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EDITORIAL

Research and Development of Radioprotective Agents: A Mini-Review

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ABSTRACT

The research and development (R&D) of radioprotective agents (RAs) have been conducting since seven decades ago, and the safety and protection against undesirable effects of ionizing radiation (IR) has become an important issue especially for the benefit of patients receiving the radiotherapy, in addition to the applications in nuclear industry, military, outer space exploration and accidental exposures. Although the technology advancement makes the radiotherapy a promising better treatment by maximizing the effect of IR to the cancer cells, there are still needs for improvement in minimizing the toxicity to the normal cells. Aiming at providing the new researchers in the radiation protection field with an overall view for R&D of the RAs, this mini-review elucidates in brief a general understanding of the IR and its effects on the cell, the concepts of radiotherapy and therapeutic ratio, the categories of RAs and their mechanisms, and discusses on the strategies and challenge for R&D of the RAs.

Key words: Radioprotective agent; Ionizing radiation; Radiation toxicity; Safety and efficacy; Research and development

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INTRODUCTION

The research and development (R&D) of radioprotective agents (radioprotectants or radioprotectors) (RAs) have been conducting since seven decades ago. A RA is a substance that reduces the side effects of ionizing radiation (IR) on the healthy tissues. RAs could be from either artificially synthetic productions or natural resources. They are designed to protect the normal cells of the organism or biological system against acute and late effects of IR[1]. RAs are capable of reducing morbidity or mortality when present prior to or

after radiation exposure[2]. Clinically, RAs should potentially protect patients during treatment and prevent the detrimental effects of radiation in order to provide therapeutic benefit and improve overall quality of life[3]. RAs could potentially counteract radiation effects from accidental or deliberate exposure hence they are also called radiation counter-measures[4].

The term of RA together with radioprotection was first introduced by Dale in 1942 during his studies on enzymes as indicative molecules[5]. Then in 1949, Patt and his co-workers were the first to investigate the radioprotective effect of amino-acid cysteine against lethal doses of X-rays according to the documented records[6, 7]. Beginning in the 1940s, R&D of the RAs was focused on treatment for internal radionuclide exposure, especially for the benefit of workers in nuclear industry[8]. From 1959 until 1973, Walter Reed Army Institute of Research had synthesized 4000 compounds and screened them in mice[9]. The most significant contribution of the institute was through the R&D of WR-2721 (S-2-3-aminopropylamino) ethylphosphorothioic acid, and many related phosphorothioates, which were first synthesized by Akerfeldt in 1959 as RAs[10]. This achievement has continued whereby WR-2721 then being introduced to cancer clinical trials to study protection against normal tissue damage caused by radiation therapy (radiotherapy)[11]. Until today R&D of the RAs for radiotherapy are still continuing with more new incoming compounds tested preclinically and clinically with a better improvement.

The need for RAs is mostly for benefit of radiotherapy and applications in nuclear industrial, military, outer space and accidental exposures. Two factors contribute the needs of effective and safe RAs are the worldwide increase in cancer disease and radiotherapy. In the next 20 to 40 years, the cancer cases will increase more than double worldwide and remain as a major disease surpassing heart disease as the leading cause of death[12,13]. Radiotherapy is a highly effective tool for the cancer treatment[14] and furthermore in patients with inoperable tumors, it is often the only option[15,16], however being accompanied by the adverse effects on normal tissues. Concern for effects from therapeutic radiation on normal tissues is due to the intimate relationship between the tumors and the normal surrounding host tissues. Although the case of death as a result of therapeutic radiation exposure is rare, in addition to the acute harmful effects, late effects of radiation sequel such as radiation-induced cancer could also severely compromise the quality of life of cancer patients[17]. Advancements in the imaging and radiotherapy technology have contributed greatly to the accuracy for targeting tumors with maximal sparing of the normal tissues. The advancements include such as the development of computerized tomography (CT), magnetic resonance imaging (MRI), positron-emission tomography (PET) and fusion PET/CT over the past two decades in the imaging, and the development of intensity modulated radiotherapy, image guided radiotherapy, helical tomotherapy, stereostatic radiotherapy, volumetric modulated arc therapy and robotic radiotherapy in recent years in the radiotherapy[18]. However, the potential or actual use of new advanced technologies also raises other questions about such as the cost, efficacy and ethics, rather than the accuracy for targeting. In fact, the economic burden of increased quality assurance is a challenge[19] as application of advanced technologies in radiotherapy also depends on the needs of the countries concerned in terms of essential infrastructure in order to allow for a smooth, incremental and safe progression to advanced radiotherapy services[20]. Therefore, R&D of the RAs is necessary to render protection to human against undesirable health consequences from radiation exposure especially in the current medical field.

