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Effect of North American Ginseng on 137Cs-induced Micronuclei in Human Lymphocytes:

A Comparison with WR-1065

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INTRODUCTION

One of the major mechanisms of ionizing radiation (IR)-induced cell death is the generation of reactive oxygen species (ROS) that attack cellular DNA (McBride *et al*, 2004; Moller and Loft, 2004; Moulder, 2002). For cancer patients receiving radiotherapy (RT), IR-induced normal tissue damage is a dose-limiting factor (Lee *et al*, 2003; Moulder, 2002). This obstacle to cancer treatment, along with the increasing threat of bioterrorism, creates an urgent need for the identification of effective radiation countermeasures (Moulder, 2002; Stone *et al*, 2004).

Ginseng is one of the most frequently purchased herbs in the United States. The two most commonly used species, *Panax ginseng* C.A. Meyer (Asian ginseng) and *Panax quinquefolius* L. (North American ginseng), have drawn widespread attention for their multifold medicinal bioactivities (anti-diabetic, anti-carcinogenic, anti-pyretic, analgesic, anti-aging, anti-stress, and anti-fatigue effects), and for their promotion of DNA, RNA, and protein synthesis (Attele *et al*., 1999; Kitts *et al*, 2000; Lee *et al*., 2005). Moreover, because of its effective antioxidant capacity, studies on the radioprotective effects of Asian ginseng have engendered interest, but the research has primarily been done in animal models (Arora *et al*., 2005; Kim *et al;* 2007; Han *et al*., 2005; Lee *et al*., 2006; Lee *et al*., 2005). We recently demonstrated in human peripheral blood lymphocytes (PBL) the ability of a crude water extract of Asian ginseng, applied at 24 h before radiation exposure to prevent ^{137}Cs-induced micronuclei (MN) formation *ex vivo* in a dose-dependent manner (Lee *et al*., 2004a). These

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findings have led us to more thoroughly investigate the potential of ginseng as a safe and natural radioprotector. Because the bioactive components of ginseng and its radioprotective potential, are linked with its ginsenoside content (Attele *et al*., 1999; Gillis., 1997; Kitts *et al*., 2000; Lee *et al*., 2005), it has become apparent that a more standardized source of ginseng than we used in our original study (Lee *et al*., 2004a) is essential to avoid batch-to-batch variations. Thus, we have switched our focus from the use of Asian ginseng to a standardized formulation of North American ginseng extract (NAGE) that contains the highest ginsenoside concentration (11.7%) currently available on the market (Hall *et* al., 2001).

Micronucleus (MN) formation is one of the standardized biomarkers for assessing IR-induced damage both *in vivo* and *ex vivo* (Fenech, 2005). To evaluate the radioprotective potential of NAGE, objectives of this study were to (1) evaluate whether the application of NAGE to culture medium at 0 h and even 90 min after radiation exposure would reduce the MN yields in human PBL *ex vivo*; and (2) assess whether this MN reduction, if present, is NAGE concentration-dependent. In addition, WR-1065, a prodrug that becomes active after dephosphorylation, is the biologically active aminothiol form of amifostine (Hoffman *et al*., 2001; McBride *et al*., 2004). Since amifostine (WR-2721) is currently the "gold standard" of radioprotectors, we compared our results with those from comparable experiments, using optimal concentrations of WR-1065, as in our previous study (Lee *et al*., 2004b), to further verify the radioprotective potential of NAGE. We believe that the information generated from this study will provide a foundation for clinical trials assessing the potential of standardized dietary NAGE supplements as an effective natural radiation countermeasure, either before or after radiation exposure; it would thus be a significant contribution to public health, national defense and environmental remediation, not only for cancer patients undergoing RT, but also for victims of accidental or terrorist exposure.

MATERIALS AND METHODS

Subjects

This study was approved by our University Medical Center Institutional Review Board. Healthy individuals (6M/6F) with a mean age of 46.4 ± 15.7 years (mean ± SEM) without known history of exposure to mutagens were included in this study; all of them signed informed consent before enrollment. No subject was currently taking any other pharmacologic agent, including medications, vitamins, or dietary supplements.

NAGE preparation and ginsenosides

We purchased the standardized NAGE powder (Lot #TKGS-010406) from the Canadian Phytopharmaceuticals Corporation (Richmond, BC, Canada). Using high-performance liquid chromatography, the vendor characterized the major ginsenosides in this NAGE powder as follows: Rb1 (5.1%), Rb2 (0.99%), Rc (1.88%), Rd (1.23%), Re (2.14%), and Rg1 (0.36%), with a total ginsenoside content (w/w) of 11.7%. To ensure stability, we stored the NAGE in a cool, dry, dark location over the course of the study. Before experimentation, we filtered a known concentration of a solution of freshly prepared lyophilized NAGE powder in RPMI 1640 culture medium through a 0.2 μm disc (Millipore, MA) under sterile conditions, to be used as the stock solution.

