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**Biology contribution**

## Protection against radiation-induced oxidative stress in cultured human epithelial cells by treatment with antioxidant agents

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**Purpose:** To evaluate the protective effects of antioxidant agents against space radiation-induced oxidative stress in cultured human epithelial cells.

**Methods and Materials:** The effects of selected concentrations of *N*-acetylcysteine, ascorbic acid, sodium ascorbate, co-enzyme Q10,  $\alpha$ -lipoic acid, l-selenomethionine, and vitamin E succinate on radiation-induced oxidative stress were evaluated in MCF10 human breast epithelial cells exposed to radiation with X-rays,  $\gamma$ -rays, protons, or high mass, high atomic number, and high energy particles using a dichlorofluorescein assay.

**Results:** The results demonstrated that these antioxidants are effective in protecting against radiation-induced oxidative stress and complete or nearly complete protection was achieved by treating the cells with a combination of these agents before and during the radiation exposure.

**Conclusion:** The combination of antioxidants evaluated in this study is likely to be a promising countermeasure for protection against space radiation-induced adverse biologic effects.

**Keywords:** Dichlorofluorescein; Antioxidant; Radiation; Oxidative stress

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## Introduction

Radiation-induced adverse biologic effects pose a significant health risk to astronauts during and after extended space travel. Two of the major components of space radiation encountered by astronauts in space are protons and high mass (H), high atomic number (Z), and high energy (E) particles known as HZE particles. We have previously demonstrated that both protons and HZE particles are capable of inducing oxidative stress in cultured human breast epithelial cells (1) and decreasing the total antioxidant concentration in serum/plasma of irradiated animals (2). We have also shown that exposure to HZE particle radiation is associated with increased cytotoxicity and malignant transformation *in vitro* (3). These findings indicated that antioxidant agents could be effective countermeasures to protect astronauts against space radiation-induced oxidative damage. The present study was undertaken to evaluate the protective effects of antioxidants against radiation-induced oxidative stress in cultured human epithelial cells.

## Methods and materials

### Chemicals, medium, buffers, and reagents

Dulbecco's modified Eagle/F12 medium, Dulbecco's phosphate-buffered saline (PBS), and 0.1% trypsin plus 0.1 mM ethylenediaminetetraacetic tetrasodium solution were purchased from Life Technologies/Invitrogen (Carlsbad, CA). Epidermal growth factor, dichlorofluorescein diacetate (DCFH) substrate, and other chemicals and reagents were purchased from Sigma Chemical (St. Louis, MO), unless otherwise specified. The PBS used in this study was supplemented with 1 mM CaCl<sub>2</sub> and MgCl<sub>2</sub>. A 100-mM DCFH stock solution was prepared in dimethylsulfoxide, stored frozen at -20°C, and diluted in PBS before use.

The antioxidants evaluated in this study included *N*-acetylcysteine, ascorbic acid, sodium ascorbate,  $\alpha$ -lipoic acid, co-enzyme Q10, l-selenomethionine, and vitamin E succinate. The stock solutions of *N*-acetylcysteine, ascorbic acid, sodium ascorbate, and l-selenomethionine were prepared in PBS. The stock solution of  $\alpha$ -lipoic acid was prepared in 0.1 M Na<sub>2</sub>CO<sub>3</sub> or tetrahydrofuran (THF). The stock solutions of co-enzyme Q10 and vitamin E succinate were prepared in ethanol or THF. These agents were diluted in medium to reach the specified final concentrations for the experiments.

### Radiation sources

The experiments were performed using four types of radiation beams: X-rays from an X-ray unit (Schneeman Electronics [Grants Pass, OR], model A-9002-100) operated at 100 kV;  $\gamma$ -rays from a Shepherd Mark I-68A <sup>137</sup>Cs source (J.L. Shepherd and Associates, San Fernando, CA); 250-MeV proton beams (vertical beam approximately 15 cm in diameter) from an accelerator in the proton radiation therapy facility at Loma Linda University Medical Center; and 1-GeV/nucleon (147 KeV/μm) and 5-GeV/

nucleon (145 KeV/•m)  $^{56}\text{Fe}$  ion beams (horizontal beam approximately 7.5 cm in diameter) from the Alternating Gradient Synchrotron at the Brookhaven National Laboratory. The measured dose rates at the position at which the samples were irradiated was 194 cGy/min for X-rays, 143 cGy/min for  $\gamma$ -rays, 80 cGy/min for the 250-MeV protons, and 100 cGy/min for the  $^{56}\text{Fe}$  ion beams.

