The use of \textit{N-t}-butyl hydroxylamine for radioprotection in cultured cells and mice

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Exposure of cells to ionizing radiation leads to formation of reactive oxygen species (ROS) that are associated with radiation-induced cytotoxicity. Therefore, compounds that scavenge ROS may confer radioprotective effects. Recently, it has been shown that the decomposition product of the spin-trapping agent \textit{\alpha}-phenyl-N-t-butylnitrone (PBN), \textit{N-t}-butyl hydroxylamine (NtBHA), mimics PBN and is much more potent in delaying ROS-associated senescence. We investigated the protective role of NtBHA against ionizing radiation in U937 cells and mice. Viability and cellular oxidative damage reflected by lipid peroxidation, oxidative DNA damage and protein oxidation were significantly lower in the cells treated with NtBHA when the cells were exposed to ionizing radiation. The modulation of cellular redox status was more pronounced in control cells compared with NtBHA-treated cells. The ionizing radiation-induced mitochondrial damage reflected by the altered mitochondrial permeability transition, the increase in the accumulation of ROS and the reduction of ATP production was significantly higher in control cells compared with NtBHA-treated cells. NtBHA administration before irradiation at 5 mg/kg daily for 2 weeks provided substantial protection against killing and oxidative damage to mice exposed to whole-body irradiation. These data indicate that NtBHA may have great application potential as a new class of \textit{in vivo}, non-sulfur containing radiation protector.

Introduction

Ionizing radiation has been shown to generate reactive oxygen species (ROS) in a variety of cells (1). When water, the most abundant intracellular material, is exposed to ionizing radiation, decomposition reactions occur and a variety of ROS, including superoxide, hydroxyl radicals, singlet oxygen and hydrogen peroxide, are generated (2). The secondary radicals formed by the interaction of hydroxyl radicals with organic molecules may also be of importance (1,2). These ROS have the potential to damage critical cellular components such as DNA, proteins and lipids and eventually result in physical and chemical damage to tissues that may lead to cell death or neoplastic transformation (3).

Since ROS appear to be mediators of the cellular damage induced by ionizing radiation, compounds that regulate the fate of such species may be of great importance in the protection of cells against radiation-induced damage. The search for agents that protect against ionizing radiation is important to those at risk by virtue of environmental exposure or health-related treatment and scientific study of the mechanism of radiation injury and cytotoxicity (4). Although no radioprotective drug available today has all the requisite qualities to be an ideal radioprotector, sulphhydril radioprotectors such as cysteine, \textit{N}-acetylcysteine, cysteamine, cystamine, aminoethylisothiourea dihydrobromide and mercaptopoetyl guanidine are the best radioprotectors known today. However, their use encounters two great difficulties: their toxicity and the short period during which they are active (5).

We recently reported that \textit{\alpha}-phenyl-N-t-butylnitrone (PBN) protects damage caused by ionizing radiation \textit{in vitro} and \textit{in vivo} (6). PBN is a spin-trapping agent that traps free radicals and protects against damage in different models such as inflammation, ischemia reperfusion and aging (7). The Ames group recently reported that the decomposition product of PBN, \textit{N-t}-butyl hydroxylamine (NtBHA), mimics PBN and is much more effective delaying senescence of IMR90 cells (8). In this study, the role of NtBHA in the cellular and \textit{in vivo} defence against ionizing radiation was investigated using U937 cells and mice. To determine differences in sensitivity to the toxic effects of ionizing radiation between U937 cells treated and untreated with NtBHA, viability, cellular redox status, oxidative damage to cells and mitochondrial damage were examined upon their exposure to ionizing radiation. NtBHA was also administered to mice, and \textit{in vivo} radioprotective effect was assessed. Our results demonstrate that NtBHA may play an important role in regulating the damage induced by ionizing radiation and NtBHA may have great application potential as a new class of \textit{in vivo}, non-sulfur containing radiation protector.