WR-1065 preparation

Dr. Robert J. Schultz (Drug Synthesis and Chemistry Branch, NIH-NCI, Bethesda, MD, USA) kindly provided WR-1065. Immediately before use, we prepared a stock solution of WR-1065 in RPMI 1640 culture medium and filtered it through a 0.2 μm disc (Millipore, MA).

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Cytokinesis-block micronucleus (CBMN) assay

Fresh peripheral blood samples were collected from each subject 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. Trypan blue exclusion showed their viability to be > 95%. The purity of mononuclear cells was > 95% as determined by Hema-3 staining (Fisher Scientific, NC, USA). The cells 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. Phytohemagglutinin (PHA, M Form, Invitrogen Corp. CA, USA) was added to 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 μg ml^{-1}. All cultures were maintained in a humidified atmosphere of 5% CO_2 at 37°C, and were terminated after another 24 h. Slides were prepared according to Fenech *et al* (1985) and stained with Hema-3 (Fisher Scientific, NC, USA).

Application of NAGE

We carried out a series of preliminary studies to ascertain the optimum radioprotective dose of NAGE; these studies showed that treatment of PBL with NAGE at 500-750 ug ml^{-1} at 0 h caused a significant reduction in ^{137}Cs-induced MN yield. Therefore, to determine a dose-response radioprotective effect of NAGE, in each experiment we applied five different NAGE concentrations (50, 250, 500, 750, and 1000 μg ml^{-1}) to mononuclear cell cultures (2-3 × 10^5 cells ml^{-1}) in RPMI 1640 at 0 h and at 90 min post irradiation for the CBMN assay.

Application of WR-1065

For each experimental condition, we serially diluted the stock solution of WR-1065 with the culture medium to the desired final concentrations (1 m*M* or 3 m*M*). We chose these two different concentrations of WR-1065 for quantification of their radioprotective effect on PBL based on our previous study (Lee *et al*., 2004b). We then applied WR-1065 (1 m*M* or 3 m*M*) to mononuclear cell cultures (2-3 × 10^5 cells ml^{-1}) at 0 h and 90 min post irradiation. After the 10-minute treatment with WR-1065, cell cultures were centrifuged, washed with phosphate-buffered saline (PBS) to remove the WR-1065, and resuspended in the RPMI 1640 culture medium for the completion of the CBMN assay.

Ex vivo irradiation

The human G_0 PBL in the presence or absence of NAGE and WR-1065 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 guarantee anonymity, and only one researcher (WW) performed the microscopy to ensure consistency of scoring. Under 400X magnification, in continuous fields from two slides prepared for each experimental check point, a minimum of 1000 consecutive nucleated PBL were evaluated for the numbers of lymphocytes that had proceeded through one or more cell cycles, including mononucleated, binucleated (with or without MN formation), and cells with more than two nuclei (>2 nuclei). Further, for the determination of MN yield at each experimental checkpoint, a minimum of 1000 binucleated (BN) cells were scored when possible. The quantification of MN yield was restricted to BN cells with distinct intact cytoplasm, including 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 cell was recorded as well. The MN yield

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was determined as MN yield = (total number of MN in BN cells/total number of scored BN cells) × 1000. Percentage reduction of MN was determined as the ratio of ^{137}Cs - induced MN yield in varying concentrations of NAGE or WR-1065 to the MN yield with radiation alone. The micronucleated (MN+) BN index was calculated as MN+BN = (Total number of micronucleated BN cells/total number of BN cells scored) × 100. The proliferation index (PI) of PBL for each experimental point was determined as PI = [(1 × number of mononucleated cells) + (2 × number of BN cells) + (3 × number of cells with >2 nuclei)] / total number of scored cells (Littlefield *et al*., 1993).

Statistical analyses

We used the software package SPSS (2001) for the data analysis; all data were blinded as to subject status. Measurements were summarized as the mean ± SEM (standard error of the mean). Statistical methods consisted of repeated measurements, with analysis of variance and linear regression, both using a mixed models approach with random intercepts. At different time points (0 h or 90 min post irradiation), linear contrasts were used to examine the effect of NAGE (0 - 1000 μg ml^{-1}) and WR-1065 (1 m*M* and 3 m*M*) on the 0 - 2 Gy radiation-induced MN yield in PBL. To evaluate the MN yield distributions relative to the Poisson distribution, the variance-to-mean ratio (δ^2/μ) and μ-parameter were defined by a goodness of fit test (Savage, 1970). Radiation doses, time points, concentrations of NAGE and WR-1065, and MN yields in different individuals were completely cross-classified in a factorial fashion. The effect of radiation on MN yield and PI of PBL with and without the presence of NAGE and WR-1065, and the interactions between radiation doses and concentrations of NAGE and WR-1065 were evaluated separately.

