

SHORT COMMUNICATION

Lack of Radioprotective Potential of Ginseng in Suppressing Micronuclei Frequency in Human Blood Lymphocyte under Gamma Irradiation

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Ginseng appears to be a promising radioprotector for therapeutic or preventive protocols capable of attenuating the deleterious effects of radiation on human normal tissue. This research addresses results on the study of radioprotective potential of ginseng on radiation induced micronuclei in lymphocyte cells *in vitro*. The peripheral blood samples were exposed to gamma rays at doses of 0.0, 0.5, and 1.0 Gy and then added with 0, 100, and 1000 ug/mL of ginseng extract. These treated samples were cultured for micronuclei (MN) examination using standard procedure. The evaluation of incubation with ginseng extract for 24 h before irradiation was also done. Our results showed that there was no radioprotective effect of ginseng addition to the frequency of MN in lymphocyte cells. Pre-incubation with ginseng extract before irradiation also did not effectively suppress the MN frequency. This research lacks to prove the ginseng's radioprotective potential that maybe related to its immunomodulating capabilities and its capabilities in scavenging free radicals induced by radiation or in attenuating the deleterious effects of radiation and its important role in increasing levels of several cytokines.

Keywords: gamma rays, ginseng, micronuclei, radioprotective effects

INTRODUCTION

Although efforts had been directed to mitigate radiation-induced normal tissue damages since the discovery of the deleterious effects of radiation, the expanding role of radiotherapy in cancer treatment creates new imperatives for developing safe and effective agents for prophylaxis and treatment of ionizing radiation-induced tissue damage (Coleman *et al.* 2004). Many radioprotective compounds had been developed over the years to reduce the levels of radiation-induced free radicals within the cell (Stone *et al.* 2004).

Ginseng is a natural product with worldwide distribution, and many reports have shown that it had a significant antineoplastic (Chang *et al.* 2003) and other pharmacological activities (Kitts & Hu 2000). Ginseng was found to increase the number of bone marrow cells, spleen cells, granulocyte-macrophage colony-forming cells, and circulating neutrophils, lymphocytes and platelets in irradiated mice. In addition, ginseng induced the endogenous production

of cytokines such as Interleukin (IL)-1, IL-6, Interferon (IFN)- γ and IL-12, which were required for hematopoietic recovery, and was able to enhance T cell helper1 (Th1) function while interfering with the Th2 response in irradiated mice (Song *et al.* 2003). These findings indicate that ginseng may be a useful agent to reduce the time necessary for reconstituting hematopoietic cells after irradiation.

Ginseng and its partially purified constituents had potential radioprotective properties (Yun *et al.* 2001; Kim *et al.* 2003; Lee *et al.* 2004). It appears to be a promising radioprotector for therapeutic or preventive protocols capable of attenuating the deleterious effects of radiation on human normal tissue, especially for cancer patients undergoing RT (Jagetia & Baliga 2002). However, reports on the radioprotective effects of ginseng, had primarily been done in non-human models.

Ionizing radiations induced chromosomal aberrations that may manifested as breaks and fragments, which appears as micronuclei (MN) in the rapidly proliferating cells. The formation of MN in cytokinesis-blocked peripheral blood lymphocytes was one of the most sensitive biomarkers for

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assessing the effectiveness of many chemicals to encounter genotoxicity or radiation damage *in situ* (Senthamizchelvan *et al.* 2009; Fenech *et al.* 2003). MNs have also been used extensively in studies as an easily evaluated indicator of deoxyribonucleic acid (DNA) damage. Study provided evidence of how analyses among genetic end points in the cytokinesis-block MN assay could provide information concerning abnormalities of cell division and possibly about structural chromosomal rearrangements induced by clastogens (IAEA 2001).

The aim of this study was to evaluate the radioprotective potential of ginseng against genotoxicity of gamma irradiation manifested as MN induced in cultured blood lymphocytes.

MATERIALS AND METHODS

Ginseng Extraction. Korean red ginseng extract was purified from *Panax ginseng* at Korea Institute of Radiological and Medical Sciences (KIRAMS) with procedures according to Ahn *et al.* (2006) and Song *et al.* (2003). In brief, fresh roots of *P. ginseng* that had grown for 6 years were washed, steamed at 100 °C for 2-3 h, and dried. The dried red ginseng roots were boiled in 4-5 volumes of water for 3 h, and the supernatants were concentrated. The concentrated extract was dissolved in phosphate buffer saline (pH 7.4). *P. ginseng* is the saponin glycosides (ginsenosides) of which there are some majors and other small amount constituents.

