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Radioprotectors in Radiotherapy

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Radiotherapy is the most common modality for treating human cancers. Eighty percent of cancer patients need radiotherapy at some time or other, either for curative or palliative purpose. To obtain optimum results, a judicious balance between the total dose of radiotherapy delivered and the threshold limit of the surrounding normal critical tissues is required. In order to obtain better tumor control with a higher dose, the normal tissues should be protected against radiation injury. Thus, the role of radioprotective compounds is very important in clinical radiotherapy.

Ionizing radiation causes damage to living tissues through a series of molecular events, such as photoelectric, Compton and Auger effects, depending on the radiation energy. Because human tissues contain 80% water, the major radiation damage is due to the aqueous free radicals, generated by the action of radiation on water. The major free radicals resulting from aqueous radiolysis are OH, H, e_{aq}^-, HO_2, H_3O^+, *etc*[1–3]. These free radicals react with cellular macromolecules, such as DNA, RNA, proteins, membrane, *etc*, and cause cell dysfunction and mortality. These reactions take place in tumor as well as normal cells when exposed to radiation.

The radiation damage to a cell is potentiated or mitigated depending on several factors, such as the presence of oxygen, sulfhydryl compounds and other molecules in the cellular milieu[2,4]. In the presence of oxygen, hydrated electrons and H atoms react with molecular oxygen to produce radicals, such as HO_2, O_2^-, apart from other aqueous free radicals[5,6]. The

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Abbreviations: OER, oxygen enhancement ratio; AET, aminoethyl isothiourea; MMS, methyl methane sulfonate; TMG, tocopherolmonoglucosid

increase in the sensitivity of cells to ionizing radiation in the presence of oxygen, compared to that in its absence, is called the oxygen effect. The ratio of the dose required to achieve a given cell survival in the absence of oxygen to the dose required for the same effect under fully oxygenated conditions is called the oxygen enhancement ratio (OER), the value of which varies between 2.5–3 for X- and gamma- radiation. The effect of oxygen is said to be dose modifying and is independent of the radiation dose. Oxidative damage to the cellular genetic material, *i.e.*, DNA, plays a major role in mutagenesis and carcinogenesis. Highly reactive oxygen radicals produced by ionizing radiation cause lesions in DNA which lead to cell killing and mutations. Enzymes such as superoxide dismutase, glutathione peroxidase and catalase protect mammalian cells from oxidative radiation damage[7]. It was recently reported that a cell line derived from a mutant strain of mouse having low cellular levels of temperature sensitive catalase activity is more sensitive to radiation and hydrogen peroxide[8].

As the tumor cells proliferate very rapidly, they usually overgrow their vascular supply, resulting in centrally necrotic and hypoxic regions, rendering radiation ineffective in these areas. To overcome this problem, higher doses of radiation must be delivered to control the tumor. This is clinically not feasible, since the normal tissues surrounding the tumor are well perfused, vascularised and remain oxygenated, and are therefore more prone to radiation damage. This necessitates the protection of normal cells surrounding the tumor from radiation injury. The identification of radiation-protecting agents is an important goal for radiation oncologists and basic radiation biologists. Work on radioprotective chemicals started more than 50 years ago in the U.S.A., at the inception of the Manhattan Project. Because the available literature on the topic is enormous, this review focuses only on those agents which are of clinical relevance and their mechanism of radioprotection.

Classification of radioprotectors

Radioprotecting agents can be classified into three groups: 1) radioprotectors, 2) adaptogens and 3) absorbents. The first group of protectors is generally sulfhydryl compounds and other antioxidants[9]. These include several myelo-, entero- and cerebro- protectors. Adaptogens act as stimulators of radioresistance. These are natural protectors, which offer chemical protection under low levels of ionizing radiation. These are generally extracted from the cells of plants and animals and have least toxicity. They can influence the regulatory system of exposed organisms, mobilize the endogenous background of radioresistance, immunity, and intensify the overall nonspecific resistance of an organism. Absorbants protect organisms from internal radiation and chemicals. These include drugs which prevent the incorporation of radioiodine by the thyroid gland and the absorption of radionuclides ^{137}Cs, ^{90}Sr, ^{239}Pu, *etc*.