## Cells and cell culture

The MCF10 human breast epithelial cells used in this study were established and characterized as previously described (4). The cells were cultured in Dulbecco's modified Eagle/F12 medium supplemented with 5% horse serum, 0.5 •g/mL hydrocortisone, 0.1 •g/mL cholera toxin, 10 •g/mL insulin, and 0.02 •g/mL epidermal growth factor and subcultured by treatment with trypsin-ethylenediaminetetraacetic tetrasodium when the cells became confluent.

## Dichlorofluorescein assay

The dichlorofluorescein (DCF) assay experiments were performed to determine whether antioxidants provided protective effects against X-ray,  $\gamma$ -ray, proton, or HZE particle radiation-induced oxidative stress in cultured MCF10 cells. Before the radiation exposure, the cells (nearly confluent) were incubated in medium containing *N*-acetylcysteine, ascorbic acid, sodium ascorbate, ethanol,  $\alpha$ -lipoic acid, co-enzyme Q10, l-selenomethionine, and/or vitamin E succinate for 12–16 h. After incubation, the cells were washed twice with PBS followed by a 30-min incubation with PBS containing 50 •M DCFH in addition to the antioxidant agent being tested. The DCF assay and the radiation experiments were carried out essentially as previously described (1 and 5), except that the radiation exposure was done while the cells were covered with PBS containing the antioxidant being evaluated. Three or four replicate wells were used for each radiation dose with each antioxidant agent in the DCF assay experiments. For experiments with HZE particle radiation, the cell plates were sealed with plastic plate sealers and held in a vertical position for radiation exposure.

## Statistical analysis

The relationships between the radiation dose and the increase in fluorescence were determined by linear regression analysis using SigmaPlot graphic software (SPSS, Chicago, IL). The slope values of the linearly fitted dose–response lines were compared between each antioxidant treatment group and PBS or relevant solvent treatment group using InStat statistical software (GraphPad Software, San Diego, CA).

## Results

The present study was undertaken to determine whether radiation-induced oxidative stress can be prevented by treatment with antioxidant agents. The concentrations of the antioxidants were selected on the basis of the results of preliminary experiments, in which each agent was evaluated at three to four different concentrations (twofold dilutions) in up to three separate preliminary experiments (data not shown). The concentrations of antioxidants shown to be effective in the DCF assay with no obvious cytotoxicity were selected and used in this study. As demonstrated by the results obtained in the experiments performed with radiation from  $\gamma$ -rays (Fig. 1), X-rays (Fig. 2), protons (Fig. 3), and HZE particles (Fig. 4), treatment with antioxidant agents affected the slope, but not the linearity, of the dose–response lines in the DCF assay. Thus, the protective effects of the antioxidant agents on radiation-induced oxidative stress could be quantified by comparing the slope values of the dose–response lines between the cells receiving antioxidant treatment and the cells without antioxidant treatment before radiation exposure.

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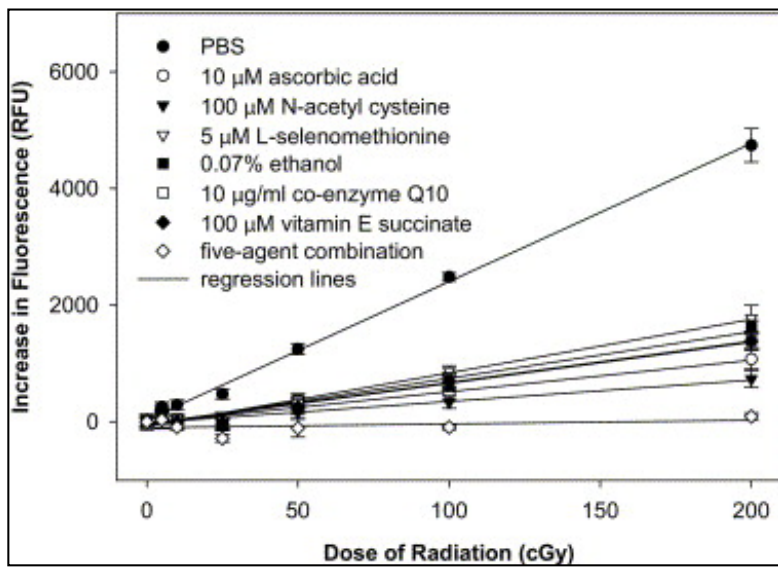


Fig. 1. Protection against  $\gamma$ -ray radiation-induced oxidative stress by ascorbic acid, *N*-acetylcysteine, l-selenomethionine, co-enzyme Q10, and/or vitamin E succinate as measured by dichlorofluorescin assay. Results expressed as mean  $\pm$  standard deviation. Slope values of regression lines presented in Table 1 ( $\gamma$ -ray radiation experiment number 3). PBS = phosphate-buffered saline.