IR AND ITS EFFECTS ON THE CELL

Radiation is the emission or transmission of energy in the form of electromagnetic waves (i.e., X-rays and gamma rays) or particles (i.e., electrons, protons, neutrons, alpha and beta particles, and other heavy ion particles) through space or through a material medium without the necessary intervention of a transporting medium[21]. Exposure to IR may come from two different sources that are either from natural (cosmic rays, terrestrial radionuclides in building materials and in air, water and food, and in the human body itself) or man-made sources (such as radiodiagnosis, radiotherapy, and nuclear accidents). IR has the ability to form ions (electrically charged particles) and deposits energy in the cells of the tissues it passes through. The gray (Gy), a unit of IR dose in the International System of Units defining as the absorption of one joule of IR energy per kilogram of matter, is used as a measure of absorbed dose. The absorbed dose plays an important role in radiobiology, radiotherapy and radiation protection, as it is the measure of the amount of energy that the incident radiation is imparting to the target tissue. The Gy measures the total absorbed dose of radiation, and the probability of damage depends on both the quality of IR (i.e., the type and energy of the individual particles or the energy of the photons) and the tissues involved. This probability is related to the equivalent dose in sieverts (Sv), which is related to the Gy by weighting factors for different quality of IR. Controlled exposure to IR is one of the most used treatments for cancer patients[22]. This deposited energy in cancer radiotherapy can kill cancer cells or cause changes of the genetic material (i.e., DNA) in the cell thus blocking the ability of the cell to divide and proliferate further, which then result in cancer cell death[23]. The rate at which energy is transferred from IR to biological system/soft tissues is expressed in terms of linear energy transfer (LET) in kiloelectron volts per micrometer (keV/μm) of track length of soft tissue. Low LET radiation is less effective than high LET radiation in producing more biological damage[24]. The penetrating power (or property) of IR depends on the quality of radiation (i.e., photon waves vs particles), the kinetic energy and the electric charge it carries. Since electron beams have a low energy level with a low penetrating power, it is used most often to treat the skin, as well as tumors and lymph nodes that are close to the surface of the body[25]. Some types of IR have more energy with higher penetrating power (i.e., high-energy photons are emitted from radioactive substances such as cobalt, cesium, iridium, particle beams produced by accelerators) than other (i.e., electron beams) and henceforth have more penetrating power into the tissue[26]. X-rays, gamma rays and charged particles are the most common types of radiation used for cancer treatment. Recent advances in radiotherapy have enabled the use of different types of radiation sources for a better cancer treatment efficacy[27].

Biological effects of IR on cellular molecules can be direct (target effects) and indirect (no target effects)[21,28]. IR could directly damage the macromolecules, i.e., proteins, lipids, and DNA, the major target of IR-induced damage presents in the nucleus and mitochondria of the cell[29]. On the other hand, IR could collide with H_2O molecules within the cell (80% of the cell is composed of water), causing the production of free radicals that are capable of damaging critical targets[30]. Free radicals can be defined as an atom, molecule or ion that has unpaired electrons[31]. Because of the presence of unpaired electrons, free radicals are highly reactive species, capable of damaging biologically relevant molecules such as proteins, lipids, and DNA in membrane and nucleus of the cell[32]. Among the free radicals generated from radiolysis of H_2O, hydroxyl radical is the most damaging species[33]. It was estimated that 60-70% of the

