Antioxidant Supplementation: A Linchpin in Radiation-Induced Enteritis

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Abstract

Radiation enteritis is one of the most feared complications of abdominal and pelvic regions. Thus, radiation to abdominal or pelvic malignancies unavoidably injures the intestine. Because of rapid cell turnover, the intestine is highly sensitive to radiation injury, which is the limiting factor in the permissible dosage of irradiation. Bowel injuries such as fistulas, strictures, and chronic malabsorption are potentially life-threatening complications and have an impact on patient quality of life. The incidence of radiation enteritis is increasing because of the current trend of combined chemotherapy and radiation. The consequences of radiation damage to the intestine may result in considerable morbidity and even mortality. The observed effects of ionizing radiation are mediated mainly by oxygen-free radicals that are generated by its action on water and are involved in several steps of signal transduction cascade, leading to apoptosis. The oxyradicals also induce DNA strand breaks and protein oxidation. An important line of defense against free radical damage is the presence of antioxidants. Therefore, administration of antioxidants may ameliorate the radiation-induced damage to the intestine.

Introduction

Radiation enteritis is one of the most feared complications of abdominal and pelvic regions.[1] Radiation-induced injury to the gastrointestinal (GI) tract has been reported since 1898, and the subsequent development and widespread use of radiotherapy made it possible to give higher and more effective doses of radiation but with more risk of intestinal damage. Since the 1980s, the incidence of acute radiation enteritis appears to have increased, because more than 50% of patients with cancer receive radiotherapy as a measure of their treatment.[2] The consequences of radiation damage to the intestine may result in considerable morbidity and even mortality. An estimated 5% to 15% of patients who receive radiotherapy develop complications such as abdominal pain, diarrhea, and fluid loss.[1,3,4]

The biological effect of radiations occurs through the production of reactive ions after their interaction with normal tissues. These ions combine with water present in the cell and induce the formation of hydroxyl radical and other free radicals. These free radicals cause cell death through single- and

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double-strand breaks in the DNA.[5] Radiation may also cause disruption of cell membrane leading to cell death.[6] However, rapidly proliferating tissues such as small intestine are particularly sensitive to radiation. Epithelial cells undergo apoptosis and shed off from the intestinal villus.[1,7]

Investigations on the molecular mechanisms underlying radiation-induced apoptosis have led to the conclusion that in the presence of air, reactive oxygen species (ROS) are involved in signal transduction cascade leading to apoptosis.[8] Thus, an important line of defense against free radical damage is the presence of antioxidants. Among the numerous substances that have the potency to influence radiosensitivity, the antioxidant vitamins deserve special attention,[1] as they are physiological compounds that have no significant toxicity when they are given at physiological concentrations.[8] One of the antioxidant vitamins is vitamin E, which protects polyunsaturated fatty acids from peroxidation. It is a singlet oxygen quencher that neutralizes these highly reactive and unstable singlet oxygen molecules.[9] Another antioxidant, vitamin C is a free radical scavenger and interacts with free radicals in the water compartment of cells as well as in the fluid between the cells, among others is BH4 and γ-tocotrienols. These antioxidant vitamins may prevent the free radical damage caused by radiotherapy to the intestine. Thus, in the present review, we will discuss the biological and molecular sequel of irradiation and also the role of antioxidant supplementation in ameliorating the damage caused by radiation-induced enteritis.

History and Recent Trend

Chronic radiation enteritis is now recognized as a frequent and clinically important sequel of abdominal and pelvic irradiation treatment for malignant disease.[10] Diarrhea with or without abdominal cramps is the most important symptom. Although in most cases intestinal strictures and associated bacterial overgrowth are well recognized in chronic radiation enteritis, the pathophysiology of diarrhea is uncertain.[11] The changes in the intestinal absorption and motility unrelated to bacterial overgrowth have been implicated in the etiology of diarrhea.[12,13]

Since the discovery of X-rays by Wilhelm Röntgen in 1897, Walsh reported a person working with a new form of energy who developed abdominal pain and diarrhea during regular X-ray exposure and these symptoms stopped when abdomen was shielded with lead.[14,15] In 1917, the first clinical report appeared of a patient who developed severe intestinal injury following radiation therapy for malignant disease. In 1930, "factitial proctitis" was described in a group of patients undergoing pelvic radiation.[15] Since then, there have been numerous reports of radiation damage to the small intestine, colon, and rectum that curative treatment of cancer can induce.[16] In the early years of radiation therapy, the amount of radiation that could be delivered to a patient by external beam orthovoltage equipment was limited by hyperemia and the induced burn of overlying skin. The advent of newer supervoltaged techniques utilizing much higher energy waves made it possible to give larger and more effective doses of X-ray, without skin injury but with more risk of injury to intestine. The addition of internally placed radiation source made it possible to deliver sufficient dose of radiation to abdominopelvic tumors either to improve response rate dramatically or to produce a cure.[17] In view of improved efficacy of radiation therapy, there has been a tremendous increase in its use as a part of overall treatment plan for a variety of malignant tumors. It has been estimated that almost half of patients with cancer will receive some form of radiation treatment.[18,19] In recent years, radiation therapy has been combined with surgery and some form of chemotherapy, both of which may lower the threshold for radiation-induced intestinal therapy.

Incidence

Many series of patients reported in 1960 and 1970 with radiation enteritis following X-ray therapy for abdominopelvic malignancy had undergone radiation therapy utilizing techniques employed during the preceding 15 years. The transient symptoms of altered intestinal function were frequent but usually dissipated. In a study of 11 patients receiving pelvic radiation therapy who had an abnormality of rectal mucosa, as indicated by biopsy, the symptoms resolved 1 month after therapy.[20,21] In similar studies, reversible abnormalities of small intestinal mucosa were found.[22-24] The incidence of last implication of radiation enteritis varied between 2.5% and 25%.[25,26] More recent evaluation suggests that the incidence of the significant late complications may be less, especially when modern computerized techniques for the delivery of therapeutic radiation are utilized.[27,28] However, other authors report no significant change or even increase in the complications.[29] In view of the absence of controlled complication studies, the exact incidence of radiation enteritis to the intestine still remains unknown. It is likely that most of the patients receiving radiotherapy remain asymptomatic.[30] These patients present with diarrhea, abdominal pain, intestinal hemorrhage, obstruction, perforation, fistula formation, and malabsorption. The morbidity can be substantial, and radiation damage to the intestine may be fatal.

