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ANTIOXIDANTS REDUCE CONSEQUENCES OF RADIATION EXPOSURE

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Abstract

Antioxidants have been studied for their capacity to reduce the cytotoxic effects of radiation in normal tissues for at least 50 years. Early research identified sulfur-containing antioxidants as those with the most beneficial therapeutic ratio, even though these compounds have substantial toxicity when given in-vivo. Other antioxidant molecules (small molecules and enzymatic) have been studied for their capacity to prevent radiation toxicity both with regard to reduction of radiation-related cytotoxicity and for reduction of indirect radiation effects including long-term oxidative damage. Finally, categories of radiation protectors that are not primarily antioxidants, including those that act through acceleration of cell proliferation (e.g. growth factors), prevention of apoptosis, other cellular signaling effects (e.g. cytokine signal modifiers), or augmentation of DNA repair, all have direct or indirect effects on cellular redox state and levels of endogenous antioxidants. In this review we discuss what is known about the radioprotective properties of antioxidants, and what those properties tell us about the DNA and other cellular targets of radiation.

1. INTRODUCTION

There are many types of radiation damage to normal tissues. The types of damage depend on the cells and organs being irradiated, the dose and dose rate of the exposure, and the time after exposure that is being assayed for a radiation effect. Many of the types of damage seen after irradiation can be ameliorated by antioxidants. This review will outline a number of radiation-related toxicological processes and discuss the role antioxidants might play in affecting these processes in terms of the likely cellular types or compartments in which an antioxidant is employed. The role that different combinations of antioxidants might play in preventing each of these individual effects will also be explored.

2. CELL COMPONENTS

Exposure of a cell to ionizing radiation results in the formation of free radicals within the cell, leading to damage of cellular components. Here we will provide some examples of how antioxidants reduce or prevent the damaging effects of radiation at three sensitive targets in the cell, the nucleus, cellular membranes and mitochondria.

2.1. Nucleus

2.1.1. Immediate Effects by Antioxidants—Radiation-induced DNA damage is the best studied effect of radiation. An oxygen enhancement ratio (OER) of 2.5 to 3 in the yield of DNA damage is observed in the presence of oxygen tensions of 5 mmHg or higher compared to

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maximally hypoxic conditions (<1 mmHg). In accordance with this difference in DNA damage, there is a 3-fold difference in cell reproductive survival measured by clonogenic assays in the presence of oxygen which is generally independent of the phase of the cell cycle.1 Prevention of immediate radiation-induced genotoxicity requires that an antioxidant be present at the time of irradiation.2 To be maximally effective the antioxidant must be present near the DNA and thus must have access to the nucleus. It must be able to either, 1) react with all the oxygen-related free radicals and detoxify them to radicals that are not themselves genotoxic and/or 2) effectively compete with oxygen to repair damage to the DNA chemically through reactions with free radicals on the DNA. Thiol-based compounds are especially good antioxidants because these compounds are capable of both scavenging oxygen radicals and affecting chemical repair of some forms of DNA damage with the subsequent formation of sulfur-based radicals, which are not reactive with DNA.[3] Incorporating one or more positive charges on the thiol-based antioxidant has the effect of changing the proximity of the compound to the DNA.[4, 5] The resulting counter-ion condensation between the positive charge of the thiol and the negatively charged sugar-phosphate backbone of the DNA binds the thiol close to the DNA, facilitating the competition of the thiol with oxygen in reactions with DNA radicals, thereby, reducing DNA damage and increasing cell survival.[5, 6]

Like the synthetic antioxidants (e.g., amifostine, captopril, and NAC), antioxidants derived from natural sources also exhibit dose-modifying effects on DNA damage and cell survival when present at the time of irradiation. This immediate protection is mediated by the scavenging of radicals. For example, there are a number of antioxidants, including caffeine, melatonin, flavonoids, polyphenols, and other phytochemicals (e.g., albana), which are shown to decrease radiation-induced damage in either plasmid or cellular DNA through the scavenging of oxygen radicals and/or peroxides.[7–12]