IR-induced cellular damage was caused by hydroxyl radicals[34]. Reactive oxygen species react with cellular macromolecules and produce biomarkers of oxidative damage, leading to cell dysfunction and mortality[35]. Damage to DNA by free radicals could be indicated by such as the chromosomal aberrations and micronuclei[36], and the formation of DNA hydroxylation products (i.e., 8-hydroxy-2'-deoxyguanosine or 8-OHdG)[37] which is also a biomarker of carcinogenesis[38,39]. In fact, IR can produce a wide spectrum of DNA lesions in cellular DNA, including damages to nucleotide bases (base damage), single-strand breaks, double-strand breaks, and DNA-DNA and DNA-protein crosslinks[40]. Lipid damage shown as the formation of malondialdehyde and thiobarbituric acid reactive substances that are lipid peroxidation products[41], could lead to changes in membrane permeability[42]. Protein structure could also be changed by hydroxyl radical and other free radicals, resulting in the change in the function of proteins[43]. Protein hydroxylation products such as oxidized amino acids are the indices of protein damage[44]. Cell repairing enzyme systems (i.e., superoxide dismutase, glutathione peroxidase, and catalase) have been formed through evolution to protect the integrity and the accuracy of heredity material[45] by neutralizing free radical-induced cellular damage[46]. However once the level of reactive oxygen species increases above the tolerable limits, the endogenous system usually can not fix such hard lesions and neither repair DNA[47].

When cells are exposed to IR, the standard physical effects on the atoms or molecules of the cells occur first and the possible biological damage to cell functions follows later[48]. In the cell structure, nucleus is widely regarded as the principle target of IR due to the fact that it is the largest cellular organelle in the cell[49]. Alteration of the chemical structure of cell components depends on the dose and duration of exposure, as well as the radiosensitivity of the organ[50]. Radiosensitivity refers to the susceptibility of cells, tissues and organ systems to the injurious action of radiation. Generally, cell radiosensitivity is directly proportional to the rate of cell division and inversely to the degree of cell differentiation[51]. The relative radiosensitivity of a cell is determined by its cell cycle phase. A cell is the most radiosensitive in the G2-M phase, less sensitive in the G1 phase, and least sensitive during the latter part of the S phase[52]. Exposure to high amounts of IR results in severe damage to haematopoietic, gastrointestinal and central nervous systems depending on radiation dose[53]. Among the three systems, haematopoietic system is the most radiosensitive in the body as it has a highest cell turnover[54]. IR-induced cell death is by various mechanisms. For examples, IR kills cancer cells through induction of apoptosis, necrosis, mitotic catastrophe, autophagy and senescence[55]. Among of these, apoptosis is a major cell death mechanism involved in cancer radiotherapy[56], which is characterized by cell shrinkage and forming of cell fragments called apoptotic bodies[57]. Induction of apoptosis in cancer cells plays an important role in the efficacy of radiotherapy[58]. Unlike apoptosis, necrosis is a form of cell injuries that could result in premature death of cells in living tissues by autolysis[59]. While apoptosis often provides beneficial effects to the organism, necrosis is almost always detrimental and can be fatal[60]. Following IR, necrosis is seen less frequently but does occur in cancer cell lines or tissues.

RADIOTHERAPY AND THERAPEUTIC RATIO

Radiotherapy is a common therapy using IR to treat cancer and prevent tumor recurrence after surgery. It could also serve as a palliative treatment when cure is not possible, aiming for local disease control or symptomatic relief. In addition, radiotherapy has several applications in non-malignant conditions. It is estimated that 50% of all cancer patients received radiotherapy during the course of their treatment[61] and around 40% of cancer cured by radiotherapy either as a single modality or combined with other treatment[62]. There are two ways to deliver radiation to the location of the cancer, and the differences relate to the position of the radiation source: delivery outside the body and inside the body, by external radiation beams, by brachytherapy (sealed radioisotopes seeded directly into the tumor site) and by infusion or oral ingestion of radioisotopes. External radiation is the most common approach in the clinical setting meanwhile internal brachytherapy is particularly used in the routine treatment of gynecological and prostate malignancies as well as in the situations where retreatment is indicated, based on its short range effects[63].

The therapeutic ratio in radiotherapy for cancer treatment refers to the ratio obtained by dividing the effective therapeutic dose with the minimum lethal dose, namely, the ratio of the maximum dose by which death of cancer cells is locally controlled to the minimum dose by which cells in normal tissues have low acute and late morbidity[64]. The aim of radiotherapy in clinical treatment is to deliver enough radiation to the tumor by means to shrink and destroy it without irradiating the adjacent normal tissue to a dose that would lead to serious complications[65]. Although it is believed over a century that cancer cells were more radiosensitive compared to the normal cells, to completely eliminate the cancer cells, higher doses must be delivered to the tumor. This leads to more toxic effect on the surrounding normal tissues[66] that could affect a patient's quality of life. Consequently, this will bring radiation dose reductions or treatment delays that can lead to poor therapeutic outcome[67].

