OXIDANTS AND ANTIOXIDANTS: THE CONCEPT OF BALANCE

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During the nearly thirty years that we have studied the superoxide dismutases, there has been a slowly progressing realization that superoxide radical may not be totally bad, and that we have evolved ways to put this generally noxious compound to a few constructive uses. 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). It is also clear that superoxide production may trigger cell division (3) and may serve as a normal physiological regulator of the process, even though the same action may contribute to malignancy (4) or apoptosis (5) 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 (6).

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 is more attractive. 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 possibility, leading to possibly 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 undoubtedly stems from our failure to recognize the schizophrenic nature of this radical metabolite.

As an example of the paradoxical roles of superoxide in physiological processes, let us consider the process of lipid peroxidation, which poses a constant threat to cellular integrity and function. Iron plays a crucial role in the initiation of new lipid-radical chain reactions. Ferrous iron can cause the reductive lysis of the oxygen-oxygen bond in a pre-existing lipid hydroperoxide molecule (LOOH) giving rise to the alkoxyl (LO[•]) radical which may then serve to initiate a new chain reaction. The overproduction of superoxide within a cell can mobilize iron from ferritin (7) or from enzymes such as aconitase that contain a 4Fe/4S center (8). This maintains a redox-active pool of iron in the reduced state capable of initiating lipid peroxidation. Ironically, the only way to eliminate a radical is by reacting it with 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 (6). 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 non-zero* value by the cellular SOD content. Therefore, it was hypothesized that O^{•-}, 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. We have found this prediction to be true (6). In the isolated perfused rabbit heart, ischemia followed by reperfusion results in serious oxidative stress. Maximal recovery of preischemic function (developed pressure) occurs when hearts are reperfused with SOD at 5 mg/L, and lipid peroxidation is reduced to a minimum. Any concentration of SOD greater than or less than the optimal leads to increased lipid peroxidation and therefore to increased oxidative stress.

As a second and more subtle example of the 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 programmed cell death, or apoptosis. Mild oxidative stress induces normal healthy cells to divide (3), 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. 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.

If a cell is genetically modified, and the resulting mutation produces a permanent oxidative shift in the cell's redox status, then the cell may be put in a mode of continuous proliferation known as malignant transformation. Tumor cells are nearly always low in Mn-SOD

and catalase, and are often low in Cu,Zn-SOD (9). Furthermore, the transfection of cultured human melanoma cells with the cDNA encoding Mn-SOD results in loss of the malignant phenotype (4).

Apoptosis is a form of programmed cell death characterized by cell shrinkage with nuclear condensation and fragmentation. It may serve in many cell types as a failsafe 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 shift is sufficient in magnitude, the failsafe device may take control and force the cell to actively commit suicide. In many or all cases, malignancy may require a failure of the failsafe device, in addition to sustained internal oxidative stress. 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.

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