Redox Modulation of Chemotherapy-Induced Tumor Cell Killing and Normal Tissue Toxicity

James H. Doroshow

The traditional view of intracellular oxidation–reduction, or redox, balance in epithelial cells, which is more than three decades old (1), emphasizes a dynamic equilibrium between the production of reactive oxygen species (ROS; these include superoxide anion, \( \text{O}_2^- \); hydrogen peroxide, \( \text{H}_2\text{O}_2 \); and chemical species with the characteristics of the hydroxyl radical, \( ^\cdot\text{OH} \)) by a variety of flavin dehydrogenases that occupy essentially every cellular compartment, and the detoxification of these species by a broad range of antioxidant enzymes and related small molecules (2). ROS are produced by the mitochondrial electron transport chain during the course of cellular respiration, by cytochrome P450–related components of microsomes (3), and, in many human tumors, by the recently described family of membrane-bound NADPH oxidases that possess a high degree of homology with components of the NADPH oxidase system of polymorphonuclear leukocytes and macrophages (4). ROS are detoxified by a complex and interactive series of proteins and small molecules—including members of the superoxide dismutase, glutathione peroxidase, and peroxiredoxin gene families, as well as catalase—that ultimately reduce \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) (or lipid peroxides) to nontoxic species, such as lipid alcohols or water (5,6).

In this perspective, ROS are toxic by-products of “normal” cell growth and metabolism that contribute to the production of oxidized DNA base lesions in both the nucleus and mitochondrion. These DNA lesions increase in frequency with age, are mutagenic if not repaired, and may contribute to the senescent phenotype (7). Similarly, pathologic “oxidant stress,” which is produced by a wide variety of conditions, such as drug-enhanced reactive oxygen metabolism, chronic inflammation, ischemia reflow, and exposure to environmental toxins, has traditionally been viewed as a form of toxic cell injury that may serve to initiate cell damage, repair, or death cascades (8–10).

Until recently, reactive oxygen metabolism was seen as synonymous with the potential for the development of tissue injury. Reactive oxygen formation was associated many years ago with the cardiac toxicity of the anthracycline antibiotics and the site-specific oxidative metabolism of these drugs in cardiac sarcoplasmic reticulum and mitochondria (11). Moreover, over the past decade, reactive oxygen production that is unrelated to known drug metabolism pathways and that usually occurs 6–48 hours following drug exposure, after cells have committed to a specific death pathway, has been demonstrated in tumors treated with a series of structurally dissimilar anticancer agents, including camptothecin, vinblastine, cisplatin, paclitaxel, cytarnabine, and histone deacetylase inhibitors (12–16). The mechanism(s) by which exposure to so many different cancer chemotherapeutic agents initiates reactive oxygen production is unclear. Developing an understanding of these mechanisms is an important area of current investigation, because the initiation of a common reactive oxygen cascade by such a wide variety of drugs questions our traditional classification of anticancer agents and the molecular targets that have been employed to build the classifiers used today.

A broader, physiologic view of the role of reactive oxygen has also developed over the past decade (17,18). There is now little question that ROS, in particular \( \text{H}_2\text{O}_2 \), perform a critical role in cell signaling following the binding of essentially all receptor tyrosine kinase ligands (19–22). Furthermore, low levels of \( \text{H}_2\text{O}_2 \) are potent proliferative, rather than antiproliferative, signals that are essential for the trophic effects of a wide variety of cytokines (23). Finally, very recent evidence suggests that ROS production is essential for the growth of p53-deficient tumors in vivo (24). These data are consistent with a large body of literature suggesting that the redox balance of many epithelial tumor cells favors an elevated oxidant set point, leading to a growth-inhibitory effect of antioxidants in these tumors (25–28).

In light of our evolving understanding of the role of ROS in tumor cells, there are at least three perspectives from which to evaluate the work of Alexandre et al. in this issue of the Journal (29). The first perspective is the traditional understanding of oxidant stress as a precursor to tissue injury, which involves the complex interaction of free radical production, detoxification, and repair of radical damage. The second perspective is the view of ROS as critical messengers of signal transduction that play an essential role in tumor cell proliferation or in the maintenance of genomic instability, which facilitates growth (24,30). The third, evolving perspective is the view of ROS as secondary “death markers” for cells that are in the process of committing to apoptotic or necrotic pathways following a toxic insult (12).

