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REVIEW PAPER

Normal Tissue Protectors Against Radiation Injury

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ABSTRACT

Radiation damages normal tissues that can adversely affect the success of cancer radiotherapy, safety of nuclear installation workers and military personnel, and public exposed to nuclear accidents. Certain chemicals are able to protect against the harmful effects of radiation. But more than 50 years of research has produced only one approved radioprotective drug, WR-2721 or amifostine. The general utility of WR-2721 is limited by its inherent toxicity and high cost. Efforts to find non-toxic radioprotectors have revealed the promising properties of some medicinal plants. This is an attempt to review the recent publications on radioprotectors and to identify the research needs relevant to developing countries.

Keyword: Radiation protection, chemical radioprotectors, herbal preparations, normal tissue toxicity

1. INTRODUCTION

Discovery of x-rays by Roentgen ushered a new era in biomedical research and also provided a powerful tool for clinical diagnosis and therapy. But it was soon realised that x-rays also bring about irreversible and detrimental changes in cells. The destructive nature of ionising radiations became widely recognised following the atomic bombings in Hiroshima and Nagasaki. Research on the biological effects of radiation, facilitated by design of new equipments and radiation sources, assumed a global interest and the need for protection against undesirable exposures was widely accepted. The protective measures adopted were: physical barriers and shielding (public and occupational workers), and fractionation of total radiation dose into several small doses (radiotherapy). Where such measures are not possible or are ineffective, use of anti-radiation drugs (chemical radioprotectors) was contemplated.

2. CHEMICAL RADIOPROTECTORS

Radioprotectors function on the premise that some chemicals when given before irradiation protect the vital biomolecules from radiation-induced lesions, either by preventing the initial damage to the vital molecules or by restituting the original structure by repair, or both. Free radicals generated by radiolysis of water are implicated in the induction of radiation damage to biomolecules. Effective free radical scavengers are, therefore, presumed to act as good radioprotectors. Research on radioprotectors also opened the possibility that the information resulting from these studies could be used in understanding the mechanism of action of radiation on cellular molecules and in bringing about tissue damage.

3. HISTORICAL BACKGROUND

Research on radioprotectors was conceived realising a need for protection of military personnel against the possible use of nuclear weapons, a consequence of the atom bomb attacks in Japan. Therefore, the early studies on radioprotectors were aimed at finding suitable drugs for soldiers at the war front and also for those who are involved in rescue work. The emphasis shifted after the non-nuclear treaty in the '60s, and protection research was directed to finding drugs that can reduce the normal tissue damage resulting from cancer radiotherapy. There has been a renewal of interest in radioprotectors for non-clinical applications in recent years.

Studies on *in vivo* radioprotection started within a few years of the end of World War II and the global effects of military detonation of nuclear bomb became known. The first decade of protection research demonstrated that the best radioprotectors were the aminothiol compounds belonging to the cysteine-cysteamine group. These studies also helped in elucidating the structure-activity relationship most suitable for *in vivo* protection[1].

3.1 Phosphorylated Aminothiols

The severe drug toxicity associated with aminothiol was a limiting factor for their human application. A turning point came when Akerfeldt[2] demonstrated that phosphorylation of cysteamine reduced its toxicity without affecting its protective efficiency. Under an Antiradiation Drug Development Program at the Walter Reed Army Institute of Research, USA, phosphorylated aminothiols (phosphorothioates) of increasing structural complexity, designated as WR compounds, were synthesised and

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tested for their radioprotective effect *in vivo*. These compounds incorporated major improvements over the earlier aminothiols in terms of protective efficacy, drug tolerance, and duration of action, but had also some undesirable side effects. One of these compounds, S-2-(3-aminopropylamino) ethylphosphorothioic acid, code named WR-2721, emerged the most successful candidate. The prodrug WR-2721 (also known as amifostein) is dephosphorylated by alkaline phosphatase to its free thiol, WR-1065[3], which is the active protective molecule. WR-2721, at the maximum tolerated dose, gave a high dose reduction factor (DRF, i.e., the ratio of the radiation doses to produce a given effect in the presence and absence of the protector) of 2.7 for 30-day mouse survival[4] in mice exposed to haematopoietic dose of X-ray.