The literature available on radiaoprotectors is enormous and the ensuing discussion focuses on different radioprotectors, depending on their molecular structure, therapeutic activity or metabolic function. Table 1 presents a list of various categories of radioprotectors and their mechanisms of action.

Table 1. Different radioprotectors and their mechanism of action

Radioprotectors	Mechanism of action	References
A. *Sulfhydryl compounds* Cysteine, Cysteamine, Glutathione, AET, WR 2127 and other WR-compounds	Free-radical sacvenging, donation of H atom	9–20
B. *Antioxidants* Tempace, Hoechst 33342, Vitamin A, E, & C, TMG, Melatonin, *etc*.	Free-radical scavenging	21–35
C. *ACE inhibitors* Captopril, Elanopril, Pencillamine, Pentoxyfylline, L-158, 809, *etc*.	Protease inhibition (through renin-angiotensin system), anti-oxidation collagen synthesis inhibition.	44–51
D. *Cytoprotective agents* Mesna, Dexrazoxane, Amifostin (WR2127)	Reduced toxicity of chemotherapuetic drugs, decrease of urothelial toxicity and nephrotoxicity	52–59
E. *Metalloelements* Manganese chloride, Cadmium salts, Bismuth Subnitrate, *etc*.	Metallothionine induction	60–66
F. *Immunomodulators* Gamma-interferon, Polysaccharides AM5, AM218, Heat killed *Lactobacillus* cells, Broncho-Vaxom, Trehalose dicoryno-mycolate, AS101	Immune stimulation, increased production of cytokines	71–81
G. *Lipopolysaccharides and Prostaglandins*	Prostaglandin synthesis, elevated levels of cyclic AMP, DNA repair	84–91
H. *Plant extracts and* isolated compounds Curcmin, Orientin, Vicinin	Free-radical scaveng ing, anti-oxidation	36–43
I. *DNA binding ligands* Hoechst 33342	Electron transfer, free-radical scavenging	92–96
J. *Other Compounds* Melatonin, Carnosin Tempace, Tempol	Free-radical scavenging Antioxidant, free-radical scavenging	21–23, 30–33

Sulfhydryl radioprotecting compounds

The early work was mainly focused on sulfhydryl compounds. Analogues of cysteine and mercaptoethylamine were the early compounds tested for radioprotection. The synthesis of aminoethyl isothiourea (AET) was an important development, and research on its radioprotective mechanisms helped us to better understand the structural features of sulfhydryl compounds which are cardinal for radioprotection[9]. The most effective compounds were those with sulfhydryl groups at one end of a 2 or 3 carbon chain and a strong basic amino group at the other end. A large number (> 4000) of these type of compounds were synthesized at the Walter Reed Army Research Center in the U.S.A. Most of them were found to be toxic and unsuitable for human use. The synthesis of WR 2721 or amifostine or ethiofos [S-2-(3-aminopropylamino) ethylphosphorothioic acid] was a major breakthrough in the development of radioprotective drugs[10,11]. WR 3689, WR 151327, WR 638, WR 77913, and WR 44923 are other important radioprotectors of this series. All of them are water soluble, which facilitates administration. Their chemical structures differ with respect only to the length of the aminoalkyl group, the presence or absence of a methyl group at the terminal end and/or a hydroxyl group at the alkyl chain. The phosphorylated aminothiols were better than other aminothiols with respect to activity, tolerance, and duration of action. However, many of them had severe side effects, such as nausea, vomiting, and hypotension[12,13]. The search for safe and non-toxic radioprotectors has yielded an enormous amount of information, and several clinical trials have been undertaken.

