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Effects of Ginseng Protein on Relative Survival and Chromosome Aberration of UV Irradiated Cells

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Abstract □ A ginseng protein fraction which has been reported to have radiation protective effect was purified from Korean ginseng and its effects on relative survival and chromosome aberration were studied in UV irradiated CHO-K1 cells. When the protein fraction (100 μg/m*l*) was added to the cells before UV irradiation at 4 J/m^2, the survival rates were increased to 53.8% from 40.6% in control. Addition of the protein (100 μg/m*l*) after UV irradiation at 4 and 8 J/m^2 raised the rates to 85.4 and 24.0% from 79.2 and 11.5% in control, respectively. When the ginseng protein (800 μg/m*l*) was added to the cells exposed to UV light at 10, 20, 30 J/m^2, the frequencies of chromosome aberration (CA) were reduced significantly to almost same level regardless of the UV dose increment and there was no significant difference between pre- and post-treatment. When the concentration of ginseng protein was increased from 200 to 800 μg/m*l*, at UV dose of 10, 20, 30 J/m^2 each, the CA frequencies were decreased consistently as the dose of ginseng protein increased, at all UV doses tested. Similar effects were observed in both cases of pre- and post-treatment. The data suggest that the protein may reduce cell damage caused by UV light, especially damage to DNA molecule, or play a role in repair processes of damaged DNA, to increase cell survival and reduce chromosome aberrations.

Keywords □ Radioprotective ginseng protein, UV irradiation, relative survival, chromosome aberration, DNA repair process.

Ginseng components have been reported to have radiation protective effects in X-ray or γ-ray irradiated ICR mouse, Wistar rats and Harteley guinea pigs. Takeda *et al.*[1,2] tested the ginseng fraction of Oura[3] to find out significant increase of 30-day survival rates and facilitated recovery of the amount of DNA, reduced by X-ray irradiation. Yonezawa *et al.*[4,5] reported similar effects by testing heat stable ginseng protein fraction, explaining the reason as being by enhanced mitosis of bone marrow. Kim and Han[6] also reported the increase of survival rates and the amount of liver DNA by examining ginseng protein fraction on γ-ray irradiated ICR mouse. This protein fraction has been shown to stabilize the DNA molecule by raising the Tm value[7] and reduce the high frequencies of sister chromatid exchanges induced by UV light in CHO-K1 cells.[8]

When cells are exposed to radiation, the survival rates are reported to be decreased and the chromosome aberrations to be increased. Puck and Marcus[9] showed the reduction of the number and size of colonies formed as the dose of radiation increases in HeLa cells. Rauth[10] found out that the colony forming ability of UV irradiated mouse L-cell was markedly decreased by the addition of caffeine and suggested that the caffeine might inhibit the recovery of the UV damaged cells. Maher[11] compared the colony forming ability of normal human fibroblast and excision repair deficient *Xeroderma pigmentosum* cells, exposed to UV light, to determine the ability to undergo excision repair. On the other hand, UV light has been known to form cyclobutylyl pyrimidine dimer in the DNA molecule, that leads to base modification,[12] sister chromatid exchanges,[13] chromosome aberrations,[14] tumor formation,[15] and necrosis.[16] Damaged DNA could be mended by cellular repair processes such as excision repair, however, unrepaired or misrepaired DNA could remain, which has been reported to cause the formation of sister chromatid exchanges or chromosome aberrations.[17,18]

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In this study, effects of the radioprotective ginseng protein were examined on cellular level using Chinese hamster ovary (CHO-K1) cells. Effects on relative survival of UV irradiated cells were determined by measuring the colony forming ability of single cells plated in culture dishes.[19] And effects on the frequencies of chromosome aberration were studied by adding the protein to UV exposed cells to see if the protein plays any role in the formation of chromosome aberrations.