Cancer radiotherapy focuses on three strategies to increase the therapeutic ratio: modification of the intrinsic radiosensitivity via such as molecular targeting, manipulation of microenvironmental factors to enhance the tumor radiosusceptibility, and improvement of radiation delivery to critical tumor locations while sparing normal tissues. Advances in understanding of the molecular mechanisms on radiosensitivity make it possible to modify the response of both the tumor and the normal tissue to radiation through targeting genes and signaling pathways involved in radiosensitivity and radioresistance. Normal cells usually can repair themselves at a faster rate to retain normal function while cancer cells in general are not as efficient as normal cells in repairing the damage caused by radiation treatment thus resulting in differential cancer cell killing[68]. However, the nature of high-energy radiation can override the ability of damaged normal cell to repair, consequently causing another risk for carcinogenesis. Advances in radiation technology lead to significant improvements in delivering the radiation dose more precisely to the shape of the tumor and at the same time limit the dose of radiation to the normal cells[69]. On the other hand, protection of the normal tissues from radiation injury remains as a major goal as both cancer cells and normal cells that have close relationship regarding their location in the organ[70], and it is not possible to exclude all normal tissues from the radiation field because the doses necessary to achieve tumor control usually overlap with those that can cause complications[71]. As an overall view, protection of the normal tissue is needed to accompany with advancement in radiation technology to improve the therapeutic ratio and to maintain the relevant of radiotherapy as one of the best cancer treatment. In fact, R&D of the RAs could improve the therapeutic ratio by means of protection to the normal tissue without demonstrable protection to the tumor tissue. In addition, this concept is also applicable for determination of the safety and toxicity of a new RA.

RA, ITS CATEGORIS AND MECHANISMS OF FUNCTION

The concept of the RA was introduced in the Induction section. It was the harmful effects of IR on human health that led to the study on RAs seven decades ago, and some reviews by such as Mönig *et al* well documented the history of R&D of the RAs, especially the early work done in the early 1940s by the international pioneers in this field[72,73]. In 1949-1950, studies showed the efficacy of cysteine, glutathione and cyanide as RAs[7,74,75]. In the early 1950s, Pihl and Eldjarn discussed the early studies on RAs and proposed the mechanisms, including their seminal theory on formation of mixed disulfides by protective thiols[76]. From the 1950s to the 1980s, thiols compounds dominated the field, later followed by the discovery of non-thiol-containing protectors, R&D of RAs appeared to be at a turning point: non-thiol protectors, including such as the protease inhibitors, vitamins, metalloelements and calcium antagonists were actually playing a large role in radioprotection. In the 1990s, interest increased in endogenous radioprotective system as opposed to chemical RAs[77]. Since the late 20th century, efforts have been made to identify and develop novel agents for radiation mitigation and therapeutic treatment regimens[78]. With the successful R&D of the RAs, there is also a shift in emphasis from prophylactics that need to be administered before radiation exposure to radiation mitigators that could be administered after radiation exposure.

The main purpose for R&D of the RAs is to protect the normal tissue from IR toxicity. Side effects of IR produced acute toxicities, and late tissue and organ reactions as consequences of cellular damage to a complex network of interacting populations of the cells[79]. As the consequence of cell killing, acute toxicities manifest not only the tissue degeneration but also the inflammation and infection process to extend beyond the treated area[80]. Depression, fatigue, dermatitis, nausea and vomit are symptoms of acute radiation effects while the side effects are usually transient and these symptoms would disappear four to six weeks after completion of treatment course[81]. Late toxicities could be observed several months to years after completion of treatment. They are different from the acute toxicities. They neither affect the survival nor being limited to the tissues treated[82]. Late effects result from a series of complex responses of the tissue towards a process of healing or failure to healing which produces fibrosis and severe organ functional failure. Hence, to reduce these toxicities and improve the therapeutic ratio, RAs are receiving significant concern. Moreover, the needs for R&D of the RAs are also with the respect to the potential application of IR in the medical practice, the planned exposures where as an attractive countermeasure to protect first responders deployed for rescue and other military operations, and the accidental exposures as well[83,84]. R&D of the RAs are also of great importance in space science, given that manned missions are increasing[85]. An ideal RA should be able to provide significant protection to the normal cells against the effects of IR without marked influence on the radiosensitivity of the tumor. The RA must have an acceptable route of administration (preferably oral or alternatively intramuscular), and an acceptable toxicity, stability profile and protective time-window effect. In addition, the RA should be compatible with a wide range of drugs that the patient may be exposed to[86].

