection from H_2O_2-induced apoptosis may be useful in establishing the boundaries of statin benefit and allowing comparison to other "antioxidants" (vitamin C doesn't perform very well under these study conditions either) but does not re-create the

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TABLE I. Reactive Species

$^{\cdot}NO$	Nitric oxide
$^{\cdot}NO_2$	Nitrogen dioxide
$ONOO^-$	Peroxynitrite
$O_2^{\cdot -}$	Superoxide
$^{\cdot}OH$	Hydroxyl radical
H_2O_2	Hydrogen peroxide
$ROO^{\cdot}$	Peroxyl radical
HOCl	Hypochlorous acid

true environment of oxidant stress or measure the impact of statins under physiological conditions. The common portrayal of ROS as purveyors of chaos upon guileless tissue with antioxidants heroically dashing about soaking up harmful unpaired electrons is misleading. This editorial is an attempt to expose the fallacy of such oversimplifications by summarizing information from more in-depth reviews[7-14] that examine the actions and reactions of reactive species and their role in both inflammation and vascular disease.

Coordinating the Inflammatory Response

In response to infection or injury, leukocytes migrate to a target location, synthesize and release digestive enzymes and oxygen radicals, and send cytokine calls for assistance. In order to provide a measured response with such destructive machinery, a tightly regulated system for localization, activation, and cessation is required. To further this end, a homing mechanism consisting of a signal/effector cell pair is used. Target identification and activation, or the signal, is the responsibility of endothelial cells and activated platelets within the region of interest. In an ordered sequence, the signaling cell lightly tethers its target cell, using molecular signposts such as P-selectin and E-selectin, followed by exposure of a 2nd or activating signal such as platelet-activating factor (PAF), interleukin-8 (IL-8) or RANTES (regulated on activation of normal T cells expressed and secreted). Activation signals trigger firm, intercellular adhesion molecule (ICAM-1)-dependent attachment, diapedesis, and preparation of the inflammatory effector cell for further cytokine stimulation. Simultaneous recognition of tether/activator combinations such as P-selectin and PAF/RANTES or E-selectin and IL-8 is required for activation.

Of course, the normal endothelial cell does not present selectins or activator signals upon its surface. Neither does the inflammatory effector cell normally circulate spouting cytokines or highly reactive oxygen radicals. In both cell types, appropriate receptor occupation results in the generation of 2nd messenger molecules that initiate phosphorylation or by other means transmit the call for action internally. The roster of 2nd messenger systems, once limited to cyclic nucleotides, has grown in recent years to include, among others, the reactive species, the actions of which extend far beyond their well-known destructive potential (Table II). Many of the above-described signal/effector actions are coordinated by a battery of nuclear transcription factors and enzymes that are influenced by oxidative modification. In fact, intracellular generation of ROS is a crucial activator of the final common pathway for the prothrombotic and proinflammatory maladaptive cellular behaviors that are associated with vascular injury.

Signaling and Reactive Oxygen and Nitrogen Species

The ability of reactive species to effect a change in cellular behavior is the product of their interaction with metalloproteins and other radical molecules, and through oxidative modification of enzymes, receptors, and G proteins. The most well-recognized radical messenger, nitric oxide or $^{\cdot}NO$, is produced by the action of nitric oxide synthase (NOS) upon L-arginine (Fig. 1). Within endothelial cells, NOS is present constitutively (endothelial NOS, eNOS). Its activity may be enhanced by phosphorylation or by a rise in endothelial calcium concentration resulting from shear stress or a variety of vasodilator stimuli. Alternatively, a 2nd form of NOS may be produced in response to specific, usually inflammatory, stimuli (inducible NOS, iNOS). Nitric oxide is freely diffusible and can travel to neighboring cells, where association with the heme iron of soluble guanylate cyclase results in increased

TABLE II. Cellular Proteins or Activities Affected by Oxidant Signaling

Endothelial cell contraction and permeability
P-selectin expression
E-selectin synthesis
Interleukin-8 synthesis
Phospholipase A_2 activation
Phospholipase C activation
Phospholipase D activation
Protein kinase C activation
L-type calcium channel inhibition
Calcium-dependent ATPase inhibition
Nuclear transcription factors affecting cellular proliferation and apoptosis
Activator protein-1 (AP-1)
Nuclear factor-kappa B (NFKB)
Mitogen-activated protein kinase (MAPK)