Among the various sulfhydryl radioprotectors, the one that has undergone a large number of clinical trials and is currently in use as an adjuvant in radiotherapy, is WR 2721[14,15]. The selective radioprotection of normal cells by this drug has been related to its differential absorption by normal and malignant tissues and its conversion into an active sulfhydryl compound (WR 1065) in normal tissues by alkaline phosphatase action[14–17]. The prior treatment of patients undergoing radiation and chemotherapy with this drug significantly reduced hematologic, mucosal and renal toxicity as well as the frequency of neuropathy. It remains one of the most promising compounds at present in clinical radiation therapy for protecting normal tissues, because it is safe and practical to administer in a clinical setting. The maximum tolerated single dose of WR 2721 is only 740 mg/m^2 body surface when it is administered over a period of 15 min. This drug provides effective radiation protection only when administered immediately prior to radiation exposure, because it has a plasma half life of less than 10 min[15]. Further, there has been no cumulative toxicity in patients who received a total amount of 6.8 g/m^2 over a period of 5 weeks in fractionated radiotherapy[14]. This drug has also been found to reduce the toxicity of a cisplatin treatment in patients with metastatic breast cancer[18]. It also protects against late radiation toxicity to pelvic organs without interfering with the beneficial effect of radiation therapy and decreases the hematological and mucosal toxicity[19]. In patients undergoing chemotherapy and radiation therapy for unresectable non small cell lung cancer, WR2721 reduces cisplatin-related nephrotoxicity and radiation induced esophagitis[20].

Free-radical scavengers and antioxidants

Several aliphatic alcohols including ethanol, ethylene glycol, glycerol, *etc* are found to be good radical scavengers. However, these are of academic interest only and not suitable for clinical applications because of their toxicities at radioprotecting concentrations. Metodiewa, *et al*[21] have reported two compounds — Tempace and Troxyl — which are 2,2,6,6-tetramethyl piperidine derivatives and act as scavengers of superoxide, inhibitors of iron and ascorbate driven Fenton reaction. These two compounds may prove to be promising antioxidants and radioprotectors in clinical settings, pending an increasing number of trials and pharmacological applications.

Recent studies on many water-soluble nitroxides have shown radioprotection in animals, when the compounds are administered prior to radiation exposure[22,23]. Tempol, a low molecular-weight nitroxide, was shown to permeate the cell membrane freely and to act as a superoxide dismutase mimic[24]. It was found that the six-membered piperidine ring nitroxides including tempol were reduced faster than the 5-membered ring nitroxides. Scavenging of free radicals and induction of hypotension and bone marrow hypoxia are thought to be mechanisms for their radioprotection[22,24].

Antioxidants like vitamin A, C and E offer radiation protection, because radiation damage mimics the oxidative stress associated with active oxygen toxicity[25,26]. Selenium (as Na_2SeO_3) and vitamin E have been shown to act alone and in an additive fashion as radioprotective and chemopreventive agents[27]. Selenium confers protection by inducing or activating cellular free-radical scavenging systems and by enhancing peroxide breakdown, whereas vitamin E offers protection by a complementary mechanism[27]. Vitamins A, E and K are lipophilic and their local concentrations in specific cellular compartments might be sufficiently high for a protective effect, unlike most of the water-soluble sulfhydryl compounds, such as cysteamines[27]. Harapanhalli *et al*[28] have reported that soy bean oil gave a substantial protection against the Auger effect of ^{125}IdU. The radioprotection offered by this oil against the effects of ^{210}Po is also significant. The presence of vitamin A in the oil further enhanced radioprotection against the effect of ^{125}IdU. A water-soluble vitamin E derivative, TMG (tocopherol monoglucoside), was found to be very effective in protecting DNA *in vitro* and also mice after oral or intraperitoneal administration against gamma-radiation[29].

Melatonin (N-acetyl-5-methyloxytryptamine), a pineal gland hormone involved in regulating the neuroendocrine axis, is a highly efficient free-radical scavenger and antioxidant[30–33]. When administered to mice prior to radiation exposure, melatonin offered significant radiation protection, as assessed by the frequency of chromosomal aberrations in spermatogonia, spermatocytes and micronuclei in bone-marrow cells[34].

A derivative of ethinylestradiol, named as E838, has been reported to be an effective radioprotector for a hemopoietic system in studies with mice[35]. It showed a significant protective effect in the colony formation units of spleen and bone-marrow nucleated cells of irradiated mice, which indicated its potential clinical application in leukocytopenia caused by radiotherapy[35]. These compounds, though highly effective in *in vitro* studies, may find little use in their clinical application, because they do not discriminate between normal and tumor cells.

