Superoxide Radical: Controversies, Contradictions, and Paradoxes (43885B)

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Abstract. **The study of free radical biology has engendered a great deal of controversy and apparently conflicting observations, particularly with regard to the use of the antioxidant enzyme superoxide dismutase as a protective or therapeutic agent. Slowly, the reasons behind the confusion are beginning to emerge. The superoxide radical, $O_2^{\cdot-}$, has a number of paradoxical physiological and pathophysiological roles. Several examples of the radical's schizophrenic behavior include its roles in bactericidal action versus inflammation, as a modulator of cell division versus malignant transformation and apoptosis, and as both an initiator and a terminator of lipid peroxidation.** [P.S.E.B.M. 1995, Vol 209]

Is it possible to have too much of a good thing? Most would answer affirmatively. It may be more provocative to ask whether it might be possible to have too little of a bad thing (or at least of a thing generally perceived as bad). Some free radical biologists are now pondering these questions with regard to the "good" superoxide dismutases and the "bad" free radicals that they scavenge. In the 25-year history of free radical biology, there has been a slowly progressing realization that things are not as simple as they first appeared to be. I am not suggesting that we were wrong—only pointing out that our level of understanding was superficial and incomplete.

The process of evolution is driven by the pressure to make the best of our situation, whatever it may be. It is not surprising then to discover that, faced with the inevitability of free radical production due to our existence in a powerfully oxidizing atmosphere, we have evolved ways to put these generally noxious compounds to a few constructive uses. Evidence now suggests that there may be several such constructive uses for the superoxide radical. It is universally accepted that the production of superoxide radical by activated polymorphonuclear leukocytes and other phagocytes is an essential component of their bactericidal armamentarium (1), even though the same action may contribute to the tissue damage associated with inflammation (2–4). It is also quite clear that superoxide production may trigger cell division (5) and may serve as a normal physiological regulator of the process, even though the same action may contribute to malignancy (6) or apoptosis (7) under other circumstances. Recently, we have found that the superoxide radical may serve the cell as a terminator of lipid peroxidation, even though it may paradoxically serve to initiate lipid peroxidation through the liberation and redox cycling of cellular iron stores (8).

The realization that superoxide is not all bad changes how we must deal with problems of overproduction of the radical under pathophysiological circumstances, including its production during the reperfusion of ischemic tissues. The view a decade ago was that complete scavenging of the radical was the best possible outcome. In view of the radical's potential beneficial actions, however, the concept of a proper balance becomes unavoidable. The best working hypothesis at the moment is to assume that in a healthy cell the optimal balance exists between superoxide production and superoxide scavenging. If this balance is upset under pathological conditions, the best therapeutic goal would be the restoration of the optimal balance. Overcorrection becomes a real possibility, and the consequences of overcorrection may lead to erroneous conclusions concerning the role of superoxide in the pathology in question. The history of free radical biology is fraught with controversy. Much of it

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undoubtedly stems from the failed recognition of the schizophrenic nature of this radical metabolite.

Bactericidal Action Versus Inflammation

As a first example of the dual nature of superoxide's roles, let us consider its production by phagocytes. These roles now seem straightforward and noncontroversial. Babior showed that neutrophils stimulated to engulf latex particles produce the superoxide radical (9). The bactericidal roles of the free radical and its metabolites, H_2O_2 and HOCl, are now well understood (1). Much subsequent work has characterized a multicomponent NADPH oxidase located in the plasma membrane of the neutrophil (10). This enzyme may be the only enzyme in the body that produces superoxide by design rather than by accident. The genetic inability to produce superoxide in this instance causes the life-threatening condition known as chronic granulomatous disease (CGD). Neutrophils from such individuals have seriously impaired ability to kill microorganisms that have been ingested, leading to multiple recurrent local infections, and often to septicemia and death at an early age (11).

The neutrophil is programed for overkill, not for caution, because so much is at risk if it fails to carry out its mission. We pay a substantial price for this ultraconservative policy. The "antibiotics" in the neutrophil's arsenal ($O_2^{\cdot -}$, H_2O_2, and HOCl) are of the broadest possible spectrum: they are not the selective silver bullets of our modern day pharmacopoeia. Therefore, an inflammatory locus becomes littered with casualties. Host tissue cells succumb to the oxidant attack, along with the microbes and the neutrophils themselves. Infected tissues display the cardinal signs of inflammation (redness, heat, swelling, pain, and loss of function) not necessarily as a result of the invading microbe, but largely due to the war waged by the attacking neutrophils and the unavoidable damage to host tissues.

