REVIEW ARTICLE

Reactive Oxygen Species as Mediators of Cardiac Injury and Protection: The Relevance to Anesthesia Practice

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Reactive oxygen species (ROS) are central to cardiac ischemic and reperfusion injury. They contribute to myocardial stunning, infarction and apoptosis, and possibly to the genesis of arrhythmias. Multiple laboratory studies and clinical trials have evaluated the use of scavengers of ROS to protect the heart from the effects of ischemia and reperfusion. Generally, studies in animal models have shown such effects. Clinical trials have also shown protective effects of scavengers, but whether this protection confers meaningful clinical benefits is uncertain. Several IV anesthetic drugs act as ROS scavengers. In contrast, volatile anesthetics have recently been demonstrated to generate ROS in the heart, most likely because of inhibitory effects on cardiac mitochondria. ROS are involved in the signaling cascade for cardioprotection induced by brief exposure to a volatile anesthetic (termed "anesthetic preconditioning"). ROS, therefore, although injurious in large quantities, can have a paradoxical protective effect within the heart. In this review we provide background information on ROS formation and elimination relevant to anesthetic and adjuvant drugs with particular reference to the heart. The sources of ROS, the means by which they induce cardiac injury or activate protective signaling pathways, the results of clinical studies evaluating ROS scavengers, and the effects of anesthetic drugs on ROS are each discussed.

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Free radicals and their nonradical reactants are recognized as critical mediators of cardiac injury during ischemia and reperfusion. They have been implicated in reversible postischemic contractile dysfunction (myocardial stunning), cardiac cell death, dysrhythmias, and in chronic cardiovascular diseases. Intensive laboratory and clinical investigative effort has focused on the capability of antioxidant therapies to attenuate deleterious effects of free radicals in the heart. Many investigators have also sought to demonstrate that anesthetic drugs can either prevent free radical formation or scavenge free radicals.

The deleterious effects of free radicals have been known for almost 50 yr. More recently, however, an essential role for free radicals in physiologic control of several aspects of cell function has been demonstrated. Free radicals are, indeed, now considered as key regulatory molecules vital for life, but they cause cellular and organ damage when produced in excess or when innate antioxidant defenses are overwhelmed.

Anesthetic preconditioning (APC) is of particular current interest to anesthesiologists. In this phenomenon, lasting protection of myocardium is elicited by brief exposure to a volatile anesthetic. Free radicals are now known to act as second messengers in the preconditioning cell-signaling pathway. Most remarkably, volatile anesthetic drugs have recently been shown to enhance generation of free radicals in cardiac cells, probably by causing mild uncoupling of the mitochondrial electron transport chain. A cardioprotective signaling pathway that is triggered by volatile anesthetics and that utilizes free radicals as essential mediators thus represents a paradigm shift in traditional anesthesiology teaching. First, effects of volatile anesthetic drugs in the heart are not evanescent but may be long lasting and, second, moderate release of free radicals may induce a protective rather than an injurious effect within the myocardium.

This review is intended to provide background information on free radical formation and elimination relevant to anesthetic and adjuvant drugs with particular reference to the heart. The sources of free radicals, the means by which they induce cardiac injury or

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activate cardiac signaling pathways, the results of trials evaluating free radical scavengers, and the effects of anesthetic drugs on free radicals are each discussed.

Free Radicals and Reactive Oxygen Species

A free radical is any species capable of independent existence that contains one or more unpaired electrons occupying an atomic orbital by itself. Radicals are therefore formed by the loss or gain of an electron from a nonradical. The presence of an unpaired electron usually makes the species highly reactive, although different free radical species differ greatly in their reactivity. "Redox" or oxidation-reduction reactions are those reactions that involve exchange of electrons between molecular species.

The diatomic "ground state" oxygen molecule has two unpaired electrons, each in a different orbital, and indeed fulfills the definition for a free radical. If this molecule accepts an electron the product is superoxide radical ($O_2^{\cdot -}$). Many of the important free radicals are derived from oxygen. Another important radical is nitric oxide ($NO^{\cdot}$). Many of these have important intermediates, such as hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$), which are not free radicals but which are highly reactive and may be responsible for some of the biologic effects attributed to free radicals. Therefore, the term reactive oxygen species (ROS) is used to encompass these species. Nonradical ROS also include ozone and singlet oxygen.

Sources of ROS

Sources of free radicals are the mitochondrial electron transport chain, the enzymes xanthine oxidase, NADPH oxidase, lipoxygenase/cyclooxygenase and nitric oxide synthase (NOS), and auto-oxidation of various substances, particularly catecholamines (Fig. 1).

The tetravalent reduction of molecular O_2 by the mitochondrial electron transport chain is necessary for generating most biologic energy. To accomplish this there are four mitochondrial complexes (I–IV) involved in energy conservation. The reduction is not 100% efficient, however, and 1%–4% of available oxygen is normally incompletely reduced and leaks from the electron transport chain in the form of $O_2^{\cdot -}$. $O_2^{\cdot -}$ is produced at complex I (NADH coenzyyme Q reductase, also called ubiquinone oxidoreductase) and complex III (ubiquinol cytochrome C reductase). This process becomes greatly accelerated at supranormal O_2 tensions or after mitochondrial injury and is believed to be the primary intracardiac source of ROS