Irradiation. Two milliliters of peripheral blood samples were collected in sterile heparinised vacutainers (Becton Dickinson) from two healthy volunteers (all males) aged of 42 and 47 years old. Each sample were irradiated with gamma rays at doses of 0.0, 0.5, and 1.0 Gy and at a dose rate of 3.16 Gy/min in ¹³⁷Cs Gamma-cell 3000 Elam Nordion International machine located in KIRAMS.

Lymphocytes Culture. After irradiation, blood samples were put into culture solution containing 8.5 mL of RPMI 1640 medium with L-glutamine and 25 mM HEPES buffer (Gibco Laboratories) supplemented with 10% fetal calf serum (Gibco) and antibiotics. Ginseng was added directly at concentrations of 0, 100, and 1000 µg/mL working doses. Purified phytohaemagglutinin (30 µg/mL; Sigma) was added as mitogen. Treated and control (without ginseng treatment) bloods were cultured at 37 °C for 48 h in a humidified atmosphere containing 5% CO₂. MN yield was determined with cytokinesis-blocked (CB) assay. After an incubation period of 72 h the cells were collected, treated with a hypotonic solution of 0.075 M KCl (cooled at 4 °C) and

prefixed with 2 mL of cold pre-fixative solution (3% formaldehyde in fresh fixative solution). Fixative was done with a mixture of methanol/glacial acetic acid. After fixation the cells were dropped onto clean slides and allowed to dry. After mounted, MN was scored according to IAEA (2001). For 24 h treatment before irradiation, blood was mixed with culture medium in conical tube and kept in incubator at 37 °C for 24 h and then irradiated, cultured and harvested for MN as above.

Micronuclei Scoring. Frequencies of MN was conducted under a Nikon microscope with 100x magnification. The MN frequencies were scored according to the criteria proposed by Fenech *et al.* (2003) and IAEA (2001) as follow: the diameter of an MN was less than one third of the diameter of the MN, it is non-refractile and is not linked to the macronucleus by a nucleoplasmic bridge. MN partly overlapping with the nucleus or with each other was also taken into account. One thousand binucleated (BN) cells were scored on one or two slides per individual.

Statistical Analysis. The results obtained from all the groups were expressed as mean. Anova test was used to find out whether mean of sample drawn from various groups deviates significantly. The significance of the results was computed at the levels of $P < 0.05$.

RESULTS

Induction of MN After Irradiation. This study showed that the yields of MN in control peripheral blood lymphocytes (PBL) (without ginseng treatment) were consistently higher for higher doses of radiation. Radiation clearly increased the MN yield in PBL that was in a dose-dependent manner. Irradiation with gamma rays at doses of 0, 0.5, and 1.0 Gy without the presence of ginseng extract yielded MN frequency of 0.008, 0.054, and 0.281, respectively, per 1000 binucleated (BN) cells. MNs appeared as rounded shape next to the BNC within a cytoplasm that painted lighter than the body of BNC and MN (Figure 1).

Ginseng Potential on MN Induction. The addition of ginseng (100-1000 µg/mL) did not reduce or suppress the MN yields even though it was in higher concentration (Figure 2). This result was unexpected since many studies reported that ginseng act as a radioprotector. Treatment with ginseng for 24 h before radiation exposure results in no suppression in MN yields for all ginseng concentrations tested. It means that pre-treated ginseng extract appeared to have no radioprotective on MN (Table 1). In general,