3.2 WR-2721, Preclinical Studies

WR-2721 and its metabolites were extensively studied during the late 1960s through the 1990s to establish their protective effect against acute and late reactions of the major dose-limiting normal tissues in radiotherapy and elucidate the mechanisms of radioprotection. *In vivo* studies using x- and γ-radiation demonstrated that WR-2721 protected against acute radiation lethality, increased hemopoietic and intestinal crypt stem cell survival and reduced chromosome damage in mouse bone marrow. WR-1065 showed anticlastogenic and antimutagenic effects, reduced DNA strand breaks and enhanced DNA repair *in vitro*[5,6] (reviewed by Capizzi[5], Uma Devi[6]). Recently, Vujaskovic[7], *et al*. reported that WR-2721 (150 mg/kg b.w.) reduced the delayed lung toxicity of high dose acute partial body irradiation (28Gy of 4 MV photons) in rats.

3.2.1 Selective Normal Tissue Protection by WR-2721

WR-2721 has been reported to selectively protect normal tissues against radiation and different cytotoxic drugs, without affecting their therapeutic efficacy. This is explained on the basis of slow and poor absorption of the drug in solid tumors compared to normal tissues. This pattern of absorption followed both intraperitoneal (i.p.) and intravenous (i.v.) administration[8]. Protection by WR-compounds also depends on the oxygenation conditions at the time of irradiation, the maximum protection being achieved at intermediate oxygen concentrations, with little or no protection under hypoxia[9]. However, the oxygen concentration at which the maximum protection was achieved varied for different tissues[10].

The differential tumor protection by WR-2721 of most normal tissues versus solid tumors can be related to the following factors:

- Reduced vascularity and perfusion of the tumors[10];
- Reduced uptake of hydrophilic radiprotectors by tumors[11];
- Active uptake of WR-2721 in normal tissues versus passive uptake in tumors[8];
- Reduced dephosphorylation of WR-2721 in tumors where the p*H* is below the optimum of 8.6-9.0 for alkaline phsophatase[12];
- Selective exclusion of WR-2721 derivatives (WR-1065 and WR-33278 disulphides) from tumor tissues in specific situations[13].

3.2.2 WR-2721-Clinical Studies

Extensive clinical trials in patients with different types of cancers have established the efficacy of WR-2721 in decreasing both radiation and chemotherapy-related toxicity. Based on the results of multicentric clinical trials, this drug was approved for use as a radioprotector and chemoprotector and is currently marketed as ethyophos and cytophos, etc.

Phase II clinical trials have shown that WR-2721 protected against radiotherapy-related xerostomia and mucositis, without compromising the efficacy of cancer therapy[14, 15]. The most common method of administration of WR-2721 is by slow i.v. infusion. Administration of WR-2721 (200mg/m^2) 30 min before each radiation fraction (2Gy/day, 5 fractions/ week for 6 weeks; total dose 60 Gy) in a 5 min intravenous infusion in patients with advanced head and neck cancers significantly reduced the radiation-related xerostomia, dermatitis, dysphagia and mucositis[16]. In addition to reducing the acute normal tissue reactions, this compound also reduced the late reactions of radiotherapy like salivary gland dysfunction[14, 17], delayed xerostomia, radiation pneumonitis, esophagitis, dysphagia, and dermatitis[18-20]. Wasserman[21] has reviewed the publications on clinical trials with WR-2721 in radiotherapy, conducted mainly in head and neck cancer patients.