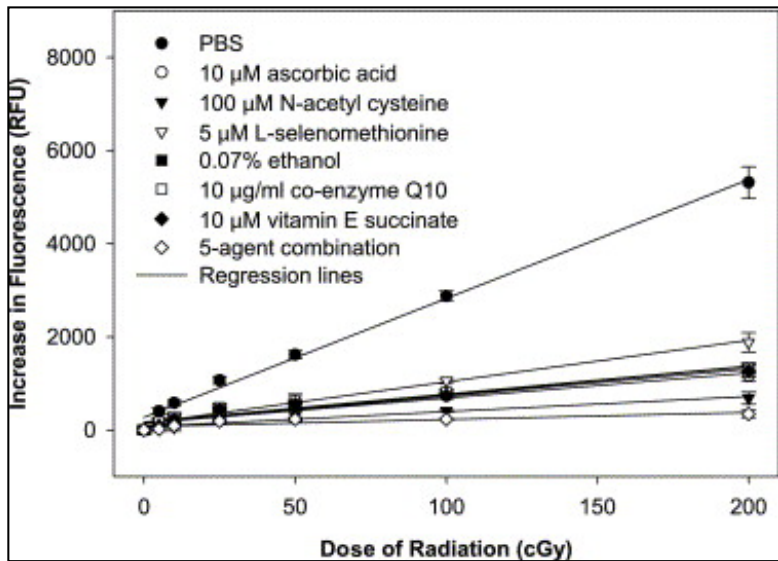


Fig. 2. Protection against X-ray radiation-induced oxidative stress by ascorbic acid, *N*-acetylcysteine, l-selenomethionine, co-enzyme Q10, and/or vitamin E succinate as measured by dichlorofluorescin assay. Results expressed as mean  $\pm$  standard deviation. Slope values of regression lines presented in Table 2 (X-ray radiation experiment number 1). PBS = phosphate-buffered saline.

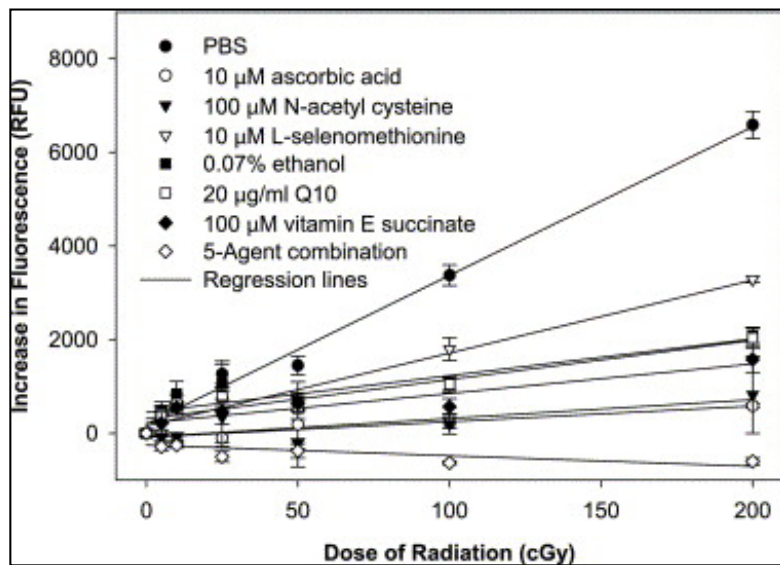


Fig. 3. Protection against proton radiation-induced oxidative stress by ascorbic acid, *N*-acetylcysteine, l-selenomethionine, co-enzyme Q10, and/or vitamin E succinate as measured by dichlorofluorescin assay. Results expressed as mean  $\pm$  standard deviation. Slope values of regression lines presented in Table 3. PBS = phosphate-buffered saline.