The radiation enteritis is a functional disorder of large and small bowel that occurs following the course of radiation therapy to the abdomen, pelvis, and rectum. The large and the small bowel are sensitive to the ionizing radiation.[31] The probability of tumor control increases with increase in the radiation dosage, so damages to the normal tissues as well.[32,33] Acute side effects to the intestine occur at approximately 10 Gy of whole-body radiation.[34] Since the curative doses for many abdominal and pelvic tumors range between 50 and 75 Gy, enteritis is likely to occur. Almost all the patients undergoing radiation to the abdomen, pelvis, or rectum show signs of acute enteritis. The cytotoxic effect of radiotherapy is mainly on rapidly proliferating epithelial cells[35] like those lining small and large bowels. Patients having acute radiation enteritis may complain of nausea, vomiting, abdominal cramps, and watery diarrhea.[1] With diarrhea, the absorptive and digestive functions of the GI tract are lost or altered, resulting in malabsorption of
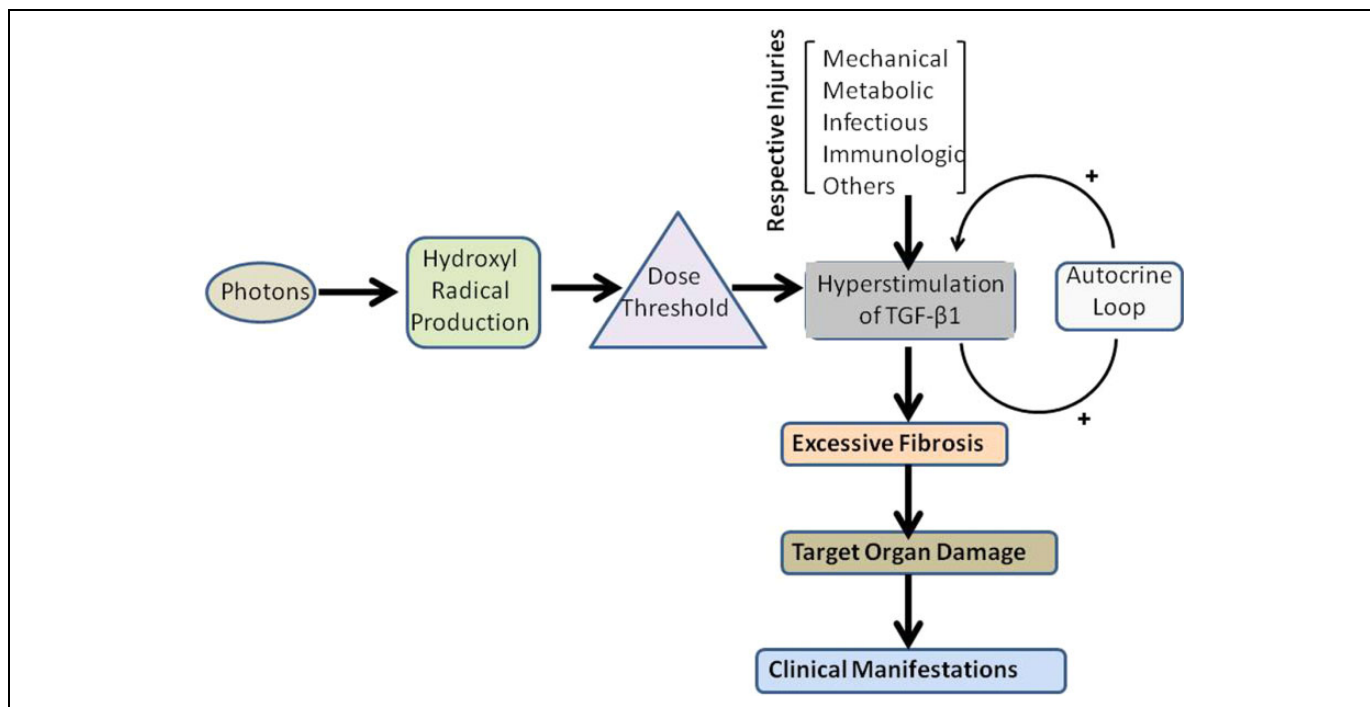
Figure 1. This is adapted from Nguyen *et al*[31] and is self-modified. It shows the postulated mechanism of radiation enteritis and other conditions characterized by excessive fibrosis: hyperstimulation of transforming growth factor β1. It also describes the mechanism how hydroxyl radical production and other injuries lead to hyperstimulation of TGF-β1 and finally to clinical manifestations. TGF-β1 indicates transforming growth factor-β1.

fat, lactose, bile salts, and vitamin B_{12}. The symptoms of proctitis including mucous discharge, rectal pain, and rectal bleeding (if ulceration is present) may result in radiation damage to anus or rectum.[36]

Biological Effects of Radiation

The current radiation therapy utilizes short-wavelength, high-frequency X-rays or γ-radiations that carry enough energy to produce ionization in the body tissues that absorb them. Ionization refers to the production of ions, atoms, or the electrical charges sufficient to cause injury to the living cell.[37] The electrons produced by the interaction of photons with the normal tissue combine with cell water and induce the formation of hydroxyl radicals and other free radicals. However, the hydroxyl radical is the main component of the radiation effect because of abundance of cellular water.[31] The radicals cause cell death through single- or double-stranded DNA damage.[38] The interaction of radicals with the cell membrane may also contribute to cell death.[39] The cellular sensitivity is dependent on the phase of cell cycle. The cells are vulnerable to the killing effect of radiation during G2 and M phases.[40] They undergo apoptosis (programmed cell death) and are shed from the intestinal villi.[7] An acute side effect of radiation enteritis such as diarrhea is seen during that phase. Consequently, rapidly proliferating tissues such as small intestine are particularly sensitive to the radiations[31] (Figure 1).

Molecular Biology of Radiation Effect on Intestine

Apoptosis is an active mode of cell death characterized by chromatin and cytoplasmic condensation, secondary to the activation of endonucleases and transglutaminases. Apoptosis is controlled by the regulator genes such as *ced-3*, *ced-4*, *p53*, *ced-9*, and *bcl-2*.[41,42] Under the normal physiological conditions, both small intestine and colonic epithelia undergo a low rate of spontaneous apoptosis.[43] In the colon, the apoptotic rate is very low because of the presence of antiapoptotic protein bcl-2, which protects the cell from undergoing apoptosis.[1]

In animal experiments, there is a rapid increase in the rate of apoptosis of the intestinal crypts when the animals were exposed to low dose of radiation (1 Gy). Apoptosis is observed mainly in the stem cells of the crypts. The rate of apoptosis is dose dependent and reaches the plateau at 1 Gy.[44] Parallel to the increased rate of apoptosis is the increased expression of tumor suppressor gene p53 in the stem cell region (Anwar M et al, 2017). Apoptosis induced by radiation is reportedly p53-dependent. In the animals devoid of p53, there was no increase in the rate of apoptosis,[45] which confirms the above fact. The small intestinal epithelial cells are more sensitive to radiation as compared to the stem cells of colon and rectum because of the presence of Bcl-2 in the latter.[7] There is an estimated 5% risk of complication at 5 years at a dose level of 45 to 50 Gy for small intestine and 60 to 70 Gy for colorectal mucosa.[46,47] Ionizing radiation also activates the translation of the genes
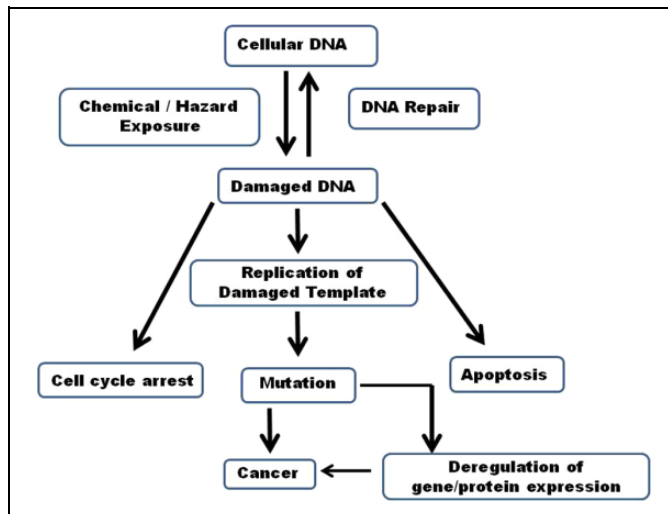
Figure 2. This is modified by us which shows the model representation of the molecular biology of radiation effect and DNA damage leading to cancer. This also depicts how different exposures to cellular DNA lead to its damage and hence carcinogenesis.

encoding transforming growth factor β (TGF-β), which is known to promote the fibrosis by stimulating expression of collagen and fibronectin genes and chemotaxis of fibroblasts[48] (Figure 2).