Intravenous administration is cumbersome, slow, causes discomfort to patients, and needs the assistance of trained hospital staff. Koukourakis[22], *et al*. have shown that subcutaneous administration of WR-2721 was well-tolerated and effectively reduced early toxicity of radiotherapy like pharyngeal, esophageal, and rectal mucositis and incidence of acute perineal skin and bladder toxicity in patients with thoracic, head and neck, and pelvic tumors. This is encouraging because subcutaneous route is easier and safer, and takes less time than intravenous administration.

3.2.3 Toxicity of WR-2721

Major toxic symptoms like hypotension, nausea and vomiting, allergic reactions and fever have been reported in patients after administration of WR-2721. The maximum tolerated dose with fractionated radiotherapy was 340 mg/m^2 given 4 days a week for 5 weeks[23]. WR-2721, 200 mg/m^2, given in a 5 min intravenous infusion 30 min before 2 Gy x 5 days/ week for 6 weeks induced side effects like nausea, vomiting and hypotension, leading to termination of drug in some patients due to severe hypotension[16]. Brizel[24] *et al*. reported nausea and vomiting in 55 per cent of patients with head and neck cancer treated with WR-2721 + radiotherapy as against 5 per cent of patients receiving radiotherapy alone. The patients also showed mild hypotension and allergic reactions.

4. CURRENT STATUS OF AMINOTHIOL PROTECTORS

Despite 4000 phosphorylated aminothiol compounds were synthesised and tested for radioprotective activity, only WR-2721 has been approved for clinical applications. However, the applicability of WR-2721 as a general radioprotector in patients is still a point of debate because of its major toxic effects discussed above. This necessitates continuous monitoring of patients, additional medication to reduce the side effects and, in some cases, cessation of treatment. The drug, being expensive, is also not affordable by all patients. Due to its inherent toxicity, need for parenteral administration and short time window of action, its utility in non-clinical situations, like nuclear accidents and warfare, is limited. These have prompted a worldwide effort to search for orally effective, non-toxic and cheaper drugs[6].

4.1 Natural Products

Many natural products, including cytokines, hormones, amino acids, carbohydrates, tissue extracts, bacterial and yeast products, herbal preparations, plant extracts and phytochemicals have been screened for protection against radiation-induced cytotoxicity *in vitro* and/or *in vivo*. Of these, immunomodulators (cytokines), antioxidant vitamins and phytochemicals have been studied in detail.

4.1.1 Immunomodulators

Neta[25], *et al.* showed that recombinant murine IL-1α, human IL-1α and IL-1β render radioprotective effects in C57 and DBA mice. The maximum protection against mouse lethality was obtained when IL-1 was administered 20 h before whole-body irradiation (WBI). They obtained a DRF of 1.2-1.25 for IL-1 protection, which was similar to that reported for lipopolysaccharide[25]. Dalmau,[26] *et al*. concluded that the cytokines such as IL-1, IL-12 and CSF, which stimulate cell cycle in stem cells, would be suitable for radioprotection, while cytokines like TNF-α, TGF-β, interferon-γ (IFN- γ) and MIP-1α, which inhibit cell cycle could be considered for protection against cell cycle-dependent drugs. The suggested mechanisms of protection by cytokines include DNA repair, production of scavengers that neutralise reactive oxygen intermediaries, and export and/or detoxification of drugs[27].

Ginsan, a polysaccharide extracted from the roots of *Panax ginseng*, has been reported to stimulate normal lymphoid cells to proliferate and to produce cytokines such as IL1, IL2, IFN and GM-CSF[28]. Treatment with 100 mg/kg b.w. of ginsan 24 h before irradiation protected mice from the lethal effects of γ-irradiation, giving a DMF of ~1.45 for 30 day survival, and significantly increased the number of bone marrow and spleen cells as well as the bone marrow GM-CFU[29]. They suggested stimulation of CFU-S toward proliferation and self-renewal and elevation of endogenous production of radioprotective cytokines as the mechanisms for ginsan-induced radioprotection.