Several plant extracts, herbal preparations and phytochemicals have been reported to have radioprotective action in *in vitro* and *in vivo* studies[36–43]. However, no clinical trials for their efficacy in clinical use have been reported so far. Their radiation-protecting abilities have been attributed to their antioxidant and free-radical scavenging properties.

Angiotensin converting enzyme inhibitors

Angiotensin-converting enzyme (ACE) inhibitors and angiotensin II type-I receptor blockers were found to be effective in the prophylaxis of radiation-induced lung and renal injury in experimental animals[44–48]. The various ACE inhibitors investigated for radiation protection included pencillamine, pentoxyfyllin, captopril, elanopril, *etc*. Studies have revealed that blockage of the angiotensin II receptor type I is sufficient for treating radiation-induced renal and lung injury, because a renin-angiotensin system could be fundamentally involved in the pathogenesis of these injuries[44–48]. Captopril (D-3-mercapto-2-methyl propanoyl-L-proline) is widely used as an antihypertensive drug[49]. Captopril has been shown to spare early lung reaction induced by fractionated hemithorax irradiation in rats[48]. Captopril's therapeutic action has been partly ascribed to the prevention of a radiation-induced increase in the pulmonary arterial pressure, resulting in less severe edema in an irradiated lung. Radiation-induced pulmonary damage leading to severe pneumonitis and excessive fibrosis is associated with a significant decrease of ACE and plasminogen activator activity and a marked increase in prostaglandins. ACE inhibitors prevented these reactions. Captopril and angiotensin II receptor type-I blockers protected lung parenchyma from an inflammatory response and subsequent fibrosis in irradiated animals. Although pencillamine is a weak ACE inhibitor, it is a strong antifibrotic agent, and showed a moderate anti-inflammatory property[50]. ACE inhibitors and angiotensin II type-I receptor blockers effectively protected lungs from radiation-induced pneumonitis and the development of lung fibrosis in rats exposed to radiation. ACE inhibitors containing SH groups were more effective than those without. The radiation-protective effect of captopril could be related to an inhibition of the angiotensin II system as well as its combined pharmacological properties, such as antioxidation, free-radical scavenging and protease inhibition[45]. The use of ACE inhibitory drugs and angiotensin II receptor blockers opens new possibilities in radiation therapy with high doses of radiation and better control of radiation-related side effects. However, the action of these drugs seems to be tissue specific, because it was reported that captopril provided no protection to irradiated animal from gastrointestinal radiation injury[51].

Cytoprotective agents used in chemotherapy

A number of cytoprotective agents have been developed that can protect normal cells, but not tumor cells, from the toxicity and damage associated with chemotherapy and radiotherapy of cancer. Mesna (2-mercaptoethanesulfonic acid), dexrazoxane and amifostin are three of the protective agents approved by the United States Food and Drug Administration which have potential chemo- and radio-protective activity in cancer treatment[52–57]. Mesna decreases the incidence of chemotherapy-induced urothelial toxicity in cancer patients. The administration of mesna eliminated the urothelial toxicity assciated with higher doses of ifosfamide and

cyclophosphamide[52,54–57]. Dexrazoxane has been found to be useful as an adjuvant in the doxorubicin-based chemotherapy of tumors[52–54]. Cardiac toxicity and the protection of tumor cells are some of the limitations of the use of dexrazoxane[52]. Amifostin reduced the nephrotoxicity in patients receiving cisplatin-based chemotherapy[52–54]. The use of these radioprotectants in several clinical trials undergoing radiotherapy revealed varying and limited success[58,59].