Cellular and Tissue Response to Radiation

The mechanism underlying ionizing radiation enteritis or killing of tissue has been extensively studied, especially in the cells grown in culture.[49,50] The radiation may produce overt injury with immediate cell death, or alternatively, the ability of the cell to sustain reproduction or division is altered.[51] However, oxidative damage to the lipid membrane may result from the ionizing radiation which can induce apoptosis in specialized cell types.[52] In response to the DNA damage, Ku-80 associates with broken DNA strand followed by the binding of inactive enzymes called DNA-dependent protein kinases (DNA-PKCs). Binding of DNA-PKC activates kinase activity, resulting in phosphorylation of the substrate molecules, and thus p53 activation leading to cell cycle arrest, apoptosis, or DNA repair.[53] There are some cells that survive radiation energy leading to abnormal cell function or altered genetic function. The number of cells that survive the radiation damage is the exponential function of radiation dosage.[51,32] Even at lower radiation dosages, each increment in the dose leads to an increase in the number of surviving cells. This type of dose–response curve shows one hit of ionizing radiation may be all that is required for inactivation of small definable number of cells. The cell survival is also critically affected by radiation dose rate, that is, whether the total dose is administered over short- or long-time exposure.[37] The rapid rate of delivery of total required dose is usually more harmful to cells than smaller dose for prolonged periods or in many separate dose fractions. The resistance of the cell to radiation dose rate probably relates

to the ability to affect concomitant repair of sublethal injury during the prolonged slow exposure or rapid repair immediately after the radiation exposure, when short fractionated doses were used.[54] One of the important factors governing the response of the cells to the ionizing radiations is the cell stage at which the cell is exposed to the radiation.[55,56]

Recently,[57] the potential of ultra-high dose rate of irradiation to lung fibrogenesis in C57BL/6J mice has been shown, which were exposed either to short pulses ($\leq$500 milliseconds) of radiation delivered at ultra-high dose rate ($\geq$40 Gy/s, FLASH) or to conventional dose rate irradiation ($\leq$0.03 Gy/s, CONV) in single doses. Thus, suggesting that FLASH irradiation protects the lung from fibrosis and also elicits a significant decrease in apoptosis in the radiation response at equivalent doses,[57] it also reported the human breast cancer HBCx-12A tumor xenografts were exposed to 17-Gy FLASH or CONV in 2 equal fractions at a 24-hour interval. They found FLASH was as efficient as CONV in repressing tumor growth.

In the proliferating cell population, the cell cycle can be divided into 4 stages mitosis (M) phase, gap (G) phase, S phase (in which DNA synthesis occurs), and another gap phase (G2) that occurs prior to mitosis and after the completion of DNA synthesis. During the mitosis, the cell divides to produce 2 daughter cells from 1. The cells are most sensitive to ionizing radiation during the mitosis.[58] The resistance to radiation injury increases progressively during G1, reaching a peak in the late S phase and then rapidly declining during the G2 prior to the mitosis.[59]

In response to the DNA damage, the cell cycle control system rapidly causes cell cycle arrest at DNA damage checkpoints.[60] Most of the cells have at least 2 checkpoints: 1 in the late G1, which prevents entry into the S phase, and other in the late G2, which prevents entry into the M phase[61] (Figure 3).

The GI checkpoint blocks the progression into the S phase by inactivating G1/S cdk and S cdk complexes.[62] In the mammalian cells, DNA damage leads to the activation of p53 which stimulates the transcription of p21, which is a cdk inhibitor that binds to G1/S-cdk and S-cdk inhibiting their activities, thereby blocking the cell cycle progression through the S phase.[63]

DNA damage activates p53 by an indirect mechanism. In the undamaged cells, p53 is bound to Mdm2 and is targeted for ubiquitination and subsequent degradation.[64] However, in the damaged cells, p53 is activated by phosphorylation under the action of upstream kinases.[65] Several cell cycle regulators such as p21, GADD 45, and members of 14-3-3 are induced by p53.[66] Other induced proteins include Bax, CD 95, and DR5, which are classical members of apoptotic pathways. However, the significance of these inductions remain obscure, as bax-I mice shows normal radiation sensitivity.[67] Most of the morphological changes that were observed are accused by a set of cysteine proteases that are activated especially in the apoptotic cells.[68] The death proteases are homologous to each other and are a part of large protein family called caspases.[69]

All the caspases possess the cysteine active site and cleaves at asp-X-X-X, a caspase distinct substrate specificity that is
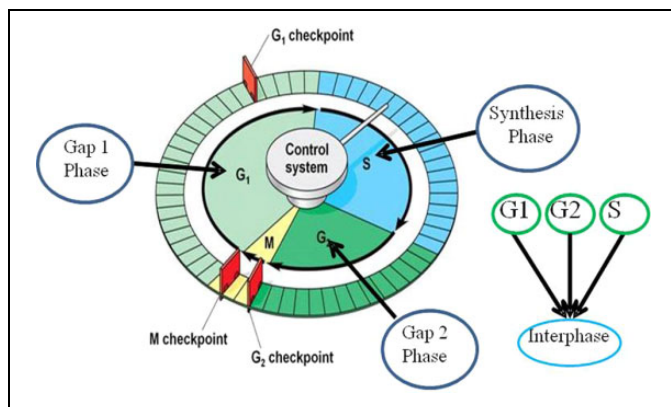
Figure 3. Adapted from Campbell and Reece[61] and self-modified. This show different phases of cell cycle, like, G1, M, G2, and S and the check points schematically.

determined by 4 amino acid residues in the cleavage site.[69] Several important caspase substrates have been identified in recent years.[70] The nuclease cuts the genomic DNA between the nucleosomes to generate DNA fragments with a length of approximately 180 base pair and its multiple is a marker for apoptotic cell death.[71] The caspases activated in response to the death signals by external signal stressed condition within the cell, resulting in cell death either by extrinsic or intrinsic pathway.[72]

The extrinsic/death receptor pathway is triggered by the members of the superfamily of CD95 and tumor necrosis factor receptor. Binding of CD95 ligand to CD95 induces receptor clustering and formation of death inducing signaling pathway.[73] This complex via adaptor molecule such as Fas-Associated protein with Death Domain (FADD) results in the activation of caspase 8 leading to apoptosis.[74]

Intrinsic pathway is the mitochondrial pathway used extensively in response to internal insults such as DNA damage.[69] The diverse responses converge in mitochondria, resulting in the activation of pro-apoptotic members of the Bcl-2 family. Unlike Bcl-2, which seems to spend most if not all of its life attached to intracellular membranes, however, many group II and III members such as Bax, Bak, Bim, and Bad can shuttle between cytosol and organelles.[75-77] The cytosolic forms represent the pools of inactive but battle-ready proteins. Proapoptotic signals redirect these proteins to the mitochondria where these fight for cells fate. Activation of proapoptotic members occurs through proteolysis and dephosphorylation.[69]

The proapoptotic and antiapoptotic Bcl-2 family members meet at the surface of mitochondria, where they compete to regulate cyt c release by the mechanism that is still debated.[76] However, it has been suggested that proproteins interact with proteins like voltage-dependent anion channel and regulate its channel activity.[78] If the genes encoding for both Bak and Bax are inactivated, cells are remarkably resistant to most apoptosis-inducing stimuli, indicating the crucial importance of proteins in the induction of apoptosis.[6] Bak and Bax are themselves activated by other apoptosis-promoting proteins of the Bcl 2 family such as Bid and Inhibitors of Apoptosis Proteins

(IAP) inhibitor of apoptosis, as these are also important family of intracellular apoptosis that are suggested to act in 2 ways: they bind to some procaspases to prevent their activation or they bind to the caspases to inhibit their activity. The intracellular cell death program is also regulated by extracellular signals which can either activate apoptosis or inhibit by regulating the levels of Bcl-2 and IAP proteins.[79]