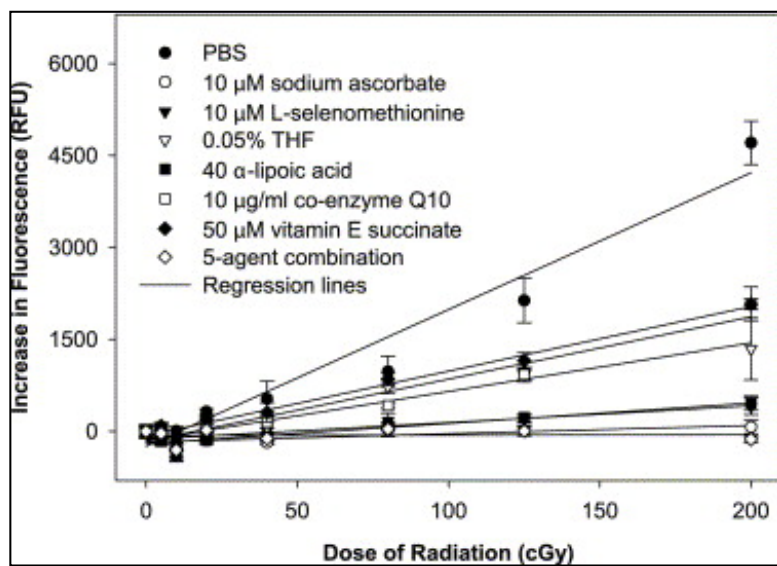


Fig. 4. Protection against 5-GeV/n  $^{56}\text{Fe}$  ion radiation-induced oxidative stress by ascorbic acid, l-selenomethionine, co-enzyme Q10, and/or vitamin E succinate as measured by dichlorofluorescin assay. Results expressed as mean  $\pm$  standard deviation. Slope values of regression lines presented in Table 4. PBS = phosphate-buffered saline.

On the basis of the slope values obtained in the experiments with  $\gamma$ -ray (Table 1) or X-ray (Table 2) radiation, water-soluble free radical scavengers, such as ascorbic acid (10, 20, or 100  $\bullet\text{M}$ ), sodium ascorbate (10 or 20  $\bullet\text{M}$ ),  $\alpha$ -lipoic acid (100  $\bullet\text{M}$ ), and ethanol (0.1% vol/vol), were highly protective against radiation-induced oxidative stress in the irradiated MCF10 cells (decreasing the slope value by  $>75\%$  compared with PBS treatment). l-Selenomethionine (5 or 20  $\bullet\text{M}$ ) and vitamin E succinate (100  $\bullet\text{M}$ ) were moderately protective (decreasing the slope value by 44–66% compared with PBS and 0.02% THF solvent treatment, respectively). The protective effect of 20  $\bullet\text{M}$  l-selenomethionine was not stronger than that of 5  $\bullet\text{M}$  l-selenomethionine, suggesting a plateau in the protective effect against radiation-induced oxidative stress by l-selenomethionine. The effects of co-enzyme Q10 (10 or 20  $\bullet\text{g/mL}$ ) and vitamin E succinate at a low (10  $\bullet\text{M}$ ) concentration could not be ascertained, because they conferred  $<16\%$  protection compared with ethanol (0.1% vol/vol) or THF (0.02% vol/vol) solvent treatment. Complete protection (100% decrease in the slope value) or near-complete protection was achieved by treatment with combinations of these antioxidant agents.

Table 1.

Protection against  $\gamma$ -ray radiation-induced oxidative stress by antioxidant treatments