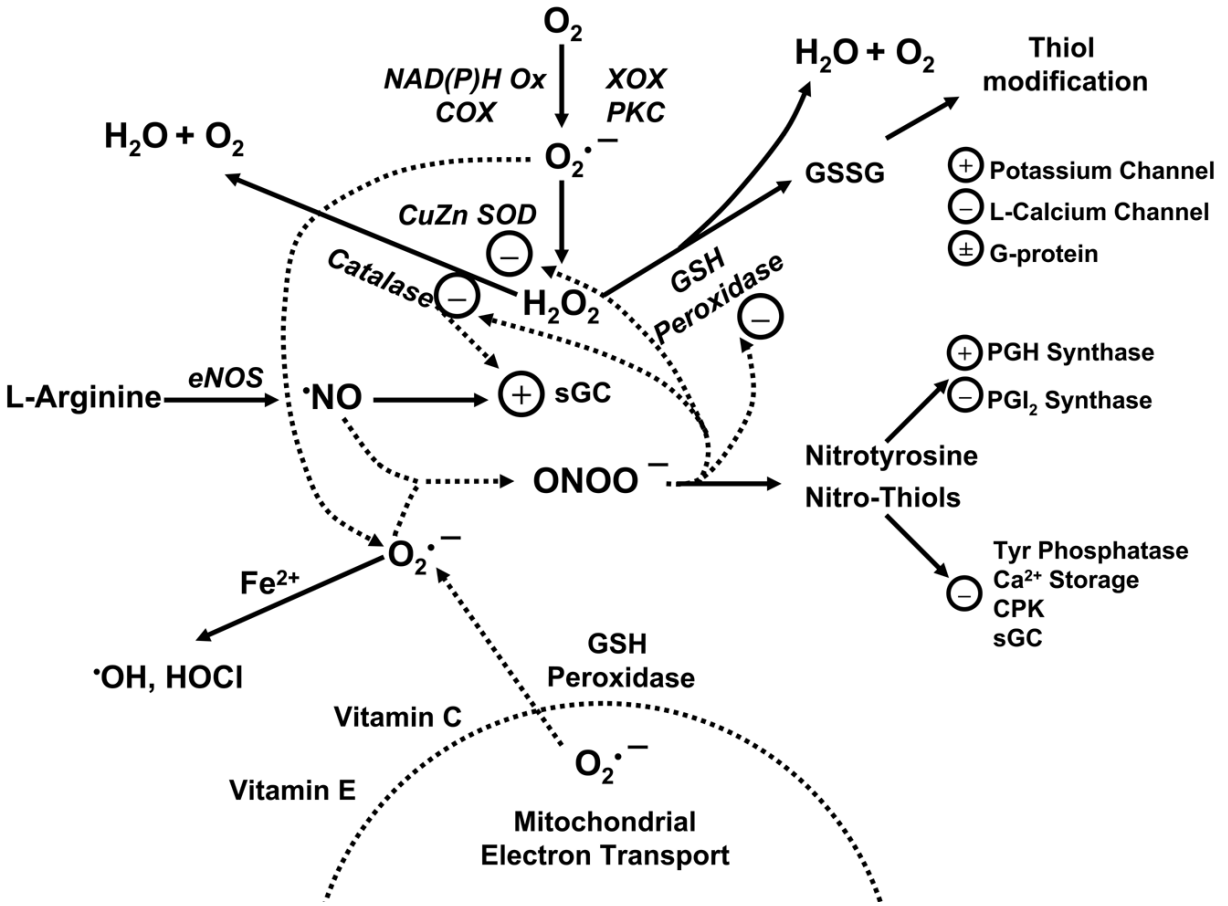

Fig. 1 *The generation, interaction, and signaling of reactive species.*

See text for details. Ca^{2+} = calcium; COX = cyclooxygenase; CPK = creatine phosphokinase; CuZn = copper-zinc; eNOS = endothelial nitric oxide synthase; FE^{2+} = iron; GSH = glutathione; GSSG = oxidized glutathione; H_2O = water; H_2O_2 = hydrogen peroxide; HOCl = hypochlorous acid; NAD(P)H OX = NAD(P)H oxidase; $^{\cdot}NO$ = nitric oxide; O_2 = oxygen; $O_2^{\cdot-}$ = superoxide; $^{\cdot}OH$ = hydroxyl radical; PG = prostaglandin; PKC = protein kinase C; sGC = soluble guanylate cyclase; SOD = superoxide dismutase; Tyr = tyrosine; XOX = xanthine oxidase

production of cGMP. Within vascular smooth muscle cells, cGMP promotes relaxation, while in platelets, both aggregation and secretory responses may be blunted. Other effects of $^{\cdot}NO$-metalloprotein interaction include inhibition of lipoxygenase (the enzyme responsible for leukotriene production), antagonism of adhesion molecule expression, and prevention of hydroxyl radical production.