Transforming Growth Factor β and Effect of Radiation

Transforming growth factor β is a multifunctional peptide growth factor with its wide range of effects on growth, differentiation, extracellular matrix deposition, and immune response. There are 3 isoforms of TGF-β—TGF-β1, TGF-β2, and TGF-β3. The location of TGF-β1 is in intestinal villi. These levels are regulated by Smad7 which binds to TGF-β1 receptor complex and prevents the phosphorylation of Smad2 and Smad3.[80]

In animal experiments, irradiation resulted in a sustained increase in TGF-β immunoreactivity in the small intestine. Transforming growth factor β also acts as a potent fibrogenic and proinflammatory cytokine. Hyperplasia of connective tissue, mast cells, and increased leukocyte migration results from activation of TGF-β in the intestinal wall. It promotes fibrosis by stimulating the expression of collagen and fibronectin genes and chemotaxis of fibroblasts.[31] The extracellular matrix is also increased as a result of inhibition of degradation by TGF-β. All the 3 isoforms are overexpressed in early postirradiation; however, at 26 weeks postirradiation, only TGF-β1 isoform remains elevated.[80] The immunoreactivity of TGF-β1 increases strongly in areas of radiation histopathologic injury to the mouse intestine as compared to sham-irradiated intestine, which showed weak intracellular epithelial staining.[31]

Transforming growth factor β also plays an important role in regulating the immune system in the intestinal wall[81,82] and observed an increase in the number of Myeloperoxidase (MPO)-positive cells after TGF-β induction in late radiation injury. However, TGF-β1 is also known for its pleiotropic immunosuppressive effects and leads to enhanced intestinal wall fibrosis due to local overexpression of TGF-β1 that is associated with enhanced inflammatory infiltration. In parallel,[82] inhibition of TGF-β with a soluble TGF-β type II receptor resulted in a reduction in the radiation injury score, enhanced mucosal surface area, and decreased intestinal wall fibrosis in a mouse model of radiation enteropathy. Thus, inhibition of TGF-β1 signaling may decrease clinical manifestations of radiation enteropathy.[83]

Oxidative stress results from an increased production of ROS or reactive nitrogen species (RNS) and/or reduced antioxidant capacity. Biological systems have developed superior antioxidant mechanisms, enzymatic and nonenzymatic, to remove ROS/RNS generated during normal metabolism or under pathological conditions. The enzymatic system includes mainly the superoxide dismutases (SODs) and catalase, while nonenzymatic system includes glutathione (GSH),

ascorbic acid, and α-tocopherol. Transforming growth factor β has been shown to increase ROS production and suppress the antioxidant system and thus induce oxidative stress, and such a stress contributes to TGF-β's pathophysiologic effects including fibrosis.[84]

Mitochondria are the major source of ROS in cells. Transforming growth factor β1 has been shown to increase mitochondrial ROS production.[85] Transforming growth factor β induces the generation of mitochondrial ROS from complex III of the electron transport chain that is required for TGF-β-mediated transcription of profibrotic genes in both normal and patient's lung fibroblasts. Because the induction of Nox4 also requires mitochondrially generated ROS, thus, TGF-β-induced ROS generation originates from the mitochondria and is sustained and amplified by cytosolic NAD(P)H oxidases.[86,87]

Effect of Radiation on p53 and p21 Expression

The p53 tumor suppressor gene is an essential component of the apoptotic program induced by anticancer agents in oncogenically transformed cells.[88] p53 protein levels are upregulated rapidly in response to DNA damage induced by radiation. p53 transcriptionally regulates many genes. This regulation may either be positive or negative.[89] p53 has been shown to regulate the expression of genes important for cell cycle arrest such as p21 apoptosis, for example, Bax.[90] The expression of p21 protein mediates p53-dependent cell cycle arrest. p21 inhibits cell cycle progression by binding to and inhibiting the function of cyclin-dependent kinases and proliferating cell nuclear antigen.[91] Following irradiation, the levels of wild-type p53 expression are elevated with time course in the small intestine similar to that for apoptosis, that is, peak levels of 3 to 6 hours postirradiation.[92] The acute apoptotic response (3-6 hours postirradiation) in the intestinal epithelia is p53-dependent since the apoptotic response is abrogated in p53 homozygous null mice. In addition, it has been demonstrated that for 12 to 24 hours postirradiation, apoptosis could occur independent of p53. The intestinal epithelia from both BDF-1 and p53 wild type showed a time- and dose-dependent increase in apoptosis and p53 and p21 imunoreactivity after exposure to 8 Gy radiation. The small intestinal crypts showed a characteristic peak in the apoptotic frequency at cell positions 3 to 5 from the bottom of crypt.[7] Changes in p53 and p21 immunoreactivity were coincident with apoptosis. Neither p53 nor p21 could be in contrast to detectable immunoreactivity of p53 within 1 hour postirradiation. Highest immunoreactivity of p53 was detected 4 hours postirradiation, which declined at 24 and 48 hours and was almost undetectable by 72 hours postirradiation,[44] while the maximum immunoreactivity of p21 was at 24 hours postirradiation. There is a slow drift in the distribution of p21-positive cells toward the top of crypt, which slowly exits from the crypts and are later found at lower positions of villi. Detectable levels of p21 are reported 96 hours postirradiation, while by 6 days, its level goes completely undetectable. The cells that are strongly immunopositive for p53 are found at crypt base, while p21-positive cells are positioned toward crypt base.[44]

The p21 expression is dose-dependent. The cells exposed to as low as 0.3 Gy of rays results in minimal expression of p21, while considerable expression of p21 is induced after exposure to 16 Gy; this suggests greater percentage of p21-positive cells in comparison with the cells exposed to 8 Gy. The expression of p21 is p53-dependent, as is suggested by the experiments with homozygous null mice which failed to increase p21 in response to radiation. The results from Potten and Grant[43] are consistent with the hypothesis that individual cells tend to undergo p53-mediated growth arrest or apoptosis in response to γ-radiation are dependent on the concentration of active p53 protein, with higher p53 expression resulting in apoptosis and low p53 resulting in growth arrest. The levels of p53 are below those that are capable of inducing either cell cycle arrest or apoptosis. p53 has been proposed to suppress the apoptosis and promote differentiation, which suggests p53-binding domains within the sequences of p53-regulated genes to display different affinities for p53 binding. In the HCT116 cell line, wild-type p53 induces apoptosis irrespective of p21 status.[93] Thus, p21 don't provide a dominant signal for the suppression of cell- and tissue-dependent efficacy of p53-mediated transcriptional activation. It appears that resistance to γ-radiation-induced apoptosis is related to reduced ability to increase functional p53 to a level sufficient to initiate apoptosis.[44]

Gastrointestinal tract exposure to radiation causes acute GI toxicity or the GI syndrome that is caused by destruction of the GI epithelium, which leads to infection and loss of fluid and electrolytes.[94,95] The small intestine integrity is maintained by constant renewal of stem cells residing in the crypts. Radiation impairs the regeneration of intestinal epithelium mainly by inducing cell death in crypt epithelial cells. Crypt cells are highly sensitive to radiation-induced apoptosis, which occur few hours postirradiation.