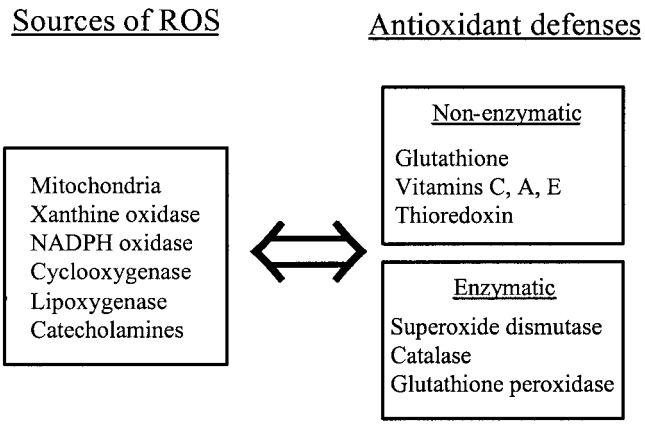

Figure 1. Formation of reactive oxygen species in cardiac myocytes. An electron is gained by molecular O_2 yielding superoxide ($O_2^{\cdot -}$). In myocytes, this reaction occurs primarily at complexes I and III of the mitochondrial electron transport chain. The electrons are derived from NADH and $FADH_2$. O_2 is converted to hydrogen peroxide (H_2O_2) in the presence of superoxide dismutase, found in the mitochondrion and in the cytosol. H_2O_2, a non-radical, but a freely permeable and highly reactive reactive oxygen species (ROS), is converted to hydroxyl radical ($OH^{\cdot}$) in the presence of copper or iron (the Fenton reaction). Alternatively H_2O_2 reacts with $O_2^{\cdot -}$ to form $OH^{\cdot}$ in the Haber-Weiss reaction. Glutathione peroxidase catalyzes the conversion of H_2O_2 to nonradical water and oxygen. ETC = electron transport chain.

during ischemia and reperfusion. Cellular hypoxia decreases the activity of complex IV (cytochrome oxidase); when O_2 is reintroduced, leakage of free radicals from more proximal complexes is greatly accelerated. Although it was previously believed that ROS formation occurred primarily or solely at reoxygenation after ischemia, it is now known that significant formation of ROS occurs during ischemia from residual O_2. This has been demonstrated in cardiomyocytes (1) and in the intact heart (2,3).

Most $O_2^{\cdot -}$ is dismutated by manganese-superoxide dismutase (MnSOD) in the mitochondrial matrix to H_2O_2, which readily diffuses through mitochondrial membranes. The remainder exits the mitochondria through anion channels in the mitochondrial membrane and is then rapidly converted to H_2O_2 in the cytoplasm, either spontaneously, or when catalyzed by copper superoxide dismutase (CuSOD). H_2O_2 is reduced to H_2O and O_2 by catalase and glutathione peroxidase. Alternatively, H_2O_2 reacts with transition metals, particularly Fe^{2+} (the Fenton reaction), to generate hydroxyl radical ($OH^{\cdot}$). These reactions are shown in Figure 2. $OH^{\cdot}$ is a particularly reactive radical, such that it will react with virtually all biomolecules at diffusion limited rates. This has important consequences, as $OH^{\cdot}$ will react at the site of its formation whereas more stable species, such as $O_2^{\cdot -}$ and H_2O_2, can react at more distant locations.

Xanthine oxidase is a major source of ROS after reperfusion of ischemic tissue in several organs, although its role in the heart remains somewhat unclear.

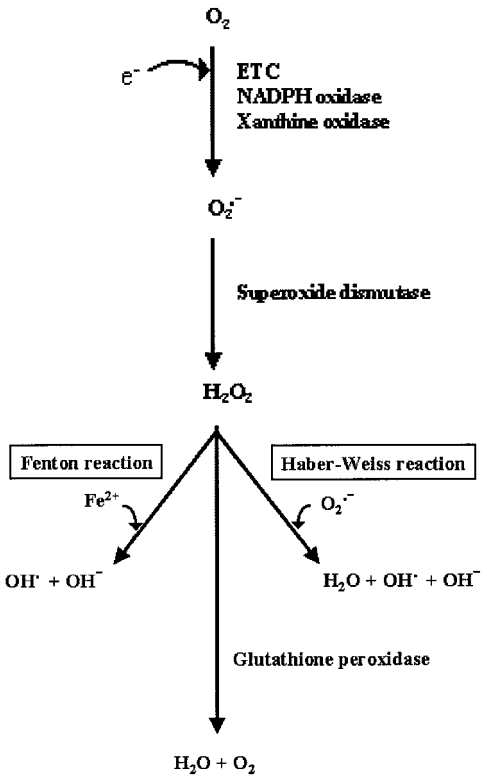

Figure 2. Reactive oxygen species (ROS) are derived from multiple sources and are counterbalanced by enzymatic and non-enzymatic antioxidants. Under pathologic conditions, such as ischemia-reperfusion, antioxidant defenses are overwhelmed by excess ROS and cellular injury results.

During ischemia, purine precursors are degraded to the nucleotide derivatives hypoxanthine and xanthine and the enzyme xanthine dehydrogenase is converted to xanthine oxidase. Xanthine oxidase catalyzes oxidation of hypoxanthine and xanthine to uric acid while reducing O_2 to $O_2^{\cdot-}$ and H_2O_2. The main source of xanthine oxidase in human hearts remains unknown. Endothelial cells are more likely candidates than cardiomyocytes, which appear to contain little of the enzyme (4).

Phagocytic cells generate $O_2^{\cdot-}$ from O_2 when these cells are activated. This is the so-called "respiratory burst," which is catalyzed by the enzyme NADPH oxidase. ROS generated from this source play a central role in host defense. Cardiopulmonary bypass (CPB) is associated with significant neutrophil activation as a result of ischemia-reperfusion injury but is also a result of a direct effect of contact of neutrophils with the extracorporeal circuit. Neutrophils therefore provide a major source of free radicals during and after CPB (5); indeed, free radicals from extracardiac sources during CPB are reported to greatly exceed those from the heart (6). Enzymes related to NADPH oxidase have been identified in nonphagocytic cells, including vascular smooth muscle cells (7).