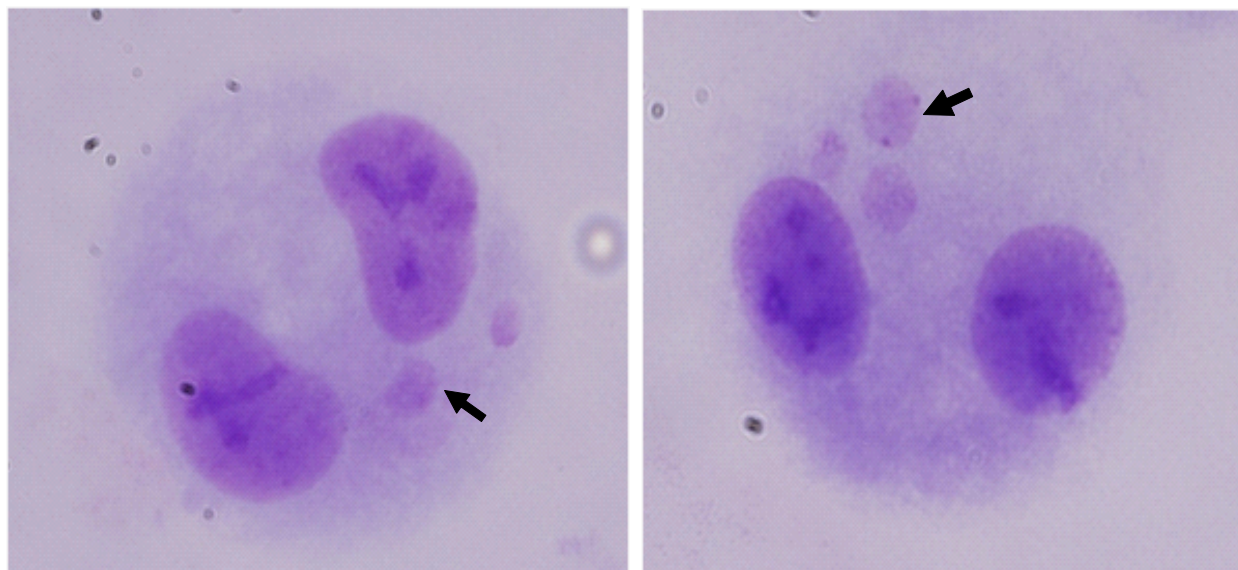


Figure 1. Microscopic view of two (left) and three (right) MNs (arrow) induced by irradiation in lymphocyte cells treated with 1000 µg/mL ginseng and pictured at magnification of 1000x.

Table 1. Total MN and its observed frequency in 1000 BNC of human peripheral blood lymphocytes after pre-incubation with ginseng at the concentration of 1000 µg/mL 24 h before gamma irradiation

Dose (Gy) pre-24 h	Total BNC counted	Total MNs	Cells with MNs						Total cells with MNs/ Total counted cells	Total MNs/Total cells with MNs	Frequency (total MNs/ total counted cells)
			1	2	3	4	5	Total			
0	1000	14	12	1	0	0	0	13	0.013	1.076	0.0014
0.5	1000	66	44	8	2	0	0	54	0.054	1.222	0.066
1.0	1000	128	98	11	0	2	0	111	0.111	1.153	0.128

BNC: binucleated cells; MNs: micronucleus.

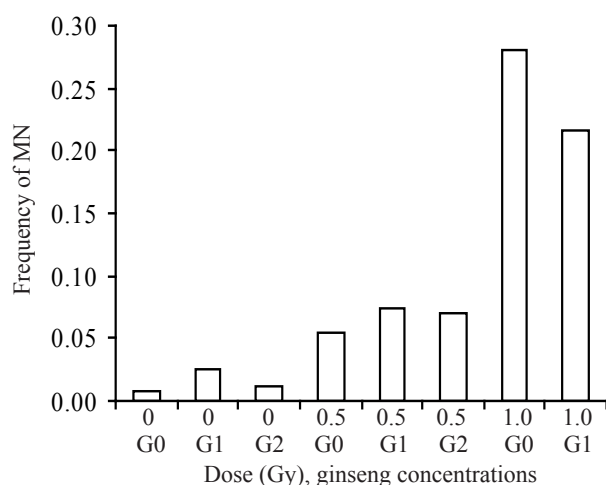


Figure 2. Frequency of MN in peripheral blood lymphocytes after addition of ginseng at various concentrations (G0:0; G1:100; and G2:1000 µg/mL) administered at 0 h after gamma irradiation with 0, 0.5, and 1.0 Gy.

the addition of ginseng at the concentrations used in this study could not clearly decreased the ^{137}Cs -induced MN in PBL. Thus, in this study we could not reveal a radioprotective potential of ginseng, even in lower dose of irradiation (0.5 Gy).

There were no data available from 1.0 Gy irradiation and 1000 µg/mL ginseng treated samples. This was

due to technical problem such as contamination in culture. Oppositely with expectation that there is a tendency that higher concentration of ginseng results in higher MN yields. This finding is not in agreement with the evidence that supplementation of antioxidants such as ginseng could inhibit the radiation induced DNA damage represented as MN in human tissues. This was supported by result of the statistical analysis that there was no significant difference among the treatments of ginseng ($P < 0.05$).