Materials and methods

Abbreviations: CMAC, \textit{t}-butoxycarbonyl-Leu-Met-7-amino-4-chloromethylcoumarin; DCFH-DA, \textit{t},\textit{t}-dichloro-fluoroscin diacetate; DHR, dihydro-rhodamine; DNP, dinitrophenyl; DPPP, diphenyl-1-pyrenylphosphine; FACS, fluorescence-activated cell sorter; FITC, fluorescein isothiocyanate; HNE, \textit{4}-hydroxynonenal (HNE)-Michael adduct antibody and anti-human \textit{4}-hydroxynonenal (HNE)-Michael adduct antibody and anti-human dinitrophenyl (DNP) antibody were obtained from Sigma Chemical (St Louis, MO). Anti-human \textit{4}-hydroxynonenal (HNE)-Michael adduct antibody and anti-human dinitrophenyl (DNP) antibody were obtained from Calbiochem (La Jolla, CA). \textit{2},\textit{t}-Dichloro-fluoroscin diacetate (DCFH-DA), \textit{t}-butoxycarbonyl-Leu-Met-7-amino-4-chloromethylcoumarin (CMAC), diphenyl-1-pyrenylphosphine (DPPP), dihydrorhodamine (DHR) 123, and LIVE/DEAD viability/cytotoxicity kit were purchased from Molecular Probes (Eugene, OR).

Materials

NtBHA, 2,4-dinitophenylhydrazine (DNPH), 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB), pyrogallol, xylene orange, anti-rabbit IgG tetramethylrhodamine isothiocyanate (TRITC) conjugated secondary antibody and anti-rabbit IgG fluorescein isothiocyanate (FITC) conjugated secondary antibody were obtained from Sigma Chemical (St Louis, MO). Anti-human \textit{4}-hydroxynonenal (HNE)-Michael adduct antibody and anti-human dinitrophenyl (DNP) antibody were obtained from Calbiochem (La Jolla, CA). \textit{2},\textit{t}-Dichloro-fluoroscin diacetate (DCFH-DA), \textit{t}-butoxycarbonyl-Leu-Met-7-amino-4-chloromethylcoumarin (CMAC), diphenyl-1-pyrenylphosphine (DPPP), dihydrorhodamine (DHR) 123, and LIVE/DEAD viability/cytotoxicity kit were purchased from Molecular Probes (Eugene, OR).
Cell culture and cytotoxicity assay

Human promonocytic U937 cells (American Type Culture Collection, Rockville, MD) were grown in RPMI 1640 culture medium supplemented with 10% (v/v) FBS, penicillin (50 U/ml), and 50 µg/ml streptomycin at 37°C in a 5% CO2/95% air humidified incubator. For cytotoxicity assay, U937 cells were first grown on a 96 well plate at a density of 2 x 10^3 cells/well. After culture for GSH fluorescence optimization, various concentrations of NtBHA were applied to the cells and cells were incubated for additional 2 h at 37°C. After incubation, cells were irradiated at room temperature with 137Cs source at a dose rate of 1 Gy/min. After 48 h of irradiation to cells, 20 µl of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS)/phenazine methosulfate solution was added and incubated for another 4 h at 37°C. The absorbance was read in an ELISA plate reader at 490 nm with a 620 nm reference. Cell viability was also observed using a fluorescent LIVE/DEAD viability assay following the manufacturer’s protocol. Cells were double-stained with calcine-AM and ethidium homodimer-1 and observed with a fluorescence microscope.

Mice

Adult male ICR mice were obtained from Hanchang Science (Taegu, Korea). The animals were housed five per cage in climate-controlled, circadian rhythm-adjusted room and allowed food and water ad libitum. The animals were, on average, 50-70 days old and weighed between 20 and 30 g at the time of irradiation. Experiments on mice were conducted according to the principles outlined in ref. 9.

