

American Ginseng Modifies ^{137}Cs -Induced DNA Damage and Oxidative Stress in Human Lymphocytes

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Abstract: The multifold bioactive medicinal properties of ginseng have been closely linked to its antioxidative ability, which is related to its ginsenoside content. Since the key mechanism of radiation-induced cell death and tissue damage is the generation of reactive oxygen species (ROS) that attack cellular DNA, this study focuses on the impact of a standardized North American ginseng extract (NAGE) on ^{137}Cs -induced oxidative stress in human peripheral lymphocytes (PBL) obtained from 10 healthy individuals (6M/4F), 42.7 \pm 4.6 years of age. At two different time points (0 h and 24 h before irradiation), we applied NAGE (250-1000 $\mu\text{g ml}^{-1}$) to mononuclear cell cultures for cytokinesis-block micronuclei (MN) assay and determination of the state of oxidative stress in PBL. We found that at both time points, NAGE significantly reduced the MN yields in PBL after irradiation (1 and 2 Gy) in a concentration-dependent manner ($P < 0.001$). Compared with radiation alone, the maximum reduction rate of MN yield were 51.1% and 49.1% after 1 Gy and 2 Gy exposures, respectively. We also found that before irradiation the presence of NAGE in the culture medium resulted in a significant increased intracellular total antioxidant capacity (TAC) in PBL. At both time points, the increment of ^{137}Cs -induced MN yields in PBL was positively correlated with the increment of intracellular ROS production ($R = 0.6-0.7$, $P = 0.002$), but negatively correlated with the reduction of TAC levels ($R = -0.4-0.5$, $P = 0.02-0.004$). However, the presence of NAGE in the culture medium significantly increased the TAC levels, while concomitantly decreasing both ROS production and MN yields in PBL ($P < 0.001$). Our findings that NAGE is effective in protecting human PBL against radiation-induced oxidative stress should encourage further *in vivo* study of dietary supplementation with NAGE as an effective natural radiation countermeasure.

INTRODUCTION

The major detrimental effect of ionizing radiation (IR) on normal tissue cells is the induction of oxidative stress, defined as a disturbance in the equilibrium status of pro-oxidant and antioxidant systems in favor of pro-oxidation *in vivo*. IR exposure causes radiolysis of water in the cell, generating free radicals, collectively known as reactive oxygen species (ROS), which attack diverse cellular macromolecules such as DNA, lipids, and proteins, eventually inducing cell death. Identifying and developing effective radiation countermeasures would be a significant medical advance, not only because IR-induced normal tissue damage is a limiting factor in radiotherapy (RT) for cancer patients, but also because growing threats of global terrorism or accidental radiation exposure have made finding such countermeasures a high priority for homeland security [1-4]. The term "radioprotector" primarily refers to free radical scavengers that

avert the initial cascades of radiochemical events in cells following IR exposure [4]. Currently, the majority of radioprotectors under investigation are designed to scavenge IR-induced intracellular free radicals [4]. However, their practical applicability is limited due to their inherent toxicity [1, 4, 5]. In contrast, natural products with an abundance of antioxidant resources have begun to receive attention as possible radiation modifiers [5-8].

Ginseng is one of the most highly valued natural dietary supplements, the term usually refers to the dried root of several species in the genus *Panax* of the Araliaceae family [9-11], including two commonly used species, i.e., *Panax ginseng* C. A. Meyer (Asian ginseng) and *Panax quinquefolius* L (North American ginseng). These two ginseng species have drawn attention worldwide for their invaluable medicinal potential including antiaging, antidiabetic, anticarcinogenic, analgesic, anti-pyretic, antistress, and antifatigue effects, as well as promotion of DNA, RNA, and protein synthesis [6, 10-12]. These multifold bioactive medicinal properties of ginseng have been closely linked to its antioxidative effects [10-15]. In addition, ginseng and its partially purified constituents also have potential radioprotective properties [8-10, 16]. However, despite the increasing interest in the clini-

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cal applications of ginseng, an in-depth analysis of its radioprotective potential has yet to be carried out what studies there have primarily used non-human models [10, 17, 18]. Our laboratory first demonstrated the unambiguous capacity of *Panax ginseng* crude water extract applied at 24 h before radiation exposure to protect against ^{137}Cs -induced micronuclei (MN) in human peripheral blood lymphocytes [9]. In contrast to the many documented citations on *Panax ginseng* C. A. Meyer (Asian ginseng), a relative dearth of studies have involved *Panax quinquefolius* L (North American ginseng) [6, 10].