DISCUSSION

In this research the ability of ginseng in protecting or suppressing the effects of irradiation was studied. We found that ginseng did not suppress MN induced by irradiation and suggested that ginseng tended to be a weak radiosensitizer in some cases. This may be due to discrepancies existed in route of treatment and fundamental mechanisms of protective action of ginseng. However, our data is in accordance with a part of research by Lee *et al.* (2010) who found that different concentrations of ginseng extract did not affect the MN yields; where radiation alone sharply

increased the MN yields. In their study, treatment with ginseng for 24 h before radiation exposure results in a significant linear decline of MN yields as ginseng concentration increases. Lee *et al.* (2010) in another experiment found that, compared to radiation alone, the extent to which ginseng water extract reduced the MN yields induced by 1 Gy exposure was 46.0% at 1500 µg/mL and 61.5% at 2000 µg/mL. MN data suggested a tendency for overdispersion relative to the Poisson model, and over the different levels of ginseng concentrations, the trend in micronucleated BN index was as similar to that of the MN yields. These results indicated that ginseng crude water extract exerted no apparent cytogenetic effect on human PBL at concentrations up to 2000 µg/mL as evaluated by the CB assay, and the protection of ginseng water extract against ¹³⁷Cs-induced MN in human PBL was concentration-dependence. Therefore, their findings indicated that ginseng might have therapeutic value as a possible radioprotector for normal tissue during radiotherapy of cancer patients.

Because of the diversity of ionizing radiation applied in medicine, agriculture, and industry, the acquisition of radioprotectors is urgent for many countries. However, the applicability of radioprotectors currently under investigation is limited due to their inherent toxicity. Moreover, a majority of potential radioprotective synthetic compounds have demonstrated limited clinical application owing to their inherent toxicity, and thus, the seeking of naturally occurring herbal products, such as ginseng, for their radioprotective capability has become an attractive alternative. Therefore, it is proposed that the modulation of antioxidant enzymes by ginseng is partly responsible for protecting the organisms from radiation, and can be applied as a therapeutic remedy for various ROS-related diseases. However, this mechanism was not confirmed in this research. Radioprotective agents are known to be most effective when they are applied before radiation exposure, and must be present in the system at the time of irradiation, thus, the time of administration of a radioprotector is critically important. In experiment by Lee *et al.* (2004), it was found that the administration of 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, it was demonstrated that the application of ginseng (100 and 1000 µg/mL) to PBL cultures obtained from 2 healthy volunteers was not radioprotective, even though in research by Lee *et al.* (2010) ginseng was effective 90 min post irradiation. The 24 h pre-

irradiation incubation of ginseng water extract is necessary to confer radioprotection inside the PBL before and during the time of radiation exposure by decreased lipid peroxidation levels.

Other investigators also proposed that ginseng might be exerting its radioprotective effects through upregulation of these immunomodulating cytokines. For instance, *P. ginseng* and another kind of ginseng (*P. quinquefolius*) had been reported to upregulate the production of cytokines, such as IL-1, IL-2, IL-4, IL-6, IL-10, IL-12, GM-CSF, interferon (IFN)- γ and tumor necrosis factor (TNF)- α in animal models (Hsu 1996; Block & Mead 2003). Beside as immunomodulator, the strong free radical scavenging effects of ginseng had been extensively documented (Kitts *et al.* 2000). The radioprotective effect of ginseng has been closely linked to its antioxidative capability through both the chelating of transition metal ions and the scavenging of free radicals responsible for DNA damage. Studies have demonstrated that ginseng root extracts exhibit both lipid-soluble and water-soluble antioxidant activity *ex vivo*, and that this antioxidant action occurs both directly through free radical scavenging and indirectly through upregulation of antioxidant enzymes, leading to the prevention of DNA degradation; but these both mechanisms are not taken place that may be related to the quality of ginseng which should relatively non-toxic to normal cells and incubation time.

In this study, we were applying an easy method to detect chromosome damage induced by ionizing radiation. Chromosome damage usually measured by the chromosome aberration (CA) technique that reliable to assess the radiation dose absorbed by cells. However, this CA technique has some disadvantages as its scoring is difficult and requires skill and experience which of these lead to low number of cell counts (Hatayoglu & Orta 2007). Therefore MN was a method of choice which it also measures chromosome losses that has easy scoring criteria leading high numbers of cell counts and therefore holds more statistical power. Thus MN technique with an easy and short-term application can be used as an alternative to the chromosome aberration technique. Moreover in application of MN assay for biological dosimetry, one should consider the condition of whether the exposure is at a part of a body or at whole body. To do that, MN yields have been analysed in PBLs of groups of patients treated with fractionated partial body radiotherapy. The doses estimated by MN analysis agree quite well with averaged whole body doses calculated from the radiation treatment plans (Thierens *et al.* 1995; Lee *et al.* 2000). It can be concluded that the yields of MN in PBL, without

ginseng treatment, are consistently higher for higher doses of radiation. There was no radioprotective effect of ginseng addition to the frequency of MN in lymphocyte cells. Pre-incubation with ginseng extract before irradiation also does not effectively minimize the MN frequency. This research lacks to prove the ginseng's radioprotective potential. It needs further research with more subjects and/or repeated treatment of radioprotective agents (pharmacological intervention) with deeper evaluation.

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