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FREE RADICALS AND CARCINOGENESIS

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The role of free radicals and active states of oxygen in human cancer is as yet unresolved. Various lines of evidence provide strong but inferential evidence that free radical reactions can be of crucial importance in certain carcinogenic mechanisms. A central point in considering free radical reactions in carcinogenesis is that human cancer is really a group of highly diverse diseases for which the initial causation and the progression to clinical disease occur through a wide variety of mechanisms. Furthermore, for many human cancers it appears that there are alternate pathways capable of tumor initiation and tumor progression. While for certain of these pathways free radical reactions appear necessary, it is unlikely that there are human cancers for which free radicals, or any other mechanism, are sufficient for the entire process beginning with the genetic alteration leading to a somatic mutation and eventually resulting in clinically overt disease. It is crucial that we view free radical reactions as among a panoply of mechanisms leading to human cancer, and consider research about the role of free radicals in cancer as opportunities to prevent the initiation or progression of human cancer.

KEY WORDS: Free radicals, tumor promotors, phorbol esters, phagocytic cells, active oxygen, carcinogenesis.

INTRODUCTION

Among the myriad reactions that participate in the many paths to clinically overt human cancer, there are certain reactions for which reasonable evidence exists to infer a role for free radicals. The most clearcut occur in radiation carcinogenesis. Much of what we know about free radical chemistry in biological systems was first worked out in irradiated solutions, including interactions at the level of nucleic acids, DNA, the nucleus, whole cells and organisms. Much of what we are trying to do in chemical carcinogenesis is to determine if the insights gained from radiation biology and radiation carcinogenesis are applicable.

Before discussing the role of free radicals in cancer, it is necessary to consider the definitions of cancer. The common thread to the various definitions is that of an unchecked growth of cells that are unresponsive to stimuli normally resulting in differentiation and eventual cell death. Note that such a definition in essence encompasses a final common pathway rather than describing a single starting point or pathway leading to the cancer. For the purposes of the present review, it should be stressed that there are many different pathways that can lead to the initial somatic mutation resulting in the autonomous cell we call a cancer cell, and many different pathways from this single mutated cell to clinical cancer. Further there is no one step that, while necessary, is sufficient by itself to be responsible for the formation of an overt cancer.

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The question of the role of free radicals in cancer must be phrased in terms of asking in which of the many pathways and multiple steps leading to cancer will free radicals be found to play a role. Based on the work briefly discussed below it appears highly likely that there are important processes central to the causation of certain cancers which one can reasonably ascribe to free radical reactions. As a corollary it is highly unlikely that free radical reactions are a necessary cause of all cancers.

Chemical Carcinogenesis

A major confounding factor in evaluating the role of free radicals in chemical carcinogenesis is the multipotential nature of the chemical carcinogen. Chemicals for which one can readily demonstrate reactions resulting in the release of free racicals also have other potential reactions. In a simple test tube study one can quantify the rates for each of the potential reactions and determine which one is dominant. However, this is difficult to do in a biological system, in part because of the complexity of the system, but more importantly because chemical rate constants for free radical generation and their subsequent reaction must be interpreted in relation to cellular defense mechanisms and in relation to the biological step that is most crucial in determining the disease outcome.

For example, in our studies on the mechanism on benzene hematotoxicity we have been focussing on the possibility that the opening of the benzene ring by hydroxyl radicals produces α,β-unsaturated aldehydes and related products responsible for bone marrow effects, including leukemia.[1-3] This is a quantitatively minor chemical pathway, originally considered to account for no more than 2% of total benzene metabolites. Other potential pathways for benzene-induced DNA damage and leukemia might involve a direct or indirect radical attack on DNA through relatively major metabolites such as hydroxylated intermediates.[4-6] However, in the face of evidence suggesting that hepatic metabolism plays a role in bone marrow toxicity,[7] we find it difficult to believe that a short-lived free radical or related active species could travel from liver to bone marrow. Accordingly, simple examination of chemical rate contants for the formation or reactivity of active species possibly derived from benzene in the liver does not necessarily provide an answer for bone marrow toxicity and leukemia.

Anatomical localization of reactions must be considered as an intracellular as well as an interorgan phenomenon. *In vitro* studies of the possible genotoxic effects of free radical generating reaction mixtures are often done simply by adding DNA to the mixture. Extrapolation of the findings from this experimental approach to *in vivo* conditions has at least three problems: will short-lived free radicals actually be generated in the cell in sufficient proximity to DNA so that reaction is likely; will normal cellular defense mechanisms be able to protect DNA *in vivo*; and will the relatively homogenous nature of the *in vitro* experimental condition lead to free radical chain lengths that are longer than are possible under the heterogenous chemical composition of the normal cell.