NOS normally generates the free radical $NO^{\cdot}$, which is required for a variety of physiologic processes reviewed elsewhere (8). Increasing evidence points to the important role of the interaction between $NO^{\cdot}$ and $O_2^{\cdot-}$ to form $ONOO^-$. This reaction may act as an endogenous scavenging system, as $ONOO^-$ is considerably less reactive than $OH^{\cdot}$, the formation of which is necessarily decreased as $O_2^{\cdot-}$ is removed. In addition, there appear to be multiple physiologic signaling pathways that require $ONOO^-$, including a cardioprotective pathway (9). Nonetheless, excess $ONOO^-$ can also damage cellular components (10). Furthermore, electron transfer by NOS can become uncoupled under certain conditions, including hyperglycemia, directly causing the generation of $O_2^{\cdot-}$ by NOS (11).

Endogenous Antioxidant Systems

There are endogenous antioxidant systems that counteract the potential for injury to cellular structures by regulating the balance of individual ROS and their reactants. These endogenous antioxidants are upregulated when exposure of the cell to ROS is increased; thus the term "redox homeostasis" has been coined. However, under pathologic conditions such as ischemia-reperfusion, ROS formation can rapidly overcome antioxidant defenses and cellular injury ensues.

Major endogenous antioxidants in cardiomyocytes include superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione, Coenzyme Q10 (ubiquinone), and vitamins C and E (Fig. 1). SOD exists in 3 isoforms: MnSOD is within mitochondria, CuZnSOD is in the cytoplasm, and extracellular SOD is located extracellularly associated with glycosaminoglycans. During CPB endogenous antioxidant systems become activated to high levels in response to increased free radicals; such is the ROS production, however, that these rapidly become depleted so that CPB leads to oxidant damage (12).

Injurious Effects of ROS in the Heart

Free radicals exert their biologic effects by obtaining an electron from any molecule that may yield it, including lipids, proteins, and DNA. Lipid peroxidation causes particularly destructive effects on cell membranes. Modification of protein structure on attack by free radicals or their reactive products can alter function or lead to enhanced susceptibility to proteolysis. Proteins that are misfolded or partially unfolded are most sensitive to oxidation; thus, oxidative denaturation of dysfunctional proteins is believed to serve a physiologic housekeeping function. At the same time, consumption of ROS in this process aids in cellular antioxidation (i.e., scavenging).

ROS are strongly implicated in the pathogenesis of postischemic myocardial stunning, necrosis, programmed cell death (apoptosis), and vascular dysfunction. They may also contribute to the pathogenesis of postischemic dysrhythmias. Myocardial stunning refers to contractile dysfunction that is reversible, as cells remain viable, but which lasts hours to days. It is reported to occur after CPB (13) and also after off-pump cardiac surgery, albeit to a lesser extent (14). Clinically evident heart failure may occur as a consequence of stunning. Animal models provide evidence of a role for ROS in the pathogenesis of stunning (15). The $OH^{\cdot}$ radical is suggested to be particularly responsible and several target molecules have been proposed. Protection from stunning using scavengers is incomplete, indicating an additional contributing mechanism.

Cell death after ischemia-reperfusion results from necrosis and apoptosis. ROS are involved in the apoptotic pathway. Indeed, they have been shown to directly induce apoptosis after ischemia-reperfusion injury (16). Severe lipid peroxidation by ROS causes myocardial necrosis (17).

Dysrhythmias represent an important form of morbidity during reperfusion after ischemia and in patients undergoing cardiac surgery. ROS have been shown to have direct electrophysiologic effects that contribute to dysrhythmias (18). An important source of ROS leading to dysrhythmias after acute myocardial infarction may be neutrophils, as activation of neutrophils correlated with the incidence of dysrhythmias (19). Overall, however, some doubt remains over the exact role of ROS in dysrhythmias after ischemia-reperfusion injury in humans (20).

In settings other than ischemia-reperfusion, ROS have been implicated in the pathogenesis of cardiovascular disease. Chronic exposure of myocardium to ROS is proposed as a hypothesis for the development and progression of congestive cardiac failure (CCF) (21). In support of this, cardiac failure occurs predictably when exogenous ROS are applied to the previously uninjured heart (22). Moreover, excess ROS generation (23) and inadequate antioxidant defenses (24) have been reported in animal models of CCF. The mechanism of the eventual myocyte contractile failure is likely to be multifactorial; for example, sarcolemmal Ca^{2+} entry (25) and adrenoreceptor sensitivity (26) are each impaired under conditions of chronic oxidative stress. In addition, evidence links oxidative damage of cardiac mitochondrial DNA to postischemic left ventricular remodeling and heart failure (27); this suggests that impaired mitochondrial bioenergetics secondary to chronic redox imbalance may play a pivotal role. Human studies have shown evidence of oxidative stress in patients with CCF (28), and a relationship between exercise tolerance and oxidative stress in such patients has been reported (29). Nonetheless, whether these findings represent a true cause-effect relationship or merely epiphenomena in the failing heart remains to be established. Clinical trials evaluating antioxidant therapies in CCF have failed to demonstrate clear benefit.

Increasing evidence points to a pathogenic role for ROS in atherosclerosis. Risk factors for atherosclerosis, including cigarette smoking, diabetes mellitus, hypertension, and hypercholesterolemia, are associated with increased ROS levels within the coronary vasculature. Endothelial apoptosis (30), vascular smooth muscle cell proliferation (31), and activation of matrix metalloproteinases (32) are among several factors that are ROS-activated and that are central to different stages of atherogenesis. Furthermore, as described above, the interaction between $NO^{\cdot}$ and $O_2^{\cdot -}$ to form $ONOO^-$ acts to deplete $NO^{\cdot}$. The vasodilating and platelet-inhibiting effects of $NO^{\cdot}$ are thereby attenuated favoring vasoconstriction and thrombogenesis. Finally, restenosis after angioplasty has been linked to an effect of ROS. Vascular $O_2^{\cdot -}$ production increases after balloon angioplasty (33) and scavengers of ROS were shown to prevent neointimal formation after angioplasty (34).