Uptake and distribution of antioxidants also plays a role in their dose-modifying effects. With amifostine, there is differential uptake of the compound in tumors and normal tissues. In tumors, the uptake is predominantly through passive diffusion, which is slow due to the hydrophilicity of the compound.13 This is in contrast to the dephosphorylated form of the compound, WR-1065, which is less hydrophilic and readily crosses the tumor cell membrane. In fact, Brown et al.[14] suggested that the hydrophilicity of the compound could be useful for designing or selecting better differential radioprotectors. This is supported by their work that showed increases in the therapeutic gain (ratio of the dose reduction factors for the hematopoietic system and tumor) for 6 hydrophilic thiols, ranging from 1.59–2.29, in comparison to values ranging from 0.88–1.59 for 5 lipophilic thiols. In normal tissues, there is active uptake of amifostine through the polyamine transport system.[15] This active transport results in a preferential uptake of the antioxidant into normal tissues as compared to tumors. 13 Another factor that aids in the differential uptake of amifostine into normal tissues is the higher concentration of alkaline phophatases in these tissues as compared to tumors, converting amifostine to WR-1065, which is then readily taken up by normal tissues.[16] However, the levels and distributions of the aminothiol can vary between and within tissues, leading to variations in the dose-modifying effects of this compound in irradiated tissues.[17] These variations can be attributed to differing degrees of negative feedback on the polyamine transport as a consequence of variable polyamine concentrations with tissues, thereby reducing or inhibiting the uptake of amifostine, and to differences in oxygen concentration within tissues.[17, 18] Also, there is a limit to which cells can take up and accumulate thiol-based antioxidants before the compounds become cytotoxic. For example, in several tumor lines, concentrations of WR-1065 greater than 25–30 nmole/10^6 cells induced significant cytotoxicity in unirradiated cells.[19] In-vivo these agents cause peripheral neuropathies and hypotension.[20] Clinically speaking, although the impact of amifostine and similar thiol-based antioxidants can theoretically be as great as a factor of 3 in dose modification, no antioxidant reaches this potential and few if any alter the in-vivo tolerance to irradiation by more than a factor of 1.3 when administered at

concentrations below those that elicit hemodynamic- or cyto-toxicities in unirradiated controls.[2, 11, 14]

2.1.2. Chronic Radioprotective Effects by Antioxidants—For many antioxidants, the impact that these compounds have on radiation-induced damage and the biological consequences of the damage within cells and tissues can extend to their direct or indirect interaction with other cellular targets. For example, melatonin has been shown to augment the activity of glutathione peroxidase in addition to stimulating the activity of glutathione reductase and increasing the synthesis of glutathione (GSH); all of which are important in reducing levels of oxygen radicals and peroxides in cells.[8] In addition, WR-1065 has been shown to induce a delayed radioprotective effect through the activation of the redox-sensitive nuclear transcription factor, NFκB, and subsequent expression of the antioxidant enzyme, manganese superoxide dismutase (MnSOD). Other thiols, such as captopril ([S]-1-[3-mercapto-2-methyl-1-oxo-propyl]-L-proline), mesna (sodium-2-mercapto-ethane-sulfonate), and NAC demonstrated similar effects to those observed for WR-1065 with respect to increasing cell survival.21 The involvement of NFκB in the induced expression of MnSOD was shown in experiments where pretreatment of human microvascular endothelial cells with Helenalin, an inhibitor of NFκB, prior to treatment with WR-1065, prevented the thiol-induced activation of NFκB and subsequent elevation in MnSOD levels.22

2.2. Membranes

The irradiation of lipid membranes is known to cause an increase in the formation of lipid radicals and peroxides that can result in damage or release of membrane proteins,23 in addition to the liberation of products formed from the peroxidation of lipids that subsequently react with and alter cellular components.[24] Various natural and synthetic antioxidants are known to decrease the peroxidation of membrane lipids. For example, pretreatment of mice with diethyldithiocarbamate (DDTC) prior to whole body dose resulted in a two-fold decrease in lipid peroxidation in isolated liver microsomes, as compared with irradiated control mice.[25] In a recent study, disulfiram, a drug that is used in treating alcohol abuse, inhibited lipid peroxidation in microsomes and reduced lipid peroxides in whole-body-irradiated mice by 65% compared with unirradiated controls.26 The flavonoid, luteolin, reduced lipid peroxidation by almost 4-fold 48 h post-radiation in comparison with radiation controls in mouse bone marrow cells when mice were pretreated with the flavonoid for 2 hours before irradiation.27 In a variation on structural design, the antioxidant, tocopherol-monoglucoside (TMG), is a water soluble derivative of the lipophilic parent compound, α-tocopherol. This structural modification allows TMG to scavenge oxygen radicals, such as peroxides, superoxides, and hydroxyl radicals, in both water and lipid phase.[28] Additionally, the compound also increases the levels of glutathione peroxidase (GPx) in treated cells.