To explore the radioprotective potential of a standardized North American ginseng extract (NAGE), we recently have shown that the ^{137}Cs -induced MN yields in human peripheral blood lymphocytes (PBL) declined significantly with increasing concentrations of NAGE [19]. Even at 90 min post irradiation, the maximum MN reduction rate in PBL after 1 Gy and or 2 Gy irradiation was 53.8% and 37.3% respectively, compared to the controls. Our results in that study also indicate that the radioprotective effects of NAGE are comparable to that of WR-1065, which is the active metabolite of amifostine. However, it is unclear as to what extent NAGE meets the characteristic requirements of an applicable radioprotector. Since dietary antioxidants are free radical scavengers that interfere with radical chain reactions [5, 7, 8], administration of exogenous antioxidants should be possible to protect cells from IR-induced oxidative stress. To test this hypothesis, and to further verify the radioprotective potential of NAGE, in this present study, we assessed the impact of different concentrations of NAGE on intracellular total antioxidant capacity (TAC) and levels of ROS production in human PBL before and after ^{137}Cs exposure. Because the formation of MN in dividing cells is the result of chromosome breakage due to unrepaired or misrepaired DNA damage, MN is one of worldwide used biomarkers for genotoxicity testing and biomonitoring studies [20]. Thus, we also evaluated the correlation between the oxidative status and MN yield in PBL in the presence and absence of NAGE at two different time points of NAGE application, both before and after radiation exposure. We believe that the information generated from this study will provide the foundation for clinical trials to assess the potential of standardized NAGE as an adjuvant natural radioprotector, not only for cancer patients undergoing RT, but also as a dietary radiation countermeasure for scenarios such as accidental exposure or terrorist threats.

MATERIALS AND METHODS

Subjects

Our University Medical Center Institutional Review Board approved this study. Ten healthy individuals (6M/4F) 42.7 ± 4.6 (mean \pm SEM) years of age, without known history of exposure to mutagens, participated in this study. No individuals were currently taking any other pharmacologic agents, including medications, vitamins, or dietary supplements. All participants signed informed consent before enrollment.

NAGE Preparation and Ginsenosides

The standardized NAGE powder (Lot-TKGS-010406) was purchased from Canadian Phytopharmaceuticals Corpo-

ration (Richmond, BC, Canada). Using high-performance liquid chromatography, the major ginsenosides in this NAGE powder were characterized by the vendor as follows: Rb1 (5.1%), Rb2 (0.99%), Rc (1.88%), Rd (1.23%), Re (2.14%), and Rg1 (0.36%) with total ginsenoside content (w/w) of 11.7%. To ensure stability, the NAGE was stored in a cool, dry, dark location over the course of the study. Before experimentation, a known concentration of the freshly prepared lyophilized NAGE powder solution in RPMI 1640 culture medium was filtered through 0.2 μm disc (Millipore, MA) under sterile conditions and was used as the stock solution.

Cytokinesis-Block (CB) MN Assay

Fresh peripheral blood samples were drawn from each individual into Vacutainer Cell Preparation Tubes (Becton-Dickson, NJ, USA). Mononuclear cells were isolated by density gradient centrifugation at 1800 g for 20 min, washed, and counted on a hemacytometer. The trypan blue exclusion showed the viability to be greater than 95%. The purity of mononuclear cells was $> 95\%$ as determined by Hema-3 staining (Fisher Scientific, NC, USA). For each culture, $2-3 \times 10^5$ cells ml^{-1} were incubated in polystyrene culture tubes containing RPMI 1640 culture medium (Sigma Chemical, MO, USA), supplemented with 10% fetal calf serum, L-glutamine, and penicillin and streptomycin. The final volume of each culture was 1 ml. Duplicate cultures were set up for each experimental point within 60 min after venipuncture. PHA (M Form, Invitrogen Corp. CA, USA) was added into each culture (1.5%, v/v) immediately after *ex vivo* radiation exposure. Cytochalasin B (Sigma Chemical, MO, USA) was applied at 44 h after the PHA stimulation, with a final concentration of $4 \mu\text{g ml}^{-1}$. All cultures were maintained in a humidified atmosphere of 5% CO_2 at 37°C , and were terminated following another 24 h. The Slides, prepared according to the method of Fenech *et al.* [21], were stained with Hema-3 (Fisher Scientific, NC, USA).

