ABSTRACT: Irradiation from diverse sources is ubiquitous and closely associated with human activity. Radiation therapy (RT), an important component of the multiple radiation origins, contributes significantly to oncotherapy by killing tumor cells. On the other hand, RT can also cause some undesired normal tissue injuries that afflict numerous cancer patients. Although many promising radioprotective agents are emerging, few of them have entered the market successfully due to various limitations. At present, the most accepted hypothesis for the radiation-caused injury involves reactive oxygen species (ROS) generation. Superoxide dismutase (SOD), the unique enzyme responsible for the dismutation of superoxide radicals, is expected to occupy an indispensable position in the treatment of ROS-mediated tissue injuries originating from exposure to radiation. This review focuses on the mechanism of radioprotection by SOD at the tissue or organ level, cellular level, and molecular level, respectively, in order to provide references for further investigation of radiation injury and development of new radioprotectors.

Keywords: Superoxide dismutase, radioprotection, radiation, reactive oxygen species

1. Introduction

Irradiation from diverse sources is ubiquitous and closely associated with human activity. As shown in Figure 1 (1), among all sources of radiation, natural radiation, including radon, thoron, cosmic radiation, and natural radioactivity in soils and food, dominates in the average doses of individual radiation adsorption. However, the dose from natural radiation is not under human control. By contrast, artificial radiation, which consists primarily of medical exposure of patients, accounts for approximately 14 percent of the individual radiation adsorption and has been attracting more and more attention over recent years. Moderate application of man-made radiation is extremely beneficial as shown by the excellent performance of radiation therapy in oncology (2). However, some adverse effects accompany the therapeutic benefit as a consequence of the unavoidable exposure of the surrounding normal tissues to radiation. In this sense, besides the skin being irradiated directly, other internal radiosensitive organs also cannot escape from being injured to different degrees (3), which become an impediment, counteracting the efficacy of radiation therapy (RT). In order to minimize these undesired side effects, many efforts have been made to improve the RT technology, such as image-guided radiotherapy, proton radiotherapy, and intensity-modulated radiotherapy. Even though these advanced techniques have the advantage of improved accuracy and control of irradiation, the patients still confront the potential risk of normal tissue injuries (2). Thus, to seek the radiation modifiers with selective protection for normal tissues has been a realm of intense investigation.

Despite the fact that many promising radioprotective candidates are emerging, amifostine (WR2721) is the only one approved for clinical use to date. In addition to its high efficacy in ameliorating xerostomia resulting from irradiation (4,5), high frequencies of deleterious
side effects (nausea, cutaneous reactions, hypotension, etc.) and even tumor radioprotection have been reported, which limit its use (6-9). Therefore, the search for other radioprotectors with high potency and low toxicity should be the primary subject of further research.

At present, various compounds are being evaluated and examined for their radioprotective properties. It has been reported that ReIB, one of the five NF-κB family members existing in mammals, can improve the radiosensitivity of prostate cancer cells through up-regulation of the mitochondria-localized manganese-superoxide dismutase (Mn-SOD) expression (10). Meanwhile, Murley et al. have carried out a series of research on the delayed radioprotection for RKO36 cells (a strain of human colon carcinoma cells). They found that pre-incubation of RKO36 cells with WR1065 (the free thiol form of amifostine) or tumor necrosis factor alpha (TNF-α) could effectively stimulate the expression of Mn-SOD, thereby enhancing the adaptive response of the cells to the subsequent radiation challenge (11,12). Additionally, in the study by Zhang et al., CpG-oligodeoxynucleotide (CpG-ODN) was shown to effectively relieve bone marrow hemopoiesis radiation injury. Interestingly, the mechanism by which CpG-ODN acted was also through activating the NF-κB pathway and elevating Mn-SOD content (13).

These several series of evidence indicate that SOD plays a significant role in radioprotection, and it is of great importance to investigate further details of its mechanism of action so as to develop it as a radioprotector. This review describes the radioprotective studies of SOD based on the hypothesis of reactive oxygen species (ROS) generation associated with radiation injury.