Antioxidant treatment	Final solvent concentration	Slope value (mean $\pm$ SD)	Protection (%)	
			PSB	Solvent
$\gamma$ -Ray radiation experiment 1				
PBS	0.1% PBS	34.12 $\pm$ 1.02	0.00	
100 $\mu$ M ascorbic acid	0.1% PBS	0.07 $\pm$ 0.15*	99.80	
100 $\mu$ M $\alpha$ -lipoic acid	0.1 $\mu$ M Na <sub>2</sub> CO <sub>3</sub>	12.14 $\pm$ 0.32*	64.41	
20 $\mu$ M l-selenomethionine	0.1% PBS	19.08 $\pm$ 1.13*	44.08	
Solvent (ethanol)	0.1% Ethanol	4.91 $\pm$ 0.35*	85.60	0.00
20 $\mu$ g/mL co-enzyme Q10	0.1% Ethanol	7.89 $\pm$ 0.38†	76.89	NAP
100 $\mu$ M vitamin E succinate	0.1% Ethanol	5.00 $\pm$ 0.29	85.34	NAP
Combination‡	0.1% Ethanol	-0.75 $\pm$ 0.25†	100.00	100.00
$\gamma$ -Ray radiation experiment 2				
PBS	0.1% PBS	26.31 $\pm$ 0.97	0.00	
100 $\mu$ M ascorbic acid	0.1% PBS	2.81 $\pm$ 0.95*	89.32	
Solvent (THF)	0.02% THF	20.80 $\pm$ 1.42*	20.95	0.00
10 $\mu$ g/mL co-enzyme Q10	0.02% THF	17.51 $\pm$ 1.35†	33.45	15.81
100 $\mu$ M $\alpha$ -lipoic acid	0.02% THF	4.52 $\pm$ 0.71†	82.81	78.25
100 $\mu$ M vitamin E succinate	0.02% THF	9.53 $\pm$ 0.87†	63.79	54.19
Combination§	0.02% THF	-0.14 $\pm$ 0.05†	100.00	100.00
$\gamma$ -Ray radiation experiment 3				
PBS	0.1% PBS	23.66 $\pm$ 0.52	0.00	
10 $\mu$ M ascorbic acid	0.1% PBS	5.40 $\pm$ 0.39*	77.17	
100 $\mu$ M N-acetyl cysteine	0.1% PBS	3.67 $\pm$ 0.44*	84.50	
5 $\mu$ M l-selenomethionine	0.1% PBS	9.17 $\pm$ 0.63*	61.27	
Solvent (ethanol)	0.07% Ethanol	7.97 $\pm$ 0.70*	66.32	0.00
10 $\mu$ g/mL co-enzyme Q10	0.07% Ethanol	7.07 $\pm$ 0.46	70.12	NAP
10 $\mu$ M vitamin E succinate	0.07% Ethanol	7.05 $\pm$ 0.81	70.21	NAP
Combination	0.07% Ethanol	0.65 $\pm$ 0.69†	97.26	91.87

**Abbreviations:** PBS = phosphate-buffered saline; NAP = no additional protection compared with solvent treatment; THF = tetrahydrofuran.

• Significantly different from PBS control ( $p < 0.001$ ).

† Significantly different from the solvent control ( $p < 0.001$ ).

‡ Consisting of 100 •M ascorbic acid, 100 •M  $\alpha$ -lipoic acid, 20 •M l-selenomethionine, 20 •g/mL co-enzyme Q10, 100 •M vitamin E succinate, and 0.1% (vol/vol) ethanol (solvent).

§ Consisting of 100 •M ascorbic acid, 10 •g/mL co-enzyme Q10, 100 •M  $\alpha$ -lipoic acid, 100 •M vitamin E succinate, and 0.02% THF (solvent).

|| Consisting of 10 •M ascorbic acid, 100 •M *N*-acetyl cysteine, 5 •M l-selenomethionine, 10 •g/mL co-enzyme Q10, 10 •M vitamin E succinate, and 0.01% ethanol (solvent).

Table 2.

Protection against X-ray radiation-induced oxidative stress by antioxidant treatments

Antioxidant treatment	Final solvent concentration	Slope value (mean $\pm$ SD)	Protection (%)	
			PSB	Solvent
X-ray radiation experiment 1				
PBS	0.1% PBS	25.93 $\pm$ 0.84	0.00	
10 •M ascorbic acid	0.1% PBS	5.37 $\pm$ 0.52•	79.29	
100 •M <i>N</i> -acetyl cysteine	0.1% PBS	3.26 $\pm$ 0.38•	87.41	
5 •M l-selenomethionine	0.1% PBS	8.93 $\pm$ 0.59•	65.56	
Solvent (ethanol)	0.07% Ethanol	6.08 $\pm$ 0.45•	76.57	0.00
10 •g/mL co-enzyme Q10	0.07% Ethanol	6.01 $\pm$ 0.56	76.84	NAP
10 •M vitamin E succinate	0.07% Ethanol	5.81 $\pm$ 0.64	77.59	NAP
Combination†	0.07% Ethanol	1.51 $\pm$ 0.38‡	94.16	75.08
X-ray radiation experiment 2				
PBS	0.1% PBS	32.23 $\pm$ 2.61	0.00	
10 •M ascorbic acid	0.1% PBS	7.00 $\pm$ 0.87•	78.28	
20 •M ascorbic acid	0.1% PBS	4.75 $\pm$ 0.80•	85.26	
10 •M sodium ascorbate	0.1% PBS	7.64 $\pm$ 1.04•	76.30	
20 •M sodium ascorbate	0.1% PBS	5.44 $\pm$ 1.32•	83.12	
Solvent (THF)	0.02% THF	24.37 $\pm$ 1.56•	24.39	0.00
10 •M $\alpha$ -lipoic acid	0.02% THF	25.92 $\pm$ 1.52	19.58	NAP
100 •M $\alpha$ -lipoic acid	0.02% THF	12.29 $\pm$ 1.05‡	61.86	49.57

Abbreviations as in Table 1.