Toxicology of NtBHA and whole-body irradiation

To determine its maximal tolerated dose, solutions of NtBHA were freshly prepared in saline. Two groups of 15 mice each received either NtBHA or saline. NtBHA was administered prior to irradiation at a dose of 5 mg/kg in volumes equivalent to 1% of each animal’s weight once daily for 2 weeks. Control mice were given saline and all injections were administered intraperitoneally. Survival was assessed up to 30 days after injection without irradiation. To determine survival after whole-body irradiation, the same protocol for the NtBHA administration was applied and then the groups of 15 mice were transferred to round Plexiglas containers (30.5 cm in diameter and 10.5 cm in height) with holes for ventilation. After irradiation with a 137Cs source at a dose rate of 1 Gy/min, the mice were returned to climate-controlled cages for observation. Survival was assessed 30 days after irradiation.

Preparation of tissue extracts

The tissue portions of mice were homogenized with a solution of 0.3 M sucrose, 25 mM imidazole, 1 mM EDTA, pH 7.2, containing 8.5 µM leupeptin and 100 µg/ml aprotinin, using a homogenizer at maximum speed for 15 s. Each sample was then centrifuged at 4000 g for 15 min at 4°C and the resulting supernatant were stored at -20°C and used for the assays. The protein concentration in the supernatant was measured by the Bradford method using the Bio-Rad Protein Assay kit.

Cellular redox status

NADPH was measured using the enzymatic cycling method as described by Zerez et al. (10) and expressed as the ratio of NADPH to the total NADP pool. The concentration of total glutathione was determined by the rate of formation of 5-thio-2-nitrobenzoic acid at 412 nm (ε = 1.56 x 10³ M⁻¹ cm⁻¹) as described by Akerboom and Sies (11), and GSSG was measured by the DTNB-GSSG reductase recycling assay after treating GSH with 2-vinylpyridine (12). The intracellular GSH level was also determined by using a GSH-sensitive fluorescence dye CMAC. U937 cells (1 x 10⁶ cells/ml) were incubated with 5 µM CMAC cell tracker for 30 min. For in vivo visualization of GSH levels in tissues, tissues were perfused with 5 mM DHR 123 and cells were double-stained with 100 nM MitoTracker Red. Cells were double-stained with 100 nM MitoTracker Red, which is a morphological marker of mitochondria. The fluorescence images at 520 nm were simultaneously obtained with a laser confocal scanning microscope. To evaluate the levels of mitochondrial ROS U937 cells in PBS were incubated for 20 min at 37°C with 5 µM DHR 123 and cells were double-stained with 100 nM MitoTracker Red. Cells were washed, resuspended in complete growth media, and ionizing radiation was applied to the cells. The cells were then incubated for an additional 40 min. DHR 123 and MitoTracker Red fluorescence were visualized by a fluorescence microscope. Intracellular ATP levels were determined by using luciferin-luciferase as described (20). Light emission was quantified in a Turner Designs TD 20/20 luminometer (Stratec Biomedical Systems, Germany).

Quantification of relative fluorescence

The averages of fluorescence intensity from fluorescence images were calculated as described (21).

Results

Cell killing

When cultured U937 cells were exposed to γ-irradiation, a dose-dependent decrease in cell viability was observed. However, the cells pre-treated with 0.1 mM NtBHA for 2 h were significantly more resistant than control cells untreated with NtBHA (Figure 1). The protective effect of NtBHA was concentration-dependent and NtBHA itself up to 1 mM was without effect on the viability of U937 cells (data not shown). The protective effect of NtBHA against ionizing radiation was also confirmed by dual staining with calcine-AM and ethidium homodimer-1. Ionizing radiation caused lethal injury to U937 cells and their nuclei were mostly stained with ethidium homodimer-1 to exhibit a red fluorescence. NtBHA (0.1 mM) pre-treatment for 2 h decreased the proportion of red fluorescent nuclei of dying cells in γ-irradiated cultures (data not shown).