To a great extent, endothelial dysfunction is underproduction or cellular insensitivity to $^{\cdot}NO$, manifest as the absence of shear-induced vasodilation, expression of adhesion molecules, and failure to inhibit thrombosis. Local production of the prototype oxygen radical, superoxide ($O_2^{\cdot-}$), which can effectively override $^{\cdot}NO$, is the most likely culprit. When dihydroethidium is used to observe intracellular $O_2^{\cdot-}$, diseased, atherosclerotic arteries show a marked increase in production throughout the vessel wall, most notably in thickened neointima.

Superoxide can be produced by several enzymes (including xanthine oxidase, the cytochrome P-450 family, and NAD(P)H oxidase) and as a result of uncoupled oxidative phosphorylation in mitochondria. Xanthine oxidase, the familiar source of uric acid and target of allopurinol, acts upon xanthine and hypoxanthine, molecules whose concentration is increased during ischemia or hypoxemia. Therefore, both xanthine oxidase and mitochondrial function make the production of $O_2^{\cdot-}$ a gauge of cellular oxygen tension.

The best recognized source of $O_2^{\cdot-}$ is the respiratory burst of activated neutrophils. Once activated, neutrophil NAD(P)H oxidase subunits assemble to produce large quantities of $O_2^{\cdot-}$, a substrate for myeloperoxidase. Vascular cell NAD(P)H oxidases are similar to

neutrophil NAD(P)H oxidase but far less prolific. They exist 1) preassembled and responsible for constitutive, intracellular activity and 2) as separate cytosolic subunits activated in a manner similar to that in which the neutrophil enzyme is activated. Superoxide produced by vascular NAD(P)H oxidase is released into the cytosol or cellular compartments in very small amounts, with effects that include the activation of kinases and the inhibition of tyrosine phosphatases. Vascular NAD(P)H oxidase activity is increased by elevated transmural pressures, hyperglycemia, angiotensin II (AT-II), tumor necrosis factor-α (TNF-α), thrombin, and platelet-derived growth factor (PDGF) receptor occupation. The production of its rate-limiting subunit is increased in early atherosclerotic lesions, even before the infiltration of macrophages.

Very low concentrations of $O_2^{\cdot -}$ are converted by superoxide dismutase to H_2O_2, which is a relatively stable molecule with diffusion characteristics similar to those of water. Hydrogen peroxide has a variety of actions. It may be converted to H_2O and O_2 by catalase or glutathione peroxidase. Catalase, primed by its action upon H_2O_2, may in turn help $^{\cdot}NO$ to activate soluble guanylate cyclase. On the other hand, glutathione peroxidase produces oxidized glutathione, which may then modify sulfur-containing amino acids to create glutathione adducts or, more importantly, sulfur bond cross-links within a protein molecule (Fig. 1). This action, known as thiol modification, is increasingly recognized as an important method of signal transduction, particularly for the inflammatory response. Human endothelial cells treated with H_2O_2 demonstrate prolonged expression of proinflammatory adhesion molecules, including P-selectin and ICAM-1, and may undergo apoptosis.

Superoxide that escapes superoxide dismutase and glutathione peroxidase combines with $^{\cdot}NO$ to create peroxynitrite ($ONOO^-$). Peroxynitrite is a member of the reactive nitrogen species not produced specifically by enzyme generation but only as a result of the interaction of $^{\cdot}NO$ and $O_2^{\cdot -}$ (Fig. 1). Its action ranges from stimulation of potassium ion channels to inhibition of energy storage enzymes, modification of tyrosine kinase pathways, and finally, stimulation of pathways central to apoptosis. Peroxynitrite is an extremely potent stimulant of prostaglandin H synthase, the enzyme that generates prostaglandin precursors. It simultaneously inhibits prostacyclin synthase, which funnels prostaglandin H to the creation of proinflammatory prostaglandins and thromboxanes. Reactive nitrogen species (RNS) such as $ONOO^-$ are capable of modifying protein structure and function analogous to thiol modification, except that the modification is the creation of nitro- or nitroso-adducted amino acids. Nitrotyrosine modification of apolipoprotein B-100 stimulates macrophage LDL uptake via a specific receptor site.