Application of NAGE

To ascertain the optimum radioprotective dose of NAGE, a series of preliminary studies were carried out (Data not shown). Treatment of PBL with NAGE at $500-750 \mu\text{g ml}^{-1}$ at 0 h was found to cause a significant reduction in ^{137}Cs -induced MN yield. Therefore, for the determination of a dose-response radioprotective effect of NAGE, in each experiment, three different NAGE concentrations (250, 500, and $1000 \mu\text{g ml}^{-1}$) were applied to mononuclear cell cultures ($2-3 \times 10^5$ cells ml^{-1}) in RPMI 1640 at 0 h (Time 1) and 24 h before exposure to ^{137}Cs - irradiation (Time 2) for CBMN assay.

Ex Vivo Irradiation

The human G_0 PBL in the presence or absence of NAGE were exposed *ex vivo* to ^{137}Cs γ - rays (Gamma Cell 40, Radiation Machinery, Ontario, Canada) with 1 or 2 Gy (0.6 Gy/min) at room temperature (22°C).

Microscopy

Slides were coded and randomized to ensure anonymity upon scoring. For consistency, the microscopy was performed by one researcher (WW). Under 400X magnification, in continuous fields from two slides prepared for each experimental checkpoint, he scored a minimum of 1000

binucleated (BN) cells where possible. The quantification of MN yield was restricted to BN cells with distinct intact cytoplasm and included those with nuclear bridges. MN with smooth edges touching the main nucleus and those with clearly defined overlap were also included in the count. The distribution of MN number in each BN cells was also recorded. The MN yield was determined as $MN\ yield = (Total\ number\ of\ MN\ in\ BN\ cells / Total\ number\ of\ scored\ BN\ cells) \times 1000$. Percentage reduction of MN was determined as ¹³⁷Cs-induced MN yield in varying concentrations of NAGE compared to that with radiation alone.

Measurement of the Intracellular Total Antioxidant Capacity (TAC) in PBL

Using the Antioxidant Assay Kit (Sigma, CS-0790), at the end of Time 1 and Time 2, PBL (1×10^6 cells ml^{-1}) before and after ¹³⁷Cs exposure and incubated with different concentrations of NAGE were measured for intracellular TAC. The antioxidant assay is based on the formation of a ferryl myoglobin radical from myoglobin and hydrogen peroxide, which oxidizes ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] to produce a radical cation $ABTS^+$, a soluble green chromogen that can be determined at 405 nm. In the presence of antioxidants, the radical cation is suppressed to an extent dependent on the activity of the antioxidant and the color intensity is decreased proportionally. Trolox, a water-soluble vitamin E analogue, serves as a control antioxidant. In brief, PBL were sonicated on ice in 1 ml of cold 1 x Assay buffer and centrifuged at 12000 g for 15 min (4°C). In a 96-well culture plate, the supernatant of PBL lysates (10 μ l) in each well were mixed with 1X myoglobin working solution (20 μ l), ABTS substrate working solution (150 μ l), and 3% hydrogen peroxide (25 μ l) and allowed to incubate at 25°C for 30 min. For the Trolox standard curve, 10 μ l of a Trolox standard and 20 μ l of myoglobin working solution were added to each well. Kit stop solution (100 μ l) was then added to each well. Samples were read immediately at 406 nm excitation/530 nm emission on a plate reader. Results were calculated using a reference curve based on the soluble antioxidant Trolox as a standard, and intracellular TAC in PBL was expressed in mM Equivalent/L.

Measurement of Intracellular Oxidative Stress in PBL

Using the Live Cell Fluorescent Reactive Oxygen Species Detection Kit (MGT-M1049, Marker Gene technologies, Eugene, OR), at the end of Time 1 and Time 2, PBL (1×10^6 cells ml^{-1}) before and after ¹³⁷Cs exposure and incubated with different concentrations of NAGE were measured for intracellular ROS level. In brief, the culture medium was aspirated after centrifugation (1000 g, 15 min) and pellets were suspended in ROS inducer, i.e. t-butyl hydroperoxide (TBHP) working solution (100 μ M), then incubated for 60 min at 37°C. After centrifugation, PBL pellets were resuspended and incubated in darkness for 45 min in 2',7'-dichlorofluorescein diacetate (25 μ M), which is a cell-permeable substrate and a reliable fluorogenic marker for ROS detection. Upon enzyme activity, the highly fluorescent dye, 2',7'-dichlorofluorescein was produced. The cellular fluorescence from each well was determined using 480 nm excitation/535 nm emission with a microplate spectrofluorometer. The values of fluorescence intensity, reflecting

the intracellular concentration of ROS in PBL, were expressed as percentage of controls.