• Significantly different from PBS control ( $p < 0.001$ ).

† Consisting of 10 •M ascorbic acid, 100 •M *N*-acetyl cysteine, 5 •M l-selenomethionine, 10 •g/mL co-enzyme Q10, 10 •M

vitamin E succinate, and 0.01% (vol/vol) ethanol (solvent).

‡ Significantly different from the solvent control ( $p < 0.001$ ).

In the experiments performed with proton radiation, ascorbic acid (10 •M), *N*-acetylcysteine (100 •M), and ethanol (0.07% vol/vol) were highly protective (decreasing the slope value by >75%) against proton radiation-induced oxidative stress (Table 3). *L*-Selenomethionine (10 •M) was moderately protective (decreasing the slope value by 50.91% compared with PBS), and vitamin E succinate (100 •M) was weakly protective (decreasing the slope value by 19.54% compared with ethanol [0.07%, vol/vol] solvent treatment). The protective effects of co-enzyme Q10 (20 •g/mL) could not be ascertained, because the results of co-enzyme Q10 did not show any additional protection compared with ethanol (0.07%, vol/vol) solvent treatment. Complete protection (100% decrease in the slope value) was achieved by treatment with a combination of these antioxidant agents.

Table 3.

Protection against proton radiation-induced oxidative stress by antioxidant treatments

Antioxidant treatment	Final solvent concentration	Slope value (mean $\pm$ SD)	Protection (%) compared with	
			PSB	Solvent
PBS	0.1% PBS	31.74 $\pm$ 1.26	0.00	
10 •M ascorbic acid	0.1% PBS	3.34 $\pm$ 0.55*	89.48	
100 •M <i>N</i> -acetyl cysteine	0.1% PBS	3.98 $\pm$ 1.27*	87.46	
10 •M <i>L</i> -selenomethionine	0.1% PBS	15.58 $\pm$ 1.17*	50.91	
Solvent (ethanol)	0.07% Ethanol	7.83 $\pm$ 1.84*	75.33	0.00
20 •g/mL co-enzyme Q10	0.07% Ethanol	8.38 $\pm$ 1.32	73.60	NAP
100 •M vitamin E succinate	0.07% Ethanol	6.30 $\pm$ 1.20	80.15	NAP
Combination†	0.07% Ethanol	-2.26 $\pm$ 0.93‡	100.00	100.00

Abbreviations as in Table 1.

\* Significantly different from PBS control ( $p < 0.001$ ).

† Consisting of 10 •M ascorbic acid, 100 •M *N*-acetyl cysteine, 10 •M *L*-selenomethionine, 20 •g/ml co-enzyme Q10, 100 •M vitamin E succinate, and 0.01% (vol/vol) ethanol (solvent).

‡ Significantly different from the solvent control ( $p < 0.001$ ).

In the experiments performed with HZE particle radiation, ascorbic acid (20 •M), sodium ascorbate (10 •M),  $\alpha$ -lipoic acid (40 •M), and *L*-selenomethionine (10 or 20 •M) were highly protective (decreasing the slope value by  $\geq 70\%$  compared with PBS or THF solvent treatment) against HZE particle radiation-induced oxidative stress (Table 4). Vitamin E succinate (100 •M) was also highly protective (87.63% reduction in slope value) when ethanol (0.07%) was used as the solvent. Co-enzyme Q10 (10 or 20 •M) and THF (0.05% vol/vol) solvent were moderately protective (decreasing the slope value by 27.82–54.30% compared with THF or ethanol solvent or PBS treatment) against HZE particle radiation-induced oxidative stress. Complete protection (100% decrease in slope value) or nearly complete protection (94.18% decrease in the slope value) was achieved by treatment with a combination of multiple antioxidant agents.

Table 4.