RESULTS AND DISCUSSION

After a mutagenic attack, the micronuclei (MN) in interphase cells are derived from the mitotic loss of acentric fragments or from lagging chromosomes that are not incorporated into the daughter nuclei (Fenech and Morley, 1985). The degree of MN formation represents a particularly interesting endpoint covering both clastogenicity and aneugenicity induced by mutagens in mammalian cells (Decordier and Kirsch-Volders, 2006). Because of its reliability and technical ease, and because of the direct correlation between MN formation and genomic damage, the cytokinesis-block MN (CBMN) assay of peripheral blood lymphocytes (PBL) is a sensitive measure of *in vivo* cytogenetic damage in patients receiving partial-body radiotherapy (Catena *et al.*, 1996; Lee *et* al., 2003). Therefore, we employed the CBMN assay of PBL in this study. The effects of NAGE and WR-1065 applied at different time points on MN yield (mean ± SEM) and MN+BN index in irradiated and non-irradiated PBL are summarized in Tables 1 - 4. We found that in the absence of NAGE or WR-1065, the mean (±SEM) baseline MN yield of PBL obtained from 12 healthy individuals at 0 Gy ranged from 14.4 ± 1.5 to 15.9 ± 1.5 per 1000 BN cells (P>0.05). At 0 h, with the increment of NAGE concentrations in PBL culture medium, a trend of decline from the mean baseline MN yield appeared, with a reduction rate of up to 31.6% when the applied concentration of NAGE was 750 μg ml^{-1}, compared to that in the absence of NAGE. A comparable trend also appeared for in the MN$^+$BN yield, but regression analysis indicated these trends were not statistically significant (P >0.05). At 0 Gy, the different concentrations of NAGE and WR-1065 applied at various time points did not affect the MN$^+$BN index in PBL (P >0.05, Tables 3 - 4). In contrast, radiation alone at 1 Gy and 2 Gy linearly increased the MN yield to 128.0 ± 7.1 (Table 2) and 247.8 ± 10.3 (Table 1) per 1000 BN cells, respectively. However, at 0 h or even at 90 min post irradiation (Figures 1 - 2), application of NAGE to PBL culture medium induced a strong decline in MN yields per 1000 BN cells as NAGE concentration increased (P < 0.0001). Compared with radiation alone at 1 Gy, NAGE reduced the MN yield by a minimum of 22.5% when the lowest concentration (50 ug ml^{-1}) was applied at 0 h, while the maximum reduction

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of MN yield was 53.8% when 750 ug ml^{-1} of NAGE was applied at 90 min post irradiation. After 2 Gy irradiation , the minimum reduction of MN yield was 19% when the lowest concentration of NAGE (50 ug ml^{-1}) was applied at 90 min post irradiation, while the maximum reduction of MN yield rose to 48.8% when 750 μg ml^{-1} of NAGE was applied at 0 h. The best fit for the relationship between increasing NAGE concentrations and ^{137}Cs-induced MN yield in BN lymphocytes was a simple linear regression model (Table 5): Y = Intercept + (Slope) *D* (*P* <0.0001), where *Y* is the MN yield per 1000 BN cells and *D* is the NAGE concentration (50-1000 ug ml^{-1}). There was evidence of a bending upward, or positive quadratic component, at 0 h (Fig. 1). However, the quadratic trend was shallow and could not be reliably estimated. A similar trend was also found in MN+BN index response to NAGE treatment afterirradiation (Table 3 - 4).

We previously found that when compared to radiation alone, a 24-hr incubation with self-prepared Asian ginseng crude water extract (500 μg ml^{-1}) reduced MN yields by 22% after 1 Gy exposure and 22.5% after 2 Gy exposure in PBL cultures obtained from four healthy individuals (Lee *et al*., 2004a). In the present study of PBL obtained from 12 healthy individuals, application of 500 μg ml^{-1} of standardized NAGE at 0 h reduced MN yields by 45.3% after 1 Gy and 45.1% after 2 Gy exposure (Table 1), compared to MN yields from irradiated, unprotected PBL. Even at 90 min post irradiation, 500 μg ml^{-1} of NAGE reduced MN yields in PBL by 37.7% and 28.6% after 1 Gy and 2 Gy irradiation respectively, compared to MN yields after radiation alone (Table 2). In both studies, the decline in radiation-induced MN yield in PBL was inversely correlated with ginseng concentration in a linear fashion, although the ginsenoside content in the previous study was unknown. As mentioned above, the concentrations of total ginsenosides (w/w) in Asian ginseng root usually falls in the range of 1.5% - 7% (Liu and Xiao., 1992), and 4% is the concentration of the most widely used standardized Asian ginseng extract G115 (Pharmaton, Switzerland). In contrast, the NAGE used in the current study was standardized to a total of 11.7% (w/w) ginsenoside content, the highest currently available (Liu and Xiao., 1992); thus, NAGE showed a much more pronounced radioprotective effect than did Asian ginseng when evaluated by the MN yield in human PBL (Lee *et al*., 2004a).