It is known that the active form of vitamin E in membranes is maintained through reactions with ascorbic acid.[29] Without this regenerative mechanism, the active form of vitamin E would be rapidly exhausted in membranes. Therefore, the optimal properties of antioxidants designed to protect cellular membranes are, 1) an ability to scavenge lipid radicals and react with lipid peroxides in membranes at concentrations that will not alter the structure or properties of the membrane, and 2) provide for the maximum interaction of the compound with cytosolic-reducing agents (ascorbic acid or GSH) to regenerate the antioxidant. This strategy also necessitates the use of multiple antioxidant therapy, for example the combination of vitamin E and vitamin C, which provide both an effective protection of membranes and increased radioresistance in cells.[30, 31]

2.3. Mitochondria

The mitochondrion is the cellular organelle responsible for energy generation in the cell through the production of ATP.[23] Mitochondria, like the nucleus, contain DNA and this DNA is required for proper mitochondrial function and for mitochondrial replication. Replication of mitochondria occurs naturally in non-dividing cells. The impact of radiation on mitochondrial DNA likely does not result in changes in reproductive integrity and thus clonogenic survival, which is perhaps why it is rarely studied. Long-term cellular health however clearly requires cells to have a continuous supply of mitochondria for normal functioning. Mitochondrial DNA has the advantage over nuclear DNA in that it is present in many replicates (instead of just duplicate), can increase the number of DNA copies in response to radiation exposure, and the mitochondria is naturally high in antioxidant capacity.[23, 32] In comparison, however, to the nuclear DNA, nucleotide excision repair of mitochondrial DNA is lacking[33] and repair is not efficient for specific classes of DNA damage, such as bulky lesions, and some types of alkaline-labile sites and single strand breaks[34, 35] (Table 1). Also, although not yet shown, the fidelity of the repair of radiation-induced damage at clustered sites in mitochondrial DNA is likely to be adversely impacted in a similar fashion to clustered lesions in nuclear DNA.[36–38]

A consequence of mitochondrial energy generation (ATP synthesis) is the evolution of heat (entropy) and the production of ROS. Mitochondria have an inherent antioxidant capacity (e.g., the interaction between GSH, GPx, glutathione reductase [GRd], and MnSOD) to counteract much of the ROS. Stressors, such as ionizing radiation, damage the mitochondrial function, likely leading to additional ROS production which can overwhelm the antioxidant capacity of the organelle. The unscavenged ROS may produce further damage to mitochondrial components, including mitochondrial DNA, leading to additional mitochondrial damage and ROS formation. Providing additional antioxidant capacity to mitochondria, either through uptake of additional antioxidant agents like vitamin E, or through increasing the levels of GSH and mitochondrial antioxidant enzymes, can provide the necessary antioxidant buffer to scavenge additional ROS produced as a consequence of exposure to radiation and thereby minimize damage to mitochondria and its DNA.

The antioxidant, melatonin, is particularly effective at protecting mitochondria by increasing the efficiency of oxidative phosphorylation, thereby reducing the leakage of electrons from the electron transport chain.[8] The reduction of electron leakage decreases the formation of ROS from these electrons and, therefore, damage to mitochondria. Additionally, melatonin induces the levels of antioxidant enzymes, such as GPx and, more importantly, also increases GSH levels within the cell. This latter effect can reduce the levels of radiation-induced oxygen radicals and peroxides in mitochondria through the increased availability of glutathione for GSH/GSSG cycling that is used in regenerating GPx.[8] A similar redox cycle has been proposed for WR-1065 to explain the regeneration of the thiol after it is converted to the disulfide form following reactions with lipid peroxides in the mitochondrial membrane. In this case, the disulfide form of WR-1065 is recycled to the reduced state through the oxidation of GSH to the disulfide, GSSG. The GSSG is then reduced to GSH by GRd.[39]