Cellular redox status

To investigate whether the difference in viability of U937 cells upon exposure to ionizing radiation is associated with ROS formation, the levels of intracellular peroxides in the U937 cells were evaluated by FACS analyses with the oxidant-sensitive probe DCFH-DA. As shown in Figure 2(A), an increase in DCF fluorescence was observed in U937 cells when they were exposed to 20 Gy of γ-irradiation. The
increase in fluorescence was significantly reduced in cells pre-treated with 0.1 mM NtBHA for 2 h. The pre-treatment of NtBHA also resulted in a significantly lower intracellular level of H$_2$O$_2$ as compared with that of untreated control with the exposure of 20 Gy of irradiation (Figure 2B). These data strengthen the conclusion that NtBHA provided protection from the cytotoxic actions of γ-irradiation by decreasing the steady-state level of intracellular oxidants. GSH is one of the most abundant intracellular antioxidants and determination of changes in its concentration provides an alternative method of monitoring oxidative stress within cells. Cellular GSH levels determined with the GSH-sensitive fluorescent dye CMAC in U937 cells treated with 20 Gy of γ-irradiation were significantly decreased (Figure 3A). However, the depletion of GSH was significantly protected by the pre-treatment of 0.1 mM NtBHA for 2 h. One important parameter of GSH metabolism is the ratio of GSSG/total GSH (GSHt), which may reflect the efficiency of GSH turnover. When the cells were exposed to 20 Gy of γ-irradiation, the ratio of cellular [GSSG]/[GSHt] was significantly higher in control cells than in NtBHA-treated cells (Figure 3B). These data indicate that GSSG in control cells was not reduced as efficiently as in NtBHA-treated cells.

**Fig. 1.** Resistance of control (filled circle) and NtBHA-treated (filled triangle) U937 cells against ionizing radiation. Cells (2 × 10$^5$/well) were grown in 96-well plates and then exposed to different doses of γ-irradiation prior to measurement of cell viability by MTS assay. Cell viability is expressed as a percentage of absorbance seen in the untreated control cells. Each value represents the mean ± SD from five independent experiments.

**Fig. 2.** Effects of NtBHA on peroxide level and ROS generation. (A) Effect of NtBHA on γ-irradiation-induced ROS production. Cells were exposed to γ-irradiation and subjected to FACS analyses. (B) Production of hydrogen peroxide in U937 cells was determined by the method described under Materials and methods. Each value represents the mean ± SD from five independent experiments. *$P < 0.01$ compared with irradiated control cells.

**Fig. 3.** Effect of NtBHA on the cellular redox status of U937 cells exposed to ionizing radiation. (A) Effect of NtBHA on GSH levels. Fluorescence image of CMAC-loaded cells was obtained under microscopy. The averages of fluorescence intensity were calculated as described (21). Each value represents the mean ± SD from three independent experiments. (B) Ratios of GSSG versus total GSH pool. Each value represents the mean ± SD from three independent experiments. *$P < 0.05$ and **$P < 0.01$ compared with irradiated control cells.
NADPH, required for GSH generation by glutathione reductase, is an essential factor for the cellular defence against oxidative damage. The ratio for \([\text{NADPH}]/[\text{NADP}^+]\) was significantly decreased in cells treated with 20 Gy of \(\gamma\)-irradiation, however, the decrease in this ratio was much less pronounced in NtBHA-treated cells (Figure 3C).