At both 0 h and 90 min post irradiation, WR-1065 treatment resulted in a significant reduction of MN yields per 1000 BN cells with a linear-quadratic trend as WR-1065 concentration increased (Table 5, *P* <0.004). The best fit regression equation of the relationship between the concentration of WR-1065 and ^{137}Cs-induced MN yield in BN lymphocytes was: Y = Intercept + (Slope)D + (Curvature)D2 (*P*<0.004), where *Y is* the MN yield per 1000 BN cells and *D* is the WR-1065 concentration (1 m*M* or 3 m*M*). Compared with radiation alone at 1 Gy, WR-1065 reduced the MN yield in PBL by a minimum of 47% when 1 m*M* was applied at 90 min post irradiation (Table 2), while the maximum reduction in MN yield was 61.2% when 3 m*M* was applied at 0 h (Table 1). After 2 Gy irradiation, WR-1065 reduced the MN yield in PBL by a minimum of 36.2% when a concentration of 1 m*M* was applied at 0 h, and the maximum reduction rose to 54.4% when a concentration of 3 m*M* was applied at 0 h (Table 1). The trend in MN^{+}BN yield response to WR-1065 treatment at different time points after irradiation was also similar to that of MN decline (Tables 3-4).

Based upon the goodness of fit tests for the Poisson distribution, the MN yield data obtained from the different experimental conditions were significantly overdispersed. Overdispersion indicates a disproportionally increased incidence of PBL carrying MN as described by a negative binomial distribution. The MN distributions in this study did not follow a Poisson distribution but deviated from it (data not shown); our results agree well with those reported in the literature (Catena *et al*., 1996). At no point in time did application of NAGE and WR-1065 to PBL culture medium show any significant effect on the PI of PBL either before or after the *ex vivo* ^{137}Cs irradiation (data not shown).

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The data we generated in this study provide strong evidence that standardized NAGE protected against ^{137}Cs-induced MN formation in human PBL *ex vivo* in a concentration-dependent manner. Across the applied 1-2 Gy radiation dose range, the yields of MN in PBL treated with NAGE were consistently lower than those without NAGE treatment at 0 h and even at 90 min post irradiation. This highly significant decline in MN yield correlated in a linear fashion with NAGE concentration (Table 5). Tables 1 - 2 show that the extent to which NAGE administered at different points in time reduced the MN yield induced in PBL by 1 Gy or 2 Gy irradiation ranged from 22.5% - 53.8% and 19% - 48.8%, respectively. Since unrepaired or misrepaired DNA damage is responsible for MN formation in human PBL (Fenech, 2005), these findings indicate the radioprotective potential of NAGE.

Because radioprotective agents are known to be most effective when applied before IR exposure, and must be present in the system at the time of irradiation (Coleman *et al*., 2004; Kumar *et al*., 2003; Moulder, 2002); thus, the time of administration of a radioprotector is critically important. In our previous preliminary report (Lee *et al.*, 2004a), we found that the administration of Asian ginseng crude water extract to human PBL *ex vivo* 24 h before radiation exposure resulted in a significant linear decline of MN yields as ginseng concentration increased**.** In the current study, we further demonstrate for the first time that the application of standardized NAGE (50 - 1000 μg m^{l-1}) to PBL cultures obtained from 12 healthy volunteers was radioprotective not only at 0 h, but also at 90 min post irradiation (Tables 1 - 2, Fig. 1 - 2). Furthermore, after 2 Gy irradiation of PBL (Table 5), the resulting regression equation is: $Y = 198.23 + (-0.06)D$, where *Y* is the MN yield per 1000 BN cells and *D* is the NAGE concentration. Therefore, this model suggests theoretically that at 90 min after a 2 Gy irradiation, the application of the lowest NAGE concentration (50 ug ml^{-1}) induced a reduction of MN yield from 198.23 to 195.23, with a reduction of 3 MN per 1000 BN cells; and the application of the highest NAGE concentration (1000 ug ml^{-1}) induced a reduction of MN yield from 198.23 to 138.23, with a reduction of 60 MN per 1000 BN cells. The observed extended NAGE radioprotection time of 90 min post irradiation in PBL indicates a longer window of protection against IR exposure *ex vivo*. We believe this new finding may be particularly important for the triage management of IR-exposed victims.

The radioprotective effect of NAGE on MN yield in human PBL is further supported by two additional MN-related parameters: MN^{+}BN index, and the number of MN per BN cell. Because MN^{+}BN index is the percentage of the total number of BN cells with a varying number of MN in the cytoplasm, it indicates the spectrum of DNA damage in BN cells (Lee *et al.*, 2004a; Lee *et al*., 2005). Because a direct correlation exists between MN yield and genomic damage (Fenech, 2005), the number of MN per BN cell is a reflection of the degree of IR-induced DNA damage within a cell. We found that in the irradiated PBL population, increasing concentrations of NAGE in the culture medium resulted in a decrease in both MN^{+}BN index and number of BN lymphocytes containing ≥ 2 MN (Table 3-4), implying the efficacy of NAGE in reducing IR-induced DNA damage. Furthermore, the radioprotection of NAGE occurred without apparent genomic toxicity, based on the fact that incubation with PBL at a concentration up to 1000 μg ml^{-1} caused no increase in MN yield (Tables 1 -2).