Protection against HZE particle radiation-induced oxidative stress by antioxidant treatment

Antioxidant treatment	Final solvent concentration	Slope value (mean $\pm$ SD)	Protection (%) compared with	
			PSB	Solvent
1-Gev/n $^{56}\text{Fe}$ ion radiation experiment				
PBS	0.1% PBS	9.35 $\pm$ 1.38	0.00	
10 $\bullet$ M ascorbic acid	0.1% PBS	0.13 $\pm$ 0.74 <sup>•</sup>	98.61	
20 $\bullet$ M l-selenomethionine	0.1% PBS	0.12 $\pm$ 0.22 <sup>•</sup>	98.67	
Solvent (ethanol)	0.07% Ethanol	3.00 $\pm$ 1.80 <sup>•</sup>	67.88	0.00
20 $\bullet$ g/mL Co-enzyme Q10	0.07% Ethanol	2.17 $\pm$ 0.59	76.81	NAP
100 $\bullet$ M Vitamin E succinate	0.07% Ethanol	-0.82 $\pm$ 0.94 <sup>†</sup>	100.00	100.00
Combination <sup>‡</sup>	0.07% Ethanol	-1.54 $\pm$ 0.58 <sup>†</sup>	100.00	100.00
5-Gev/n $^{56}\text{Fe}$ ion radiation experiment				
PBS	0.1% PBS	22.32 $\pm$ 2.01	0.00	
10 $\bullet$ M Sodium ascorbate	0.1% PBS	1.34 $\pm$ 0.66 <sup>•</sup>	94.00	
10 $\bullet$ M l-selenomethionine	0.1% PBS	2.73 $\pm$ 0.70 <sup>•</sup>	87.77	
Solvent (THF)	0.05% THF	10.20 $\pm$ 2.48 <sup>•</sup>	54.30	0.00
10 $\bullet$ g/mL Co-enzyme Q10	0.05% THF	5.81 $\pm$ 1.76	73.97	NAP
40 $\bullet$ M $\alpha$ -Lipoic acid	0.05% THF	3.06 $\pm$ 2.24 <sup>†</sup>	86.29	70.00
50 $\bullet$ M Vitamin E succinate	0.05% THF	11.14 $\pm$ 1.92	50.09	NAP
Combination <sup>§</sup>	0.05% THF	1.30 $\pm$ 2.04 <sup>†</sup>	94.18	87.25

Abbreviations as in Table 1.

<sup>•</sup> Significantly different from PBS control ( $p < 0.001$ ).

<sup>†</sup> Significantly different from solvent control ( $p < 0.001$ ).

<sup>‡</sup> Consisting of 10  $\bullet$ M ascorbic acid, 20  $\bullet$ M l-selenomethionine, 20  $\bullet$ g/mL co-enzyme Q10, 100  $\bullet$ M vitamin E succinate, and 0.01% (vol/vol) ethanol (solvent).

<sup>§</sup> Consisting of 10  $\bullet$ M sodium ascorbate, 10  $\bullet$ M l-selenomethionine, 10  $\bullet$ g/mL co-enzyme Q10, 40  $\bullet$ M  $\alpha$ -lipoic acid, 50  $\bullet$ M vitamin E succinate, and 0.05% (vol/vol) THF (solvent).

## Discussion

We have previously demonstrated that X-ray,  $\gamma$ -ray, proton, and HZE particle radiation are all capable of inducing oxidative stress in cultured MCF10 human breast epithelial cells in a linear dose-dependent manner as determined using the DCF assay (1 and 2). The present study was done to evaluate the effects of *N*-acetylcysteine, ascorbic acid, sodium ascorbate, co-enzyme Q10,  $\alpha$ -lipoic acid, l-selenomethionine, and vitamin E succinate on oxidative stress induced in cells exposed to radiation with X-rays,  $\gamma$ -rays, protons, and HZE particles. *N*-acetylcysteine is a small-molecular-weight thiol that serves as a precursor to intracellular glutathione (6), which is a potent antioxidant involved in the cellular response to radiation exposure (7 and 8). Ascorbic acid is a water-soluble antioxidant that protects cells by interacting with hydroxyl radicals to form less toxic ascorbate free radicals that can be detoxified by enzymes that reduce ascorbate free radicals back to ascorbic acid (9).  $\alpha$ -lipoic acid is a B vitamin that reacts with

hydroxy radicals, singlet oxygen, peroxy, and hypochlorous radicals and has been called a “universal antioxidant” (10). It is an excellent free radical scavenger in both oxidized and reduced forms. The results of the present study have indicated that these agents are highly effective in protecting against radiation-induced oxidative stress as determined by the DCF assay method.