Protection of the mitochondria can be further facilitated through the development of antioxidants that are designed either for increased uptake into mitochondria, or to increase the activity of antioxidant enzymes. Linking the positively-charged functional group, alkyl-triphenyl-phosphonium ion, to vitamin E or ubiquinone (CoQ) increased the uptake of these antioxidants into the mitochondrial matrix.[23] However, studies to determine how this structural modification might influence the radioprotection of mitochondria have not yet been performed. Increasing the levels of antioxidant enzyme activity in mitochondria has been shown to occur with the administration of SOD mimetics or through over-expression of MnSOD by transfection of a transgene.40 Another approach to increasing mitochondrial content of an antioxidant is to take advantage of the low pH outside of the inner membrane of the

mitochrondrion whereby functional groups on the compound undergo protonation to change the charge on the molecule and, thereby, prevent the elimination of the compound from the mitochondrion.

3. APOPTOSIS

Reactive oxygen species play a pivotal role in the initiation of apoptosis, and antioxidants have been shown to have the ability to inhibit apoptosis. This inhibitory effect appears to occur through a number of pathways but has as a common result, the preservation of mitochondrial membrane integrity and the electrochemical gradient (ΔP) across the membrane. It is suggested that scavenging of ROS by antioxidants interferes with the initiation of apoptosis by depleting ROS levels in cells and maintaining membrane integrity.[41] Also, antioxidants like the water soluble vitamin E derivative, trolox, reduce both lipid membrane peroxidation and the post-irradiation uptake of calcium, thereby inhibiting apoptosis.42 Reduction in lipid peroxides and decreased apoptotic indices were also found in irradiated mice treated either with SOD or, more effectively, with the combination of catalase and trolox.[43]

Antioxidants also have the ability to affect apoptosis through inhibiting proteins in the apoptotic cascade or modification of gene expression. By inhibiting the cleavage of caspase-3 and its substrate, poly(ADP-ribose) polymerase, the green tea polyphenol, (−)-epigallocatechin, was found to prevent apoptosis in HaCaT human keratinocytes when pretreated 16 h before irradiation.44 Pretreatment of human microvascular epithelial cells with WR-1065 thirty min prior to irradiation was found to down-regulate a host of genes associated with apoptosis.45 A greater than two-fold reduction in the expression of 12 genes was observed, including the caspases 2, 4, and 9; the cyclins A, G1, G2, and D3; the DNA check damage/checkpoint proteins, ATM, DNA-PK, and RAD 23B; TNF receptor 1; and FAST kinase. Also, treatment with WR-1065 significantly reduced the accumulation of cells in an apoptotic sub-G1 population 1–2 days following irradiation to levels that were not statistically distinguishable ($p<0.05$) from non-irradiated cells. What is not clear from these results are the relative contributions of the radical scavenging properties and the modifying effect that amifostine has on gene expression to the observed reduction in apoptosis.

4. TISSUE-BASED RADIATION EFFECTS

Late fibrovascular effects of radiation include vascular dysfunction. Ischemia itself injures tissue, and ischemia followed by reperfusion is thought to further the injury through the production of a rapid burst of ROS. This rapid production of ROS can overwhelm the antioxidant capacity of the tissue and lead to free radical-mediated damage to all intracellular and tissue compartments.[46] This can be especially problematic under conditions of chronic ischemia where recurrent injury to tissues is expected to occur. The involvement of chronic oxidative stress, and concomitant production of ROS, has been suggested as a driving force in the amplification of late radiation effects such as fibrosis, chronic inflammation, and oncogenesis in irradiated tissues.[47, 48] Consequently, increasing the antioxidant capacity of the involved tissues is expected to reduce tissue injury due to ROS-mediated late radiation effects. However, the use of antioxidants to reduce the effects of chronic ROS-mediated injury in post-radiation treatments has not been sufficiently studied and therefore the mechanisms by which these effects are mediated are ill-defined. Below, we provide some examples of what is known from studies of post-radiation administration of protein and non-protein based antioxidants on late radiation effects in tissues.