2. Reactive oxygen species and radiation injury

Reactive oxygen species (ROS), *in vivo* byproducts of oxygen metabolism, comprise a multitude of family members such as superoxide radical (O₂⁻), hydrogen peroxide (H₂O₂), singlet oxygen (¹O₂), hydroxyl radical (OH) and so on (14). Though the physiological levels of ROS are critical for a variety of cellular functions, such as cell growth, stress adaption, injury response, and cellular phenotype development (15), inevitable toxicities can be induced by overdoses of ROS under some pathological conditions (16). Homeostasis of ROS is not only affected by endogenous factors but also by exogenous ones (Table 1).

Table 1. Summary of diverse sources of ROS

<table>
<thead>
<tr>
<th>Endogenous sources</th>
<th>Exogenous sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAD(P)H oxidase</td>
<td>Radiations</td>
</tr>
<tr>
<td>Xanthine oxidase</td>
<td>Pathogens</td>
</tr>
<tr>
<td>P-450 monoxygenases</td>
<td>Metals: Fe, Cu, Zn</td>
</tr>
<tr>
<td>Lipooxygenases</td>
<td>Xenobiotics, etc.</td>
</tr>
<tr>
<td>Cyclooxygenases, etc.</td>
<td></td>
</tr>
</tbody>
</table>

Note: This table summarizes the diverse sources of ROS based on the authors’ interpretation of the references (14) and (17). Readers can refer to the web version of these articles.

Ionizing radiation (IR), possessing great strength of penetration, usually exerts its harmful effects on organisms and biomolecules through both direct and indirect effects. The former is referred to the irreversible injuries caused by radiation selectively impacting certain biomolecules, of which DNA damage is the most notable one. The latter is associated with the condition, in which radiation interacts with non-targeted molecules, induces abnormal levels of ROS exceeding the capacity of the organism to clear them, and, consequently, leads to oxidative stress-mediated damaging effects (2). Owing to the highly oxidative activity, •OH, the radiolysis product of water, contributes a lot to the adverse reactions immediately after radiation by breaking chemical bonds and promoting lipid peroxidation. This explains the significant role of water in the indirect effects of IR (18). Moreover, it has been demonstrated that the radiation-exposed organisms perpetuate elevated levels of ROS (19-21), caused to a large extent by the mitochondrial dysfunction. Under such condition, molecular oxygen (O₂) is partly reduced to generate considerable amounts of O₂⁻ and H₂O₂ (22). Then, by the way of Fenton and Haber-Weiss reactions, respectively, both O₂⁻ and H₂O₂ can be further converted to OH (23), which is the most toxic of all ROS responsible for the majority of IR-mediated adverse reactions. Depending on the photochemical reaction between radiation and some endogenous photosensitizers localized in the cellular or mitochondrial membrane, such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and xanthine oxidase (24), huge doses of ROS are generated, overwhelming the antioxidant defense system in organisms, which result in a series of serious adverse effects.

Depending upon the level of injury, the IR-induced damages are usually categorized as follows: tissue or organ level effects, cellular level effects and molecular level effects (see Figure 2).

3. Superoxide dismutase and its radioprotective effects

Approximately eighty years ago, Keilin et al. isolated a blue protein containing copper from bovine erythrocytes for the first time and named it erthrocuprein. However, they had no idea about its bioactivity. Not until 1969 did...
McCord and Fridorich discover that this protein has the enzymatic activity of catalyzing $\cdot O_2^-$ dismutation and formally denominate it superoxide dismutase (SOD) (25), which is regarded as a thumping breakthrough that triggered extensive research on SOD from then on.

As naturally present antioxidant enzymes, SODs exist in mammals in diverse forms. There are Cu, Zn-SOD in cytoplasm and nucleus, Mn-SOD oriented in mitochondria, and EC-SOD present mainly in extracellular spaces (25-27). Their three-dimensional structures are depicted in Figure 3.