Synthesizing Divergent Stimuli

Considered broadly, the primary influence of $^{\cdot}NO$ is vasodilatory, anti-inflammatory, and antithrombotic. Its influence can be augmented by low and damped by high $O_2^{\cdot -}/H_2O_2$ generation. In low concentrations, preferential metabolism of H_2O_2 by catalase increases cGMP generation and vasodilation: an effect that may be very important in myocardial autoregulation. Increased $O_2^{\cdot -}$ and H_2O_2 concentrations stimulate proinflammatory, prothrombotic cellular responses as oxidized glutathione begins thiol modification, leaving $^{\cdot}NO$ to take the role of spoiler. Meanwhile, should $O_2^{\cdot -}$ and $^{\cdot}NO$ mingle, their reaction product, $ONOO^-$, initiates even more potent activation of proinflammatory, prothrombotic cellular responses and apoptosis, all the while favoring its own production by inhibiting the conversion of $O_2^{\cdot -}$ to H_2O_2.

The production of $^{\cdot}NO$ and $O_2^{\cdot -}$ is subject to the effect of specific physical and chemical stimuli, although the stimulus may differ between cell types. Their production and the variable metabolism of H_2O_2, along with the capacity for $ONOO^-$ production and interaction with G-proteins and other signaling molecules, allow the reactive species to integrate multiple and sometimes contradictory stimuli. With such incredible complexity, the linear concept of stimulus-response becomes stimulus- and condition-dependent. If I do A, then B will occur, as long as receptors C and D are occupied and enzymes X and Y are active. If things are just a little bit different, option K may occur. This may sound a bit like the predicting the national champion for collegiate football in division I. It is, only worse.

Dysfunctional Signaling

Superoxide generation is not regulated in the hypoxic cell. It is the product of the mitochondrial electron transport chain and the activity of xanthine oxidase. However, oxidant signaling may be deranged by means other than hypoxemia. Some means that are well known include dysfunction of cellular antioxidant systems (glucose-6-phosphate dehydrogenase [G6PD] deficiency), inappropriate or excessive stimulation of NAD(P)H oxidase by physical (pressure, shear stress) or chemical (ATR_1 occupation, hyperglycemia) forces, and accidentally produced proinflammatory molecules. Deficiency of folic acid deprives NOS of a necessary cofactor, allowing the paradoxical generation of $O_2^{\cdot -}$ rather than $^{\cdot}NO$. The resultant imbalance in RNS and ROS generation can produce cellular responses to stimuli that are inappropriate, favoring the schemes triggered by ROS and thiol modification.[15,16] Glutathione peroxidase dys-

function may permit the generation of highly reactive nitrogen species, triggering an inflammatory response or apoptosis. Single nucleotide polymorphisms that alter the function of NAD(P)H oxidase and glutathione peroxidase have been linked to disease extent and activity in atherosclerosis.[17-19] Angiotensin II antagonism, which deprives NAD(P)H oxidase of 1 stimulus for activation, is associated with a reduction in the incidence of acute coronary events and may retard the progression of atherosclerosis.[20,21]

An intriguing product of and probable source of oxidant stress within endothelial and smooth muscle cells appears to be an unintended consequence of interaction between reactive species and phospholipids. Oxidation of phospholipids may produce molecules with PAF-like activity or with metabolism that results in increased production of PAF precursors.

Phospholipids are constructed from a spine of glycerol with an attached polar function at the 1st carbon and long-chain fatty acids or fatty alcohols attached via ester or ether bonds to the 2nd and 3rd carbons. Beyond serving as a physical and chemical barrier in cell membranes and micelles, specific phospholipids may act as a storehouse for potential lipid-derived chemical messengers such as arachidonic acid or the ether phospholipid that gives rise to PAF. Phospholipase A_2 (PLA_2) hydrolyzes the ester bond in the 2nd position of specific phospholipid molecules (Fig. 2). In so doing, it produces arachidonic acid, the precursor for prostaglandin production, as well as the remaining phospholipid, lyso-PAF. Lyso-PAF can then receive an acetyl group at its unoccupied site, creating the potent inflammatory activator PAF. Lyso-PAF appears to have proinflammatory properties in its own right.