Alexandre et al. extended their prior observation (31) that mangafodipir, a contrast agent used clinically for magnetic resonance imaging, possesses antioxidant (specifically, \( \text{O}_2^- \)- and \( \text{H}_2\text{O}_2 \)-detoxifying) properties, to evaluate the role of ROS in the therapeutic activity and toxicity of oxaliplatin, paclitaxel, and 5-fluorouracil. They examined the effect of mangafodipir on the growth-inhibiting properties of these chemotherapeutic agents against mouse colon cancer cells and on their hematologic toxicity in the mouse, as well as their toxic effects against human leukocytes in vitro. The strongest portion of the work demonstrates that mangafodipir is protective against the hematologic toxicity of paclitaxel in a murine model. Two other well-known chemicals, MnTBAP and CuDIPS—which, like mangafodipir, have superoxide dismutase–like activity (that is, the ability to catalyze the reduction of \( \text{O}_2^- \) to \( \text{H}_2\text{O}_2 \)) but, unlike mangafodipir, lack...
known effects on H₂O₂—were ineffective in the same model. The glutathione precursor N-acetylcysteine (NAC), which can function directly in the detoxification of H₂O₂ and as a thiol donor to protect critical sulfhydryl groups throughout the cell, also prevented the hemato logic toxicity of paclitaxel. On the other hand, mangafodipir, but not NAC or MnTBAP or CuDIPS, appeared to improve the therapeutic activity of paclitaxel against CT26 mouse colon cancer cells in vivo. Mangafodipir and NAC, but not MnTBAP or CuDIPS, also protected normal leukocytes from the toxic effects of oxaliplatin and 5-fluorouracil in vitro. Alexandre et al.’s results are of interest primarily because they suggest that at least some of the toxic effects of secondary ROS production following prolonged exposure to chemotherapeutic agents can be ameliorated. Because mangafodipir has been used clinically as a contrast agent, this drug might be appropriate for study as a chemoprotective compound in human trials.

However, several important issues regarding these results, in particular the authors’ interpretation of the effect of antioxidants on tumor cells, require clarification. Although it has been recognized for many years that antioxidants, including NAC, can blunt the cytotoxicity of platinum-containing chemotherapeutic agents against both tumor cells and normal tissues (24–26,35), as was confirmed in the present paper, NAC itself has profound anti-proliferative effects across a wide spectrum of tumor cell types (24,26,36). Furthermore, other thiol-containing antioxidants have been shown to dramatically enhance the therapeutic activity of 5-fluorouracil in vivo (28). These antitumor effects may well be due to the ability of antioxidants to interfere with the critical growth factor–dependent oxidant proliferation pathways outlined above. Thus, depending on the model system, therapeutic benefit may arise either by increasing reactive oxygen production in tumors, as suggested by the work of Alexandre et al., or by decreasing the intracellular reactive oxygen levels required by a variety of signal transduction pathways essential for tumor cell replication. Also, although the in vivo results in this study seem clear, the data regarding both the protective effects of mangafodipir in leukocytes in vitro and the enhancement of ROS production in drug-treated tumor cells by mangafodipir are less compelling because the concentrations of paclitaxel, oxaliplatin, and 5-fluorouracil used for these experiments were far in excess of those achieved in routine clinical practice.

Overall, this study contributes to our rapidly developing understanding of tumor cell redox balance and to the possibility that therapeutic approaches to the modulation of oxidant-mediated growth control may be possible in the near future, perhaps with mangafodipir or with other redox modulators in development. However, as this and other recent efforts demonstrate, a remarkable degree of variation in the redox status of current model systems exists, indicating that the optimal study venue for agents that target the intracellular oxidant milieu of tumors may well be early-phase clinical trials.

References

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