Statistical Analyses

All measurements were represented as the mean and standard error of the mean (\pm SEM) and were blinded as to subject status. We used the software package SPSS for the data analysis [22]. Statistical methods consisted of repeated measures of ANOVA and linear regression using a mixed-model approach with random intercepts. At different time points (0 h or 24 h before- irradiation), and at each radiation dose (0 - 2 Gy), linear contrasts were used to examine the effect of NAGE (0 -1000 μ g ml^{-1}) on radiation-induced MN yield in PBL, and were completely cross-classified in a factorial fashion. The effect of radiation on MN yield of PBL with the presence and absence of NAGE, and the interactions between radiation doses and concentrations of NAGE were evaluated separately. Associations between MN yield, and intracellular TAC and ROS levels in PBL were assessed by Pearson's correlation test.

RESULTS

1. Effect of NAGE on MN Yield in PBL Before ¹³⁷Cs Irradiation

Before radiation exposure and in the absence of NAGE, mean (\pm SEM) baseline MN yield of PBL obtained from the 10 healthy volunteers was 15.1 ± 2.1 and 15.3 ± 1.4 per 1000 BN cells at Time 1 and Time 2, respectively. The presence of NAGE (250-1000 μ g ml^{-1}) in PBL culture medium at different time points did not affect the MN yield significantly (Figs. (1A, 2A), $P > 0.05$).

2. Effect of NAGE on MN Yield in PBL After ¹³⁷Cs Irradiation

Radiation alone (1 Gy and 2 Gy) sharply increased the MN yield in PBL in a dose-dependent manner ($P < 0.001$). However, at both time points, NAGE significantly reduced the MN yields as NAGE concentration increased (Figs. (1A, 2A), $P < 0.001$). The best fitting line for the relationship between increasing NAGE concentrations and ¹³⁷Cs-induced MN yield was $Y = C + \alpha D + \beta D^2$ ($P < 0.001$), where Y is the MN per 1000 BN cells and C is the intercept (spontaneous MN frequency), D is the NAGE concentration (μ g ml^{-1}), α and β are the linear and quadratic coefficients, respectively. Our results also suggested that the optimal radioprotective NAGE concentration appears to be 500 μ g ml^{-1} (Figs. 1A, 2A). Based on the results of multivariate analyses, the reduction of MN yield in PBL was more significant (F-value = 3.92, $P = 0.02$) when NAGE (500 μ g ml^{-1}) was applied at 24 h before irradiation (Fig. 2A). Compared with radiation alone, Table 1 shows that application of 500 μ g ml^{-1} NAGE at 0 h reduced MN yield by 42.1% after 1 Gy and 27.2% after 2 Gy exposure, respectively. When NAGE (500 μ g ml^{-1}) was applied at 24 h before radiation exposure, reduction rate of MN yield increased to 51.1% and 49.1% after 1 Gy and 2 Gy exposure, respectively.

3. Effect of NAGE on Intracellular TAC Status in PBL Before and After ¹³⁷Cs Irradiation

Figs. (1B, 2B) illustrate the variations of intracellular TAC levels in PBL under different experimental conditions.