Metalloelements and Metallothionin

Metallothionin, which is a low molecular-weight protein of 60 amino acids, of which one third is cysteine, and has been shown to offer protection to animals and cells exposed to ionizing radiation[60–66]. The administration of metalloelements to animals results in increased synthesis of this protein in various tissues. It is involved in the regulation of metabolism of metalloelements, the detoxification of excess metalloelements and the scavenging of free-radicals[60]. The oral administration of bismuth subnitrate to mice reduced the radiation-induced lethal effects and bone-marrow injury following whole body irradiation; this radiation protection has been attributed to an induced synthesis of metallothionin in bone-marrow cells[64]. The pretreatment of mice with manganese chloride and cadmium salts increased the level of metallothionin in various tissues of the animal and reduced lethal effects of whole-body irradiation[60]. The induction of metallothionin by metalloelements is organ/tissue specific. It was found that a manganese chloride treatment did not result in increased levels of metallothionin in the skin and small intestine, and did not protect these tissues in mice[66]. Tungstate, vanadate, and molybdate salts have insulin-like effects, because they increase the basal fructose-2,6-bisphosphate levels, counteract the effects of the glucagon and fructose-2,6-bisphosphate concentrations and 6-phosphofructo-2-kinase activity, and stimulate the glycolytic flux[67]. These salts also stimulate adenyl cyclase activity[68]. Studies have shown that these compounds at low nontoxic levels protected experimental animals from lethal effects of ionizing radiation through their effect on the hemopoietic system[69,70].

Cytokines and Immunomodulators

Various immunomodulators in combination with radiotherapy or chemotherapy have been reported to control tumor growth in experimental animals as well as in clinics. Human cytokine interferon gamma induces immune response in humans, including T-cell activation and the expression of class II major histocompatibility complex and receptor for the Fc portion of the immunoglobulin on monocytes. In a randomized clinical study it was demonstrated that the administration of recombinant interferon gamma resulted in immune stimulation in patients who had complete remission of small cell lung cancer after radiotherapy and chemotherapy[71].

Protein-associated polysaccharides, such as AM5 and AM218, heat killed *Lactobacillus* cells, bacterial extracts, and synthetic trehalose dicorynomycolate have immunomodulating properties. These compounds are also shown to have protective action against radiation[72–78]. The bacterial extract Broncho-Vaxom, when administered in combination with indomethacin, an inhibitor of prostaglandin production, to mice prior to lethal irradiation, exerted an addi-

tional radioprotective effect[78].

Ammonium trichloro-(dioxyethylene-0,0')telluride (AS101) is a synthetic compound exhibiting immunomodulating properties and minimal toxicity undergoing clinical trials in cancer patients[79,80]. AS101 showed a radioprotective effect on the hemopoiesis of irradiated mice and mice treated with various chemotherapeutic drugs. Studies have revealed that the administration of AS101 elevated levels of serum amyloid A (SAA) in the sera of treated mice. There was also an increase in SAA, factor B and ceruloplasmin in the sera of patients treated with AS101. It was shown that interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha) are the important mediators of changes in SAA concentrations induced by AS101[79,80]. The cytokines IL-1, IL-6 and TNF-alpha and the stem cell factor had an important role in the radioprotection by AS101, since the administration of antibodies against the receptors of IL-1 and IL-6 and TNF-alpha or stem cell factor completely abrogated the ability of AS101 to increase the survival of lethally irradiated mice[79,80]. Various immunostimulatory substances have been reported to afford radioprotection to mammalian organisms[81]. The intraperitonial administration of interleukin-I protected mice from lethal effects of gamma irradiation[82,83].

Lipopolysaccharide and Prostaglandins

Lipopolysaccharide has been shown to protect the intestine and bone marrow from radiation injury resulting from whole-body radiation exposure in mice[84] It was reported that a parenteral administration of lipopolysaccharide 2-4 hr prior to radiation exposure resulted in a 2-fold increase in the number of surviving crypts 3.5 days after 14 Gy of whole-body gamma-irradiation. Studies with cyclooxygenase-2 inhibitors and mutant strains of mice having a defective gene for cyclooxygenase-2 revealed that lipopolysaccharide offered radioprotection in mice through a prostaglandin-dependent pathway[84]. Prostaglandin and OK-432 have protected mice against radiation injury[82,86].

Prostaglandins are synthesized by almost all tissues in response to a wide range of stimuli, such as hormones, trauma, inflammation, and allergic reactions, and have influence on the number of cellular functions associated with normal and pathological events[87]. Prostaglandins have been found to offer radioprotection to several tissues, including gut, bone marrow, hair follicles and male germinal epithelium[88,89]. Hanson *et al*[90] have reported the radioprotective action of misoprostol, a prostaglandin E1 analogue. It was found that this compound selectively protected normal cells from radiation injury while sparing tumor cells. Recent studies on misoprostol with DNA repair proficient and DNA repair deficient cell lines indicated that the radioprotection property was dependant on the cell cycle, and that DNA repair could be facilitated by this compound[91]. The induction of a radioresistant state has also been found to be a mechanism of radioprotection by isoproterenol, which elevates the cellular cyclic AMP levels. Cyclic nucleotides have been found to alter the cellular radiosensitivity.

