REVIEW ARTICLE

ROLE OF FREE RADICALS IN GASTROINTESTINAL CANCER

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ABSTRACT

Incomplete reduction of oxygen in vivo generates oxygen-derived free radicals, which are highly reactive species capable of extensive cellular injury. The experimental and clinical evidence for a role of free radicals in the process of development and spread of gastrointestinal cancer is examined in the present review article.

Keywords: neoplasia, oxyradicals, permissive, survival, metastases

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INTRODUCTION

Considerable interest continues to revolve around the aetiopathological significance of oxygen-derived free radicals. In fact, the relevance of oxyradicals to clinical medicine cannot be overestimated at a time when researchers repeatedly identify new roles for oxidants, reactive oxygen species and free radicals in human disease. Oxygen is required for the production of energy, oxidation of endogenous compounds and detoxification of xenobiotics. However, its incomplete reduction leads to the formation of a series of reactive chemical intermediates including oxygen-derived free radicals[1-6]. These radicals are highly reactive species capable of widespread, indiscriminate oxidation and peroxidation of protein, lipids and nucleic acids, which can lead to significant cellular injury and even organ failure[6-10]. The regulation of the oxidative environment of cells is a highly complex process in which a fine balance exists between free radical production via metabolism and the activity of a multilevel antioxidant system which limits the concentration of these cytoxic species[1,2,4]. While the study of free radicals is a rapidly growing field, the central question remains: are these radicals a mere consequence of the various pathological processes responsible for disease development or are they actually the initiators of these processes?

FREE RADICALS AND CANCER

Tumour development

High-energy radiation may lead to malignant transformation by free radical reactions[11]. Most of the energy taken up by tissues exposed to radiation is absorbed by the cell water causing one of its oxygen-hydrogen covalent bonds to split. Thus, a single electron is left on the hydrogen and another on the oxygen, creating the hydrogen and hydroxyl radicals[11]. The latter radical is most reactive and as such, does not remain for more than a few microseconds before combining with another molecule in its vicinity. However, during its lifetime, it can attack and injure almost every molecule found in living cells. The reactions of the hydroxyl radical, like any other radical, leave behind a legacy in the cell in the form of propagating chain reactions[1,2]. Therefore, if the hydroxyl radical attacks DNA, free radical chain reactions spread through the DNA causing chemical alteration of the bases that can lead to mutations as well as strand breakage. Imperfect repair of such damage may lead to oncogene activation and carcinogenesis[11]. Radiation-induced oxidative damage in experimental animals can be reduced by the free radical scavengers dimethyl sulphoxide and dithiothreitol, suggesting that such scavengers may act as radioprotective drugs[12,13].

The permissive theory for carcinogenesis

Tumour development and progression can be considered a microevolutionary process based on sequential changes in multiple determinants[14]. It may be viewed as the gradual emancipation of a clone of somatic cells from the complex controls that regulate its growth. Tumour progression has been defined as the "independent reassortment" of multiple "unit characteristics" that influence the neoplastic phenotype.

By the early 1980's, a torrent of new information about the protein products of viral and cellular oncogenes moved the principal locus of neoplastic action towards the periphery of the cell - the cytosol and the plasma membrane - where growth factor receptors, protein kinases, and guanosine triphosphatases that transduce external signals normally reside[15]. In the model of carcinogenesis which emerged from these findings, excessive amounts of cytoplasmic or extracellular growth regulating proteins, or mutant versions of them, would perturb the control network by sending persistent signals for unbridled growth to the nucleus, through still unidentified messengers. Ultimately, the transcriptional programme would be affected, but as a final common pathway mediated by epigenetic events, not as a primary mechanism for neoplastic change through mutations of transcriptional regulation genes[15].

Cells are normally controlled by the opposing effects of growth stimulating and growth inhibiting factors. When the balance between the positive and negative regulation is upset, the result may be the uncontrolled growth of cancer cells[16]. Transforming growth-factor-alpha (TGF - α) is a classic stimulant of cell growth, whereas transforming growth-factor-beta (TGF - β) inhibits the division of most types of cells, with the exception of fibroblasts[16]. The outer cell membrane is the site of the TGF-β receptor[16]. It thus appears that failure to produce TGF-β or loss of responsiveness to its inhibitory effects might allow oncogene expression and cancer development.