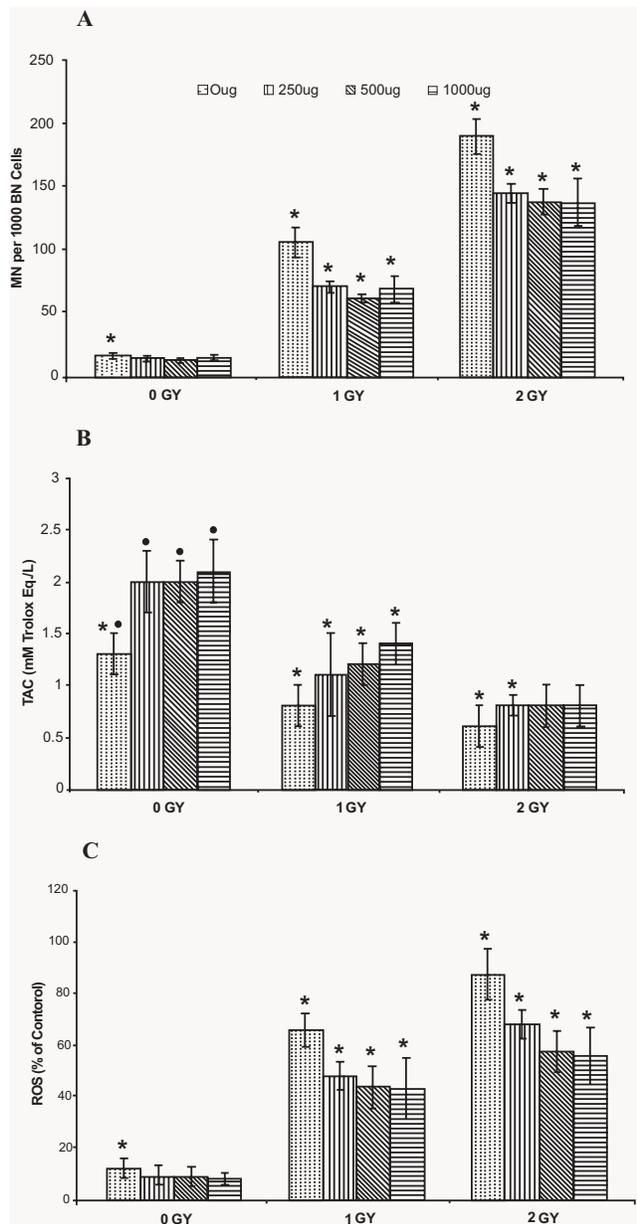


Fig. (1). Effect of NAGE ($\mu\text{g ml}^{-1}$) applied at 0 h (Time 1) on (A) ^{137}Cs -induced MN yields in PBL; (B) intracellular TAC levels (mM Trolox Eq/L) in PBL before and after ^{137}Cs irradiation; and (C) intracellular ROS levels (% of control) in PBL before and after ^{137}Cs irradiation. Each bar represents the mean \pm SEM of duplicate independent measurements pooled from each individual compared to their respective irradiated controls (* $P < 0.001$, • $P < 0.02$).

Before ^{137}Cs irradiation and in the absence of NAGE in PBL culture medium, the baseline TAC level (mM Trolox equivalent/L) in PBL was 1.3 ± 0.2 (Time 1) and 1.1 ± 0.4 (Time 2). However, the baseline TAC level increased significantly in PBL before irradiation with increments in NAGE concentration (250-1000 $\mu\text{g ml}^{-1}$) in a concentration-dependent manner ($P < 0.02$). In contrast, after the ^{137}Cs exposure (1 Gy and 2 Gy) of PBL at both time points, the baseline TAC levels in PBL decreased with increased radiation dose in a linear manner ($P < 0.001$). As compared with radiation alone (1 Gy and 2 Gy), the TAC levels in irradiated PBL at both time points increased with the increments of NAGE concentra-

tion, although this increment of TAC was significantly lower than that in PBL before irradiation.

4. Effect of NAGE on Intracellular ROS Status (% of Control) in PBL Before and After ^{137}Cs Irradiation (Figs. 1C, 2C)

In the absence of NAGE at 0 Gy, the baseline fluorescent ROS level (% of control) in PBL was 12.2 ± 3.8 (Time 1) and 10.8 ± 2.1 (Time 2). After ^{137}Cs exposure of PBL, it increased significantly with radiation dose to 65.7 ± 6.4 (Time 1) and 57.3 ± 6.9 (Time 2) after 1 Gy, and to 87.2 ± 9.9 (Time 1) and 78.6 ± 7.5 (Time 2) after 2 Gy exposure. However, at both time points and with the presence of NAGE in culture medium, ROS in irradiated PBL decreased significantly with the increments in NAGE concentrations ($P < 0.001$).

DISCUSSION

Since ionizing radiation (IR) initiates intracellular oxidative stress through enhanced ROS formation, there is great interest in developing interventions with natural antioxidants as possible radiation modifiers [5-8, 23, 24]. However, most results from different studies on the supplementation of dietary antioxidants to prevent oxidative DNA damage were inconclusive [23-25]. In proliferating cells, damage to chromosomes is manifested as breaks and fragments, which appear as micronuclei (MN). Due to the high phospholipid content of the cell membrane, human peripheral blood lymphocytes (PBL) are vulnerable to oxidative damage [12].

We have reported our preliminary results on the radioprotective effects of both Asian ginseng [9] and American ginseng [19] on human PBL evaluated by CBMN assay. However, the exact radioprotective mechanism of ginseng is unclear [9, 10], probably it is related to the antioxidative properties of ginsenosides, which protect the outer membrane of mammalian cells [12], and scavenge free radicals responsible for DNA damage [10, 17, 18, 26]. These actions may be mediated by various ginsenosides or their metabolites through different pathways [16, 27].

Intracellular oxidative stress occurs when ROS levels exceed the antioxidant capacity for scavenging them. Thus, by removing ROS, cellular antioxidants play an important part in limiting damage to DNA, protein and lipids. In this study, to explore the effect of a standardized North American ginseng extract (NAGE) on ^{137}Cs -induced oxidative stress in human PBL, we measured the MN yields along with the levels of intracellular total antioxidant capacity (TAC) and ROS production in PBL at two different time points of NAGE application, i.e. 24 h before and at 0 h of ^{137}Cs exposure. Intracellular TAC level reflects a state of dynamic balance between oxidant and antioxidant elements in the cell [28]. Intracellular ROS level indicates the presence and degree of oxidative stress, and thus it provides an indication of the cellular redox state [29]. At both time points, we found that across the applied 1-2 Gy radiation dose range, the yields of MN in PBL in the presence of NAGE were consistently lower than those in the absence of NAGE ($P < 0.001$, Figs. (1A, 2A)).