DNA binding ligands

The role of Hoechst 33342 as a radioprotector has been investigated. It was found that radioprotection is mediated by electron transfer, and that the radioprotective activity may be

improved by the addition of electron-donating substituents to the ligand[92,93]. Hoechst 33342 binds in the minor groove of DNA at discrete sites characterized by 3–4 consecutive AT base pairs[94,95]. Studies have shown that although maximum protection against radiation-induced strand breaks is at the binding sites, there is also some protection of the intervening DNA due to a global radiation protection resulting from reduction by the bound ligand of transient radiation induced oxidizing species of DNA[93,96].

Mechanisms of radiation protection

The radioprotectors can elicit their action by various mechanisms, such as: 1) by suppressing the formation of reactive species, 2) detoxification of radiation induced species, 3) target stabilization and 4) enhancing the repair and recovery processes.

As a corollary to the oxygen effects, protectors were initially thought to bring about a reversal of the oxygen effect. Pharmacological agents, capable of altering hemodynamics *in vivo*, were found to be radioprotective by interfering with the delivery of oxygen into irradiated tissues. Physical blockage of blood perfusion by microsphere embolization of the intestine or kidney and perfusion with deoxygenated dextran haemoglobin decreased the sensitivity of the tissues[97] by altering the distribution of blood supply, and thereby the delivery of oxygen. Biogenic amines, histamine, serotonin, nor-epinephrine and epinephrine also manifest their radioprotective action by inducing local hypoxia.

The chemical or biochemical consumption of oxygen can bring about hypoxia in cells and tissues. This may be one of the mechanisms by which sulphydryl compounds (RSH), which can undergo an oxidation reaction with molecular oxygen, manifest radioprotection. Metal ions, such as Fe^{+++}, can catalyze the oxidation reaction[98].

$$2\ RSH + 2\ O_2 \Rightarrow RSSR + H_2O_2 + O_2$$

Of late, interest has been focused on thiol-induced hypoxia by WR 2721, which offers selectivity in protecting normal cells *vs* tumor cells[99].

The removal of radiation-induced reactive aqueous free-radical species with short lifetimes in the nano-second range can significantly mitigate radiation damage. Several free-radical scavengers are known to interact with aqueous free radicals and to prevent the radiation-induced lethality of cells. The radioprotectors may also react with water radicals or radicals of bio molecules ($X^{\bullet}$) by donating hydrogen atoms to repair the radical species:

$$2\ X^{\bullet} + 2\ RSH \Rightarrow 2\ XH + RSSR$$
$$2\ RSH + 2OH \Rightarrow RSSR + 2H_2O$$

Radioprotectors can also interact with cellular targets, like DNA, by forming mixed disulfides and prevent radiation damage by stabilizing the target. Several amino thiol radioprotectors, such as cysteamine, guanidoethyldisulfide and glutathione disulfide, bind to DNA and their DNA binding paralleled their radioprotective potency. However, diamines like cadaverine, pentamethylene diguanidine bind well to DNA, but offer no radioprotection[100]. The radioprotective activity of a number of thiol compounds (RSH, R'SH *etc*) has been correlated with the ratio and extent of mixed disulfide (RSSR') formation[101]. The regeneration of

native proteins can be achieved by a thiol disulfide exchange with glutathione, possibly catalyzed by thiol transferase, and subsequent action of glutathione redox system coupled with glutathione reductase and NADPH.

$$\text{Protein-S-S-R} \Rightarrow \text{Protein-S} + {}^{\bullet}\text{SR}$$
$$\text{RSH} + \text{R'SSR'} \Leftrightarrow \text{RSSR'} + \text{R'-SH}$$
$$\text{RSSR'} + \text{RSH} \Leftrightarrow \text{RSSR} + \text{R'SH}$$

The mixed disulfide hypothesis is limited to the protection of enzymes and proteins, and fails to explain the radioprotection of nucleic acids, because the SH group is restricted only to proteins.