Thus, in the case of bactericidal action and inflammation we see paradoxical actions of superoxide. We also see the logic of the concept of balance. In the case of autoimmune diseases and allergies the war may be waged at a perceived, but nonexistent or nonthreatening enemy. The result of this overproduction of superoxide is damage to the organism. In the case of any immune compromise there may be underproduction of superoxide, and again the result is damage to the organism. The balance may be remarkably delicate. The neutrophils of patients with Down's Syndrome (trisomic for Chromosome 21) possess 50% more Cu,Zn-SOD than normal due to the gene dosage effect (12). This extra SOD in the neutrophil results in more scavenging of superoxide produced by these cells, or in less net production of the radical. Accordingly, they seem less able to kill microbes, and the patient is again put at risk (13).

Proliferation Versus Malignant Transformation Versus Apoptosis

As a second example of apparently paradoxical roles of superoxide, let us consider the effects of the radical on the rate of cell growth and proliferation, on the process of malignant transformation, and on the phenomenon of programed cell death, or apoptosis. Much evidence supports the view that mild oxidative stress induces normal healthy cells to divide (5, 14–16), even though cell growth inevitably declines when the oxidative stress becomes severe. This response to mild stress makes good sense in the context of the oxidative events associated with the inflammatory process, as discussed above. At an inflammatory locus there is often physical destruction of cells (e.g., a wound that must be healed). Hence, the oxidative shift resulting from an infiltration of activated phagocytes serves as an ideal signal for surviving cells in the area to proliferate to replace lost cells, and for fibroblasts to migrate into the area, to proliferate, and to lay down scar tissue to repair the wound. As the phagocyte infiltration subsides and oxidative status returns to normal, the cells cease their proliferation and return to a quiescent state. This normal response is illustrated as the range between Point A and B in Figure 1.

What happens if a cell is genetically modified, and the resulting mutation produces a permanent oxidative shift in the cell's redox status? The cell may be put in a mode of continuous proliferation known as malignant transformation (illustrated by a cell at Point B in

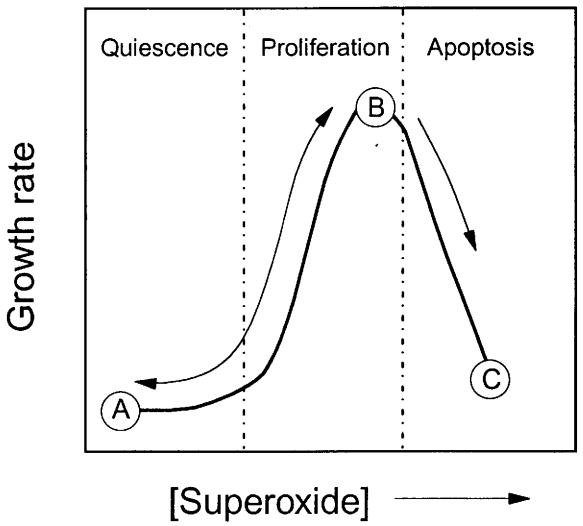

Figure 1. Hypothetical response of a cell to increasing exposure to the superoxide radical. Under normal physiological conditions, cells may move reversibly between Point A and B. At higher oxidative stress, however, the threshold inducing apoptosis may be crossed, leading to irreversible programed cell death (Point C).

Figure 1, unable to return to quiescence). Tumor cells are nearly always low in Mn-SOD and catalase, and are often low in Cu,Zn-SOD (14, 17). This relationship is only apparent when tumor cells are compared with the specific cell type from which the tumor arose. Different cell types within a tissue may vary greatly in their content of antioxidant enzymes, depending on the nature of their individual metabolic roles. The genetic loss of Mn-SOD serves to raise the steady-state concentration of superoxide within a cell, and may be interpreted as a perpetual signal to proliferate (18). Conversely, overexpression of Mn-SOD causes a permanent reductive shift in the cell's redox status. St. Clair *et al.* (19) showed that mouse cells transfected to overproduce Mn-SOD were about twice as resistant to radiation-induced malignant transformation as the control cells. Furthermore, the transfection of cultured human melanoma cells with the cDNA encoding Mn-SOD results in loss of the malignant phenotype (6). Clones showing a 5-fold or greater increase in Mn-SOD activity completely lost the ability to form tumors when injected into nude mice (0/16), whereas the nontransfected melanoma cells caused tumors 100% of the time (18/18).

Apoptosis is a form of programmed cell death characterized by cell shrinkage with nuclear condensation and fragmentation (20, 21). It differs from necrosis (characterized by cell swelling) in that apoptosis is an active process, on the part of the cell, that may be triggered by any of a number of internal or external stimuli. Apoptosis may serve in many cell types as a fail-safe device to prevent cells from running amok and proliferating uncontrollably in the face of a persistent oxidative stress. Hence, as redox status shifts toward the oxidative, a cell may go from quiescent to proliferative. If the oxidative stress is internal (e.g., resulting from a genetic mutation) rather than transiently external, the proliferative state may be perpetually sustained and the cell becomes malignant. If the oxidative shift is sufficient in magnitude, the fail-safe device may take control and force the cell to actively commit suicide (apoptosis, illustrated by Point C in Fig. 1). In many or all cases, malignancy may require a failure of the fail-safe device, in addition to sustained internal oxidative stress.