Compared with *N*-acetylcysteine, ascorbic acid, sodium ascorbate, and  $\alpha$ -lipoic acid, co-enzyme Q10, l-selenomethionine, and vitamin E succinate were only moderately effective in protecting against radiation-induced oxidative stress as determined by the DCF assay method. The difference in effectiveness was probably related to mechanisms of action and localization of these agents as antioxidants in the cells. It is expected that the generation of free radicals and reactive oxygen species by radiation exposure is instantaneous and occurs mainly in an aqueous environment. Water-soluble free radical scavengers, such as *N*-acetylcysteine, ascorbic acid, sodium ascorbate, and  $\alpha$ -lipoic acid, are expected to be highly effective and react quickly to quench the radiation-generated free radicals and reactive oxygen species, because they are present in the same aqueous microenvironment in which free radicals and reactive oxygen species are generated. Co-enzyme Q10 is an essential electron and proton carrier that functions in the production of biochemical energy in aerobic organisms (11). Vitamin E succinate is a derivative of vitamin E that functions as a chain terminator to protect lipid membranes from free radical damage (12 and 13). l-selenomethionine is an organic compound of selenium, which is an essential component of several important antioxidant enzymes, such as glutathione peroxidase and thioredoxin reductase (14 and 15). Co-enzyme Q10 and vitamin E succinate are not water soluble and their protective effects may not come into play until the radiation-initiated free radical chain reaction is propagated from the aqueous environment to membrane lipids. l-selenomethionine is not a free radical scavenger by itself, and its antioxidant activity is likely to be mediated through the selenium-containing antioxidant enzymes. Thus, the observed differences in the effectiveness of the antioxidant agents could be interpreted as an indication that the effects of co-enzyme Q10, l-selenomethionine, and vitamin E succinate on radiation-induced oxidative stress are relatively slow and/or occurred in a different microenvironment than that in which the bulk of the free radicals and reactive oxygen species were initially generated after radiation exposure. However, these results do not necessarily suggest that co-enzyme Q10, l-selenomethionine, and vitamin E succinate are less effective in protecting against radiation-induced oxidative stress and other downstream biologic damage.

An important observation in the present *in vitro* study, and in our previous animal study (2), is that combinations of antioxidant agents were more effective than individual antioxidant agents in protecting against radiation-induced oxidative stress. This was not surprising, because different antioxidants can replenish one another (16 and 17), thereby increasing the pool of total antioxidant power available to respond to the sudden surge of free radicals and reactive oxygen species after radiation exposure. For example, glutathione at a high concentration was able to increase ascorbate-mediated Trolox (a water-soluble homolog of vitamin E) regeneration from the Trolox radical (18).  $\alpha$ -lipoic acid is involved in the regeneration of ascorbic acid from dehydroascorbic acid (19), which in turn recycles vitamin E (10, 20 and 21). Treatment with  $\alpha$ -lipoic acid, together with vitamins C and E, has been shown to protect against lens damage in an *in vitro* system that simulates the low-level radiation exposure of astronauts (22). On the basis of the expected additive effects of antioxidant agents with a similar mode of action and the synergistic effects of antioxidant agents with different mechanisms, treatment with multiple antioxidant agents in combination is likely to be more effective than individual antioxidant agents in protecting against radiation-induced oxidative damage.

The radiation doses used in these studies covered the radiation doses expected for relatively short missions to exploration class space missions, as well as the doses from protons expected during solar particle events (23). As one example, the dose expected to be received by an astronaut during a 3-year mission to Mars is estimated to be approximately 100 centisieverts (cSv) (24), which is equivalent to a dose of 50 cGy, assuming a relative quality factor of two. In general, the dose rates of the HZE particle radiations evaluated in these studies were generally greater than those of the radiation exposure expected for space travel. The effects of dose rate on the biologic effects expected from space radiation and their interactions are unknown.

The results of the present study demonstrate that treatment with multiple antioxidant agents is highly effective in protection against radiation-induced oxidative stress, including the oxidative stress induced by protons and HZE particles, which are the main components of space radiation of concern for the health of astronauts. Because radiation-induced oxidative stress is likely to occur instantaneously at the time of the radiation exposure and precede other downstream events manifested by unrepaired oxidative damage to important macromolecules, a combination of antioxidant agents is likely to be a promising countermeasure against space radiation-induced adverse biologic effects.

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