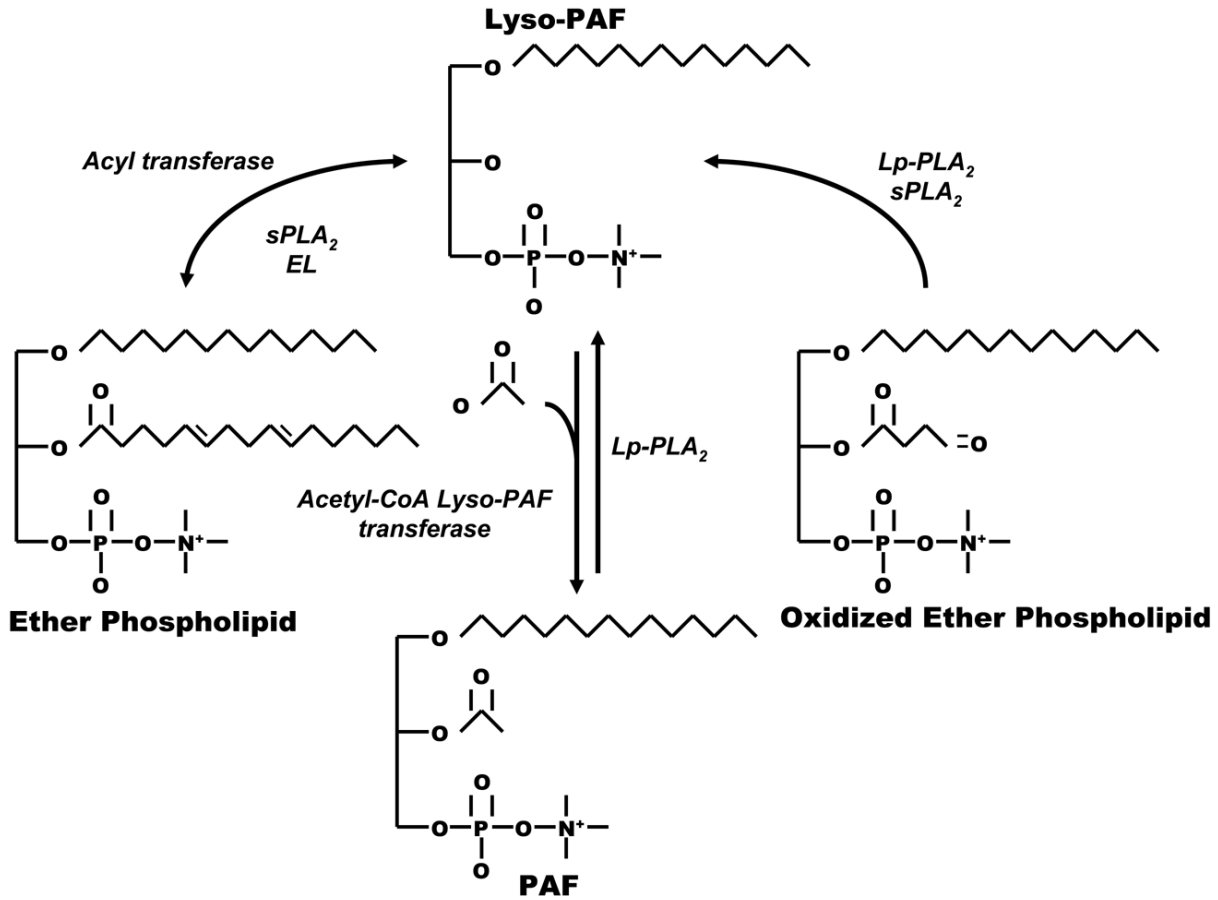

Fig. 2 *Phospholipid oxidation and platelet-activating factor (PAF).*

Specific ether phospholipids are subject to the action of phospholipase A_2 (PLA_2) and endothelial lipase (EL), producing the intermediary molecule lysophosphatidylcholine or lyso-PAF. A transferase may attach an acyl group at the 2nd carbon position, creating PAF or a long-chain fatty acid, returning lyso-PAF to a storage or barrier function. The principle action of lipoprotein-associated PLA_2 ($Lp\text{-}PLA_2$) is removal of the acyl group from PAF. However, oxidized phospholipids may also be attacked by $Lp\text{-}PLA_2$ to generate oxidized free fatty acid and lyso-PAF, thus usurping a regulatory step in the generation of PAF and lyso-PAF, 2 inflammatory mediators.

CoA = coenzyme A; $sPLA_2$ = secretory-phospholipase A_2

Because of the dangerous potential of PAF, its generation is supposed to be tightly regulated; however, it, or very close look-alike molecules, may be generated accidentally. Oxidative cleavage of the fatty acid attached to the 2nd carbon of a phospholipid, leaving a chain of 5 or fewer carbons, produces these PAF mimics. Platelet-activating factor and its mimics are subject to rapid detoxification by PLA_2, leaving lyso-PAF available for recycling to genuine PAF or for eliciting its own response. Phospholipase A_2 comes in many forms, one of which has the primary responsibility to detoxify PAF. This enzyme, platelet-activating factor acetylhydrolase (PAF-AH), is membrane associated and is found in large concentrations in the coating of lipoproteins. Unfortunately, PAF-AH is not specific for PAF but also hydrolyzes ether phospholipids with virtually any oxidatively modified adducts in the 2nd carbon position (Fig. 2). Therefore, many investigators prefer the name lipoprotein-associated phospholipase A_2 (Lp-PLA_2) rather than PAF-AH. Some aspects of this enzyme's substrates or activity appear to be closely linked to atherosclerosis. Elevated serum concentrations of Lp-PLA_2 are associated with an increased risk for atherothrombotic events.[22]