**Cellular oxidative damage**

As indicative markers of oxidative damage to cells, the occurrence of oxidative DNA damage, protein oxidation and lipid peroxidation were evaluated. The increase in lipid peroxidation is proportional to the relative degree of oxidative stress imposed on the cells. It is well established that oxidative stress in various cells usually leads to accumulation of potent, cytotoxic lipid peroxides such as MDA and HNE (22). Exposure of 20 Gy of \(\gamma\)-irradiation increased the level of MDA 5-fold in control cells, however, the increase in MDA content of NtBHA-treated cells was significantly lower than that of untreated cells (Figure 4A). To determine whether NtBHA pre-treatment decreased the sensitivity to protein damage, we performed carbonyl content measurements for protein oxidation after cellular exposure to ionizing radiation. Control cells elicited an \(-2\)-fold increase of carbonyl groups, as compared with unirradiated cells when 20 Gy of \(\gamma\)-irradiation was exposed. Although the carbonyl content of NtBHA-treated cells also increased with irradiation, the increase was significantly lower than that of control cells (Figure 4B). Protein oxidation was also visualized by immunocytochemical method using anti-DNP antibody. As shown in Figure 4(C), the fluorescent intensity, which reflects the endogenous levels of carbonyl groups in proteins was significantly increased when U937 cells were exposed to 20 Gy of \(\gamma\)-irradiation and the increase of protein oxidation was markedly reduced in NtBHA-treated cells. Lipid peroxidation was visualized by immunocytochemical method using anti-HNE antibody. The fluorescent intensity, which reflects the endogenous levels of HNE adducts in proteins was significantly increased when U937 cells were exposed to 20 Gy of \(\gamma\)-irradiation and the

![Fig. 4. Cell damage of U937 cells. (A) Lipid peroxidation of U937 cells after exposure to ionizing radiation. The level of MDA accumulated in the cells unexposed and exposed to \(\gamma\)-irradiation was determined by using a TBARS assay. Each value represents the mean ± SD from four independent experiments. (B) Protein carbonyl content of U937 cells exposed to ionizing radiation. Protein carbonyls were measured in cell-free extracts by the method of Levine et al. (15) with the use of DNPH. Each value represents the mean ± SD from five independent experiments. (C) Relative intensities of DNP-FITC, HNE-TRITC, DPPP and avidin-TRITC fluorescences in U937 cells. Protein carbonyl content of U937 cells detected with DNP-specific antibody and anti-human IgG FITC conjugate as a secondary antibody. Lipid peroxidation in U937 cells was detected with polyclonal anti-HNE Michael adduct antibody and anti-human IgG TRITC conjugate. To visualize lipid peroxidation in U937 cells exposed to \(\gamma\)-irradiation, cells (1 \(\times\) 10^6/ml) were stained with 5 \(\mu\)M DPPP for 15 min. Fluorescence images were obtained under microscopy. In order to estimate 8-OH-dG levels in U937 cells, the cells were fixed and permeabilized immediately after exposure to \(\gamma\)-irradiation. 8-OH-dG levels reflected by the binding of avidin-TRITC were visualized by a fluorescence microscope. The averages of fluorescence intensity were calculated as described (21). Each value represents the mean ± SD from three independent experiments. *P < 0.01 compared with irradiated control cells.\]
The increase of lipid peroxidation was markedly reduced in NtBHA-treated cells. Recently, it has been shown that DPPP is a suitable fluorescence probe to monitor lipid peroxidation within cell membrane specifically (17). DPPP fluorescent intensity was increased markedly in untreated cells, whereas it was increased slightly in NtBHA-treated cells after exposure to ionizing radiation. 8-OH-dG, the most abundant and most studied lesion in DNA generated by intracellular ROS, has been used as an indicator of oxidative DNA damage in vivo and in vitro (23). Recently, it has been shown that 8-OH-dG level is specifically measured by a fluorescent binding assay using avidin-conjugated TRITC (18). The fluorescent intensity, which reflects the endogenous levels of 8-OH-dG in DNA was significantly increased in untreated cells upon exposure to ionizing radiation. In contrast, the overall DNA appeared to be markedly protected in NtBHA-treated cells even after exposure to the same dose of ionizing radiation. These results indicate that NtBHA appears to protect cells from oxidative damage caused by ionizing radiation.