RAs can be classified into 3 different categories based on the time when the agent is administered: prophylactics, mitigators, and treatment[87] as models for evaluating RAs intend for the prophylaxis, mitigation and treatment of IR injuries. Radioprophylactics are administered before radiotherapy (exposure) to protect against radiation-induced injury. This class includes the compounds with thiol (sulfhydryl) groups and compounds with antioxidant properties or function through free radical scavenging[7]. Among the compounds that belong to this class, amifostine is the most famous example[88]. Mitigators are given during or shortly after exposure before the appearance of radiation symptoms, aiming to minimize toxicity and prevent or reduce the negative effects of IR on cells or tissues[89]. They target a series of cellular insult recognition/repair responses initiated following exposure to IR, including DNA repair, apoptosis and regulation of signal transduction cascades that stimulate proliferation and immunoinflammatory responses[90]. Mitigators are mostly developed for space research to reduce carcinogenesis of total body exposures and for possible accidents and terrorism as well[91]. Sodium orthovanadate, as a potent p53 inhibitor, could mitigate the hematopoietic damage from IR exposure[92]. Treatment or therapeutic preparations are applied after IR exposure following the clinical appearance of normal tissue toxicity to cure or ameliorate the radiation damage. As an example of this class, pentoxifylline (PTX) showed a significant beneficial effect in the prevention of late radiation effects, and recent evidence also suggested that it might be beneficial for the treatment of acute lesions[93].

There are different theories propounded regarding the mechanisms of radiation protection, such as free radical scavenging, calcium channel blocking, inhibition of lipid peroxidation, enhancement of DNA repair and stimulation of stem cell proliferation are considered important[94]. In fact the mechanism-based approaches are the strategies for R&D of the RAs. Starting from the timing before exposure of the cell to IR, through activation of signal cascades for dealing with damage of the cell, until the appearance of cell death or tissue disorganization, RAs could target any aspect in the whole process to intervene or even to reverse the destructive process. For low LET IR such as X-rays and gamma-rays, 60% of cellular damage is caused by the indirect effect[95]. For high LET particle IR, though the physical damage to DNA due to direct mechanism is much severe, indirect mechanism (free radicals formation) also plays an important role in causing damage to DNA, cytoplasmic organelles and endoplasmic reticulum. As most of the IR-induced biological damage arises from the interaction of IR-induced free radicals with the biomolecules, scavenging free radicals could consequently reduce the occurrence of damage, thus the mode of the cell damage caused by indirect mechanism has the potential to be modified by the use of free radical scavenging or antioxidant compounds that could neutralize IR-induced radicals. Sulfhydryl compounds especially the amino thiols and phosphorothiates containing a sulfhydryl group, are particularly suitable for free radical scavenging because of their propensity to donate a hydrogen atom for the reduction of radical species[96]. Since free radicals are short-lived, free radical scavenging or antioxidant compounds are used as prophylactics as it is necessary for such RAs to be present in the cellular milieu in sufficient concentration at the time of exposure[97]. Amifostine is a sulfhydryl-containing compound. It is the only RA that has been approved by Food and Drug Administration of the USA[98] and the most commonly used RA in clinic, protecting the normal tissue via the scavenging of free radicals[99,100]. Having the characteristic of an ideal RA, it is of note that amifostine does not protect the tumor tissue[101]. It concentrates more rapidly in the normal tissues than in the tumor tissues in studies of tumor-bearing animals[102]. To date, amifostine has been applied for clinical protection against xerostomia during radiotherapy of head and neck cancer[103]. Interestingly, in addition to the radical scavenging, amifostine could also enhance DNA repair[104] and induce hypoxia[105]. Despite its approval and

radioprotective effects (which are short term), amifostine is limited in use due its cumulative toxicity that is expressed as transient hypotension, nausea, vomit and allergic reactions. The reaction may be localized or generalized and is characterized by skin rashes, urticaria, erythema, multiforme, and rare reports of anaphylactoid reactions[106]. On the other hand, other non-sulfhydryl-containing chemicals that can prevent the formation of free radicals or destroy free radicals by reacting with them, hereby inhibiting the reaction of free radicals with biomolecules, could also function as RAs[107].