However, p53-mediated signaling plays an important role in promoting apoptosis of crypt epithelial cells because crypt cells in p53$^{-/-}$ mice are resistant to radiation-induced apoptosis and surprisingly p53$^{-/-}$ mice are more sensitive to the radiation-induced GI syndrome. Furthermore, time course studies have shown that radiation exposure to p53$^{-/-}$ mice have a delayed onset of cell death in crypt epithelial cells. Thus, possible loss of p53 sensitizes crypt epithelial cells to mitotic death.[96]

There is a close relationship between mitosis and apoptosis.[90] when considering initial mitotic inhibition induced by dose of radiation such as 8 Gy (G2 block) and the burst of regenerative proliferation associated with damage induced by 8 Gy which is significantly high at 24 hours postirradiation. This occurs at the time of overshoot in crypt cellularity and cell proliferation. Thus, even though the crypt has attained cellularity, the proliferative stimuli continue to trigger cell division and homeostatic process that regulate the crypt level of apoptosis to remove unnecessary additional crypt cells.[97] It is more likely that the apoptosis is indicative of cell number homeostatic mechanisms operating to remove essentially healthy cells. This is further supported by the observations that late

apoptosis occurs in p53 knockout animals, while the earlier apoptosis is absent, suggesting that late apoptosis don't involve DNA damage recognition and damage processes involved in early cell death are completely p53-dependent.[92]

Bcl-2 Family Protein and Radiation

The Bcl-2 gene encodes a family of related proteins consisting of survival genes such as Bcl-2 and death genes such as Bax. There are interactions involving homodimers and heterodimers between various members and is actually protein–protein interactions that determine whether the cells will survive or die. Low apoptosis yield is correlated with sites where Bcl-2 is expressed, although the ability to detect Bcl-2 protein in mouse colon tends to be variable. In Bc1-2 knockout animals, the apoptosis induced by low doses of radiation in the small intestine where Bcl-2 is not expressed but is dramatically elevated in the stem cell position in the murine large intestine where Bcl-2 is weakly expressed.[7] The low evidence of small intestinal tumors despite the rapid cellular division in contrast to a relatively huge rate of neoplasia in colon implies a more effective eradication of malignant precursor lesions via apoptosis in small intestine in comparison with large intestine.[98] The absence of Bcl-2 expression and the presence of proapoptotic Bax protein in small intestine crypt favor proapoptotic threshold helping to facilitate apoptosis of genetically altered stem cells. The studies by Koumenis *et al*[99] suggested an increase in Bak levels in both p53 wild-type and null cell lines, while the Bax levels were suggested to be increased in p53 wild-type cell in a dose-dependent manner. However, no such evidence of increase is suggested with an increase in radiation dose in p53 mutant cell lines.[100,101]

Histology of Radiation Enteritis

One of the first events that can be detected in the crypts after irradiation is the appearance of histological cell death, which is usually observed toward the base of the crypts within 2 to 3 hours postirradiation.[102] At about the same time, there is generally a decline in the number of mitotic cells due to dose-dependent blockage of cell cycle progression through G2, which is most commonly referred to as mitotic inhibition and delay.[60] These include marginal condensation of chromatin, often into characteristic crescent shapes, general cytoplasmic condensation with the maintenance of organelle structure, fragmentation of nucleus and cytoplasm, followed by engulfment of these fragments by the surrounding healthy cells. Some fragments may also be extruded into crypt lumen.[7] Using 0.5 to 1.0 Gy showed that some cells near the crypt base are very sensitive to radiation and express this by undergoing fragmentation. Since most epithelial cells move up the crypt with time and onto the villi, the apoptotic fragments tend to be found at higher cell position at later times.[103]

During the immediate postirradiation period, the cells continue to emigrate from the crypt and onto the villi tip.[104,105]

Since there is some cell death and the absence of cell proliferation and yet a continued cell emigration, the crypts become noticeably smaller. The reduction in the size is most noticeably smaller in diameter or circumferential dimensions of the crypt, and the height is relatively unchanged. The total cellularity of the crypt is reduced to about 140 cells per crypt, 24 hours postirradiation with 8.0 Gy dose.[106] The crypt appears smallest at 14 to 15 hours postirradiation when the reduction may be as high as 70%.[106] If the crypt contains viable regenerative cells, then the crypt is soon reestablished as the clonogenic population of cells themselves begin to repopulate the crypt, which they do within 12-hour doubling time after the dose-dependent lag phase in growth.[107] By the third day, such sterilized crypt disappears and the output of cells onto villi is consequently and drastically reduced. One of the striking features of this crypt shrinkage is that it is achieved without dramatic changes in the number of pyknotic cells. By the third to fourth day, after high doses of radiation, the villi epithelia may appear to be discontinuous or lost. After a dose as high as 13.0 Gy, many but not all the crypts are sterilized and there is a severe depletion of villi cell from third to fourth day postirradiation. This depletion of villi cell exposes the animal to infectious contents of the gut and results in loss of fluid barrier, which significantly attributes to cell death within 3 to 4 days.[108] These symptoms are referred to as GI radiation syndrome.

The current treatment of patients with radiation enteritis is only supportive and is ineffective since the pathogenesis of the disease at the molecular level is not known. However, the radioprotective agents, such as interleukin (IL)-11 and IL-1, and the growth factors, such as R-spondin1, keratinocyte growth factor (KGF), Transforming growth factor beta (TGFb), and Basic fibroblast growth factor (bFGF), are known to protect the intestine from radiation or other cytotoxic injury by increasing the crypt cell proliferation and reducing apoptosis.[109-111] The exogenous prostaglandins including Prostaglandin E2 (PGE 2) analogues, misoprostol, and dimethyl prostaglandin are also radioprotective. Lipopolysaccharides (LPS) has recently being found to protect intestine from radiation, where radioprotective effects are mediated by the prostaglandins produced through cyclooxygenase-2 (COX-2).[112,113]

Role of Antioxidants in Radiotherapy

Belief in the medicinal effect of dietary antioxidants as protectors of human health continues to prevail. National Academy of Science defined a dietary antioxidant in the manner "a dietary substance in food that significantly decreases the adverse effect of ROS, RNS (NOS), or both on normal physiological functions in humans."[9] Even though a balanced diet provides antioxidants, some people regularly take antioxidant supplement to prevent disease by slowing down the biological oxidative processes which contribute to aging and disease risk. Dietary antioxidants are known to take part in cellular oxidation–reduction reactions in which they act either as an antioxidant or prooxidant depending upon the physiological environment and oxidation state.[114,115] Reactive oxygen species are normal

metabolic by-products that are generated continuously in the mitochondria in most cells.[42] Although ROS are essential for various cell defense mechanisms, they can also cause oxidative damage to DNA, proteins, and lipids, resulting in potentially enhanced disease risk.

Consumption of a typical balanced diet provides not only antioxidant vitamins and minerals, such as vitamin C, vitamin E, selenium, and so on, but hundreds of phytochemicals that may accumulate within the cells which can also act as antioxidant/pro-oxidant in the cellular environment.[42] Pomegranate fruit possesses strong antioxidant and anti-inflammatory properties. It may have cancer-chemo preventive as well as cancer-chemotherapeutic effects against prostate cancer in humans, as pomegranate fruit extract treatment of human prostate cancer PC3 cells resulted in induction of Bax and Bak (proapoptotic) and downregulation of Bcl-XL and Bcl-2 (antiapoptotic) proteins.[116] Moreover, fiber-enriched defined formula diets may effectively protect intestinal structure against radiation-induced damage by improving mucosal integrity.[117] However, antioxidant supplements are not always safe because toxicity can occur at very high intake levels of some commonly consumed antioxidants. There is an overall lack of consistency among studies as to the types of adverse effects observed.[118] Selenium is viewed as an antioxidant because of its essentiality for GSH peroxidase activity, but it can also become toxic if ingested in sufficient quantities even though the quantity needed to bring about symptoms of chronic selenium intoxication is unknown. The effects of selenite and selenomethionine, both commonly used dietary supplements in combination with either vitamin C or $CuSO_4$, on oxidative damage in the DNA of normal human keratinocytes have been observed. Administration of vitamin C and copper sulfate protected normal human keratinocytes from selenite-induced DNA damage, whereas selenomethionine alone did not induce any DNA damage. Such data suggest that possible pro-oxidant behavior of some dietary component is needed to evaluate the merits of antioxidants,[119,120] showing that selenium as selenomethionine is the major component of dietary selenium that regulates the redox state of p53 protein leading to increased efficiency of DNA excision repair. However, selenium has also been reported to modulate carcinogen-DNA binding, suppress cell proliferation, and enhance apoptosis.