These results have confirmed our previous findings that NAGE renders protection against ^{137}Cs -induced DNA damage in human PBL in a concentration-dependent manner

Table 1. Effects of NAGE (500 µg ml⁻¹) on ¹³⁷Cs-Induced MN Yield in BN Lymphocytes

Time	Gy	NAGE	Tot. BN	Tot. MN	MN per 1000 BN Cells		
					Mean	SEM	Reduction (%)
1	1	0	8671	915	105.5	11.8	
		500	9846	601	61	3.5	42.1
	2	0	9554	1817	190.2	13.9	
		500	9960	1379	138.5	10.2	27.2
2	1	0	8822	997	113	8.1	
		500	7276	402	55.3	9.1	51.1
	2	0	11370	2341	205.9	14.7	
		500	7157	750	104.8	7.6	49.1

NAGE was applied at Time 1 (0 h) and Time 2 (24 h before radiation exposure). Reduction of MN yield (%) was determined as ¹³⁷Cs-induced MN yield in 500 µg ml⁻¹ NAGE compared to that with radiation alone.

[19]. Furthermore, we found that the increment of ¹³⁷Cs-induced MN yields is positively correlated with the increment of intracellular ROS production but negatively correlated with TAC levels (Figs. 3, 4). Under our experimental conditions, the intracellular TAC represents the cumulative antioxidant capacity in PBL, including both NAGE-derived antioxidants and those of endogenous origin. Both in the absence of radiation exposure and at both time points before radiation exposure, the presence of NAGE in the culture medium (250-1000 µg ml⁻¹) resulted in a significantly increased TAC in PBL compared with controls (Figs. 1B, 2B), which strongly suggests the presence of NAGE antioxidative micronutrients in PBL. The rise in ROS generation began at the time of ¹³⁷Cs exposure in a radiation dose-dependent manner (Figs. 1C, 2C); the significantly decreased TAC levels in the absence of NAGE (Figs. 1B, 2B) indicate that the endogenous TAC in PBL was not able to compensate totally for the new pro-oxidant/antioxidant imbalance. However, compared to the controls, the application of NAGE significantly increased intracellular TAC levels in irradiated PBL and was accompanied by a significant decrease in both ROS and MN yields in a concentration-dependent manner (Figs. 1, 2). This direct evidence supports our hypothesis that the radioprotective effect of NAGE on human PBL is related to its antioxidative activities.

Antioxidants are substances that either directly or indirectly against harmful ROS effects [7] and thus maintain a pro-oxidant/antioxidant balance in the cells. Our findings concerning the reverse correlation between elevation of TAC and reduction of ROS in PBL in the presence of NAGE indicate the importance of supplementation *ex vivo* for strengthening cellular TAC antioxidant defense systems.

The antioxidative ability of ginseng is closely related to its ginsenoside content. The standardized NAGE we used for this study contains the highest total ginsenoside concentration (11.7%) currently available on the market [19]. Ginsenosides in NAGE have the ability to intercalate into the plasma membrane, change its fluidity, and inhibit lipid peroxidation by chelating transition metals and scavenging ROS [12, 14, 15]; ginsenosides thus affect membrane function, eliciting cellular responses to cytotoxic stresses [11].