In the case of free radical reactants acting from the outside of the cell, the initial contact will be the cell membrane and a likely, but not sole, consequence will be lipid peroxidation. Damage to DNA caused by lipid peroxidation has been ascribed to 4 mechanisms: the action of free radicals derived from the decomposition of peroxidized lipid, of lipid hydroperoxides, of carbonyl derivatives, and through a second message mechanism resulting in clastogenic activity.[8,9] In our view, it is unlikely that

either free radicals or lipid hydroperoxides derived from the oxidative decomposition of cell membranes can travel to the nucleus, particularly in view of the presence of efficient cellular defense mechanisms, and particularly at sublethal reaction rates. (Note that a dying cell can not be responsible for the initiation of cancer). We prefer to consider a role for carbonyl derivatives[10–13] or some of the more recently explored mechanisms by which membrane processes may be translated into DNA alterations through programmed messages.[14–16]

There are a number of possible pathways in the causation of cancer by known human and animal carcinogens in which one can propose a role for free radicals for those processes in which direct effects on DNA occur: free radicals can be involved solely in a metabolic step eventually resulting in an ultimate chemical carcinogen; the chemical itself or one of its metabolites can be converted into a radical that can react directly with DNA by an addition reaction; the compound through its unwanted reactions or through "normal" metabolic pathways may cause the formation of free radicals which attack DNA; or, following addition to DNA, the adduct can itself generate free radicals in proximity to genetic matieral (Table I; see references [8,9,18–30] for reviews and illustrative examples).

Part of the indirect evidence supporting a role for free radicals and active states of oxygen in carcinogenesis is the inhibitory effects of antioxidants in a number of systems.[17,31–36] This has led to some encouraging but inconclusive, epidemiological studies and ongoing prospective trials of antioxidant treatment in humans.[37,38]

Carcinogenic compounds that lead to damage to DNA are known as whole carcinogens or genotoxins. A special subclass are compounds which have co-carcinogenic activity in that they themselves do not produce genotoxic effects but can enhance the carcinogenic action of a simultaneously administered genotoxic agent. A role for free radicals in one such interaction has been suggested for cigarette smoke in which radicals derived from quinone components of tar are capable of activating polycyclic aromatic hydrocarbons (PAH) to a carcinogenic intermediate.[23,24] Similarly, sulfite autooxidation has been suggested to have a cocarcinogenic role in PAH activation.[39]

Tumor Promotion

The above discussion is based on the assumption that the mechanism of chemical carcinogenesis is primarily through reaction with DNA. However, the concept of tumor promotion is now well established.[40] It has been identified experimentally through a number of different types of approaches in different organs and laboratory

TABLE I
Possible Roles of Free Radicals and Active States of Oxygen in Chemical Carcinogenesis

INITIATION	
1.	Activation of procarcinogen
2.	Binding of carcinogen to DNA
3.	Direct damage to DNA without binding
4.	Formation of genotoxic radical following binding to DNA
PROMOTION	
1.	Processes leading to enhanced cellular expression of somatic mutation
2.	Processes leading to suppression of extracellular inhibitors of growth

animals. The basic design is to give a single subcarcinogenic dose of a known genotoxic carcinogen, the initiator, and to follow this treatment with repetitive doses of an agent, the promoter, which by itself will not produce cancer. A role for free radicals and active states of oxygen in tumor promotion is supported by studies showing that free radical generating compounds act as promoters, that free radical scavengers act as promotion inhibitors, and that endogenous cellular antioxidant defenses undergo changes upon promoter treatment.

Some of the earliest and most thoroughly studied tumor promoters have been the phorbol esters present in croton oil.[40] The inflammatory properties of these potent tumor promoters have long been recognized.[41] However, the lack of correlation between the tumor promoting ability and inflammatory activity of the various phorbol esters had appeared to rule out a role for inflammatory activity in tumor promotion. Inferential evidence suggestive of a role of free radicals in tumor promotion, as well as our studies demonstrating that antiprotease compounds that inhibit tumor promotion also inhibited the production of superoxide anion radical by PMA-activated phagocytic cells,[42,43] led us to reexamine the correlation between the tumor promoting abilities of phorbol esters with one specific aspect of the inflammatory process, that of the production of active oxygen species by phagocytic cells. The excellent correlation observed[36,44] led to the hypothesis that free radicals might play a role in tumor promotion, a hypothesis that has been advanced by the studies of Emerit, Marnett, Cerutti and Slaga.[9,21,45,46] Some of the evidence supporting a role for free radicals and active states of oxygen in tumor promotion is outlined in Table II. Compounds such as benzoyl peroxide are tumor promoters; organic peroxides and active oxygen species mimic certain effect of tumor promoters in cell systems; tumor promoters affect cellular antioxidant enzyme activities; and, various antioxidants inhibit tumor promotion and progression.[21,31,34,36,45,47–49,51]