Amifostine (WR-2720), the most comprehensively studied radioprotector, is the only radioprotective drug that is FDA-approved for the prevention of xerostomia in head and neck cancer patients undergoing radiotherapy. Because MN induction in PBL is an accurate parameter for measuring the radioprotective potential of aminothiols (Littlefield *et al*., 1993), we used it as the end point in comparing the radioprotective efficacy of NAGE with that of the synthetic aminothiol WR-1065, the active metabolite of amifostine. MN reduction is the ratio of relative reduction of MN yield in PBL incubated with different concentrations of NAGE or WR-1065, compared to MN yield with radiation alone. We found that at 0 h (Table 1) after 1 Gy irradiation, the maximum MN reduction caused by NAGE and WR-1065 was 49.2% and

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61.2%, respectively; after 2 Gy irradiation, it was 48.8% and 54.4%, respectively. At 90 min post irradiation (Table 2) of 1 Gy, the maximum MN reduction caused by NAGE and WR-1065 was 53.8% and 59.2%, respectively; after 2 Gy irradiation, it was 37.3% and 42%, respectively. Our results indicate that the radioprotective potential of NAGE against ^{137} Cs-induced MN formation in PBL is comparable with that of WR-1065.

Since the proliferative index (PI) in culture cells reflects cell cycle kinetics, we further determined the PI in PBL before and after ^{137}Cs exposure to determine whether different concentrations of NAGE (50 - 1000 μgml^{-1}) and WR-1065 (1 or 3 m*M*), or their metabolites might stimulate or inhibit mitosis. We found that PI was not altered significantly in the presence of NAGE or WR-1065 at different time points (data not shown), suggesting that neither before nor after irradiation did NAGE or WR-1065 alter subsequent cell cycle progression or the ability of PBL to respond to PHA stimulation. These results are in agreement with those reported in the literature (Lee *et al*., 2004a; Littlefield *et al*., 1993). Further, we found that the intercellular distribution of MN in PBL follows a non-Poisson distribution in all cases.

It is known that IR induces DNA damage in mammalian cells predominantly by direct ionization and through generation of hydroxyl radicals that attack cellular DNA. After radiation exposure, WR-1065 and other non-protein thiols provide cytoprotection by their ability to scavenge highly reactive free radicals formed by oxidative stress (Littlefield *et al* 1993; Weiss and Landauer, 2003). Antioxidants may interfere with the initial apoptosis induced by IR exposure (Weiss and Landauer, 2003). Antioxidants may interfere with the initial apoptosis induced by IR exposure (Weiss and Landauer, 2003). The antioxidative and free radical scavenging effects of Asian ginseng have been well documented (Attele *et al*., 1999; Han *et al*., 2005; Kumar *et al*., 2003; Kitts *et al*., 2000; Lee *et al*., 2005; Lee *et al*., 2006). The cell membranes of PBL have a very high phospholipid content, rendering them vulnerable to oxidative damage (Block and Mead, 2003); however, the exact radioprotective mechanism of the standardized NAGE on human PBL is unclear (Lee *et al*., 2005). The radioprotective effect of NAGE on human PBL, like that of WR-1065, is probably related to its scavenging of radiation-induced free radicals, based on the following evidence: (1) ginsenosides function as antioxidants that protect the outer membrane of mammalian cells (Block and Mead, 2003); (2) NAGE inhibits lipid peroxidation through transition metal chelation and scavenging of hydroxyl and superoxide radicals (Kitts *et al*., 2000; Lee *et al*., 2005); (3) ginsenoside Rb1 inhibits peroxyl radical-induced DNA breakage (Kang *et al*., 2007; Kitts *et al*., 2000); (4) NAGE has a markedly higher ginsenoside Rb1/Rb2 ratio than Panax ginseng (Kitts *et al*., 2000), and (5) the total ginsenoside concentration (w/w) of the standardized NAGE we applied in this study is 11.7%, which is much higher than the 4% total ginsenoside concentration that usually appears in the literature (Hall *et al*., 2001).

Taken together, the results generated from this study strongly support our hypothesis that standardized NAGE protects human PBL *ex vivo* against IR-induced DNA damage, as evidenced by a NAGE dose-dependent reduction in the ^{137}Cs-induced MN yield. Under similar experimental conditions, the radioprotective effect of NAGE is comparable with that of WR-1065. The novelty of the present report lies in our finding that this NAGE radioprotection extends from 0 h to 90 minutes after radiation exposure. This long window of protection could have major implications for clinical radiotherapy and for victims of accidental or deliberate radiation exposure. Compared with WR-1065, NAGE is a low-cost and relatively non-toxic natural product with numerous medicinal properties that can be administered easily as a dietary supplement. Therefore, we believe NAGE to be an excellent candidate for further trials. Our subsequent studies will investigate the possible mechanisms underlying the radioprotection conferred by NAGE, including how NAGE modulates the redox homeostasis in human PBL.