Low-density lipoprotein is an ideal environment for oxidant mischief by virtue of its access to the subendothelial space, its long residence time there, and its phospholipid coat. Exposure of LDL to strong oxidants results in the oxidation and fragmentation of specific phospholipids and unregulated production of molecules with PAF-like activity. Strong oxidants are easy to come by in the desolate neighborhood of the activated macrophage. Here, then, is the target for antioxidant therapy in atherosclerosis; however, there is a catch. Early atherosclerotic lesions demonstrate oxidant stress and progress quite readily with no macrophages present. So, are their strong oxidants really the problem or just additional fuel for the fire?

Lipid peroxides are generated within cell membranes as side products of enzymes such as lipoxygenase or cyclooxgenase. Transfer of lipid peroxides from the cell to the neighboring membrane of LDL may be all that is required to initiate a chain reaction of phospholipid modification. Oxidized ether phospholipid that by good fortune has too long a carbon chain to allow PAF mimicry may still be problematic when the misdirected Lp-PLA_2 cleaves the oxidized fatty acid to produce lyso-PAF. This may be the principle danger of LDL oxidation, which has effects that mirror PAF. Under conditions of greater oxidant stress, marked by the production of $ONOO^-$, nitromodification of apolipoprotein B-100 allows these dangerous particles unregulated entry into macrophages via a scavenger receptor. Among its many harmful effects, oxidized LDL reduces receptor-mediated $^{\cdot}NO$ release, down-regulates eNOS synthesis, inactivates $^{\cdot}NO$ by stimulating $O_2^{\cdot -}$ generation, increases the expression of adhesion molecules such as ICAM-1, and enhances monocyte–endothelial cell adhesion (Fig. 3).

Cigarette smoke produces extracellular oxidant stress, supplying ozone, cyanide, nitrosoamines, and molecular targets of cytochrome P-450 enzymes. In an animal model of tobacco-smoke exposure, the spontaneous association of activated platelets and monocytes into active, secretory aggregates appeared

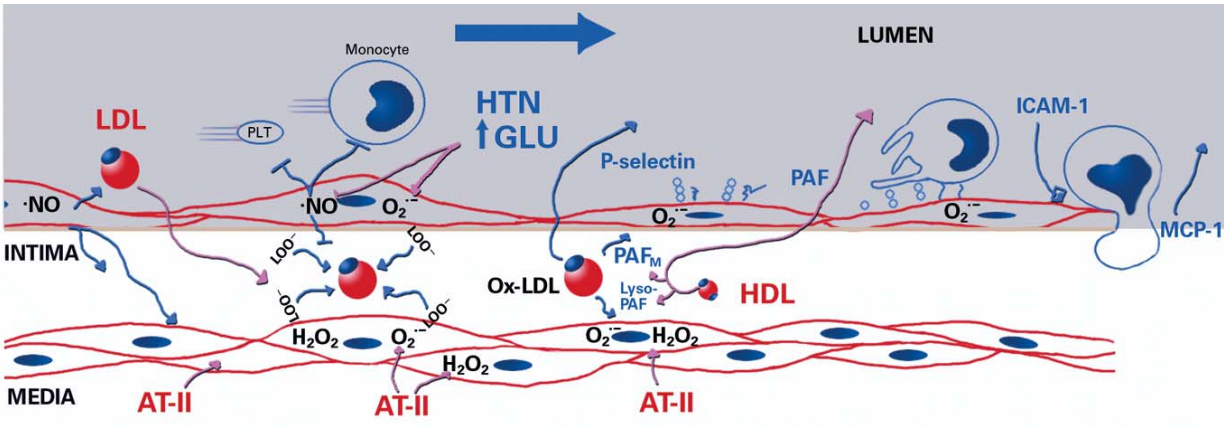

Fig. 3 *Reactive species, oxidized phospholipids, and the progression of atherosclerosis.*

See text for details. AT-II = locally produced angiotensin II; ↑GLU = increased glucose; HDL = high-density lipoprotein; H_2O_2 = hydrogen peroxide; HTN = hypertension; LDL = low-density lipoprotein; ICAM-1 = intercellular adhesion molecule; LOO^- = lipid peroxides; Lyso-PAF = lyso–platelet-activating factor; MCP-1 = monocyte chemoattractant protein-1; $^{\cdot}NO$ = nitric oxide; $O_2^{\cdot -}$ = superoxide; Ox-LDL = oxidized LDL; PAF = platelet-activating factor; PAF_M = oxidized phospholipids capable of mimicking PAF; PLT = platelet

to be the result of PAF-like molecules originating from radical-induced oxidation of phospholipid. Monocyte and polymorphoneutrophil rolling is seen with increased frequency after exposure to cigarette smoke and is prevented by pretreatment with vitamin C. Circulating and cellular antioxidant molecules are depleted in smokers. Depletion of antioxidant protection mechanisms and diffuse activation of inflammatory and procoagulant systems may underlie the close association between cigarette-smoking, vascular disease, and thrombotic events, particularly when placed in the context of pre-existing endothelial dysfunction or plasma enriched with susceptible lipoprotein.