4.1.2 Neutraceuticals and Phytochemicals

The last decade has seen a number of publications on the radioprotective effects of micronutrients and dietary and medicinal plants. A recent update of the literature by Weiss and Landauer[30] has brought out the increasing importance of the natural products, especially the antioxidant vitamins and phytochemicals, for *in vivo* radiation protection.

Vitamins: Vitamin A and β-carotene, vitamin C (ascorbic acid), vitamin E (α-tocopherol) and different vitamin formulations have been demonstrated to have significant radioprotective effect *in vitro* and *in vivo*. Vitamin A and β-carotene are known antioxidants, with demonstrated radioprotective effects *in vivo*[31]. Protection of esophagus and intestine against local irradiation damage by dietary vitamin A has been reported[32]. β-carotene has been demonstrated to protect against radiation-induced chromosome damage in mice[33] and in human lymphocytes[34] and weight loss in rats[35]. Vitamin A supplemented diet reduced lung inflammation after high dose thoracic irradiation[36]. Administration of ascorbic acid to mice bearing fibrosarcoma before whole body irradiation protected against radiation lethality and skin desquamation, but did not affect the tumor response to radiation[37]. Post-irradiation administration of vitamin C protected against wholebody irradiation-induced chromosome damage in mouse[38]. Dietary antioxidant supplementation with vitamins E, C, β-carotene, rutin, zinc and selenium reduced the frequency of radiation-induced micronuclei in human lymphocytes[39]. Combined administration of vitamins E, C and β-carotene before or after irradiation significantly reduced the radiation-induced micronuclei in mouse bone marrow[40]. Injection of vitamin E (α-tocopherol) produced a higher post-irradiation survival than vitamin E given as a dietary supplement[41]. Vitamin E was effective during an extended period, which included pre- and post-irradiation administration[42, 43]. A synthetic analogue of vitamin E 2-(α-D-glucopyranosyl) methyl-2, 5, 7, 8-tetramethylchroman-6-ol (α-TMG), is highly hydrophilic and has nearly the same kinetics of radical scavenging as that of vitamin E[44]. Rajagopalan[45] *et al*. demonstrated that TMG protected against radiation-induced damage in plasmid DNA. Intraperitoneal injection of TMG 15 min before or 5-15 min after irradiation gave significant protection against radiation-induced chromosomal damage in mouse bone marrow *in vivo*. Injection of 600 mg/kg b.w. of TMG 5 min after irradiation provided the maximum protection[46], giving a DMF of ~1.1 for mouse survival[47], which is similar to that reported for vitamin E[41]. DMFs of ~1.5 and ~2 were obtained for reducing chromosomal aberrations and micronuclei, respectively, in mouse bone marrow[47].

Plant extracts and phytochemicals: Several medicinal and dietary plants and fruits possess very good antioxidant properties. Many of them are rich in antioxidant chemicals like flavones, flavonoids like quercetin, hesperidin, etc and other polyphenolic compounds, vitamins A, C, and reducing sugars. Such plants can be good natural sources

of effective radioprotectors. Preliminary studies on several plants have shown radioprotective effect in mouse tissues or cultured mammalian cells. These include the medicinal plants used in *Ayurvedic* (Indian traditional system of medicine) preparations and Chinese medicines, commonly used spices, green vegetables and salad plants, fruits, different types of tea, wines, etc.[48]. However, most of these studies are incomplete in the sense that the toxicities of the preparations were not evaluated and doses for protection studies were selected arbitrarily. Moreover, it is not possible to do a comparative evaluation of the published data, as the different studies have used different radiation doses and parameters, and dose reduction factors are not reported in most of the cases.

Some of the important studies in this area and their findings are reviewed below.

Soyabeans: Soybeans are very widely used in diet throughout the world, in the form of pulses, cereal, cooking oil and for making bread and other preparations. Soyabeans are rich in vitamin A and also contains an isoflavone, genistein. Genistein and miso (a soybean product) protect against radiation damage to the intestine[49]. Subcutaneous administration of a nontoxic dose of genistein 24 h before gamma irradiation protected mice, giving a DMF of 1.16; multiple oral doses significantly increased protection against radiation lethality. Protection is mainly attributed to its antioxidant activity[50].