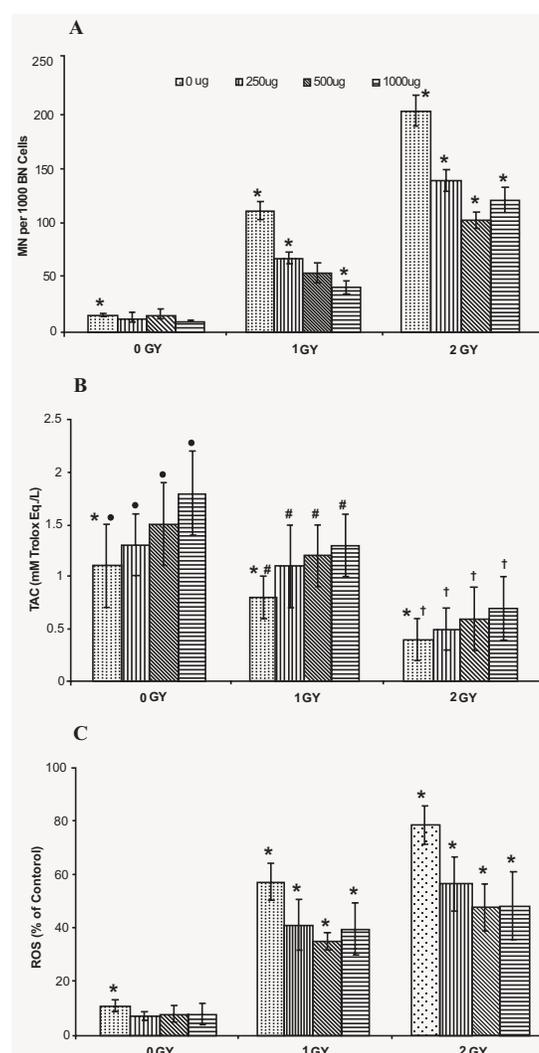


Fig. (2). Effect of NAGE (ug ml⁻¹) applied at 24 h before irradiation (Time 2) on (A) ¹³⁷Cs-induced MN yields in PBL; (B) intracellular TAC levels (mM Trolox Eq./L) in PBL before and after ¹³⁷Cs irradiation; and (C) intracellular ROS levels (% of control) in PBL before and after ¹³⁷Cs irradiation. Each bar represents the mean ± SEM of duplicate independent measurements pooled from each individual compared to their respective irradiated controls (**P*<0.001, **P*<0.02, #*P*<0.01, †*P*<0.05).

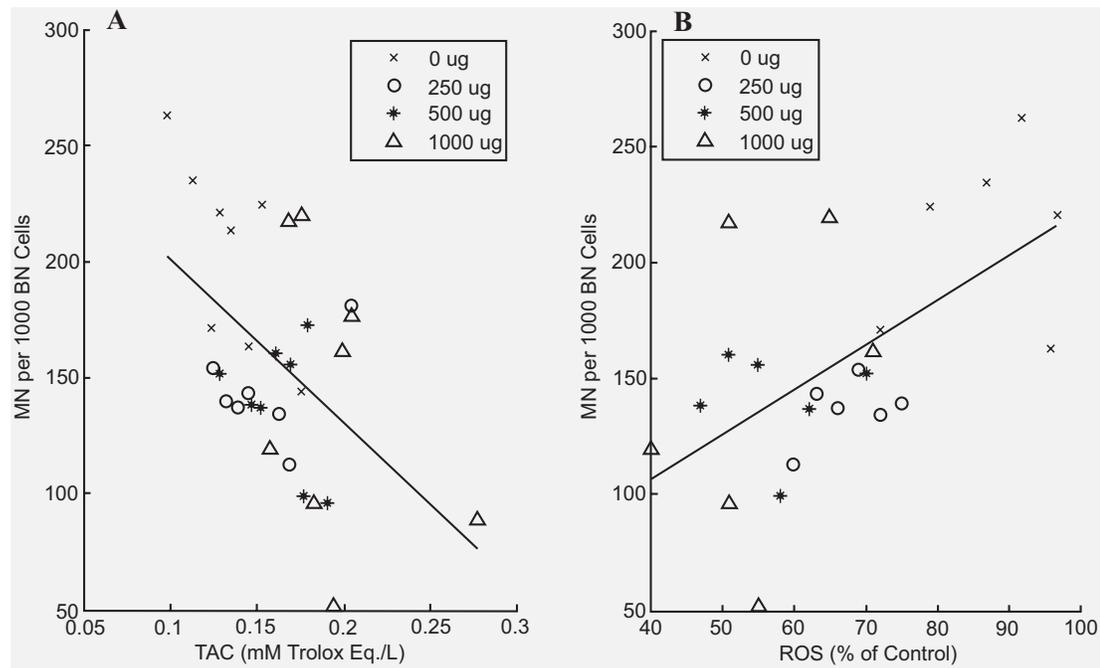


Fig. (3). Correlation between IR-induced MN yields and intracellular (A) TAC levels (mM Trolox Eq./L, $R = -0.5$, $P = 0.004$) and (B) ROS levels (% of control, $R = 0.6$, $P = 0.002$) in PBL with the presence of NAGE at different concentrations applied at 0 h (Time 1).