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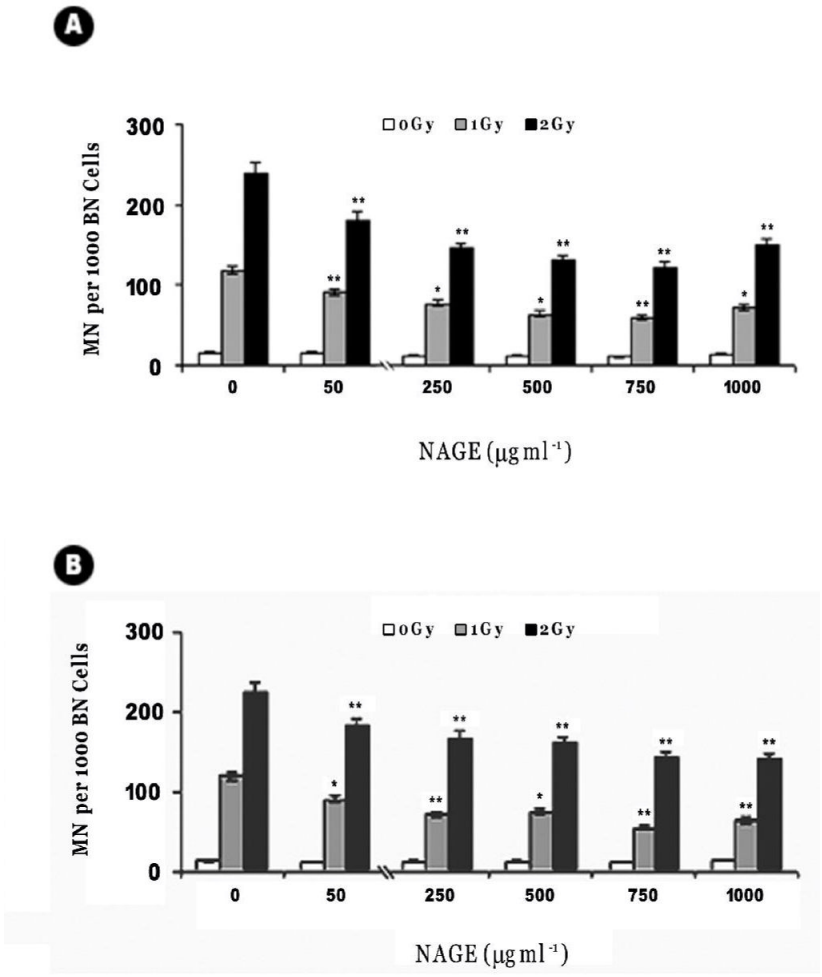

Figure 1.

Effect of NAGE (μg ml^{-1}) applied at 0 h (A) and 90 min after radiation exposure (B) on ^{137}Cs-induced MN yields in binucleated (BN) lymphocytes, compared to their respective irradiated controls (**P*<0.005, ***P*<0.001). Each bar represents the mean ± SEM of two independent determinations pooled from 12 individuals.

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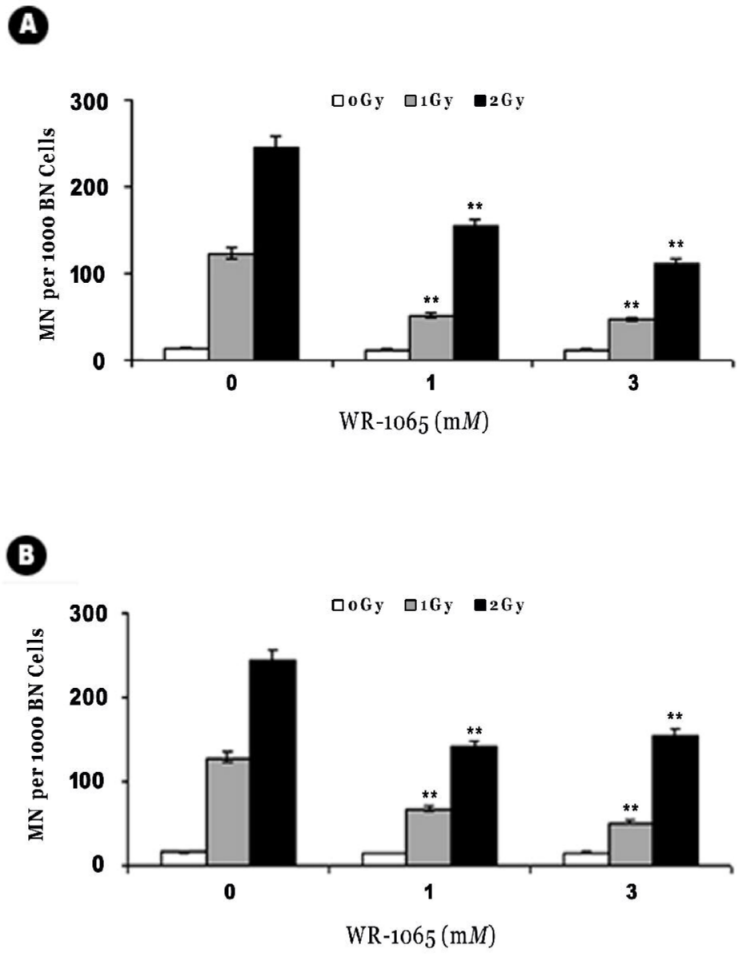

Fig.2.