All of the SODs can accelerate the dismutation of $\cdot O_2^-$ with a powerful potency to yield $O_2$ and $H_2O_2$; the latter is further decomposed into nontoxic products by catalase (CAT) or glutathione peroxidase (GPX). Besides the conventional enzymes mentioned above, some other catalysts such as thioredoxins (TRXs) and glutaredoxins (GRXs) can also facilitate the degradation of $H_2O_2$ via modulating the redox balance of disulfides (see Figure 4) (28). Hence, a naturally occurring antioxidant enzymatic defense system is established in the organism involving SOD, CAT, GPX, TRXs, GRXs, and so on.

With the growing interest in SOD, it has become clear that SOD acts fundamentally for defeating ROS-mediated diseases such as carcinoma, inflammation and aging (29,30). However, these diseases are beyond the scope of this review. Herein, we focus on the protective effects of SOD against IR-induced normal tissue injury at the tissue or organ level, cellular level, and molecular level, respectively.

3.1. Radioprotection at the tissue or organ level

Generally, large doses of radiation can lead to a remarkable reduction of parenchymal cells and development of tissue fibrosis, while the vessel wall of mesenchyma becomes thickened and microcirculation gets blocked. All of these together may provoke the burst of organ dysfunction eventually, if not repaired promptly (31). However, due to the dissimilar radiosensitivity, different tissues or organs show marked diversity in their responses to the radiation.

Skin, the firstly affected target of radiation, is one of the most acknowledged tissues with respect to the IR-induced injury. During the early period following the
radiation, it is common to observe the appearance of local hyperemia and erythema on skin surface. Thereafter, owing to the long-term, persistent and epigenetic effects of radiation, the lesion may evolve into a chronic skin injury which has no efficient treatment (31). In the study by Yan et al., after a single dose of 37 Gy was given to mice on their right hind legs, AAV2-Mn-SOD-hrGFP, a recombinant adenovirus vector expressing Mn-SOD tagged with humanized recombinant green fluorescent protein, was injected subcutaneously in the experimental group and an equal volume of AAV2-IRES (internal ribosome entry site)-hrGFP in the control group. Although all the irradiated mice demonstrated severe skin injury initially, the mice in the experimental group displayed pronounced mitigation and accelerated healing process ($p < 0.05$) two weeks after radiation compared with the control group. These results indicate the marked relief of IR-induced skin injury provided by Mn-SOD expression (32).

Because of the high radiosensitivity, it is obvious that hematopoietic cell proliferation can be suppressed upon the exposure to radiation (33). Numerous studies have been performed to explore the role of SOD in the adaptive response of the hematopoietic organ to the radiation. It was observed that intravenous injection of bovine SOD to mice could significantly promote the recovery of erythrocytes, reticulocytes and white blood cells from X-irradiation-induced loss (34,35). Moreover, Eastgate et al. verified that interleukin-1 (IL-1) administration was able to provide radioprotection of the irradiated mice from myelotoxicity as well as the explanted murine bone marrow cells from the damaging effects of IR mainly due to the effect of increased Mn-SOD expression triggered by IL-1 treatment (36).

Lung, another organ vulnerable to radiation damage, is subjected to injury regularly during the radiotherapy of patients with lung carcinoma or other thoracic cancers. Depending on the time of onset of lung injury as well as the pathological features exhibited, there are two kinds of IR-induced lung injury: acute radiation pneumonitis manifesting exudative inflammation and interstitial edema, and lung fibrosis characterized by the thickening of alveolar wall due to long-time exposure to radiation (37). However, these two types of lung injury are thought to be independent (38). Provided that lung fibrosis arising from radiation was a result of a cascade of cytokines among which TGF-$\beta_1$ was a critical one (39), Machtay et al. used RT-PCR to detect the level of TGF-$\beta_1$ expression in mouse lung homogenate and demonstrated that the group of mice treated with PEG-AOE (PEG-antioxidant enzyme composed of PEG-SOD and PEG-CAT at the ratio of 1:1) showed a notably diminished level of TGF-$\beta_1$ compared with the group that received irradiation alone. In addition, further analysis indicated a remarkable reduction in the lung hydroxyproline content by PEG-AOE administration (40). The data above supports the concept that PEG-AOE has a great potential to reverse radiation-induced lung fibrosis.