Physiologic Effects of ROS in the Heart

The first quarter-century of ROS research focused on damage inflicted on cellular substituents as outlined above. Then, in the mid-1970s, ROS were shown to activate guanylate cyclase, causing formation of the well characterized second messenger cyclic guanosine monophosphate (35). Gradually, as other second messenger systems were shown to be modulated by ROS, a biologic role for ROS in cell function became accepted. Indeed, it is now known that ROS act as important mediators in signal transduction processes involved in multiple aspects of cardiac cellular function.

For several of these processes, ROS are produced after activation of cell-surface receptors. Included among these receptors are cytokine and G protein-coupled receptors. ROS generated in response to cytokine receptor activation are implicated in the control of apoptotic pathways. Conversely, ROS generated in response to activation of G protein-coupled receptors are involved in triggering cell proliferation and hypertrophy. These findings have lead to the suggestion that ROS are central in biologic control of cell life/death determination. In support of this, ROS generation after activation of the growth factor receptor TGF-β1 was shown to directly regulate phase G_1 of the cell cycle (36). More recently, differentiation of embryonic stem cells into cardiomyocyte cell lines was shown to be triggered by ROS (37), suggesting that they have an essential role in cardiac embryogenesis.

A free radical theory of development has therefore been proposed in which a change in redox state is a key embryologic signal.

Two major determinants of oxygen delivery have lately been shown to be redox regulated. The carotid bodies control ventilatory responses to changes in arterial oxygen. The oxygen-sensing apparatus of the carotid bodies is now believed to rely on changes in mitochondrial ROS production in response to altered oxygen tension in glomus type I cells (38). In the liver and kidney, the oxygen sensor that leads to production of the hormone erythropoietin, the regulator of red cell mass, is now known to sense changes in local ROS levels (39). Indeed, the transcription factor that codes for the erythropoietin gene was shown to be unaffected by changes in oxygen tension but was dose-dependently regulated by ROS (40). Similarly, in the coronary vasculature, there is evidence that vascular responsiveness to changes in oxygen tension is, in fact, mediated by superoxide (41).

The target molecules for these and other physiologic effects of ROS are a matter of dispute, for the broad range of redox-sensitive substituents greatly complicates an accurate appraisal. Ca^{2+} channels, mitogen-activated protein kinase enzymes and tyrosine phosphatases (which contain a ROS-sensitive cysteine residue in their active site) have each been shown to be activated by ROS. Upregulation of transcription factors, including nuclear factor-κB and AP-1 (42), occurs after application of H_2O_2. Consequent to transcription factor activation is altered gene expression. Indeed, DNA microarray studies show induction of nearly 100 genes in response to oxidant stress (43). In almost all instances the details of these ROS-dependent processes require further elucidation. Figures 3 and 4 illustrate cell signaling pathways and physiologic consequences in which ROS have a demonstrated role.

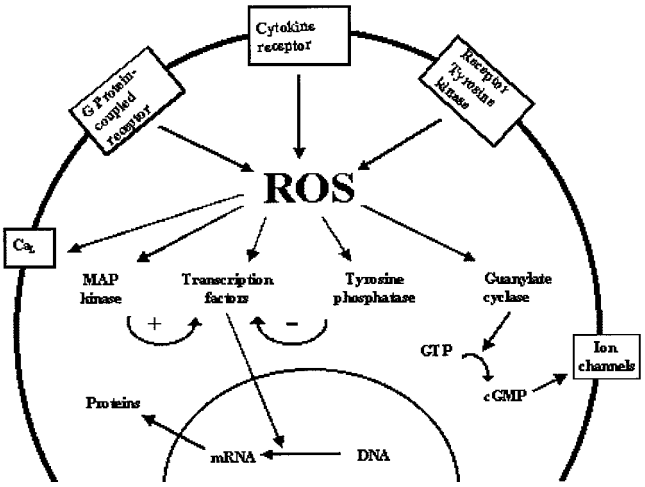

Figure 3. Cell signaling pathways in which reactive oxygen species (ROS) have a demonstrated role. Activation of cell surface receptors leads to the generation of ROS which have multiple intracellular effects including modulation of ion channels and activation of several enzyme systems. Activated transcription factors cause synthesis of proteins with wide ranging cellular effects. Mitogen-activated protein (MAP) kinase enzymes and tyrosine phosphatase, respectively, upregulate and downregulate trancription factors. Guanylate cyclase serves multiple functions including modulation of cell surface ion channels. Ca_L = L-type calcium channel.

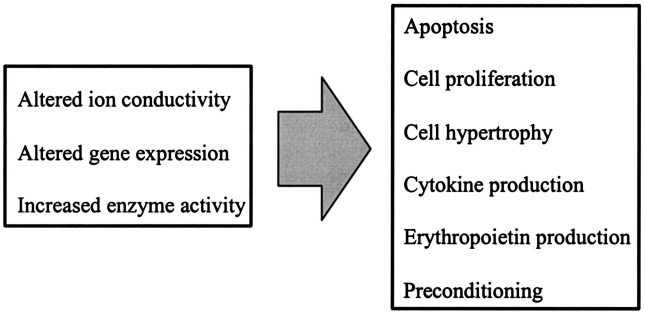

Figure 4. Reactive oxygen species (ROS)-mediated pathways utilize different effector mechanisms to exert biologically essential effects that appear to be particularly involved in the cell life/death cycle (apoptosis, cell proliferation) and adaptation to stress (cell hypertrophy, cytokine production, preconditioning).