Antioxidant and Anti-Inflammatory Therapy

During prolonged ischemia, uncontrolled generation of ROS and later production of $ONOO^-$ may trigger the inflammatory defense mechanisms of vascular cells while in the extracellular environment or within neighboring, susceptible membranes; the stimulus for inflammation is augmented by the generation of PAF and its mimics. In atherosclerosis, interaction between reactive species or even between normally produced lipid peroxides and lipoprotein residing within the arterial wall can produce PAF mimics that usurp the normal regulatory framework of the inflammatory response (Fig. 4). In both atherosclerosis and ischemia–reperfusion injury, ROS are associated with aberrant cellular behavior; however, the simplistic view of radical generation as a diffuse, unregulated process in need of suppression ignores their diverse origin and action.

The goals of antioxidant therapy are to contain uncontrolled radical generation, to divert intracellular oxidant-stimulated pathways in targeted cell populations, and to remove oxidation products with independent effects of their own, such as lyso-PAF and the PAF mimics. Surprisingly, or maybe not so surprisingly, one of the most important native antioxidant molecules—viewed from the perspective of vascular disease—is high-density lipoprotein (HDL). The concentration of HDL is negatively correlated with the incidence of atherosclerotic vascular disease and its complications. The mechanism by which HDL affords this protection is not clear but may involve cholesterol transport away from the arterial wall or, more importantly, may involve antioxidant protection for resident LDL particles. High-density lipoprotein, par-

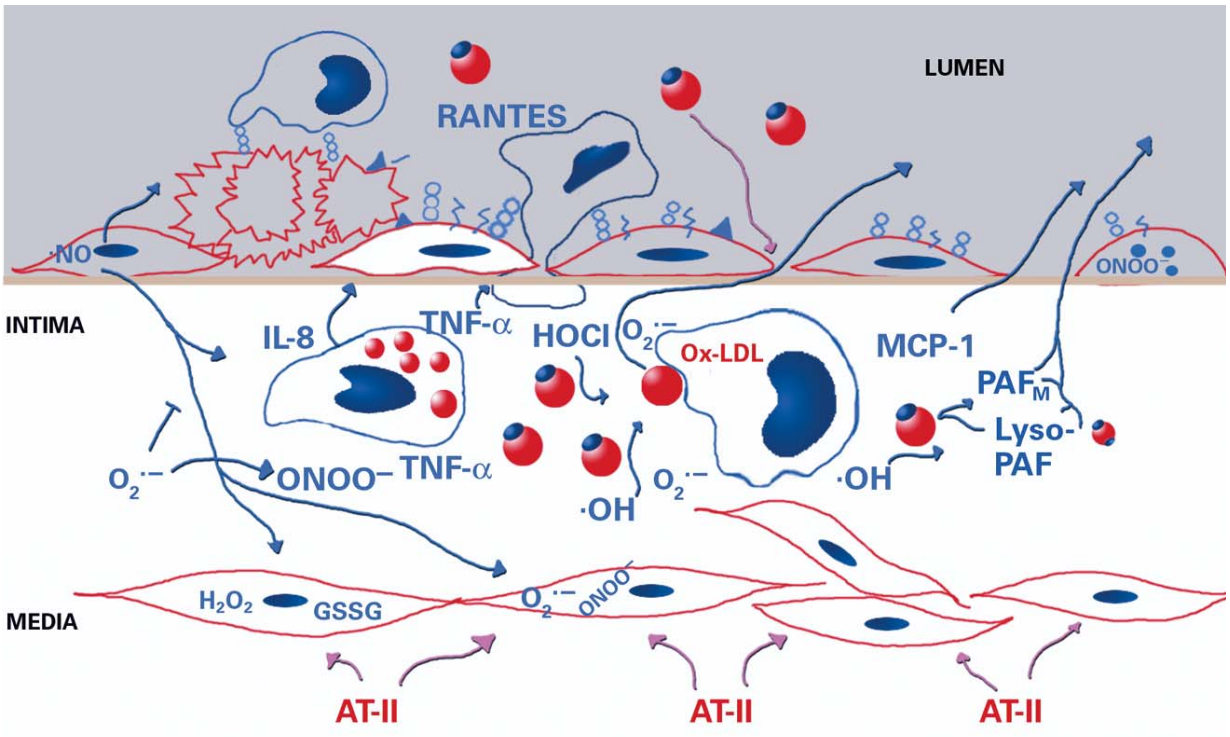

Fig. 4 *Reactive oxygen species' impact on inflammatory response within the arterial wall.*