4.1. Inflammatory Mediators

Under conditions of chronic oxidative stress, as would be encountered in irradiated tissues, the generation of ROS triggers an inflammatory response through the activation of cytokines and

other inflammatory mediators.[49] The administration of antioxidants in animal and human studies has the effect of reducing the inflammatory response through the modulation of cytokine levels in tissues. For example, epicatechin, trans-resveratrol, and theaflavin were shown to reduce the production of interleukin 1-β (IL-1β), tumor necrosis factor-α (TNF-α) and interleukin-8 (IL-8), respectively, after stimulation of an inflammatory response.[50–52] Conversely, catalase and NAC have the ability to upregulate interleukin-10 expression which, in turn, decreased the synthesis of other cytokines.53 Evidence also exists suggesting the role of antioxidants in reducing ROS and inflammation in late radiation-induced tissue injury. Protection of late radiation-induced lung injury was observed in mice over-expressing a transgene for human MnSOD.54 In another approach, increasing SOD levels through the treatment of rats 15 min prior to irradiation and 5 days post-radiation with a SOD mimetic resulted in both a reduction of collagen deposition in lung tissue and a 1.2–2.1 fold reduction in transforming growth factor-β (TGF-β) 10 to 14 weeks post-radiation.55 In a pig model, treatment with Cu/Zn SOD or MnSOD 3 times a week for 3 weeks post-radiation resulted in a softening and shrinkage of fibrotic tissue in a cutaneous radiation field that had received a single 160 Gy dose 6 months prior to treatment with antioxidant.56 Also in a pig model, the co-administration of pentoxifylline [PTX] and α-tocopherol for 26 weeks, after a 26 week post-radiation development of a subcutaneous fibrosis, resulted in a decrease of TGFβ-1 levels in residual scar tissue (26 weeks post-irradiation) as compared with groups receiving pentoxifylline + irradiation or irradiation alone.57 In human studies, the combined treatment with PTX and α-tocopherol post-irradiation appeared more effective at reducing radiation-induced fibrotic tissue in skin than when PTX or α-tocopherol were given alone.58 PTX is expected to reduce reperfusion injury and was shown in clinical studies to lower the levels of circulating bFGF (basic fibroblast growth factor) and TNF-α toward non-irradiated control levels.59 In cultured fibroblasts harvested from normal or radiation-induced fibrotic human skin, treatment of the fibroblasts harvested from fibrotic tissue with liposomal Cu/Zn SOD resulted in increased expression of MnSOD and decreased levels of TGF-β1, but no significant changes in the levels of these parameters were observed in treated fibroblasts harvested from normal skin.60 Thus, it can be seen that strategies for increasing SOD levels in post-irradiated tissues result in the protection of the tissues from late radiation-induced effects through an apparent reduction in the ROS-mediated damage and the decreased expression of at least one cytokine, TGF-β. Finally, the best treatment for many chronic radiation-induced soft tissue injuries is hyperbaric oxygen. The mechanisms of action of hyperbaric dives is not certain but includes natural induction of SOD and other antioxidants, and is associated with inhibition of inflammation and improved tissue vascularization.

Interestingly, cytokines can be radioprotective through induction of SOD levels. For example, pretreatment of mice with interleukin-1 twenty hours before receiving a lethal dose (8 Gy) of radiation was found to enhance the radioresistance of bone marrow cells.[61] It has been suggested that one reason for the radioprotective effect of the cytokine is the increased expression of MnSOD in bone marrow cells that resulted from the cytokine pretreatment. Similarly, tumor necrosis factor-α (TNF-α) has been shown to induce MnSOD in hematopoietic stem cells with a concomitant radioprotective effect.62

At the level of tissue vasculature, irradiation of endothelial cells results in the increased expression of intercellular cell adhesion molecule-1 (ICAM-1). Expression of ICAM-1 contributes to an inflammatory response that mediates the adhesion and movement of leucocytes to and through the vascular endothelium. ROS are assumed to be involved in the increased expression of ICAM-1, presumably through the AP-1 signaling pathway.[63, 64] Therefore, it is expected that a reduction in ROS by reactions with antioxidants, for example, should reduce radiation-induced expression of ICAM-1. However, pretreatment of human umbilical vein endothelial cells with the thiols NAC and pyrrolidine dithiocarbamate (PDTC) followed by a 7 Gy dose found that neither thiol reduced the radiation-induced expression of

ICAM-1 at a post-radiation time of 48 h. Instead, thiol treatment increased expression by up to 2-fold over cells irradiated alone.[64] In fact, just pretreatment of cells without exposure to radiation resulted in increased expression of ICAM-1 that was 1–2 fold higher than cells irradiated alone within 48 h post-treatment. This latter result suggests that these thiols can be considered, under certain conditions, as pro-inflammatory agents. However, it is not known whether this extends to other antioxidants, especially thiol-based antioxidants including amifostine.