Antioxidant Therapies

Administration of exogenous antioxidants has been extensively investigated as a means to attenuate myocardial ischemia-reperfusion injury and to treat or prevent chronic cardiovascular diseases. Investigations in a variety of animal models have shown beneficial effects of several drugs. However, clinical trials have furnished inconsistent results. The following paragraphs summarize experience with those antioxidant therapies that have evoked the most interest. These include drugs that scavenge formed ROS (superoxide dismutase [SOD], deferoxamine, vitamins C and E, acetaminophen) and drugs that prevent or alter the formation of ROS (deferoxamine, allopurinol).

Superoxide Dismutase

SOD decreases infarct size in animal models when given during ischemia and reperfusion (44), or in animals given a gene transfer for SOD (45). Human trials have been disappointing, however. Although one small clinical trial involving 23 patients reported protective effects of SOD against premature ventricular contractions after thrombolysis (46), a larger trial involving 120 patients undergoing angioplasty in the setting of acute infarction found no such change, nor was there any improvement in global function, i.e., SOD did not attenuate stunning (47). Conflicting results between animal studies and human trials may be

the result of species differences. Perhaps more importantly, the low cellular permeability of exogenously administered SOD likely limits potential for this therapeutic approach. Much of the production of ROS that is theorized to be deleterious is intracellular. SOD has a molecular weight of 32 kd and an overall negative charge. This largely limits exogenously administered SOD to the extracellular space. Furthermore, exogenous SOD undergoes rapid renal clearance (48).

Deferoxamine

Deferoxamine is a chelator of iron. By forming a stable complex with ferric iron it decreases the availability of iron for production of ROS by the Fe^{2+} reaction. There is also evidence that deferoxamine directly scavenges free radicals. Like exogenously administered SOD, its ability to penetrate cell membranes is poor (49). Nonetheless, studies in a variety of animal models have shown protective effects of deferoxamine against ischemia-reperfusion injury with respect to contractile function (50,51) and dysrhythmias (52). Studies in humans have shown protective effects but trials were underpowered to demonstrate any meaningful outcome differences. For example, Ferreira et al. (53) and Drossos and Lazou (54) included deferoxamine in cardioplegic solution for adults undergoing coronary artery bypass grafting (CABG) and valve surgery; they found that myocardial free radical production and lipid peroxidation were decreased but could show no differences in functional indices.

Mannitol

Mannitol, commonly added to pump prime for CPB, acts as a free radical scavenger, and is reported to decrease infarction after ischemia-reperfusion injury in some animal models (19). One small trial, underpowered to show differences in functional outcome variables, found that mannitol decreased plasma H_2O_2 in patients undergoing CPB (55). A further small clinical trial found no beneficial effects of mannitol added to cardioplegia in patients undergoing CABG (56). Once again, this may be related to the fact that mannitol remains in the extracellular compartment and therefore fails to penetrate to the site of ROS generation where initial damage occurs.

Allopurinol

The enzyme xanthine oxidase is an important source of ROS during reperfusion after ischemia in several tissues, with the possible exception of cardiac muscle, which contains little of the enzyme. Nonetheless, inhibition of xanthine oxidase activity by allopurinol was reported to decrease myocardial infarct size in dogs (57). One explanation for this discrepancy is that inhibition of extracardiac xanthine oxidase prevents release of ROS from tissues remote from the heart and thus secondarily attenuates cardiac injury. In support of this hypothesis, Nielsen et al. (58), in a rabbit model, demonstrated that activation of this enzyme in hepatoenteric tissue induced significant myocardial injury.

A literature review revealed 10 clinical trials of allopurinol in patients undergoing CABG, making it one of the most intensively investigated antioxidants in such patients. In eight of these, including the largest trial which involved 169 patients (59), improved functional indices and/or decreased CK-MB release were reported. Nonetheless, allopurinol has yet to gain acceptance in the routine management of cardiac surgery patients, likely because benefits in trials have been largely limited to less compelling end-points such as reduced inotrope use after termination of CPB. Demonstration of a decrease in hospital mortality, if there is such an effect with allopurinol, would require a considerably larger trial than has been performed to date.

Acetaminophen

Acetaminophen is a phenol and, like many other phenols, it has antioxidant properties. The studies of Merrill et al. (60,61) demonstrated protective effects of acetaminophen in animal models. Acetaminophen attenuated production of $OH^{\cdot}$ radical in the postischemic myocardium and improved postischemic recovery in guinea pig hearts (60). Acetaminophen also protected against exogenous H_2O_2, suggesting that it might act by inhibiting the Fe^{2+} reaction (61). There are no human trials of acetaminophen and human myocardial injury.

Vitamins C and E

Vitamin C (ascorbic acid) and E (α-tocopherol) are water- and lipid-soluble endogenous antioxidants, respectively. Several investigations have evaluated their effects on myocardial ischemia-reperfusion injury when given exogenously. Both these vitamins attenuated myocardial injury in animal models (62). Clinical trials have shown benefits of these vitamins in patients undergoing CABG. Dingchao et al. (63) reported that vitamin C led to decreased cardiac enzyme release and decreased intensive care unit and hospital stays compared with control patients. Yau et al. (64) reported that vitamin E improved contractile function and decreased cardiac enzyme release. Sisto et al. (65) found that a mixture of vitamins C and E and allopurinol decreased perioperative ischemic events and cardiac enzyme release. In contrast, Lassnigg et al. (66) found no benefit of parenteral vitamin E in patients undergoing heart surgery, and Westhuyzen et al. (67) found no benefit of a mixture of oral vitamins C and E in

patients undergoing CABG. The variety of preparations used, routes of administration, and dosages makes it difficult to draw conclusions from these disparate results. As a result, antioxidant vitamins have not become routine in cardiac surgery. Investigation of the effects of antioxidant vitamins on chronic cardiovascular disease states has been more extensive and study design has been more uniform. Placebo-controlled trials have repeatedly failed to demonstrate any useful benefit in patients with CCF or when used for secondary prevention of ischemic heart disease (68,69).