It is known that radioprotective agents are most effective when applied before radiation exposure and must be present in the system at the time of irradiation [1, 2, 30]. We found in this study that when NAGE ($500 \mu\text{g ml}^{-1}$) was applied at 24 h before radiation exposure (Table 1), the reduction rate of MN yield in PBL was 51.1% and 49.1% after 1 Gy and 2 Gy irradiation, respectively; in contrast, when the same concentration of NAGE was applied at 0 h, the reduction rate of MN yield was 42.1% and 27.2% after 1 Gy and 2 Gy irradiation,

respectively. These results suggest that NAGE should be inside the PBL before and during the time of radiation exposure to exert a radioprotection effect. As we used a lysed PBL preparation for the evaluation of oxidative stress, the removal of possible co-operative and synergistic intracellular constituents may diminish the antioxidant effects [31], however, our results unambiguously demonstrated the anti-oxidative effect of NAGE.

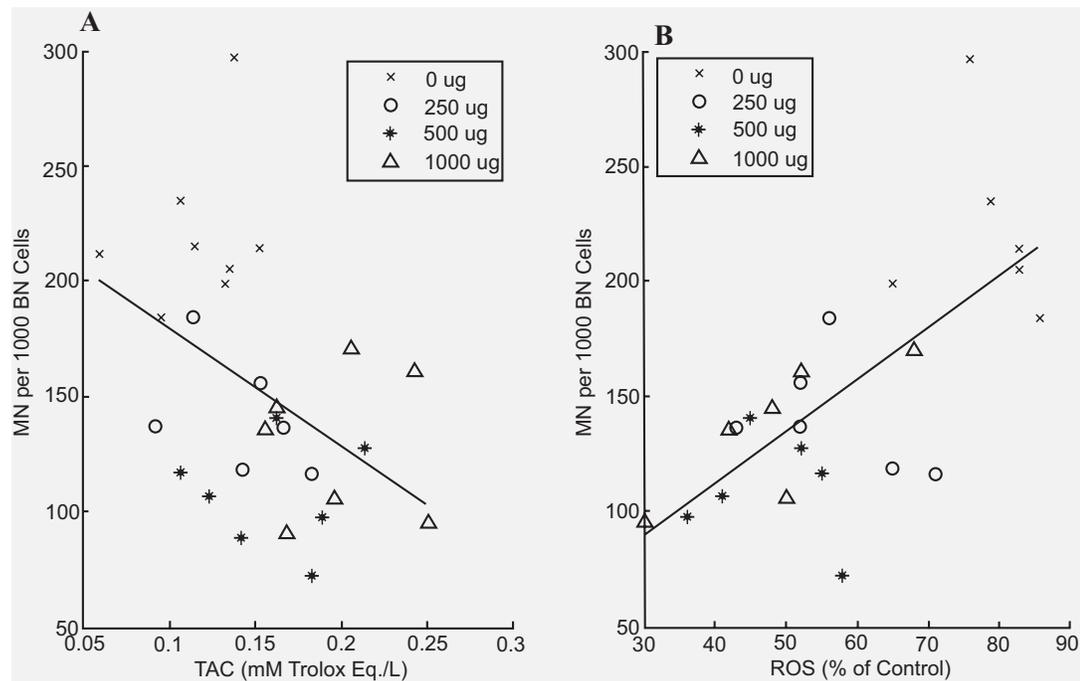


Fig. (4). Correlation between IR-induced MN yields and intracellular (A) TAC levels (mM Trolox Eq./L, $R = -0.43$, $P = 0.02$) and (B) ROS levels (% of control, $R = 0.70$, $P = 0.002$) in PBL with the presence of NAGE at different concentrations applied at 24 h before ^{137}Cs irradiation (Time 2).

There are various intracellular antioxidant mechanisms of DNA protection [31]. Koren *et al.* [25] recently stated that the intracellular TAC is maintained under tight regulation, and that supplementation with exogenous antioxidants fails to enhance the overall intracellular TAC. Their assumption was based on the effects of uric acid and other low molecular weight antioxidants on four human cell lines, including three cancer cell lines cells. However, we found that the intracellular TAC in PBL increased significantly along with NAGE concentrations, either before or after radiation exposure (Figs. 1B, 2B). Our results show the effectiveness of *ex vivo* supplementation by an antioxidant in improving the cellular antioxidant defense system. Our results are in agreement with other reports that antioxidant supplementation reduces chromosomal aberrations in human PBL and other mammalian cells [23, 24, 31, 32]. We believe that it is logical to speculate that plant-derived antioxidative compounds enhance intracellular TAC [33, 34] and may ameliorate IR-induced normal tissue damage *in vivo* [5-8].

In conclusion, the results generated from this study provide strong evidence that a standardized NAGE is a relatively non-toxic natural compound with antioxidative and radioprotective activities, which is a precondition for further assessing its efficacy as a countermeasure during accidental irradiation or acts of terrorism scenarios.

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