Reactive oxygen intermediates (ROIs) play an important role in intracellular signal transduction for growth, survival, and apoptosis by inducing oxidative stress and activate cell by phosphorylation of target protein. Inversely, oxidative stress by radiation and anticancer agents induces cell impairment and apoptosis through peroxidation of protein and the DNA and activation of molecules in the death signaling pathways.[121,122] But the details of the ROI-associated apoptotic signaling pathway are unknown.

Ueta et al[123] investigated the effect of ROI scavenger (Mn-SOD) on apoptosis of squamous cell carcinoma (SCC) by anticancer drug and γ-radiation by studying the proapoptotic and antiapoptotic cell cycle markers. These workers reported that Mn-SOD expression is advantageous for apoptosis

induction in SCC cells by anticancer drugs and γ radiations through induction of apoptotic Bcl-2 family proteins and suppression of anti-apoptotic proteins. They suggested the importance of ROIs in apoptosis induction and Mn-SOD antisense transfection and therapeutic tool for malignancies.[123]

Many patients with cancer take vitamin supplements, and the majority combine them with conventional therapy.[124] Similarly among women with early stage of breast cancer, 60% used megavitamin therapy along with surgery, chemotherapy, and/or radiotherapy.[125] However, data from a study in women with breast cancer have prescribed various high-dose combination of 3 to 6 vitamins and minerals, namely, vitamin C, β-carotene, selenium, niacin, zinc, and coenzyme Q10 in addition to standard therapies.[126] Such findings suggest that until more is known about the effects of antioxidant vitamins in patients with cancer, supplementation should be used cautiously.

Among the numerous substances that have the potential to modulate the cellular antioxidant defense and thereby influence radio sensitivity, the antioxidant vitamin C, E, β-carotene, tetrahydrobiopterin (BH4), and γ-tocotrienol deserve special attention for a number of reasons.

1. The antioxidant function, which results from the reaction with superoxide, hydroxyl radicals, and singlet oxygen, is well documented.[127]
2. All 3 antioxidant vitamins are physiological compounds that have no significant toxicity when they are given at physiological concentrations.[8]
3. Antioxidant vitamins have been administered to patients to treat side effects of radiotherapy for decades[8] and reported that the effects of vitamin (vitamin C, E, and β-carotene) combination in vitro were similar to those of the individual ones. The lack of additive effect was remarkable since vitamin C can be located in the cytoplasm and nucleus while β-carotene and vitamin E are localized in the membrane compartment of the cells and therefore are considered to detoxify different kinds of ROS. However, there are some potential risks or benefits of administering antioxidant vitamins during radiotherapy because:

   i. It might improve the outcome of radiotherapy through a direct growth inhibitory effect on tumor cells and might allow a higher dose to the target volume by exerting a radioprotective effect on normal tissue. In addition, there might also be a radiosensitizing effect on tumor cells.
   ii. It might reduce the efficiency of radiotherapy by scavenging radiation-induced ROS not only in normal tissues but also in the tumor cells.

However, the protection by these antioxidant vitamins is highly dependent on concentration and occurred only in the micromolar and submicromolar concentrations, suggesting that low concentration could lead to the tumor cell radioprotection mentioned in the second hypothesis, while a higher

concentration of vitamin would not cause such protective effect and could even increase radiation-induced apoptosis, as is assumed. Therefore, a careful consideration of vitamin concentration is required when vitamins are given to patients receiving radiotherapy.

The use of antioxidants during cancer therapy is widely debated. Controversy exists in the literature regarding whether the use of antioxidants such as vitamin C, E, and β-carotene inhibits or enhances the antitumor effects of radiotherapy.[128] Discussion revolves around general use during therapy, dose, and timing of antioxidant use (prior/during/after antineoplastic therapy). Some researchers suggested that pharmacological doses of antioxidants may protect the tumor, thereby decreasing the effectiveness of cancer therapy.[129] The impact of antioxidants on the effectiveness of cancer therapies depends on the type and dosage of the antioxidant and therapeutic agent involved as well as the tumor type.[130] However, the evidence that the antioxidants actually decrease antitumor effects of cancer therapies is limited[131,132] and has indicated that antioxidants actually enhance radiotherapy effectiveness by increasing tumor response to therapy and decreasing toxicities. Antioxidants did not reduce the efficacy of radiotherapy. Because antioxidants protect healthy cells against free radical damage, there are fewer adverse events when antioxidants were provided.[9] The specificities of dose and timing are important variables in the study design and clinic intervention.

Antioxidant Role of Vitamin C

Vitamin C is an important aqueous phase antioxidant. Ascorbate functions as a reductant for many free radicals, thereby minimizing the damage caused by oxidative stress.[114,133]

As an antioxidant, ascorbate will react with superoxide, hydrogen peroxide, or the tocopheroxyl radical to form monodehydroascorbic acid and/or dehydroascorbic acid. The reduced forms are recycled back to ascorbic acid by monodehydroascorbate reductase and dehydroascorbate reductase using reducing equivalents from Nicotinamide adenine dinucleotide phosphate (NADPH) or GSH, respectively. Dehydroascorbate may decompose into tartrate and oxalate.[114]

Thus, the indirect role of ascorbate as an antioxidant is to generate membrane-bound antioxidants, such as α-tocopherol, that scavenge peroxyl radicals and singlet oxygen, respectively.[134]

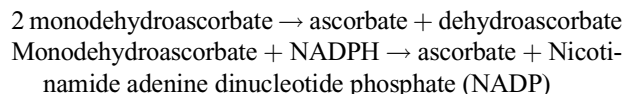The reaction of ascorbic acid with superoxide is

$$2O_2^- + 2H^+ + \text{ascorbate} \rightarrow 2H_2O_2 + \text{dehydroascorbate}$$
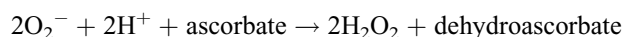
The reaction with hydrogen peroxide is catalyzed by ascorbate peroxidase

$$H_2O_2 + 2 \text{ ascorbate} \rightarrow 2H_2O + 2 \text{ monodehydroascorbate}$$
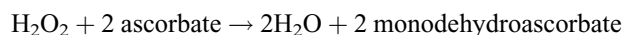
The above reactions indicate that there are 2 different products of ascorbate oxidation, that is, monodehydroascorbate and dehydroascorbate, that represent 1 and 2 electrons,

respectively. The monodehydroascorbate can either spontaneously dismutase or is reduced back to ascorbate by NADPH monodehydroascorbate reductase.

$$2 \text{ monodehydroascorbate} \rightarrow \text{ascorbate} + \text{dehydroascorbate}$$
$$\text{Monodehydroascorbate} + NADPH \rightarrow \text{ascorbate} + \text{Nicotinamide adenine dinucleotide phosphate (NADP)}$$

The dehydroascorbate is unstable at pH greater than 6 decomposing into tertarate and oxalate. To prevent this, dehydroascorbate is rapidly reduced to ascorbate by dehydroascorbate reductase using reducing equivalents from GSH.