Effect of WR-1065 (m*M*) applied at 0 h (A) and 90 min after radiation exposure (B) on ^{137}Cs-induced MN yields in binucleated (BN) lymphocytes, compared to their respective irradiated controls (**P*<0.005, ***P*<0.001). Each bar represents the mean ± SEM of two independent determinations pooled from 12 individuals.

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Table 1

Comparison of the effect of varying concentrations of NAGE (μg ml^{-1}) and WR-1065 (m*M*) on ^{137}Cs-induced MN yield in BN lymphocytes. NAGE and WR-1065 were applied at 0 h after radiation exposure. Reduction of MN yield (%) was determined as ^{137}Cs-induced MN yield in varying concentrations of NAGE and WR-1065 compared to that with radiation alone

	Gy	Tot. BN	Tot. MN	MN per 1000 BN Cells		
NAGE				**Mean**	**SEM**	**Reduction (%)**
0	**0**	10789	170	15.9	1.5	0
50		7565	121	16	2.3	0
250		9814	117	11.9	1.4	24.7
500		10565	125	11.8	2.3	25.3
750		10241	111	10.8	0.8	31.6
1000		7795	106	13.6	2.6	13.9
0	**1**	11586	1377	118.9	10.2	0
50		9248	853	92.2	7.6	22.5
250		9053	694	76.7	9.9	35.5
500		9586	623	65	10.2	45.3
750		9037	546	60.4	6.2	49.2
1000		8970	639	71.2	12.6	40.1
0	**2**	11894	2856	240	12	0
50		6043	1096	181.4	18.1	24.4
250		8899	1302	146.3	7.6	39
500		8768	1155	131.7	12.9	45.1
750		9980	1226	122.8	33.3	48.8
1000		8643	1310	151.6	25.2	36.8
WR-1065						
0	**0**	10484	161	15.4	1.4	0
1		7445	104	14.0	0.9	9.1
3		4892	68	13.9	0.4	9.7
0	**1**	11801	1470	125.3	6.8	0
1		8206	436	53.1	6.2	57.5
3		4919	239	48.6	11.4	61.2
0	**2**	11584	2871	247.8	10.3	0
1		6317	999	158	20.5	36.2

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NAGE	Gy	Tot. BN	Tot. MN	MN per 1000 BN Cells		
				Mean	SEM	Reduction (%)
3		5409	611	113	20.4	54.4

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Table 2

Comparison of the effect of varying concentrations of NAGE (μg ml^{-1}) and WR-1065 (m*M*) on ^{137}Cs-induced MN yield in BN lymphocytes. NAGE and WR-1065 were applied at 90 min after radiation exposure. Reduction of MN yield (%) was determined as ^{137}Cs-induced MN yield in varying concentrations of NAGE and WR-1065 compared to that with radiation alone

	Gy	Tot. BN	Tot. MN	MN 1000 BN Cells		
NAGE				**Mean**	**SEM**	**Reduction (%)**
0	**0**	10937	157	14.4	1.5	0
50		6826	84	12.3	2.5	14.5
250		8645	117	13.5	2.1	6.3
500		10404	139	13.4	2.8	6.9
750		10230	122	11.9	1.1	17.2
1000		7348	111	15.1	2.3	0
0	**1**	11769	1407	119.6	10.1	0
50		7248	655	90.4	13.2	24.4
250		8086	582	72	11.1	39.8
500		6874	512	74.5	9.9	37.7
750		10806	598	55.3	15.8	53.8
1000		8476	542	63.9	7.2	46.6
0	**2**	9963	2246	225.4	10.5	0
50		7086	1294	182.6	12.1	19
250		7705	1293	167.8	14.8	25.6
500		9286	1495	161	18.3	28.6
750		11599	1652	142.4	19.2	36.8
1000		7326	1035	141.3	21.9	37.3
WR-1065						
0	**0**	10942	175	15.9	1.2	0
1		4697	69	14.7	1.8	6.9
3		4240	64	15.1	1.8	5.1
0	1	11421	1460	128	7.1	0
1		4352	295	68	9.6	47
3		5560	290	52.2	9.2	59.2
0	**2**	9855	2400	243.5	8.1	0
1		5416	763	140.9	20.2	42

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NAGE	Gy	Tot. BN	Tot. MN	MN 1000 BN Cells		
				Mean	SEM	Reduction (%)
3		4225	653	154.6	18.9	36.8

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Table 3

Comparison of effect of varying concentrations of NAGE (μg ml^{-1}) and WR-1065 (m*M*) on MN+BN index and MN distribution in BN lymphocytes induced by ^{137}Cs. NAGE and WR-1065 were applied at 0 h