See text for details. AT-II = locally produced angiotensin II; GSSG = oxidized glutathione; H_2O_2 = hydrogen peroxide; HOCl = hypochlorous acid; IL-8 = interleukin-8; Lyso-PAF = lyso–platelet-activating factor; MCP-1 = monocyte chemoattractant protein-1; ·NO = nitric oxide; $O_2^{\cdot -}$ = superoxide; ·OH = hydroxyl radical; $ONOO^-$ = peroxynitrite; Ox-LDL = oxidized LDL; PAF = platelet-activating factor; PAF_M = oxidized phospholipids capable of PAF mimicry; RANTES = regulated on activation of normal T cells expressed and secreted; TNF = tumor necrosis factor

ticularly the small dense form, HDL_3, is very effective in its protection from LDL oxidation, offering enzymes (Lp-PLA_2 and paraoxonase) and a chemical environment that has the principal action of removing lyso-PAF and lipid oxidation products.[23]

In addition to direct action upon radicals or their oxidation products, several very effective drug therapies display antioxidant properties by antagonizing stimulants of NAD(P)H oxidase (angiotensin-converting enzyme inhibitors and angiotensin receptor blockers) or by influencing subcellular protein trafficking that is normally coordinated by oxidant-stimulated pathways. The latter is thought to be the mechanism of statin antioxidant activity.[4,6,12,24] Reduced LDL concentrations deprive the arterial wall of a source of false inflammatory messengers and stimulus for ROS generation, but reduced ROS generation resulting from statin therapy can be observed within hours of administration—much too soon to be the effect of LDL cholesterol lowering alone. Farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) are precursors in cholesterol synthesis that are also used for the post-translational modification of proteins to label them for subcellular localization and intracellular trafficking. The production of FPP and GGPP is diminished by HMG-CoA reductase inhibition, thereby influencing the localization and activity of a battery of enzymes. Many of the enzymes influenced by FPP and GGPP deprivation are participants in the ROS-triggered inflammatory response or participants in stimulated ROS production. In the absence of proper labeling, incorrect localization or impaired enzyme activity—or both—result in the up-regulation of eNOS, inhibition of adenosine diphosphate, and release of adenosine triphosphate by platelets after thrombin activation, which can impair stimulated NAD(P)H-oxidase superoxide production.[24] Statins inhibit AT-II-induced and epidermal growth factor-induced ROS production, enhance coronary blood flow, and attenuate P-selectin expression and leukocyte adherence.[4,5,20,25-28]

Conclusion

Ischemia–reperfusion injury is the result of unregulated and high-capacity generation of ROS, and may be reduced by treatment with radical scavenging molecules.[29] From Al-Ruzzeh's article,[1] we learn that cerivastatin cannot be counted among the contenders for this roster, at least when the endpoint used to measure treatment effect is apoptosis in cell culture, and the ROS is H_2O_2. In atherosclerosis, macrophage radical production may be viewed as high capacity and relatively unregulated, which makes it a target for radical scavenging, but this view has yet to produce therapeutic success. Antioxidant therapy directed against macrophage-derived radicals may be analogous to draining the mercury from a barometer in hope of rain: a strike against association rather than cause.

Successful antioxidant treatment is both subtle and complex. It has to reduce the quantity of lipoprotein available for intimal deposition and inadvertent oxidation, improve clearance of oxidized phospholipid false messengers, inhibit vascular NAD(P)H oxidase activation, and manipulate subcellular signaling systems influenced by ROS generation. It is unlikely that currently available radical scavenger antioxidants can be administered in a fashion that will achieve any of these goals. Therefore, brute-force antioxidant therapy is slowly fading into the horizon. The success of the statins and AT-II antagonists has shown us the road to the Emerald City. On it, we may soon see manipulation of nuclear factor kappa B, the mitogen-activated protein kinases, and peroxisome proliferator-activated receptors. We may encounter inhibitors of lipoxygenase and Lp-PLA_2 or PAF receptor antagonists, perhaps even targets for focal gene transfection. But that, as they say in Oz, is a horse of a different color.

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