Flavonoids: Flavonoids are polyphenolic compounds, present in many edible plants, and are good antioxidants. A number of flavonoids (genistin, quercetin, luteolin and green tea flavonoids) were found to reduce the frequency of micronuclei induction in peripheral blood cells in irradiated mice[51]; their radioprotective effect has been correlated to their antioxidant activity[52].

Extracts prepared from *Podophyllum hexandrum* and *Hippophae rhamnoides* have been studied extensively for their ability to render *in vivo* radioprotection. Fractionated extract from *P. hexandrum* rhizome have been shown to protect cellular DNA[53], scavenge radiation and chemical-induced free radicals[53] and modulate cell cycle progression. The fractionated extract rendered more than 90 per cent survival in mice with lethal WBI and an estimated DRF of 1.62 for 30 day survival[54] has been reported. It also protected against radiation-induced chromosomal damage[55], enhanced liver and intestine antioxidant levels[54] and also protected the hematopoietic system[53]. The effective radioprotective dose for *P. hexandrum* extract is well below the maximum tolerable dose[54] and both intraperitoneal and intramuscular routes have been demonstrated to impart significant radioprotection, at least in animal models. An extract prepared from the berries of *Hippophae rhamnoides* has shown similar (> 90 %) survival in mice with lethal WBI[56, 57]. It protected DNA *in vitro*[58, 59], reduced bone marrow cytogenetic damage[60], scavenged radiation and chemical-induced free radicals[55], reduced cytotoxicity in cultured cells[61]. Another extract prepared from the leaves of *H. rhamnoides*, has been reported to be less toxic besides being radioprotective[62-64]. It protected genomic DNA at very low concentrations as well as effectively scavenged free radicals[62]. Leaf extract of *H. rhamnoides* has been reported to protect the liver and hemopoietic system in whole-body irradiated mice[63, 64]. Though both the plant extracts seem highly promising for application as *in vivo* radioprotectors, their toxicity is yet to be studied.

Intraperitoneal injection of an aqueous extract of the leaves of the Indian medicinal plant *Ocimum sanctum* gave significant protection against radiation lethality, giving a DMF of 1.28 for 30-day mouse survival at the optimum protective dose of 50 μg/kg b.w. ($<1/20^{th}$ of its LD_{50})[65]. The extract gave almost equal chromosome protection as that of 400 mg/kg of WR-2721[66], showed good free radical scavenging activity *in vitro*[67] and enhanced the cellular glutathione and antioxidant enzymes *in vivo*[68]. Orientin and vicenin, the water-soluble flavonoids isolated from the extract, were equally effective in protecting mice against radiation lethality, giving DMFs of 1.3 for orientin and 1.37 for vicenin at the optimum dose of 50 μg/kg b.w.[69]. The flavonoids gave equal or better protection to mouse bone marrow chromosomes against 2 Gy WBI compared to that given by 150 mg /kg of WR-2721[70]. Both compounds possess strong OH-radical scavenging[71, 72] and anticalstogenic activities *in vitro*[73] and *in vivo*[74] anti-lipid peroxidative activity *in vivo*[71]. OH-radical scavenging, directly and through stimulation of intracellular glutathione and antioxidant enzymes, are suggested to be the main mechanisms of protection by ocimum extract and its flavonoids; metal chelation also may have a role in membrane protection. The extract as well as the flavonoids are also effective when given orally[75], but did not protect solid tumors[76].