Thus, oxidative stress is one of the conditions that can trigger apoptosis (22–24). The literature provides many studies concluding that oxidative stress (usually rather severe) causes a decrease in cell growth rate, although few if any of these studies have looked for apoptotic cells.

In these examples, we see that the superoxide radical may play an integral role in the regulation of essential physiological processes (proliferation and apoptosis) but may also play a central role in the pathological process of malignant transformation.

Initiation Versus Termination of Lipid Peroxidation

As a final example of the paradoxical roles of superoxide in physiological processes, let us consider the process of lipid peroxidation. The oxidation of polyunsaturated fatty acyl side chains in membrane phospholipids poses a constant threat to cellular integrity and function. These structures are by nature prone to react with molecular oxygen, especially in the presence of redox active transition metal ions, via free radical chain reactions. The process may be initiated by any oxidant capable of abstracting an allylic hydrogen from a polyunsaturated side chain:

$$X + LH \rightarrow XH^{\cdot} + L^{\cdot}$$

The lipid radical $L^{\cdot}$ adds molecular oxygen extremely rapidly to produce a lipid dioxyl radical, which is a sufficiently good oxidant to abstract an allylic hydrogen from another unsaturated side chain, producing a lipid hydroperoxide (LOOH) and propagating the chain:

$$L^{\cdot} + O_2 \rightarrow LOO^{\cdot}$$

$$LOO^{\cdot} + LH \rightarrow LOOH + L^{\cdot}$$

(The $LOO^{\cdot}$ radical may alternatively rearrange and break down to produce malondialdehyde and other radical products, rather than yielding exclusively a lipid hydroperoxide product, but these options do not affect the present discussion.) Thus, every cell must deal with this ongoing process. Glutathione peroxidases can reduce the hydroperoxides to alcohols to prevent their reaction with reducing agents such as ferrous iron (25), and the antioxidant vitamins E and C can safely scavenge the lipid dioxyl radical to break the chain cycle (26). Iron plays a crucial role in the initiation of new lipid-radical chain reactions (27, 28). Ferrous iron can cause the reductive lysis of the oxygen-oxygen bond in a preexisting lipid hydroperoxide molecule (LOOH) giving rise to the alkoxyl ($LO^{\cdot}$) radical, which may then serve as an initiating radical (29):

$$LOOH + Fe^{2+} \rightarrow LO^{\cdot} + Fe^{3+} + OH^{-}$$

$$LO^{\cdot} + LH \rightarrow LOH + L^{\cdot}$$

How then does superoxide radical affect this process? The overproduction of superoxide within a cell can encourage lipid peroxidation by mobilizing iron from the tissue storage protein ferritin (30, 31) and by keeping this redox-active pool of iron in the reduced state so that it can initiate lipid peroxidation as described above:

$$O_2^{\cdot -} + \text{Ferritin-}Fe^{3+} \rightarrow O_2 + \text{Ferritin} + Fe^{2+}$$

and

$$O_2^{\cdot -} + Fe^{3+} \rightarrow O_2 + Fe^{2+}$$

Hence, it is well-established that superoxide can indirectly initiate lipid peroxidation. Ironically, the only way to eliminate a radical is by reacting it with another radical. (Because radicals have an odd number of electrons, any reaction with a nonradical must yield a product that is another radical.) From a teleological perspective, it would be useful if a cell had available a continuous supply of a relatively unreactive free radical for the purpose of promoting a radical-radical annihilation to eliminate the very dangerous lipid dioxyl radical. We have suggested that superoxide meets these criteria (8). It is produced as a by-product of oxygen metabolism, and is therefore continuously available in all cells. As free radicals go, it is relatively unreactive. Its concentration is maintained at a very low, but nonzero value by the cellular SOD content. If alkoxyl or dioxyl radicals were scavenged by $O_2^{\cdot -}$, then entire chains of reactions would be prevented or terminated:

$$LO^{\cdot} + O_2^{\cdot -} + H^+ \rightarrow LOH + O_2$$

$$LOO^{\cdot} + O_2^{\cdot -} + H^+ \rightarrow LOOH + O_2$$

Therefore, it was hypothesized that $O^{\cdot -}$, in addition to being able to liberate iron and to initiate lipid peroxidation, may also serve as a terminator of lipid peroxidation, such that over scavenging the radical may increase net lipid peroxidation (8, 32). If this hypothesis is correct, then indices of lipid peroxidation should be high at both low concentrations of SOD (where initiation would be high) and at high concentrations of SOD (where termination would be low). At some intermediate SOD concentration, initiation by superoxide would be largely suppressed, but termination by superoxide would still be making a contribution, and net lipid peroxidation would be at a minimum. We found these predictions to be true (8), and experimental verification is shown in Figure 2. In this model of the isolated perfused rabbit heart, 60 min of no-flow ischemia followed by reperfusion results in serious oxidative stress. As the concentration of SOD in the perfusate is increased, a new balance is restored when the concentration reaches 5 mg/l. Note that maximal recovery of preischemic function (developed pressure) occurs when lipid peroxidation is reduced to the minimum. These results suggest that for any given level of oxidative stress, a single optimal concentration of SOD exists that will restore the superoxide level to yield the best combination of initiation and termination (i.e., the one producing minimal net lipid peroxidation). Any concentration of SOD other than the optimal leads to increased lipid peroxidation and therefore to increased oxidative stress.

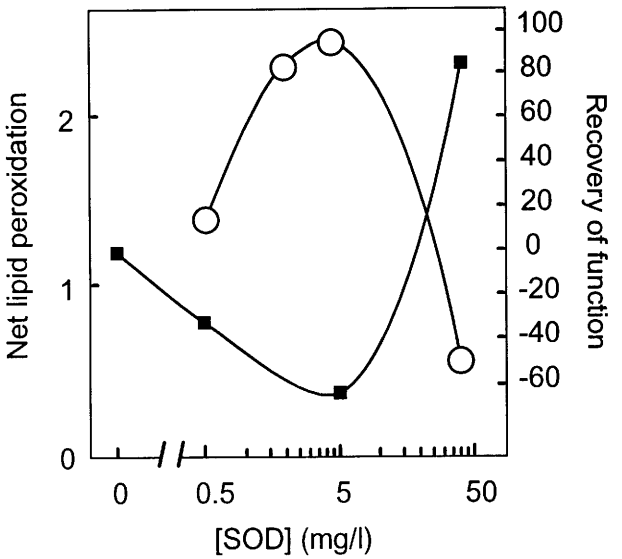

Figure 2. Relationship of net lipid peroxidation and functional recovery of isolated rabbit hearts to administered dosages of superoxide dismutase. Recovery of developed pressure correlates inversely with net lipid peroxidation. Maximal protection is seen at a dosage of 5 mg/l in the coronary perfusate. Data are replotted from Ref. 8.

Relationships to Pathophysiology

As discussed in the previous section, free radical production following ischemia and reperfusion is particularly problematic, because the production peaks very rapidly upon reoxygenation (33) and appears to decline significantly even during the first minute. Because there is a single concentration of SOD that provides maximal protection for any given rate of radical production (32, 34), the dosage of exogenously administered SOD would have to follow the fluctuation of superoxide production precisely to maintain maximal protection—an impossibly difficult task. During the initial burst of superoxide production a significant amount of cellular iron may be mobilized. Even so, lipid peroxidation may not be a major problem early on because lipid hydroperoxides (with which ferrous iron reacts) have not yet accumulated, and because the superoxide concentration is high, chain length will be kept quite short. Later on, the rate of lipid peroxidation may steadily increase as superoxide production diminishes (chain length increases) and the concentrations of mobilized iron and hydroperoxides steadily accumulate. Furthermore, low molecular weight antioxidants such as vitamins E and C may now be exhausted, also contributing to greater and greater chain length (35). As reperfusion injury progresses, there is clear evidence that the inflammatory process begins to contribute as neutrophils begin to infiltrate the damaged tissue (36). As a result, oxidative stress may begin a new rise.

The term apoptosis was first used by Kerr *et al.* (20) to describe the death of certain hepatocytes in the

ischemic liver. In the central zone of complete ischemia it was clear that cells died of necrosis, with swelling and bursting. At the periphery of the ischemic zone, however, the morphology was quite different; the cells shrank and died. In retrospect, of course, these are the cells exposed to the greatest oxidative stress, where injured cells are still oxygenated and superoxide production is elevated. More recent work has indicated a role for apoptotic cell death in cerebral ischemia and reperfusion (37, 38).

Summary

Active oxygen intermediates, including superoxide, are capable of inflicting severe damage to tissues. At the same time, these species serve as important physiological signals and play vital protective roles. Is superoxide radical good or bad? The question is an oversimplification. It is either and both, depending on where, when, and how much is produced. For these reasons, the literature on free radical biology is conflicted, contradictory, and controversial. Our view is much clearer than it was two decades ago, but we have probably seen only the tip of the iceberg.

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