For the head-and-neck cancer patients, local radiotherapy always results in xerostomia and oral mucositis (41), which bring them serious discomfort. By means of flow cytometry, it was found that the sharp reduction of salivary secretion in the mice irradiated at head-and-neck region was caused by the overproduction of ROS resulting from radiation. However, this phenomenon was not observed in the group of mice with PC-SOD (lecithinized SOD) treatment due to the capability of PC-SOD to scavenge $\sigma^\cdot$ during the whole experimental process (42). Subsequently, another study by Nagler et al. showed a similar trend in the Wistar rats administered with Mn-SOD, which showed a dramatic resistance against hyposalivation induced by local head-and-neck radiation (43). Collectively, these findings suggest that SOD can effectively protect the saliva gland from radiation injury and neutralize IR-mediated hyposalivation.

Esophagitis is a major complication developed in the non-small cell lung cancer patients receiving RT. Stickel et al. found that intraesophageal injection of Mn-SOD-PL (plasmid liposome) prior to radiation could significantly prevent the development of vacuole in the esophageal squamous lining cells ($p < 0.001$) and elevate the mouse survival ($p = 0.0099$) suggesting the protective effect provided by Mn-SOD-PL-mediated SOD expression against esophagitis (44).

3.2. Radioprotection at the cellular level

It is well accepted that cell cycle is susceptible to radiation which can induce G1 arrest, S delay, and G2 accumulation (45). Nonetheless, attributable to the long-time evolution, the cell has developed a series of cell cycle checkpoints including G1/S checkpoint, S checkpoint, and G2/M checkpoint. All the checkpoints above collaborate to initiate related repair mechanisms and to guarantee the normal transition from one phase to another depending upon the activity of cell phase-specific cyclins and cyclin-dependent protein kinases (CDKs). A representative profile for the regulation of mammalian cell cycle is described in Figure 5 (46).

During S phase, the cell needs to absorb appropriate amounts of ribonucleosides to maintain nucleic acid synthesis. In the study by Epperly et al., 5-bromo-2-deoxyuridine (BuDR) was given to the pre-irradiated mice by intraperitoneal injection and the intake of BuDR by oral cavity mucosal cells was measured one hour later to estimate the state of S phase. It was found that the groups of mice irradiated or irradiated with WR2721 administration alone showed BuDR intake multiple times higher than the normal control. However, this phenomenon was suppressed significantly in the mice treated with Mn-SOD-PL alone or Mn-SOD-PL combined with WR2721, which verified the hypothesis that SOD could suppress the radiation-induced G1 arrest to exert its protective effects (47).
Figure 5. Regulation of mammalian cell cycle. G1: 1st gap; S: DNA synthesis phase; G2: 2nd gap; M: mitosis and cytokinesis. As shown in this figure, cell cycle consists of mitotic phases and interphases which are further classified into G1, S, and G2. G1 is also called presynthetic phase during which DNA pre-replication complexes accumulate and prepare for DNA synthesis. S is regarded as the core of interphase. In this phase, DNA is replicated adequately to enable the final cell division. G2 is referred to post-synthetic phase, during which DNA replication is completed and some early mitotic events are activated to be ready for the entry to M phase. M comprises metaphase, anaphase, telophase and cytokinesis. After separation of both chromosomes and cytoplasm, two new daughter cells carrying the same genetic material as that of the parent cell are obtained. Obviously, the ordered transition among different phases cannot do without the assistance of corresponding cyclins specific for every phase. It is these cyclins that act as the key regulatory factor in respective checkpoint.