Mitochondrial damage

MPT is a very important event in both necrosis and apoptosis, and ROS is one of the major stimuli that change MPT (24). To answer whether NtBHA modulates the MPT upon exposure to ionizing radiation, we determined the change in MPT by intensity of fluorescence emitting from a lipophilic cation dye, rhodamine 123. Significantly less rhodamine 123 dye was taken up by the mitochondria of untreated cells, compared with NtBHA-treated cells (Figure 5A). The intracellular peroxide levels in the mitochondria of U937 cells were evaluated by confocal microscopy with the oxidant-sensitive probe DHR 123. As shown in Figure 5(B), the intensity of fluorescence was significantly lower in cells pre-treated with 0.1 mM NtBHA for 2 h when compared with that in the mitochondria of untreated cells upon exposure to 20 Gy of γ-irradiation. Mitochondrial injury is often followed by the depletion of intracellular ATP level. As shown in Figure 5(C), the decrease in ATP level was markedly prevented in NtBHA-treated cells when U937 cells were exposed to 20 Gy of γ-irradiation.

In vivo radioprotection

ICR male mice treated with NtBHA at 5 mg/kg daily for 2 weeks survived with no apparent adverse effects after 30 days. Mice were injected intraperitoneally with saline or NtBHA at 5 mg/kg daily for 14 days, and then they were subjected to 8 Gy of whole-body irradiation. Survival was monitored for 30 days, and the results are displayed in Figure 6. NtBHA administration before irradiation provided significant protection compared with control animals receiving saline. The kidneys from NtBHA-administered mice prior to γ-irradiation showed the lower level of hydrogen peroxide (Figure 7A) compared with mice not administered NtBHA. The ratio for [GSSG]/[GSH] was lower (Figure 7B) and the ratio for [NADPH]/[NADPH + NADP+] was higher (Figure 7C) in the kidneys of NtBHA-administered mice before γ-irradiation. The livers and lungs showed the similar results (data not shown). In the kidneys of untreated mice, TBARS and carbonyl groups in proteins were increased significantly after whole-body irradiation. Administration of NtBHA markedly prevented the
increase in TBARS and carbonyl groups in proteins (Figure 8A and B). As shown in Figure 8(C), the kidneys from non-NtBHA-administered mice showed the lower level of ATP compared with untreated mice, suggesting the role of NtBHA against the loss of mitochondrial function caused by ionizing radiation. The livers and lungs showed the similar results (data not shown).

Discussion

Ionizing radiation is toxic to living cells and organisms because it induces deleterious structural changes in essential biomolecules. A significant part of the initial damage done to cells by ionizing radiation is due to formation of hydroxyl radicals, which reacts with almost all cellular components to induce oxidative damage to DNA, lipid and protein (3,25,26). DNA is a particularly important target, suffering double- and single-strand breaks, deoxyribose damage and base modification (27). Of the total damage to DNA caused by ionizing radiation, as much as 80% may result from radiation-induced water-derivative free radicals and secondary carbon-based radicals (1). In addition to the generation of hydroxyl radicals, the hydrated electrons formed by ionizing radiation can reduce it to superoxide. Superoxide can dismutate to hydrogen peroxide with the possibility of additional hydroxyl radicals production by metal-catalyzed Fenton reaction (2). Therefore, there is considerable literature to suggest that free radical scavengers can be used to prevent oxidative damage caused by ionizing radiation. GSH, GSH precursors and thiol compounds have been used as radioprotectors. However, toxicities including the possible biological actions of sulfur-centered radicals produced by loss of hydrogen radical (H•) from thiols must not be ignored (27). Recently, it has been reported that several non-thiol compounds including tempol (4), flavonoids (28) and Zn-desferrioxamine (29) are identified as potential radioprotectors.