Strong positive correlation exists between the total dietary fat intake and the mortality from major cancers such as colon, breast, endometrial, ovarian and prostate cancers[17]. Fat

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consumption differs between individuals and among communities and may, consequently, be one of the variables which determine the incidence of cancer development in man. Current evidence indicated that the effect of high polyunsaturated fat intake is due to peroxidative reactions involving phospholipids in plasma low density lipoproteins and polyunsaturated fatty acids in membranous structures[17]. The peroxidation of phospholipids in plasma low density lipoproteins generates free radicals[18-20]. These radicals combine preferentially with antioxidants (eg α-tocopherol and vitamin C), which minimise the consequences of lipid peroxidation by terminating the chain reaction[18-20]. Excessive generation of the hydroxyl radical close to cellular membranes enables it to attack the fatty acid side chain of the membrane phospholipid, producing lipid hydroperoxides[1,2]. Accumulation of these agents in a membrane inflicts oxidative damage which reduces its fluidity and permeability, disrupts its function and can cause it to collapse. In addition, lipid hydroperoxides can decompose to yield a range of highly cytotoxic products such as the aldehydes[1,2], which can further perpetuate the tissue damage. This damage may be an essential pre-requisite for enabling carcinogens to initiate neoplasia.

The fact that not all individuals exposed to chemical carcinogens respond similarly in terms of their potential for tumour development is demonstrated in factory workers handling carcinogenic agents where the incidence of tumours varies so much among those working under similar conditions and for comparable lengths of time. This observation suggests a role for an additional factor necessary for the development of cancer besides the carcinogenic agent itself. It is unlikely in other than experimental circumstances and industrial accidents, that biological tissues are suddenly exposed to the large doses of carcinogenic agents that are capable of producing tumours, but that tissues are constantly exposed to carcinogenic agents, which are prevented from producing harmful effects by the growth regulating factors situated in their membranes. These factors could be damaged by non-carcinogenic agents such as the free radicals resulting from the metabolism of dietary fat which lead to lipid peroxidation of cellular membranes with subsequent injury that enables oncogene expression and carcinogenesis.

This hypothesis was examined in detail in the rat by the use of the free radical scavengers dimethyl sulphoxide and allopurinol[21-23]. The results demonstrated that the incidence of colonic cancer is directly dependent on dietary fat intake and that carcinogen doses producing colonic cancer in the presence of high fat intake are rendered harmless when low fat is being consumed[21]. This permissive role of dietary fat in colonic carcinogenesis appeared to be mediated by oxygen-derived free radicals[21].

Perhaps the best characterised biological damage caused by oxyradicals is their ability to stimulate the free radical chain-reaction known as lipid peroxidation. The products of this peroxidation, and in particular lipid hydroperoxides and lipid alcohols, have been shown to stimulate DNA synthesis and cell proliferation in colonic epithelium[24,25]. It is thus construed that the free radical attack on cellular membranes damages regulating mechanisms which are responsible for controlling the cell's growth. On the basis of the results mentioned above, it appears that these mechanisms also afford protection against carcinogens by maintaining a balanced cell growth. More precisely, the colon was exposed to carcinogenic agents without tumour development until the free radicals produced by lipid metabolism initiated lipid peroxidation of cellular membranes, thereby damaging growth control mechanisms and allowing carcinogenesis.

It is, therefore, proposed on the basis of the observations made in the animal study[21] that living tissues are continuously exposed to doses of carcinogens that are unable to produce tumours until a non-carcinogenic factor damages the growth regulating mechanisms situated in their cellular membranes. Such a factor appears to be the oxygen-derived free radicals which attack cellular membranes and initiate the lipid peroxidation that damages the growth regulating factors, thus allowing carcinogenesis to be triggered by the already existing carcinogens (ie permissive).