4.2 Combination of Protectors

Combining protective chemicals with different structural, pharmacological, and toxicity profiles may be a reasonable approach to increase protection and reduce the toxicity of the aminothiol protectors. Floersheim and Bieri[77] reported synergistic increase in protection by combining WR-2721 and other thiol protectors like AET and cysteamine with small doses of zinc aspartate. Weiss,[78] *et al*. observed a synergistic increase in protection and reduction in drug toxicity by combination of selenium and WR-2721. Patchen[79] *et al*. demonstrated that therapeutically administered granulocyte colony-stimulating factor (G-CSF) accelerated the hemopoietic reconstitution from WR-2721-protected stem cells in mouse spleen and bone marrow.

Combination of the optimum protective dose (20 mg/kg b.w.) of the antioxidant aminothiol MPG (2-mercaptopropionylglycine) with WR-2721 before WBI of mice synergistically increased the bone marrow stem cell survival[80] and chromosome protection, reduced the delayed chromosome toxicity of WR-2721[81] and significantly increased the intestinal protection[82]. Similarly, the protective effect of WR-2721 on mouse bone marrow chromosomes was increased synergistically by combined treatment with an optimum dose (50 mg/kg) of Ocimum extract. The protection

factor increased 2-fold (to ~6 from ~3 for 400 mg/kg WR-2721), at the same time eliminating the delayed chromosome toxicity of WR-2721[66].

4.3 Post-irradiation Protection or Mitigation

Radioprotective chemicals are effective when administered before exposure and therefore, they will not be useful in unplanned exposures, as in nuclear accidents and spillage and nuclear warfare. In such cases, the agents have to be effective when administered after irradiation and such agents are called radiation-mitigators, but it is not important that they should preferentially protect normal tissues. Very few compounds have shown such effect. The vitamins C and E have been reported to protect mouse bone marrow chromosomes even when administered shortly after irradiation[38]. Srinivasan,[42] *et. al.* and Kumar[43] *et. al.* found that vitamin E was effective post-irradiation administration scenario. α-TMG gave maximum protection against whole-body irradiation when administered within a short time after irradiation[46, 47]. The possibility of developing these into drugs for human application in nuclear emergencies is worth considering.

4.4 Protection Against Radiation-induced Genomic Instability

The most serious stochastic (probabilistic) effect of radiation recorded in man is the development of leukemia and solid tumors[83]. Prenatal irradiation has been shown to increase the risk of cancers in experimental animals as well as humans[84]. Radiotherapy of pediatric cancers was reported to induce second cancers in children and adults[85-87]. Genomic instability has been strongly implicated in radiation carcinogenesis[87]. Therefore, agents that can protect against radiation-induced genomic instability may also be able to protect against radiation carcinogenesis. The *Ocimum* flavonoids, orientin and vicenin, were demonstrated to protect against the induction of genomic instability in fetal hemopoietic stem cells and to reduce the chromosomal abnormalities in adult bone marrow and also to significantly reduce and delay the incidence of solid tumors in adult mice after prenatal gamma irradiation[88]. This, probably, is the first experimental study to evaluate the radioprotective potential of chemicals against the long-term stochastic effects of in utero irradiation. Genomic instability is considered to be the earliest critical event in radiation carcinogenesis[89]. Therefore, it is an attractive hypothesis that, by protecting against radiation induced genomic instability, these compounds may also be able to prevent radiotherapy-induced second cancers, which is worth investigating.

5. CONCLUSIONS

More than 50 years of research has yielded only one drug, WR-2721, which is approved and marketed for normal tissue protection in cancer therapy. The systemic toxicity on repeated administration with fractionated radiotherapy and its high cost are major deterrents in its routine use in cancer patients. Continuous search for cheaper and nontoxic radioprotectors has led to the discovery of many natural antioxidant nutrients and plant products with promise. More research is needed to explore the possibility of utilising the vast natural dietary and medicinal resources to develop economically viable and clinically acceptable radioprotectors for human application. The promising properties of Ocimum products–non-toxic nature, preferential normal tissue protection, protection against genomic instability, oral effectiveness and low cost are encouraging and warrants clinical evaluation to establish their applicability in cancer patients.

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