G2/M checkpoint is devoted to prohibit the cell with damaged DNA from entering M phase directly. In particular, compound Cdc2(Chk1)-CyclinB involved in the transition from G2 to M is inactivated either by ATM-Chk2-Cdc25 or ATR-Chk1-Cdc25 pathway \((48)\). Gao et al. showed that radiation induced considerable DNA damage and apoptosis as measured by the large increase of the percentage of cells with sub-G1 content. Yet, this outcome was not observed in the irradiated cells overexpressing Cu, Zn-SOD, where the protein CyclinB1 content decreased by 60-70% compared with the control and the percentage of cells accumulated in G2 phase increased significantly. The results demonstrate that Cu, Zn-SOD is able to provide radioprotection by the way of down-regulating CyclinB1 activity, retarding the G2/M transition and promoting DNA repair \((46)\). Additionally, in accordance with the finding above, Kalen et al. obtained a similar conclusion that Mn-SOD could efficiently initiate the G2/M checkpoint to produce cytoprotection \((49)\).

On the other hand, SOD is envisioned to perform its cellular level radioprotection through suppressing the abnormal proliferation. It is well accepted that stem cells usually multiply slowly to make the exact response to the signal from the external environment and determine whether to proliferate directly or differentiate for the sake of reducing DNA mutation and preventing tumorigenesis \((50)\). Take the mouse esophageal side population (SP) stem cells as an example: samples from the mice irradiated alone showed a significant increase in the content of PCNA (proliferating cell nuclear antigen) compared to the background \((p < 0.0001)\), while in contrast, the esophageal SP stem cells from mice given Mn-SOD-PL kept the PCNA level commensurate to the normal control without any perturbation of multi-direction differentiation capacity, which confirms the theory described above regarding the action of SOD \((51)\).

Clearly, the cellular level radioprotection provided by SOD can be ascribed to its capacity of either regulating the cell cycle checkpoint or inhibiting the abnormal proliferation of the cell. Therefore, it is concluded that SOD is able to act as a promising radioprotector to maintain the steady-state of the cell proliferation and restrict the inclination to carcinogenesis.

3.3. Radioprotection at the molecular level

As described previously, based on the interaction between radiation and \textit{in vivo} biomolecules, excessive ROS are generated, which lead to the various pathological symptoms, implicating the significance of the molecular level radioprotection conducted by SOD.

Through direct interaction with radiation, lipids, the major constituents included in the construction of biomembrane, can be oxidized to produce considerable peroxidized lipids and malonaldehyde (MDA), which pose a threat to the integrity of the membrane structure \((52)\). Early in 1976, a related study was conducted to determine the role of SOD involved in preserving the phospholipid biomembrane \textit{in vitro} when it was exposed to radiation. The data revealed a notable increase in the amount of peroxidized lipids in the irradiated biomembrane as evidenced by the increased absorbance at 232 nm. Adversely, the same phenomenon was not observed in the biomembrane pre-incubated with bovine SOD at an extremely low concentration of 1 ng/mL, demonstrating the striking protection effect of SOD on biomembrane \textit{in vitro} against lipid peroxidization caused by radiation \((53)\). Recently, Epperly et al. used the irradiated mouse model transfected with Mn-SOD-PL to verify the hypothesis that the \textit{in vivo} lipid peroxidization was partly regulated by the cytokines involved in the cell division to substitute the injured cells. By means of RNase protection assay, a detectable up-regulation of cytokines such as IFN\(\gamma\) and TNF\(\gamma\) was observed in the irradiated control mice but not in the mice with Mn-SOD-PL administration. Consistent with that, the latter mice also showed relatively lower level of peroxidized lipids after receiving radiation as compared with the irradiated control value, which confirmed their hypothesis successfully \((54)\).

In addition, it is also worthwhile to mention the SOD-initiated radioprotective effect on mitochondria, the essential organelle in which membrane depolarization is responsible for numerous detrimental reactions such as the release of cytochrome c, the activation of caspase, the uncoupling of oxidative phosphorylation \((55)\). Over the past years, it has been proved that overexpression of Mn-SOD in irradiated cells significantly decreased the...
occurrence of mitochondrial membrane depolarization which was commonly seen in the control group (56). Besides, when Gorman et al. investigated the bystander effect of radiation, a remarkable genomic instability coupled with mitochondrial membrane depolarization was observed in the bystander cells. However, after transfection with Mn-SOD-PL, the biological reactions above were significantly inhibited. These results demonstrate the efficient radioprotection by SOD on mitochondria (57).