The significance of oxygen-derived free radicals in colonic cancer may be much more than hitherto realised. Babbs[26] suggested that the radicals generated in faecal material next to colonic epithelium may play a role in the aetiology of colonic cancer. This hypothesis was based on the earlier conjecture by Graf and Eaton[27] who proposed a connection between hydroxyl radicals and colonic carcinogenesis coupled with the observation that highly reactive hydroxyl radicals can be produced abundantly by suspensions of faeces under aerobic conditions[26]. The relatively high concentrations of iron in faeces, together with the ability of bile pigments to act as iron chelators that support a Fenton reaction, may permit the generation of hydroxyl radicals from the superoxide and hydrogen peroxide produced by bacterial metabolism[26]. Consequently, Babbs[26] argued that such free radical generation in faeces could be an important factor in the aetiology of colonic cancer: the oxidation of procarcinogens either by faecal hydroxyl radicals or by secondary peroxyl radicals to form active carcinogens or mitogenic tumour promoters. Moreover, intracolonic free radical formation may explain the observed correlations of a higher incidence of colonic cancer with red meat in the diet, which increases stool iron, and with excessive fat in the diet, which may increase the faecal content of procarcinogens and bile pigments[26].

Formation of hydroxyl radicals within the colon depends on the superoxide driven Fenton reaction. The necessary iron for this reaction is provided from the diet, since only a small fraction of dietary iron is absorbed in the upper gastrointestinal tract, whereas the faecal source of the superoxide radical is the respiratory activity of bacteria, notably *E coli*[28]; perhaps together with spontaneous autoxidation of ferrous iron chelates that have been previously reduced by metabolites of the anerobic subpopulation of faecal flora[26]. Colonic superoxide radicals may also be generated by the lipoxygenase activity of normal or sloughed colonic epithelial cells[29].

Free radical mechanisms may be an important pathway for metabolic activation of carcinogens in the intracolonic environment. The required unsaturated lipids may derive from dietary sources, from the turnover of epithelial cells or from synthesis by faecal micro-organisms and/or epithelial cells[30]. Also relevant to the intracolonic environment is the requirement for detergent stabilization of the hydrophobic substrate, such as benzo[a]pyrene, as well as unsaturated fatty acids from dietary or other sources[26].

The hydroxyl radicals generated within the colon could easily trigger a variety of carcinogenic mechanisms. They could participate in aromatic hydroxylation reactions to form carcinogenic products[31,32], or abstract hydrogen atoms from indoles to form radicals that subsequently dimerize to produce potential carcinogens[33]. In the case of benzo[a]pyrene, free radical oxidations mimic those produced by the cytochrome P450 system in the liver, which produces active carcinogens from originally less toxic substrates[34-36]. In particular, the metabolic activation of benzo[a]pyrene hydrodiol to a carcinogenic agent occurs by epoxidation to form the diolepoxide. The resultant diolepoxides are then believed to act as ultimate carcinogens through DNA adduct formation[35,36]. Such epoxidation reactions occur by free radical mechanisms, in which the lipid hydroperoxides in the presence of ferrous iron act as epoxidizing agents[35,36]. The key step in this novel pathway for activation of

polycyclic aromatic hydrocarbons is the non-enzymatic epoxidation of isolated double bonds by lipid peroxyl radicals, a mechanism known to occur effectively at temperatures between 30°C and 60°C and at pO_2 as low as 10 mmHg[37]. In addition to polycyclic aromatic hydrocarbons like benzo[a]pyrene, aromatic amines may also be oxidised to mutagenic derivatives by peroxyl radicals[38]. It is thus apparent that one electron oxidations and epoxidations of procarcinogens by free radical mechanisms can lead to their activation to proximate carcinogens.

The conclusions gathered is that oxygen-derived free radicals formed within the colon could enhance the production of carcinogens while these radicals and those generated as part of normal human metabolism exhibit a permissive role in the mechanism of colonic carcinogenesis. Moreover, the permissive role of free radicals may be the key factor for carcinogenesis since living tissues can be exposed to carcinogens without developing cancer.