$$\text{Ascorbate (ASH)} + \text{dehydroascorbate} \rightarrow \text{Glutathione disulfide (GSSG)} + \text{ascorbate}$$
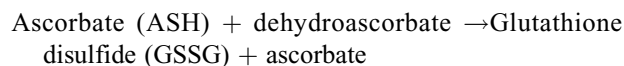
Besides cytosol and chloroplast, the cell wall is also an important site of ascorbate metabolism because it contains millimolar concentrations of ascorbate. Here, ascorbate may play a role in cell wall biosynthesis. The cell wall does not contain ascorbate peroxidase but contains ascorbate oxidase. This enzyme contains 8 to 12 copper molecules per enzyme and catalyzes the reaction:

$$2 \text{ Ascorbate} + O_2 + 2H^+ \rightarrow 2 \text{ dehydroascorbate} + 2H_2O$$
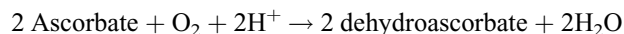
Since the enzymes to recycle oxidized forms of ascorbate are not present in the cell wall, it has been proposed that the plasmalemma may have an ascorbate translocator to shuttle oxidized and reduced forms between the cytosol and cell wall.[135]

Antioxidant Role of Vitamin E

The tocopherols, specifically α-tocopherol (vitamin E), have been studied extensively in mammalian research as membrane stabilizers and multifaceted antioxidants that scavenge oxygen free radicals, lipid peroxyl radicals, and singlet oxygen.[9] Vitamin E appears to be the first line of defense against the peroxidation of polyunsaturated fatty acids contained in cellular and subcellular membrane phospholipids.

The auto-oxidation of membrane phospholipids proceeds as a chain reaction and occurs in 3 steps:

1. Initiation phase—During this phase, the primary event is the production of R (carbon centered radical), that is, polyunsaturated fatty acid (PUFA) radical or ROO (lipid peroxyl radical) by the interaction of PUFA with free radicals generated by other means.
   $$RH + OH \rightarrow R + H_2O$$
   $$ROOH \rightarrow ROO + H^+$$
2. Propagation phase—The carbon centered radical rapidly reacts with molecular oxygen forming a peroxyl radical (ROO) which can attach another polyunsaturated lipid molecule.
 $$R + O_2 \rightarrow ROO$$
   $$ROO + RH \rightarrow ROOH + R$$
   $$ROO + R \rightarrow ROOH + R$$

This generates a chain reaction. The progression of this chain of events destroys PUFA present in membrane lipids.

3. Termination phase—The reaction proceeds unchecked till a peroxyl radical reacts with another peroxyl radical to form inactive products.

$$ROO + ROO \rightarrow RO–OR + O_2$$
$$R + R \rightarrow R–R$$
$$ROR + R \rightarrow RO–OR$$
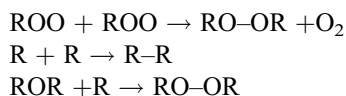
Vitamin E acts as a chain-breaking antioxidant as a result of its ability to transfer a phenolic hydrogen to a peroxyl free radical of a peroxidized PUFA.

$$ROO + Toc\ OH \rightarrow ROOH + Toc\ 0$$
$$ROO + TocO \rightarrow ROOH + non\text{-}free\ radical\ product$$
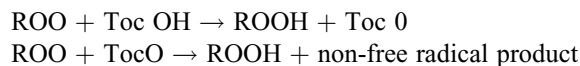
Because the active oxygen of the tocopherol is located near the surface of the bilayer and because it readily diffuses laterally in the plane of the bilayer, tocopherol can react with peroxyl radicals formed in the bilayer as they diffuse into the aqueous phase. This position also allows the tocopheroxyl radical to be reduced by ascorbate in the aqueous phase to regenerate α-tocopherol.
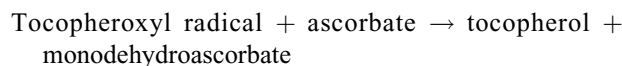
$$Tocopheroxyl\ radical + ascorbate \rightarrow tocopherol + monodehydroascorbate$$

Thus, the indirect role of ascorbate as an antioxidant is to generate membrane bound antioxidants, such as α-tocopherol, that scavenge peroxyl radicals and singlet oxygen, respectively.[134]

Regeneration of tocopherol is also done by coenzyme Q10. It reduces the tocopheroxyl radical by adding hydrogen. So, coenzyme Q10 cosupplementation with vitamin E eliminates the pro-oxidant potential of vitamin E, resulting in greatly reduced lipid peroxidation.[136] The coenzyme Q10 radical is readily regenerated in mitochondria by the readily available succinate. Moreover, vitamin E is particularly protective against exercise-induced free radicals.

The antioxidant effect of vitamin C and vitamin E is increased when they are cosupplemented as vitamin C scavenges free radicals from cytosol and vitamin E from membrane phospholipids. Moreover, vitamin C regenerates vitamin E from the tocopheroxyl radical.[137]

Antioxidant Role of BH4

Tetrahydrobiopterin is a crucial cellular nonenzymatic redox-sensitive antioxidant and plays a critical role in diverse biochemical pathways. It acts as a cofactor for a number of enzymes, such as aromatic amino acid hydroxylases and nitric oxide synthases (NOSs).[138] Tetrahydrobiopterin when supplemented ameliorates endothelial NOS uncoupling and leads to restoration of endothelial function in animal models of hypertension, diabetes, hypercholesterolemia, and organ transplantation. When used in humans, BH4 is generally administered orally. The small intestines are the principal site of absorption after oral administration of BH4. However, when administered orally to humans, it is absorbed in small intestines, a principal site of absorption.[139]

Although the effect of IR on BH4 is limited as reported by the literature, recent in vivo studies have shown that IR causes decreased BH4 level in tissue[140]. Berbee et al[139] showed that total body irradiation (TBI) of mice with 8.5 Gy of γ-ray suppressed BH4 bioavailability in lung tissue samples at 3.5 days.

Antioxidant Role of γ-Tocotrienol

γ-Tocotrienol, a vitamin E analog, is a potent protector against radiation injury. In mice, a single dose of γ-tocotrienol (400 mg/kg) greatly reduced radiation-induced injury and upcoming mortality.[141] γ-Tocotrienol decreased vascular and intestinal radiation injury and also improved hematopoietic recovery after total body irradiation of mice. Radioprophylactic effects of γ-tocotrienols are not only governed by their antioxidant properties but also by their inhibition of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase (HMGCR). However, in comparison with statins, which directly inhibit the activity of HMG-CoA reductase, γ-tocotrienol decrease HMG-CoA reductase activity by enhancing proteasomal degradation of the enzyme. It also prevents radiation-induced vascular peroxynitrite production in a HMG-CoA reductase–dependent manner.[139]

Hauer-Jensen lab, Kumar lab, and others have demonstrated that inhibition of HMGCR protects lung and vascular endothelium from radiation injury in vivo, thus pointing out this particular property of GT3 as a possible basis of its unique efficacy as a radioprotector.[142-144]

Antioxidant nutrients have been shown to prevent chemo-induced oral microsites and GI toxicity, cisplatin-induced nephrotoxicity, and doxorubicin-induced cardiotoxicity with inhibiting the antitumor effects of these agents.[145] One study even demonstrated prolonged survival among patients who received antioxidant in combination with radiation.[146] No study has examined the long-term effects of using antioxidant in combination with radiotherapy in humans.[131,147] Ortmann et al[8] have reported that protection by β-carotene and vitamin E against radiation-induced apoptosis was highly dependent on concentration and occurred only in the micromolar and submicromolar concentration, which suggested that low extracellular vitamin concentration could lead to the tumor cell radioprotection whereas higher extracellular vitamin concentration would not cause such a protection and could even increase radiation-induced apoptosis. Therefore, a careful consideration is required while administration to patients receiving radiotherapy.