DNA repair and cell recovery processes

The cellular recovery or repair processes have been found to be enhanced by radioprotectors in several studies. The most important vital target damaged by radiation is the genomic DNA of a cell. The types of damage suffered by DNA due to ionizing radiation include strand breaks of single and double-strand types, base damage and sugar damage. The frequencies of double-strand breaks in DNA is inversely correlated with cell survival[102,103]. Base damage and DNA single-strand breaks may also hold deleterious consequences for cells[104].

All of these DNA lesions could lead to cell death, mutagenecity or altered function, or may be repaired and cells may recover from radiation-induced effects[105,106]. Endogenous radioprotective substances have been investigated with respect to their role in cellular recovery from radiation and chemical onslaughts[107–110]. Thiols, such as glutathione, may be involved in the repair of DNA single-strand breaks. Cells genetically deficient in GSH synthesis or cells in which GSH deficiency is produced by *dl*- Buthionine-sulfoxime or by hypoxia or misonidazole show a lack of DNA single-strand break repair[111–115]. Cells genetically deficient in GSH synthase have an increased amount of gamma glutamyl cysteine, cystine and other low molecular weight thiols, but these can not substitute GSH in supporting DNA single-strand break repair. Several compounds that stress cells are reported to induce DNA repair enzymes. These include H_2O_2, methyl methane sulfonate (MMS), nickel compounds, and dinitropyrene. When cells are irradiated and immediately treated with inhibitors of protein synthesis or cell division, they exhibit increased radiation resistance. This phenomenon is called potential lethal damage recovery. Holding cells under conditions that are sub-optimal for growth could also lead to similar results[116]. The mechanism and molecules involved in this recovery process are not well understood.

The cellular defense mechanisms against radiation and chemical stresses elicit an early SOS response to damage and subsequent adaptation. The SOS response is for eliminating lesions in DNA and an adaptation response is for restoring cellular metabolism and normal functioning. In protecting the vital targets, SOS repair has a very important role, which comprises the activation or synthesis of several proteins, DNA precursor synthesizing enzymes and DNA precursors[117]. Mammalian ribonucleotide reductase has been reported to be a DNA-damage inducible enzyme having a role in excision repair, because the concentration of

deoxyribonucleotides is important for repair synthesis[118–120]. Drugs and chemicals, which stimulate or increase the activity of DNA precursor-synthesizing enzymes, such as ribonucleotide reductase, could function as radioprotectors. The administration of the drugs indralin and indometaphen, prior to radiation exposure, to animals (mice and dogs) resulted in a higher survival of animals from lethal doses of gamma-radiation[117]. An increase in the activity of the enzyme, ribonucleotide reductase, occurred in several organs of these animals. As a result of the activation of the DNA, precursor-synthesizing enzymes increase in the extent of DNA repair could be expected. Further, higher cellular pools of DNA precursors could prevent the formation of new lesions. The stimulated synthesis of deoxyribonucleotides as well as DNA and protein synthesis in irradiated animals has been reported to be the mechanism underlying radioprotection by these drugs[117].

Perspectives

Early research on radiation protection has unraveled the basic mechanisms and yielded a large number of radioprotecting compounds. However, most of the compounds failed in their transition from laboratory to clinic. Acute toxicity and their inability to differentiate between tumor and normal cells are the main reasons for their failure in clinical applications. One approach to overcome problems associated with the toxicities of these compounds would be to use nontoxic amounts of several radioprotecting compounds having different mechanisms of action, in combination. Another approach would be to chemically modify the compound to make it nontoxic and more acceptable to the biological system. To confer an ability to protect only normal tissue cells and to differentiate tumor cells, the compound has to be modified by taking advantage of the euoxic environment of the normal cells and the hypoxic environment of the tumor cells as well as their associated biochemical characteristics. Future research for an effective radioprotector may be directed towards compounds which can protect normoxic cells and afford no protection under a hypoxic environment, which is a common feature in all solid tumors. Also relevant are compounds which become enzymatically converted into toxic derivatives in hypoxic tumor cells while remaining unchanged and protecting normal cells.

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