When IR-induced damage is unrepaired and the signal pathways of cell death are activated, potential RAs should be able to enhance DNA repair and inhibit cell death signal pathway. Repair process could usually result in either the survival of the cell or triggering cell death pathway leading to such as apoptosis. Damage of the cellular compartment could cause the early dysfunction and eventually a chronic phase, resulting in organ destruction. Inadequate survival of cells with mutations could lead to carcinogenesis. Each of these steps can be intervened or blocked via pharmacological or molecular interference[108]. As massive cell loss that occurs in radiosensitive tissues after exposure to IR is due to the activation of apoptosis pathway through the signaling of p53[109], a transcription factor, responding to IR by initiating cell cycle arrest, DNA damage repair, senescence, and apoptosis[110], thus p53 inhibitors could be the candidates used as mitigators. Using animal models it showed that sodium orthovanadate, a p53 inhibitor, is not only a potent radioprotector but also a promising mitigator to IR-induced damage in the hematopoietic system of mice[92]. In addition, as p53 is one of the most commonly mutated genes in cancer[111], p53 inhibitors could selectively protect the normal tissues if applied to the radiotherapy for the p53-mutated cancer.

Agents that could modify radiosensitivity of the normal tissue or bear protective effect on the normal tissue could also be the candidates of RAs. As an example, hypoxia is an important mechanism for tumor radioresistance in hypofractionated radiotherapy, which must be considered in the treatment planning process[112], while an agent that could temporarily create hypoxic condition in the normal tissue may have radioprotective effect[113]. Hall et al. outlined others clinically used chemical RAs that have a beneficial effect in reducing the risk of radiation toxicity. For examples, glutamine, a non-essential amino acid was widely studied for its potential protective effect on mucositis, dermatitis and oespahagitis[114-117]; pentoxifyline, a methyl xanthine derivative, decreased the risk of radiation toxicity in lung[118]; benzydamine could prevent and treat oral mucositis[119]; and sulfasalazine was evaluated for its effects on radiotherapy-induced enteritis[120]. To date, there are still a limited number of chemical RAs clinically used to minimize the severity and duration of toxicities associated with radiotherapy. Some other agents such as palifermin[121], genistein[122], angiotensin I-converting enzyme inhibitors[123], melanin[124], hemocyanin[125] and Vitamin D[126] showed a promising effect against the radiation toxicity, however there is a lack of high quality clinical studies for most of the agents described. The evaluation of safety and efficacy of these emerging agents are needed and their effects on tumor sensitivity should be verified[117].

Phytochemicals and extracts of herbs and medicinal plants are good RA candidates. Agents from natural sources especially the edible medicinal plants or herbs have been traditionally used and consumed by humans, and many agents are considered safe and non-toxic even at higher concentration. In many countries such as Malaysia, Japan, India and China, medicinal plants and herbs are used as the main ingredient in the medicine preparation by the traditional practitioners. Ayurvedic treatment and Chinese traditional medicine have a long record in application of medicinal plants and herbs. Different classes of phenolics and flavonoids having radioprotective and anti-cancer properties are the most abundant phytoconstituents in the extracts of the medicinal plants and herbs[127]. So far, a number of medicinal plants either in the form of whole extracts or polyherbal formulations, fractionated extracts or as isolated constituents have been evaluated for their radioprotective efficacy against IR-induced damage[128]. Most of them showing an anti-inflammatory, antioxidant, antimicrobial, immunomodulatory, or anti-stress properties, may act as potential RA candidates[129]. Medicinal plants or herbs successfully used in the treatment of free radical mediated diseases such as rheumatoid arthritis, cancer, Parkinson's disease, Alzheimer's disease, aging and inflammatory diseases[130, 131] could be potential RAs. In fact, more and more plant extracts and plant-derived compounds were verified in the *in vitro* and *in vivo* models of radiological injury[132]. Today there is an increasing tendency of promoting R&D of the RAs from the herbs and medicinal plants due to the low toxicity, easy availability, low cost and good radioprotective efficacy exhibited in preclinical studies[133]. It should be noticed that although herbs and medicinal plants are generally considered as natural, safe and human friendly, their use as RAs still needs scientific evaluation and validation.