Radioprotectors and Radiosensitizers

The aim of successful and efficient radiation therapy is to maximize the radiation damage in tumor cells and reduce the
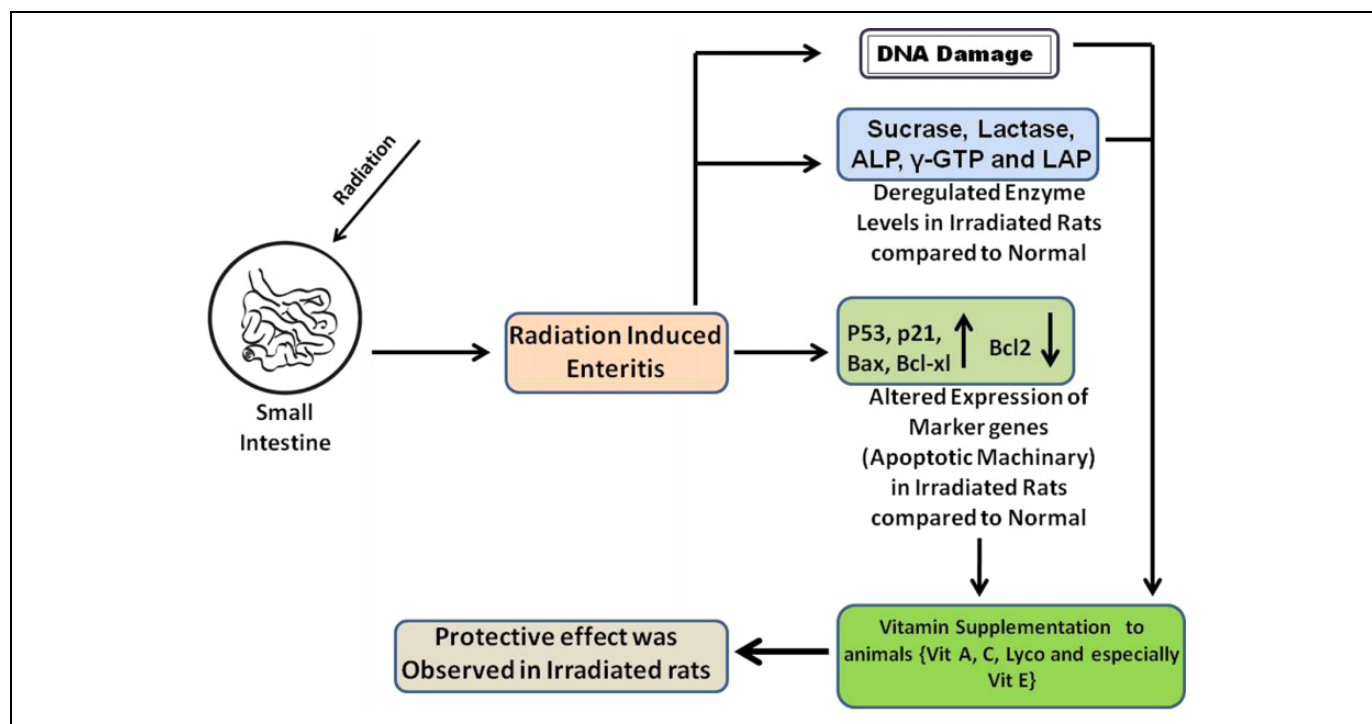
Figure 4. Proposed model of radiation-induced enteritis and its protection using antioxidants and can be explained as: when radiation is given to small intestine, then radiation-induced enteritis is observed. After that various manifestations could be observed as DNA damage, physical changes such as body weight, mortality, deregulated levels of brush border enzymes, namely, sucrose, lactase, Alkaline phosphatise (ALP), Gamma-Glutamyl Transpeptidase (γ-GTP), and Leucine aminopeptidase (LAP), and altered expression of marker genes/proteins (ie, of apoptotic machinery genes/proteins) were observed. Upon antioxidant supplementation, the effect of radiation-induced enteritis could be reversed especially with that of vitamin E that proved to be very potent in ameliorating the radiation-induced damage, hence protection.

damage in normal cells at the same time. This may be possible either by better localization of radiation dose or by using differential radioprotectors for normal cells and/or radiosensitizers of tumor cells.[148]

The agents that sensitize the tumor cells to radiation are known as radiosensitizers. These compounds apparently promote fixation of the free radicals produced by radiation damage at the molecular level. The mechanism of action is similar to the oxygen effect, in which biochemical reactions in the damaged molecules prevent repair of the cellular radiation damage. Free radicals such as OH^+ are captured by the electron affinity of the radiosensitizers, rendering the molecules incapable of repair.[149]

Radioprotectors are compounds that are designed to reduce the damage in normal tissues caused by radiation. These compounds are often antioxidants and must be present before or at the time of radiation for effectiveness. Other agents, termed mitigators, may be used to minimize toxicity even after radiation has been delivered.[150]

Hyperbaric Oxygen

The reactions of oxygen with aqueous as well as organic-free radicals induced by ionizing radiations may lead to the production of very toxic and relatively stable peroxy radicals and hydrogen peroxide, resulting in the damage to biomolecules and structures. Therefore, the simplest approach to enhance the

radiosensitivity of hypoxic tumor cells would be to increase the oxygen tension in the tumor.

Hyperbaric oxygen has been observed to be effective in relatively small tumors, whereas the advanced tumors do not show an increased radiosensitization.

Hyperthermia

Hyperthermia alone or in combination with ionizing radiation has been used in the treatment of radioresistant tumors. It has been observed to enhance cell killing.

Nicotenamide

Hypoxic cell radiosensitizers such as the nitroimidazoles were designed primarily to overcome chronic hypoxia that is diffusion-limited hypoxia resulting from the inability of oxygen to diffuse further than 100 μm through respiring tissue. However, hypoxia also arises through acute mechanisms (intermittent blockage of blood vessels). Nicotinamide, a B3 analog, has been shown in mouse tumors to prevent the transient fluctuations in tumor blood flow that lead to the development of acute hypoxia.

Radioprotectors

Tetracyclines and fluoroquinolones, which share a common planar ring moiety, were found to be radioprotective by Kim

et al.[151] Tetracycline protected murine hematopoietic stem and progenitor cell populations from radiation damage and allowed 87.5% of mice to survive when given before and 35% when given 24 hours after lethal TBI. Interestingly, tetracycline did not alter the radiosensitivity of Lewis lung cancer cells. Tetracycline and ciprofloxacin also protected human lymphoblastoid cells, reducing radiation-induced DNA double-strand breaks by 33% and 21%, respectively.

Conclusion

Thus, it is apparent that antioxidant supplementation, especially vitamin E administration, proved to be very efficacious and may be compelling in minimizing the intestinal damage caused by radiation as a side effect.[1] Also high doses of vitamin E supplementation have essentially no adverse side effects. Based on conclusion and other unpublished data, a model of radiation-induced enteritis and its protection using antioxidants was developed, which explains the mechanism of radiation damage and its protection schematically (Figure 4).

Future Prospects

Whether vitamin E can reduce damage to target malignancies requires further study. One future key goal will be to determine how posttranslational modifications of apoptotic machinery (p53, p21, Bax, Bcl-xl, and Bcl-2) and associated proteins lead to modulation of p53 activity in these processes, especially during physiological events when antioxidant vitamins especially vitamin E will be given.

It will be of significant value to define how the antioxidant vitamins and brush border enzymes and transport proteins work in accordance with each other and that function affects p53 signaling leading to DNA damage, as well as to determine the mechanism and relationship of molecular machinery and the chemical reactions used by antioxidant vitamins to fight radiation-induced damage and other malignancies. Whether the different sites of p53 posttranslational modification function cooperatively or antagonistically in regulating p53-dependent DNA damage signaling events will be of interest for future studies.

Authors' Note

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Declaration of Conflicting Interests

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