Similarly, DNA also seems to be the target biomolecule of radiation. It is well established that after radiation, a broad range of DNA damages are induced such as base damage, single strand breaks (SSBs), and double strand breaks (DSBs) if initial damage on DNA is not repaired properly. Among them, DSBs is regarded as the most deleterious one for its ability to arouse homologous recombination (HR) (58-61). Then, HR can further generate base insertion, deletion, translocation along with high carcinogenicity (59-61). Peroxynitrite, the product of the reaction between nitrogen monoxidum and superoxide, is able to trigger the formation of genomic rearrangement directly, indicating the key role of ROS scavenging in blocking cancer generation (see Figure 6). In the study by Niu et al., fluorescent yellow direct repeat (FYDR) mice were employed to estimate the incidence of HR in vivo measured by the number of fluorescent recombinant cells using flow cytometry. The data showed a significant increase in fluorescent recombinant esophageal cells in the irradiated FYDR mice compared to the control, which indicated that a large degree of HR was stimulated by radiation. Whereas, in the irradiated FYDR mice with Mn-SOD-PL administration, the level of fluorescent recombinant cell counts was almost near the normal value (62). This study provides a powerful evidence for the potential of SOD to prevent HR induced by radiation. Furthermore, using agarose gel electrophoresis, Liu et al. found that the radiation-induced plasmid DNA damages, such as the increased amount of open circular, could be effectively suppressed by the pre-incubation of Hep-SOD (heparin-SOD conjugate) in vitro (63).

4. Conclusion

As the unique enzyme capable of dismutating \( \cdot O_2^- \), SOD is expected to occupy an indispensable position in the treatment of ROS-mediated normal tissue injuries originating from exposure to radiation. Although SOD-related antiradiation research has been continued for nearly forty years and many positive outcomes have been obtained, hardly any drug based on SOD has been approved for radioprotective use in the clinic. Up to now, Orgotein is the only SOD product used as a radioprotector mainly in animals because of its inclination to induce allergic reaction in human (64). Other factors restricting its entry into clinical treatment include its large molecular weight, inability to pass the cell membrane freely, short half-life (65), rapid metabolic rate, narrow time-window of action (66) and so on. To solve these problems, scientific community has resorted to the investigation of SOD-based gene therapy, SOD conjugates, and non-enzymatic SOD mimics.

SOD-based gene therapy mediated by the plasmid liposome or recombinant virus vector showed positive outcomes in numerous research studies as described in this paper. This strategy addresses the poor membrane permeability and low expression of native SOD found in organisms. Additional work needs to be done to explore the availability of SOD-based gene therapy in human subjects. SOD conjugates obtained through the way of chemical modification have advantages of prolonged half-life, improved cell membrane permeability, augmented bioactivities and efficient targeting compared with the native SOD. An outstanding example of these compounds is Hep-SOD which has been verified for its superior radioprotection owing to its long half-life, and enhanced tolerance to high temperature, strong acid/base, and enzymolysis of trypsin (67,68). Besides these advanced forms of SOD, non-enzymatic SOD mimics have also become a favorite form of radioprotective agent to some researchers in recent years. This class of synthetic low molecular-weight compounds containing a metal ion as the active center also shows prolonged half-lives and widened time-windows compared to native SOD. Among them, M40403 (a manganese (II) complex with a bis (cyclo-hexylpyridine) substituted macrocyclic ligand) has been approved by FDA as a radioprotector for cancer patients (69).

From the discussion above, we firmly believe the great potential of SOD-based compounds to be developed as novel radioprotectors in the future. At present, the most important task is to continue studying further their pharmacokinetics, toxicity, optimal route of administration and to strive for their radioprotective application in the clinic as soon as possible.

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References


66. Bafana A, Dutt S, Kumar A, Kumar S, Ahuja PS. The basic and applied aspects of superoxide dismutase. J Mol...


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