5. CONCLUSION

The radioprotective effects of antioxidants and the mechanisms by which these effects are mediated depend on the properties of both the antioxidant and the compartment (e.g., cellular or tissue targets) where the radioprotective effects are measured. There is a large volume of data on the radioprotective effects of antioxidants at the cellular level, especially at the level of nuclear DNA, where the radical scavenging by the antioxidant protects this and other sensitive cellular targets. Many antioxidants have been shown to also protect the cell by acting to increase cellular antioxidant capacity through their ability to elevate the levels of natural antioxidants (e.g., GSH) and antioxidant enzymes (e.g., GPx, GRd and MnSOD). Interestingly, exposure to chronic, low-dose-rate ionizing radiation can also lead to the induction of antioxidant enzymes. For example, exposure of mice to a 0.5 Gy at a dose rate of 1.2 mGy/h for 23 days increased the gene expression of catalase and MnSOD by a factor of 2.5.[65] However, at higher doses of 1.0 and 1.3 Gy accumulated at the same dose rate, gene expression either increased by only approximately 1.4 or was not significantly different from unirradiated controls, respectively. Therefore, care is needed in low-dose-rate studies in discerning to what extent various agents, like antioxidants, have on modifying the levels of antioxidant enzymes. Even so, based on what is currently known, specific chemical and/or physical properties of antioxidants can be designed to take advantage of biochemical properties or a specific cellular target. In addition, there are a number of *in vitro* and *in vivo* studies that show increased radioresistance in normal tissues when antioxidants are given in combination compared with antioxidants given individually.[30, 31] There are a number of hypotheses that have been suggested to explain the enhanced radioprotective effect of combined antioxidant treatments related to the regulation and response to ROS, including the regeneration of vitamin E and other antioxidants by vitamin C, induction of cellular antioxidant systems, and interaction with inflammatory mediators.

The impact of radiation on the mitochondrial DNA and thus long-term reproductive health of the mitochondria, reproduction of the cell, and on cellular redox and energy state has not been studied in detail. The long-term consequences of radiation may be very dependent on this mechanism of radiation toxicity and may be greatly alleviated by properly designed antioxidants.

Regarding what is known about the radioprotective effects of antioxidants on late radiation effects in tissues, especially for non-protein antioxidants, there is only a limited understanding of these effects at a mechanistic level. Therefore, additional studies are needed of current and new antioxidant compounds to look at these and other radioprotective effects in antioxidants in irradiated cells and tissues to support rational approaches in the design of antioxidants as radioprotectors.

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Table 1

Characteristic differences between DNA in the nucleus and mitochondria.

Parameter	Nucleus	Mitochondria	Advantage
Target Size	Under 30,000 genes	37 genes	*Mitochondria*
DNA/Gene Ratio	High	Low	*Nucleus*
Oxygen Tension	Normoxic	Potentially Hypoxic	*Mitochondria*
Repair Capacity	>99.9% SSB and 98% DSB repaired	Low repair	*Nucleus*
Gene Copies	One duplicate copy per cell	High number of replicates per cell	*Mitochondria*
Radical Levels	Low radical environment	High radical environment	*Nucleus*
Antioxidant Level	Moderate antioxidant environment	High antioxidant environment	*Mitochondria*

The DNA in the nucleus and mitochondria have different oxidative environments and mechanisms for repair of oxidative damage. This leads to different temporal and functional DNA damage responses following irradiation. Mitochondrial DNA has an advantage in the case of radiation due to its small mass, its large number of replicates, and its naturally high antioxidant capacity. Nuclear DNA enjoys a powerful set of enzymatically mediated DNA repair pathways; mitochondrial DNA instead relies more on the presence of antioxidants. Due to the lower degree of repair capability and fidelity of direct damage in mitochondrial DNA, continuous low dose rate radiation and very late manifestation of radiation damage might be a relative disadvantage to mitochondrial DNA compared with nuclear DNA. After therapeutic radiation or other high dose or high dose rate exposure, early cytotoxicity is probably not due to DNA damage of the mitochondria. There are no comprehensive studies of late radiation toxicity to the mitochondria, so the degree to which this organelle impacts certain radiation scenarios months or years after exposure remains unknown.