N-acetylcysteine

N-acetylcysteine (NAC) is a glutathione precursor and direct scavenger of several radical species. It was shown to decrease postischemic stunning, but not infarct size, in animal hearts (70). Small studies in the middle 1990s found that NAC decreased the neutrophil oxidative burst in patients undergoing CPB (71). In addition, when given in combination with nitroglycerin to patients with acute myocardial infarction, NAC decreased lipid peroxidation and improved the cardiac index (72). Interest in this drug for therapeutic use in patients with myocardial ischemia appears to have waned despite these promising results.

Measures to Decrease Neutrophil-Derived ROS During CPB

ROS produced by activated neutrophils via the respiratory burst cascade are believed to contribute to cardiac injury during and after CPB. Thus, measures that decrease neutrophil activation during CPB are proposed to decrease ROS formation and to improve cardiac outcome. Aprotinin, the widely used serine protease inhibitor, decreases neutrophil activation during bypass and has been shown to decrease ROS production by neutrophils *in vitro* (73). In an *in vivo* dog model, aprotinin was found to decrease postischemic contractile dysfunction (74) and in a clinical trial of patients undergoing CPB, aprotinin was found to decrease cardiac enzyme release (75). Effects of aprotinin are complex, however, and it is impossible at this point to determine if cardioprotective effects result from alterations of coagulation, fibrinolysis, cytokine formation, ROS formation, or a combination of each of these.

Leukocyte depletion by filtration was shown to decrease cardiac enzyme release and to preserve stores of glutathione (an endogenous antioxidant) in patients undergoing CPB (76). Two trials have shown that heparin-coating of extracorporeal circuits decreases evidence of ROS in blood (77,78). Corticosteroids have been shown to protect against cardiac reperfusion injury in animal hearts (79) and were found to decrease myocardial apoptosis in a pig model of CPB, possibly because of a decrease in ROS (80). Multiple trials have evaluated effects of corticosteroids to improve outcome after CPB, but despite a reduction in lipid peroxidation products and the largely universal finding of attenuated levels of inflammatory mediators, evidence does not support routine administration.

Summary: Antioxidants in Cardiac Injury

Laboratory studies have conclusively demonstrated that excess ROS cause functionally significant injury to cardiac tissue. In a variety of clinical settings, measurement of peroxidation production has shown clear evidence of ROS-mediated cellular injury, and it appears reasonable to suppose that ROS are causing important functional impairment similar to that shown in animal studies. Unfortunately, however, the clinical use of exogenous antioxidant substances has generally failed to live up to their early promise.

There are a number of possible reasons for this difference between laboratory and clinical studies. First, there is a fundamental difficulty in successfully delivering the drug to myocardial tissue at risk for injury, for ischemic tissue is poorly accessible to drug delivery; delivery of the drug solely at reperfusion may provide suboptimal effect, as significant ROS production is known to occur during ischemia, not just at reperfusion. Even at reperfusion, incomplete perfusion related to intravascular thrombosis and extravascular compression is likely to compromise drug delivery. A second major limitation for many of these drugs is poor cellular permeability. Scavenging of ROS will be largely limited to the extracellular space, but, as ROS will have exerted much cellular injury before the excess leaks out of the cell, limited cellular protection can be expected. Furthermore, within the reperfused myocyte, the mitochondrial electron transport chain is the predominant site of ROS production, and injury to mitochondrial components may be a key determinant of postischemic cellular function. Thus, permeability for subcellular compartments may be a prerequisite for effective action of antioxidants in this setting.

Finally, some of the lack of congruence between laboratory and clinical studies may relate to the fact that most laboratory studies entail subjecting a previously normal heart to an ischemic insult. In contrast, human studies generally occur in the setting of injury to a heart that has already been significantly altered by disease processes and by senescence.

Anesthetics: Interactions with Cardiac Redox Chemistry

Propofol

Propofol has a structure similar to phenol-based scavengers such as the endogenous antioxidant vitamin E, and its scavenging activity has been demonstrated in

animal and human models. It is effective against a broad range of radicals, including $ONOO^-$, the product of $O_2^{\cdot -}$ and $NO^{\cdot}$ (81). Intralipid, the solvent for propofol, also has scavenging activity, but this is negligible at clinical concentrations (81).

Propofol has been shown to be protective in experimental models of injury in several organs, including brain, liver, and heart (82,83). In intact organ models, at least some of this protection may result from preservation of endothelium-dependent vasodilation, which is impaired by oxidative stress. In heart models, propofol was found to be protective against peroxidative damage and functional impairment induced by exogenous H_2O_2 (84) and by ischemia-reperfusion (85). It has been suggested that propofol-induced cardioprotection may partly result from a direct effect on myocardial calcium influx (86), or from inhibition of mitochondrial permeability transition (MPT) (87). Opening of the MPT pore uncouples mitochondria and is involved in determining pathways that lead to apoptosis and necrosis. This latter effect may not be independent of the radical scavenging effect, however, as free radicals are believed to modulate the MPT (88).

Although propofol protects against ischemia-reperfusion injury when given before initiation of ischemia (85), its administration on reperfusion alone may be ineffective (89); this possibly reflects the occurrence of oxidant damage during the ischemic period (1).