As shown in Table I, there are two basic ways that free radicals may be involved in tumor promotion: through growth of the cancer cells by enhancing the expression of the somatic mutation or other growth-promoting processes between initiation and the development of a clinically recognizable tumor; or by interference in the extracellular processes that normally inhibit cancer cell growth, for example by preferentially killing non-cancer cells.[52] It must be emphasized that tumor promoters such as phorbol esters have many different effects on cells. Proof of a role for free radicals in tumor promotion, particularly in human cancer, is still lacking.

Our further work has focused on developing an animal model in which the effects of tumor promoters on phagocytic cells could be investigated *in vivo*.[10,53–57] Intraperitoneal injection of tumor promoters into CD-1 mice with the subsequent

TABLE II
Free Radicals in Tumor Promotion and Progression

1.	Free radical generating compounds, such as organic peroxides, are tumor promoters and progressors.
2.	Promoters stimulate the endogenous production of reactive O_2 species.
3.	Reactive O_2 generating systems mimic the action of tumor promoters in cell culture.
4.	Tumor promoters provoke rapid and sustained changes in cellular antioxidant enzyme activities.
5.	Antixoidants inhibit tumor promotion and progression.

harvesting of peritoneal macrophages and the evaluation of their status in regards to the production of superoxide anion radical by staining with nitroblue tetrazolium in the presence and absence of SOD, has provided a valuable model. The complete promoter PMA and incomplete promoters such as mezerein and the calcium ionophore A23187 are capable of stimulating $O_2^{\cdot}$ production in peritoneal macrophages *in vivo*. The response is dependent on the physiological state of the macrophage.[53,54] The inactive PMA analog phorbol diacetate (PdA) inhibited oxy radical production in peritoneal exudate cells stimulated *in vivo* by mezerein when co-administered with this second stage promoter. Based on these results, a two-stage promotion bioassay was conducted which showed that PdA also inhibits second stage promotion by mezerein in SENCAR mice.[54] These studies indicate that the peritoneal macrophage system can serve as a model system for identifying promoters and antipromoters, and be a tool in studies on the mechanism of tumor promotion.

Experimentally, tumor promotion has generally been considered to be a process that begins with an initiated cell and ends with the appearance of a benign tumor, such as a skin papilloma. This process has been shown to have at least two stages.[58] In addition, to be analogous to human cancer, studies of carcinogenesis must extend beyond the papilloma stage to full blown malignancies, a process known as tumor progression in which a role for free radicals has also been suggested.[59] Pursuit of the goal of secondary prevention of cancers, i.e. early detection in a treatable stage, has led to an improved understanding of the processes by which an early human malignancy or a benign tumor becomes transformed into clinically overt cancer. Again, a wide degree of biological variability is apparent in that certain early tumors rapidly metastasize while others remain dormant for many years. In many tumors distinct stages can be observed which seem to mimic different stages in the process of tumor induction observed in laboratory animals. However, much more needs to be known about the biological processes responsible for these different changes. In certain instances there is inferential clinical evidence suggesting a role for inflammatory processes ranging from such observations as the appearance of skin tumors on the bridge of the nose of eyeglass wearers to the much greater frequency of colon tumors in inflammatory bowel diseases such as ulcerative colitis. An interesting model of bowel inflammation leading to the promotion of tumors has been reported in which a role for free radicals from activated phagocytes can be inferred.[60] The hypothesis that free radicals play a role in human cancer, while having some support, is far from proven.[8,17,61]

Conclusion

There is a need for a cautionary note of skepticism. The role for free radicals in many of the biological processes involved in the causation of cancer by chemicals seems very likely. However, proof of that role including the detection of free radicals of active states of oxygen in biological systems *in vivo* is difficult to obtain. We must be rigorous in search for such proof. It is not appropriate to blithely accept a role for free radicals simply because of inhibition by antioxidants at some dose level in some experimental system. Many new and exciting approaches are underway including assessing the mechanisms by which reactive intermediates including oxidant species may activate oncogenes, and searching for changes in DNA or other macromolecules capable of serving as specific biomarkers of exposure or of the effect of free radicals and active states of oxygen on the human genome.[62,63]

Not only does the field contain fascinating scientific challenges but, of crucial

importance, if a role for free radicals can be unequivocally obtained, then prevention of cancer is possible either through removing the offending source or through clever use of appropriate antioxidants to hinder the process.

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