NAGE	Gy	Tot. BN	MN+BN		MN/BN				Tot. MN
			Tot.	%	1	2	3	4	
0	**0**	10789	155	1.4	140	15	0	0	170
50		7565	104	1.4	88	15	1	0	121
250		9814	105	1.1	93	12	0	0	117
500		10565	112	1.1	101	9	2	0	125
750		10241	103	1	95	8	0	0	111
1000		7795	96	1.2	86	10	0	0	106
0	**1**	11586	1248	10.8	1129	109	10	0	1377
50		9248	728	7.9	619	95	12	2	853
250		9053	618	6.8	548	64	6	0	694
500		9586	562	5.9	505	54	2	1	623
750		9037	513	5.7	482	29	2	0	546
1000		8970	584	6.5	535	43	6	0	639
0	**2**	11894	2336	19.6	1874	409	48	5	2856
50		6043	919	15.2	763	135	21	0	1096
250		8899	1139	12.8	988	139	12	0	1302
500		8768	968	11	806	139	21	2	1155
750		9980	1048	10.5	903	115	27	3	1226
1000		8643	1095	12.7	896	183	16	0	1310
WR-1065									
0	**0**	10484	146	1.4	131	15	0	0	161
1		7445	98	1.3	94	2	2	0	104
3		4892	62	1.3	56	6	0	0	68
0	**1**	11801	1297	11	1148	125	24	0	1470
1		8206	380	4.6	330	44	6	0	436
3		4919	217	4.4	197	18	2	0	239
0	**2**	11584	2355	20	1895	409	46	5	2871
1		6317	864	14	742	110	11	1	999

Phytother Res. Author manuscript; available in PMC 2009 February 13.

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NAGE	Gy	Tot. BN	MN+BN		MN/BN				Tot. MN
			Tot.	%	1	2	3	4	
3		5409	549	10	489	58	2	0	611

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Table 4

Comparison of the effect of varying concentrations of NAGE (μg ml^{-1}) and WR-1065 (m*M*) on ^{137}Cs-induced MN yield and MN distribution in BN lymphocytes. NAGE and WR-1065 were applied at 90 min after radiation exposure.

NAGE	Gy	Tot. BN	MN+BN		MN/BN				Tot. MN
			Tot.	%	1	2	3	4	
0	0	10937	143	1.3	131	10	2	0	157
50		6826	76	1.1	68	8	0	0	84
250		8645	105	1.2	93	12	0	0	117
500		10404	120	1.2	104	13	3	0	139
750		10230	114	1.1	106	8	0	0	122
1000		7348	100	1.4	89	11	0	0	111
0	1	11769	1228	10.4	1071	135	22	0	1407
50		7248	560	7.7	473	81	4	2	655
250		8086	515	6.4	453	57	5	0	582
500		6874	454	6.6	401	49	3	1	512
750		10806	543	5	497	37	9	0	598
1000		8476	500	5.9	458	42	0	0	542
0	2	9963	1920	19.3	1625	267	25	3	2246
50		7086	1072	15.1	863	196	13	0	1294
250		7705	1108	14.4	940	151	17	0	1293
500		9286	1282	13.8	1086	179	17	0	1495
750		11599	1424	12.3	1211	198	15	0	1652
1000		7326	892	12.2	758	125	9	0	1035
WR-1065									
0	0	10942	158	1.4	142	15	1	0	175
1		4697	63	1.3	58	4	1	0	69
3		4240	53	1.3	43	9	1	0	64
0	1	11421	1287	11	1136	131	18	2	1460
1		4352	259	6	226	30	3	0	295
3		5560	275	4.9	262	11	2	0	290
0	2	9855	1972	20	1590	338	42	2	2400
1		5416	653	12	554	88	11	0	763

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NAGE	Gy	Tot. BN	MN+BN		MN/BN				Tot. MN
			Tot.	%	1	2	3	4	
3		4225	562	13	479	77	4	2	653

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Table 5

Regression coefficients of radiation dose-response relationship of MN yield in BN lymphocytes exposed to ^{137}Cs in medium containing NAGE or WR-1065 applied at different time points

Time		Gy	Intercept	Slope	Curvature	*P*-value
1	**NAGE**	0	14.12	-0.00	---	0.267
		1	98.05	-0.04	---	0.000
		2	197.88	-0.07	---	0.000
	WR-1065	0	15.39	-0.69	-0.004	0.591
		1	125.34	-96.41	23.79	0.001
		2	250.4	-114.5	22.78	0.002
2	**NAGE**	0	13.57	-0.00	---	0.754
		1	97.99	-0.04	---	0.000
		2	198.23	-0.06	---	0.000
	WR-1065	0	15.48	-1.92	0.39	0.451
		1	127.97	-71.41	15.59	0.000
		2	242.88	-139.27	36.37	0.000

Time 1: 0 h.

Time 2: 90 min after ^{137}Cs irradiation.