STRATEGIES FOR AND CHALLENGE OF RA R&D

Clinical testing of potential RAs could be initiated only after the radioprotective efficacy is convinced by preclinical studies that simulate, as much as possible, the conditions under which the agent will be used clinically. A three-stage approach to screening and assessment of RAs is recommended for preclinical R&D of the RAs[134]. In stage I, maximum tolerated dose (MTD) and toxicity of the agent are determined using Good Laboratory Practices or GLP[135], a quality system of management controls for laboratories to ensue the uniformity, consistency, reliability, reproducibility, quality and integrity of chemical nonclinical safety tests from physiochemical properties through acute to chronic toxicity tests in nonclinical research. In the study using experimental animals, the MTD is defined as the highest dose that could be tolerated for the study duration. In stage II, protective or mitigative effects are determined using both *in vitro* and *in vivo* models testing in both normal tissues and tumors whenever possible. If both presence of sufficient normal tissue protection/mitigation and absence of tumor protection were observed, then the mechanisms of action would be identified, if not already available. In stage III, comprehensive toxicological and pharmacological testing is performed to address the regulatory requirement for data on absorption, distribution, metabolism, excretion and toxicity profiles before proceeding to the clinical investigation[136].

The gold standard for the evaluation of radioprotective activity is the 30-day survival test in rodents (i.e., the mouse). The studies using mice with death as the end point are the most confirmatory, and the 30-day survival after lethal whole body IR could clearly indicate the capacity of the agent. In the 30-day survival test, the modulation by the agent of the recovery and regeneration of the gastrointestinal epithelium and the haematopoietic progenitor cells in the bone marrow could be evaluated, which are two of the most radiosensitive organs essential for sustenance of the life[137,138]. The gastrointestinal (GI) syndrome in mice can be assessed by determining the survival up to 10 days (measure of GI death) after exposure of the animals to comparatively high doses of IR, whereas haematopoietic syndrome

can be assessed by monitoring the survival of irradiated animals up to 30 days post-IR[137,139,140-142]. The intestinal crypt cell assay or functional changes also serve as indicators of GI damage[143]. With sufficient loss of intestinal epithelium and haematopoietic stem cells, death follows due to such as infection, haemorrhage, and anaemia. An effective RA should improve a 30-day survival in mice by protecting against GI syndrome, haematopoietic syndrome, or both. In the studies using experimental animals, dose reduction factor (DRF) is typically determined by irradiating animals, usually the mice, with or without administering RA at a range of radiation doses and then comparing the protective efficacy using the same endpoint of interest. For an example, the survival lethal dose 50/30 ($LD_{50/30}$), which is the exposure dose to IR that kills half of the exposed animals within 30 days, is often used to estimate the DRF of the RA by dividing the $LD_{50/30}$ of the RA-treated group with the $LD_{50/30}$ of the vehicle-treated group to quantify protection of the haematopoietic system[139,140]. The predominant laboratory animal used in the models of radiation syndrome for study of IR effects is the mouse due to the low cost, easy handling and ability to manipulate the phenotype and genotype[144,145]. The advantages of using the mouse as an animal model are their anatomical, physiological and genetic similarity to humans, i.e., over 95% of the mouse genome matches the human genome[146]. The most common mouse strains used are the BALB/c, C3H/HeN, B6D2F1/J, CD2F1 and C57BL/6. These strains show considerable variation in their response to IR, as demonstrated by their range of $LD_{50/30}$ values (6.5-9.0 Gy): with the BALB/c mice being the most sensitive and the C57BL/6 mice the most resistant. On the other hand, drift in the dose-response relationship and $LD_{50/30}$ may occur in any laboratory. In addition, it should be noticed that the wild type mouse may not always mean “normal” and on the other hand, the radiosensitivity of the mouse organs may not always accord with the degree of radiosensitivity of the mouse strain. For examples, the BALB/c mice have a double-stranded DNA repair defect that may account for their enhanced radiosensitivity[147], and thymocytes, splenocytes and crypt cells of the C57BL/6 mice are more sensitive to IR-induced apoptosis than those of the C3H/HeN mice[148].

In many studies, in particular those using the *in vitro* models, the intermediate biomarkers of damage or protection, such as those regarding to the lipid peroxidation or antioxidant activity including glutathione, catalase, and glutathione peroxidase, and as well as the expression of genes that are related to radiation effects, such as p53 down stream genes, nuclear factor-kappa B (NFkB) and caspases, are used to evaluate the radioprotective effect of the candidate agent[149-152]. As an agent capable of inhibiting lipid peroxidation and scavenging free radicals may act as a possible RA, testing lipid peroxidation for screening a potential RA and assay of alterations in free radicals and antioxidant status could also provide information regarding the radioprotective potential, in addition to the testing for DNA strand breaks, apoptosis, cell survival, micronucleation, chromosome aberration.