Clinical studies support a protective effect of propofol against peroxidative injury. Propofol reduced levels of malondialdehyde, a marker of lipid peroxidation, after tourniquet-induced lower limb ischemia-reperfusion injury (90), and propofol decreased oxidative damage measured in platelets (91) from surgical patients. In a study by Sayin et al. (92), 24 patients were randomized to receive propofol 3–6 $mg \cdot kg^{-1} \cdot h^{-1}$ or fentanyl 10–30 $\mu g \cdot kg^{-1} \cdot h^{-1}$ during CABG operations. Atrial biopsies were taken at several time points during the bypass period, and lipid peroxidation was measured using thiobarbutiric acid substances. Compared with fentanyl, propofol suppressed lipid peroxidation, but the study was underpowered to detect differences in functional outcome measures. Further studies will be required to determine if the cellular protective effects of propofol translate into meaningful improvements in perioperative outcome.

Barbiturates

Barbiturates have been shown to have scavenging activity in laboratory models, although to a lesser degree than propofol. The majority of published work concerns protection against cerebral or spinal ischemia-reperfusion injury. Barbiturates differ in scavenging potency in neuronal models, as thiopental is more potent than either methohexital (93) or pentobarbital (94). Similarly, respiratory burst activity of neutrophils was suppressed more by thiopental than by methohexital or by phenobarbital (95). ROS scavenging effects of thiopental have been used to explain protective effects against bowel ischemia (96), but there is scant literature concerning scavenging effects on myocardial ischemia-reperfusion injury. No effect of thiamylal on altering radical production was reported using an isolated heart ischemia-reperfusion model (97). However, sodium amytal (amobarbital) is known experimentally to reversibly block complex I (NADH oxidoreductase) electron flow.

Clinical studies similarly show that the antioxidative effects of barbiturates are questionably significant. No change in oxidative stress was measurable in platelets of surgical patients after anesthesia with thiopental, in contrast to propofol, which decreased oxidative stress (91). In summary, free radical scavenging effects of barbiturates, particularly thiopental, likely contribute to the well-validated neuroprotective effects of these drugs, but there is insufficient evidence to support significant scavenging effects after myocardial ischemia-reperfusion injury.

Etomidate

Etomidate had a modest effect (98) or no effect (99) on respiratory burst activity of neutrophils at clinical concentrations. No effect of etomidate on adhesion of neutrophils to coronary endothelium was found in postischemic hearts (100).

Ketamine

Ketamine has a weak scavenging effect (4) and a weak effect to suppress ROS production by neutrophils that is probably minimal at clinical concentrations (101). The effect is not stereoselective, suggesting that specific receptor interactions are not involved (101). Conversely, Reinke et al. (102) showed that ketamine could generate free radicals *in vivo*. This was an incidental finding when rats were anesthetized with ketamine for later experiments on liver extracts; ROS formation also occurred when ketamine was added directly to the liver extracts. The radical product was thought to be a relatively stable ketamine-nitroxide radical. Interestingly, ketamine interfered with the ability to measure other free radicals, suggesting that this radical product was reacting with other radicals, possibly accounting for the reported scavenging effect of ketamine. This may have importance for investigators of ROS when laboratory animals are anesthetized with ketamine.

Benzodiazepines

Radical scavenging activity of midazolam is significantly less than that of propofol in *in vitro* preparations (103) and it is likely insufficient to impact on *in vivo* ROS formation. After limb-tourniquet ischemia-reperfusion, a large increase in ROS production was observed in patients sedated with midazolam whereas no increase was seen in patients sedated with propofol (104). Data on effects of benzodiazepines on neutrophilic respiratory burst activity are conflicting. Investigators have reported substantial effect (105), modest effect (106), or no effect (98) of midazolam on neutrophil-derived ROS at clinical concentrations.

Volatile Anesthetics

Volatile anesthetics have been shown to protect against cardiac ischemia-reperfusion injury by mechanisms that are apparently independent of their effects to cause coronary vasodilation (5) or to depress cardiac contractility (3,4). Free radical release was decreased after ischemia-reperfusion in animal hearts exposed to halothane (107,108), isoflurane (108), or sevoflurane (3,9). The specific mechanism of this decrease in ROS formation is unclear but it is likely to result from initiation of an intrinsic protective effect.

Brief exposure of the heart to a volatile anesthetic has been shown to induce a state of protection against the effects of ischemia-reperfusion injury in animal models (3,9,109–111). This has been termed APC because it resembles ischemic preconditioning in many respects, including functional and structural outcome. There is also evidence of its occurrence in humans. Belhomme et al. (112) reported that brief pretreatment with isoflurane decreased cardiac enzyme release in patients undergoing CPB. In a subsequent study, improved outcome in cardiac surgery patients given volatile anesthetics, rather than propofol (113), was considered suggestive evidence of a meaningful preconditioning effect of volatile anesthetics (114). Experimental and clinical studies on APC have been reviewed recently (115).

APC has been shown to lead to decreased cardiac ROS formation during and after ischemia and reperfusion (3,9); specifically, mitochondrial generation of ROS on reperfusion after ischemia was attenuated by APC (116). It is difficult to discern, however, based on existing reports, if reduced ROS generation merely accompanies cardioprotection with APC or represents a fundamental mechanism of protection mediated by APC. It is also difficult to determine if decreased ROS production occurs solely as a result of APC or if volatile anesthetics have additional direct antioxidant effects when present in the heart during ischemia and reperfusion. This latter possibility appears unlikely, as exposure to isoflurane (117) or sevoflurane (3) directly induces generation of $O_2^{\cdot -}$ in the intact heart. These observations suggest that ROS generation is essential for the initiation of APC, i.e., the free radicals are components, possibly the initiators, of the signaling cascade that leads to cardioprotection. This is the subject of a recent review (118).