It must be stressed that apart from the abovementioned mechanisms, oxygen-derived free radicals may directly attack DNA leading to strand breakage, destruction and fragmentation of bases, and chromosomal aberrations which produce the mutations that can enable oncogene expression[1,2,4]. Whether this action is involved in the process of malignant transformation as an intracellular initiator or helper is not known. Consequently, the possibility remains that free radical-induced carcinogenesis is a multifactorial event of both intra- and extracellular mechanisms.

The observations that dietary fat is a tumour promoter raise questions pertinent to bile pigments and salts. The increased bile flow effected by the consumption of high fat diets may have implications in the process of carcinogenesis independent of the role of free radicals generated by lipid peroxidation. The relatively high concentration of iron in faeces, together with the ability of bile pigments to act as iron chelators that support a Fenton reaction, may well permit the generation of hydroxyl radicals from the superoxide and hydrogen peroxide produced by bacterial metabolism. Such an enhanced free radical generation in faeces could participate in colonic carcinogenesis as described above. On the other hand, the detergent-like bile salts may also emulsify procarcinogenic aromatic compounds.

Tissues normally have a number of protective enzymes which inhibit lipid peroxidation, eg superoxide dismutase, catalase and glutathione dismutase. There are also present, a number of naturally occurring antioxidants such as alpha-tocopherol, ascorbate and glutathione, which also slow these processes and act as breakers in the chain reactions that propagate peroxidation. The rat study mentioned above[21] shows that despite such protective mechanisms, high amounts of dietary fat damage tissues and allow carcinogenesis.

The influence of free radicals on survival

The influence of the oxygen-derived free radical scavengers allopurinol and dimethyl sulphoxide on the development of hepatic metastases and on survival in the rat bearing colonic cancer was studied[39]. Administration of these agents after producing the colonic tumours prevented the development of hepatic metastases three months later and significantly extended survival. The results propose that scavenging oxyradicals impair the hepatic metastases of colonic carcinoma and prolong survival. The fact that free radicals are involved in the mechanism of tissue injury[1-6] suggests that the destruction of tissues by cancer is mediated by oxyradicals and that scavenging radicals sustain the integrity of tissues and increase their resistance to the malignant metastases. This would, therefore, incur a survival advantage[39].

Clinical implications

The post-operative influence of oxygen-derived free radicals on survival in gastric and colonic cancer patients was assessed in prospective randomised controlled double-blind trials[40-42]. Throughout these trials, scavenging oxyradicals significantly hindered the development of metastases and prolonged survival. The results suggest that free radicals are directly involved in the mechanism of gastric and colonic cancer and that removing these radicals impairs the spread of cancer, thereby affording a survival advantage. It follows from the results that the ability of gastric and colonic cancer to practise a detrimental effect on normal tissues and to impair their integrity is mediated by free radicals.

CONCLUSIONS

Oxygen-derived free radicals are produced by various metabolic processes in the body. The fine balance between these radicals and the activity of a multilevel antioxidant system regulates the oxidative environment of cells and limits the concentration of toxic chemical species within them. Excessive formation of oxidants can cause tissue injury. However, this injury can itself generate more oxidants which may contribute to a worsening of the damage already sustained.

The clinical trials carried out to evaluate survival rates of patients undergoing long-term therapy with free radical scavengers following potentially curative or palliative surgery for gastrointestinal cancer have yielded promising results regarding the efficacy of these scavengers. The implication of such results is twofold. First, the realisation of a significant role for oxygen free radicals in the pathogenesis and spread of gastrointestinal cancer and, second, the introduction of a new weapon to our armamentatrium against this disease. Further randomised studies are obviously needed to establish the definitive role and optimal application of radical scavengers in the management of gastrointestinal cancer. It is perhaps extremely important to stress the need for increasing our knowledge of the various physiological and biochemical systems that are responsible for controlling the oxidative environment of cells. This knowledge may help explore the depth of contribution to the management of gastrointestinal cancer that can be achieved by antioxidant therapies.

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