R&D of the RAs have been facing many challenges and there are still a lot of issues to be concerned. Many reviews showed an increasing number of RAs with successful results demonstrated in the laboratory. However, most of the RAs failed in the transition from laboratory to clinical study. Toxicity of the RA and the inability to differentiate between the tumor and the normal cells are the main reasons. Finding ways to prevent both acute and late toxicity resultant the normal tissue injuries is of great importance since the devastation resulting from the side effects could sometimes be far worse than the initial lesion that was treated[153]. One approach to overcome the problems associated with the toxicities is by using a combination of several RAs bearing different mechanisms of action at their nontoxic dosages[154]. For an example in the study using animal models, the radioprotective effect of *Sipunculusnudus* L. polysaccharide in combination with WR-2721, rhIL-11 and rhG-CSF was investigated in mice. The results suggested that the combined administration could increase the efficacy of these drugs for acute radiation sickness, protect immunity, haematopoiesis and the reproductive organs of the mice, as well as improve oxidation resistance in the body[155]. Another approach for protecting the normal tissues is by chemically modifying the structure of the RA to make it non-toxic to the normal cells and at the same time afford no protection to the tumor cells. An ideal RA should differentiate between the euboxic environment for the normal cells and the hypoxic environment of the tumor cells. Recent work is showing a particular promise including the understanding of the difference in redox chemistry and metabolism of oxygen between euboxic regions and hypoxic regions[156-162]. To date, there are only a limited number of RAs used clinically to minimize the severity and duration of toxicities associated with radiotherapy. Although there are a number of promising agents emerging, regrettably, almost them could not meet all the requirements of the RA due to that they are not always suitable for the oral administration and their toxicity cannot be totally eliminated.

While early research has focused on small molecule RAs, with the advances in delivery, gene therapy is applied to radioprotection. As an alternative biological strategy, gene therapy could be combined with cellular therapy by using cellular therapeutic agents as vectors. This would allow a dual approach to radioprotection through both cell-mediated and transgene-mediated mechanisms[163]. On the other hand, nanoparticles are a potential non-biological vector that could be used in radioprotection[164]. Since the majority of RAs are effective only for a limited duration, this situation must be improved by pharmaceutical combination to ensure prolonged retention of the RA in the body. Exploration of new RA candidates from the natural resources such as medicinal plants and herbs has been promising a new perspective in the process of RA R&D. The steps suggested initials from the methodical approach starting with the understanding of the phenomenon of radioprotection, following by selecting the plant species, taxonomical identification, extraction and fractionation of the plants, preservation, chemical characterization, evaluation of the toxicity and efficacy in several model systems, and finally caring to meet all regulatory requirement for R&D of a safe RA[165].

A candidate agent must produce significant radioprotective effects in preclinical study before it could be approved as a RA by authority such as the FDA in the United States. Like other pharmaceutical drug development programs, getting a patent for the new RA is one of the goals for the inventors but it requires a huge amount of funding. To overcome this problem, R&D of the RAs have to be in line with the government interest for the purpose of research grant and incentive. In addition to that, support from public-private partnership especially giant pharmaceuticals company also must be taken into consideration. According to the Animal Efficacy Rule[166], potential products must undergo rigorous testing utilizing acceptable and well-controlled animal models to establish the safety and efficacy of the product under development. Technology advances together with the R&D of the RAs and improvement in the clinical treatment are important to improve the quality of life of the patients receiving the radiotherapy. With the increasing application of high LET IR in radiotherapy, R&D of the RAs are facing new challenge as high LET IR causes different type of damage to the normal tissues and the involved mechanisms under the biological effects should also be taken into account.

CONCLUSION

The R&D of the RAs are of great importance to especially protect the patients from the side effects of radiotherapy as well as the public from the effects from unwanted exposure to IR. Both technology advancement in radiotherapy and R&D of the RAs play critical roles in reducing the toxicity from radiation injuries due to radiotherapy. Diverse understanding on strategies and mechanism to increase protection to the normal tissue as well as to increase the sensitivity of the tumor cells to radiation should be improved. Safety and efficacy of product development also play an important role and should not be compromised. The success in development of new RAs also depends on the supportive from the government agencies and pharmaceutical companies for funding.

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