APC can be abolished by ROS scavengers. Mullenheim at al. (111) found that ROS scavengers, given with isoflurane to rabbits, abrogated effects of isoflurane to induce APC. Novalija et al. (9) similarly abrogated sevoflurane-induced APC effects on mechanical and vascular function using ROS scavengers, and additionally abrogated preconditioning using the $NO^{\cdot}$ scavenger L-nitroarginine methyl ester; this suggested that $NO^{\cdot}$, or possibly the $NO^{\cdot}+O_2^{\cdot -}$ product $ONOO^-$, is also required for APC. Kevin et al. (3) found that myocardial ROS generation was reduced during both ischemia and reperfusion after sevoflurane APC and restored when a SOD mimetic was given during sevoflurane exposure. Thus free radicals appear to play a dual, and apparently paradoxical, role in APC as formation of a small quantity of ROS is required to trigger APC, while decreased free radical formation during subsequent ischemia and reperfusion may underlie, at least in part, the functional and structural preservation.

Although direct generation of free radicals during (3) and after (117) anesthetic exposure was demonstrated in animal heart models, previous studies in isolated coronary (119) and mesenteric (120) blood vessels suggested indirectly that anesthetics might cause ROS formation. How do volatile anesthetics generate free radicals? Hepatotoxicity attributable to halothane may result partly from conversion of halothane to free radicals by cytochrome P450 enzymes (121), but there is no evidence of similar reactions involving other volatile anesthetics in other organs. Studies by Cohen (122) in the 1970s, demonstrated that volatile anesthetics mildly depressed oxidative phosphorylation. Later, Kissin et al. (123) showed that NADH was increased in a dose-dependent manner by each of four different anesthetics.

The above studies strongly implicate the electron transport chain of mitochondria as the most likely source of anesthetic-induced ROS generation, as inhibition of mitochondrial enzymes may be expected to concurrently increase stores of reducing equivalents and to generate free radicals at corresponding electron transport chain complexes. Indeed, Hanley et al. (124) demonstrated recently that halothane, isoflurane, and sevoflurane directly inhibited complex I (NADH: ubiquinone oxidoreductase) activity in cardiac mitochondria. Riess et al. (125), moreover, found that a sevoflurane-induced increase in NADH level was abolished by 5-hydroxydecanoate, a putative inhibitor of the mitochondrial ATP-sensitive K^+ channel; their results indicate that the effect of volatile anesthetics on mitochondrial enzymes might also be indirect and

mediated by altered mitochondrial ion flux. Therefore, although volatile anesthetics do appear to generate ROS in mitochondria, the exact mechanism of this activity is unclear.

Volatile anesthetics alter several variables of neutrophil function, including adhesion to coronary endothelial cells and microbicidal activity. In 1911, Graham (126) demonstrated impaired neutrophil function after exposure to ether. Halothane (127–129) and enflurane (128) are reported to decrease ROS release from leukocytes. Isoflurane, in contrast, has been reported to have little effect (127,129) or even to increase ROS release by neutrophils (128). Nakagawara et al. (128) reported inhibition of calcium mobilization in neutrophils in a dose-dependent manner with halothane and enflurane. This inhibition correlated with effects on $O_2^{\cdot -}$ formation; Ca^{2+} is believed to act as second messenger in the activation of NADPH oxidase. Protein kinase C is also involved in this activation pathway. Frohlich et al. (130) reported that direct protein kinase C activation resulted in ROS formation that was unaltered by volatile anesthetics. This seems to exclude a direct effect of anesthetics on NADPH in neutrophils and also excludes a direct scavenging effect on formed free radicals. Thus the current evidence suggests volatile anesthetics interfere with the neutrophil respiratory burst activation pathway by interfering with a step proximal to protein kinase C activation, most likely at the stage of capacitative Ca^{2+} influx. Nonetheless, the reason for differential effects of various volatile anesthetics is unknown.

As discussed above, ROS from neutrophils are believed to contribute to cardiac injury related to CPB, ischemia, and reperfusion. Attenuated ROS release from neutrophils exposed to volatile anesthetics may contribute to cardioprotective effects of these drugs. Interestingly, one study indicates that suppression of neutrophil ROS formation resulting from volatile anesthetics exhibits a memory phase, i.e., is present even after the anesthetic is discontinued; thus this may also contribute to the cardioprotection observed with APC (131).

Summary

During and after cardiac ischemia-reperfusion, and during CPB, widespread ROS formation occurs in cardiac and vascular cells and in neutrophils. ROS are central to the pathophysiology of cardiac ischemia-reperfusion injury; they contribute to the genesis of myocardial stunning, necrosis, and apoptosis, and possibly to dysrhythmias. They have also been linked to the development of chronic cardiovascular diseases including CCF and atherosclerosis. Multiple animal investigations have demonstrated attenuated cardiac injury when scavengers of ROS were administered before ischemia or in the setting of chronic cardiovascular diseases. Clinical studies have demonstrated decreased ROS-mediated tissue damage, but have failed to conclusively demonstrate important outcome benefits of such therapies. Some IV anesthetic drugs act as ROS scavengers. Propofol is a particularly effective scavenger. Laboratory and clinical trials have demonstrated cardioprotective effects, but whether this property of propofol provides an advantage for patients at risk of myocardial ischemia-reperfusion seems doubtful.

Among the most intriguing findings in recent cardiovascular anesthesia research is the observation that volatile anesthetics generate ROS, an effect that paradoxically confers cardioprotection. There is now some evidence to suggest that this property can be effectively harnessed to the patient's advantage. Finally, one of the main lessons that recent free radical research teaches us is that free radicals should no longer be considered as only producing damage. $NO^{\cdot}$ is essential to normal vascular function. Other ROS, like $O_2^{\cdot -}$, H_2O_2, $OH^{\cdot}$, or $ONOO^-$, may induce or mediate APC and also play a part in signaling cascades essential to normal cardiac function. Free radicals interact with each other and with endogenous antioxidant systems. It is the balance of all these constituents that determines their beneficial versus deleterious effects.

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