The spin-trap PBN has been shown to protect against oxidative damage in different biological models (30–32). NtBHA and benzaldehyde are the breakdown products of PBN. NtBHA reacts with ROS and reactive aldehydes, and thus could prevent oxidative damage and lipid peroxidation-mediated damage to cellular macromolecules (33). NtBHA
delays senescence of IMR90 cells at 20 times lower concentration compared with PBN to produce a similar effect. Recently, we have shown that PBN is an effective radioprotector in vivo and in vitro (6). Therefore, it was plausible to assume that NtBHA may play a role in preventing oxidative damage caused by ionizing radiation in cells and animals at the much lower concentrations compared with PBN. The aim of the present work was to evaluate the role of NtBHA in protecting U937 cells and mice from ionizing radiation in regards to cell death, animal mortality, cellular redox status and oxidative damage to cells and tissues.

Biological systems have evolved to develop an effective and complicated network of defense mechanisms including antioxidant enzymes and small molecular weight antioxidants to cope with lethal oxidative environments. GSH is known to play a role in protecting cells against ionizing radiation. Treatment with buthionine sulfoximine to inhibit GSH synthesis increases radiosensitivity (34). The depletion of intracellular GSH and the increase in the ratio of [GSSG]/[GSH], which reflects the efficiency of GSH recycling were significantly reduced by NtBHA. The ratio for [NADPH]/[NADP+ + NADPH], the other parameter that reflects the cellular redox status and the availability of the reducing equivalent for GSH turnover by glutathione reductase, was significantly increased by NtBHA. These results indicate that ionizing radiation results in the perturbation of cellular redox balance presumably by depletion of GSH and NADPH pools and NtBHA may shift the balance to antioxidant condition. Whether the presence of NtBHA induced concomitant alterations in the activity of major antioxidant enzymes was investigated. The activity of antioxidant enzymes such as SOD, catalase, glutathione peroxidase, glucose 6-phosphate dehydrogenase and glutathione reductase was decreased upon exposure to γ-irradiation, however, NtBHA did not significantly affect the activity of antioxidant enzymes (data not shown). These results indicate that NtBHA may act as a direct scavenger of ROS.

Mitochondria are vulnerable to oxidants because they are the major source of free radicals in the cells and are limited in their ability to cope with oxidative stress (35). Therefore, improving the mitochondrial status could cause a significant decrease in the level of oxidants in the cells (8). It is well established that mitochondrial dysfunction is directly and indirectly involved in a variety of pathological states. All the changes caused by ionizing radiation are compatible with mitochondrial failure, encompassing reduced production of ATP, generation of ROS, and accumulation of rhodamine 123, which reflect mitochondrial swelling or changes in the mitochondrial inner membrane. It has been suggested that mitochondria are a potential primary target for NtBHA in delaying senescence of IMR90 cells (8). NtBHA is an antioxidant that is recycled by mitochondrial electron transport chain and prevents radical-induced toxicity to mitochondria (33). A clear suppression of such damages indicates that NtBHA prevents a deterioration of the bioenergetic state.

NtBHA is not only an in vitro but in vivo radioprotector. Radioprotective effect of NtBHA reflected by suppression of lethality was evident 30 days after exposure to radiation. The measurement of lipid peroxidation and protein oxidation in kidneys, livers and lungs from mice exposed to γ-irradiation indicates that the damage caused by ionizing radiation was similar in these tissues and that NtBHA protects damage in tissues in a similar manner. To confirm oxidative damage in irradiated mice due to the pro-oxidant status, the formation of ROS in tissues was measured. NtBHA administration showed the tendency to lower the formation of ROS. The observed beneficial effects of NtBHA in mice supported here offer the possibility of developing antioxidant approaches to treating damage caused by ionizing radiation. The use of NtBHA can avoid benzaldehyde, which is both mutagenic and carcinogenic (36), formed when PBN decomposes. In conclusion, NtBHA is effective in protecting cells and mice from oxidative stress caused by ionizing radiation and alleviated damage suggests that further study of NtBHA or similar compounds is warranted.

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References


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