EXPERIMENTAL METHODS

Materials

Bovine serum albumin (BSA), CM-Cellulose, Sephadex G-75, Tris-(hydroxymethyl) amino-methane, N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), and Giemsa stain were purchased from Sigma Chem. Co., Folin-C-phenol reagent was the product of Merck Co., and Eagle's minimum essential medium (EMEM), Fetal bovine serum (FBS), Trypsin-EDTA, Dulbecco's phosphate buffered saline (PBS), Penicillin-Streptomycin solution, and Colcemid were purchased from Gibco Inc.

Isolation and purification of ginseng proteins

This process was carried out by Tris-HCl buffer extraction, 70% ammonium sulfate fractionation, CM-Cellulose column chromatography, heat inactivation and Sephadex G-75 column chromatography, as described earlier.[20] The same process was repeated to obtain enough amount of the protein for the following experiments.

Cell culture

Chinese hamster ovary cells (CHO-K1) were cultured in growth medium consisting of EMEM supplemented with 10% FBS, penicillin-streptomycin solution, at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. The cells were maintained routinely at the logarithmic phase of growth through subculturing twice a week using 0.05% trypsin-EDTA.

UV irradiation

Cells in normal growth in 60 mm petri dishes were exposed to 254 nm UV light at an incident dose rate of 2.6 erg/mm^2/sec. The medium was removed, the cells were rinsed with PBS twice and the dish cover was off for irradiation. To avoid a shadowing effect caused by the edge of the dishes, the lamp was placed as far as possible above the dishes and the dishes were rotated on a turntable during irradiation to equalize exposure.

Preparation of ginseng protein solution

Ginseng protein fraction was dissolved in D.W. and filtered with 0.22 μm membrane filter. Serum-free medium was added to make the final concentrations of the protein to treat cells before and after UV irradiation.

Cell survival test[21,22]

In 60 mm petri dish, 100-400 single cells were plated. In the case of pre-treatment, ginseng protein at the dose of 100 μg/ml was added and then incubated for 24 hrs, followed by UV irradiation at 0, 4, 8, 12 J/m^2. In the case of post-treatment, seeded cells were incubated for 24 hrs, followed by UV irradiation at the same dose as above with the addition of ginseng protein at the dose of 100 μg/ml immediately after. Cells were then cultured until right size colonies develop. Colonies formed were fixed with methanol and stained with Giemsa solution. The rate of cell survival was calculated by the formula shown below.

$$\% \text{ Survival} = \frac{\text{Plating efficiency of ginseng treated cells}}{\text{Plating efficiency of control}} \times 100$$

$$\text{Here, Plating efficiency} = \frac{\text{No. of colonies formed}}{\text{No. of cells plated}}$$

Chromosome aberration

In the case of pre-treatment, ginseng protein at the dose of 200, 400, 600, 800 μg/ml each was added to the cells and incubated for 24-26 hrs, followed by UV irradiation at 10, 20, 30 J/m^2. In the case of post-treatment, UV light at the same doses was irradiated to the cells, followed by the addition of ginseng protein at the same doses as above and then incubated for 24-26 hrs. Cultures were harvested after 2 hr-exposure to Colcemid (0.08 μg/ml) by shaking the dishes to dislodge the loosely attached mitotic cells. Collected cells were treated with isotonic solution and fixed with methanol-acetic acid (3:1). Chromosome preparations were made by air drying technique and the slides were stained with 4% Giemsa solution for 15 min. Chromosome aberrations were scored in 100 meta-phases in each experiment, according to the criteria of Evans[23] and the same experiment was repeated three times.

RESULTS AND DISCUSSION

Purification of ginseng proteins

As shown in the previous paper,[13] two fractions, named GI and GII, were obtained from the last step

of the purification procedure. The relative yield of the GI fraction was 2.33%(w/w) as compared to the amount of the total protein in crude extract. This fraction has been reported to have radio-protective effect[6] and was used for the following experiments.

Cell survival

In the case of pre-treatment, survival rate of CHO-K1 cells, at UV dose of 4 J/m^2, was 40.6% in control and 53.8% in ginseng protein (100 μg/m*l*) treated cells, showing a significantly higher ($p < 0.05$) rate in ginseng treated cells as compared to the control, (Fig. 1). In the case of post-treatment, at UV dose of 4 and 8 J/m^2, the rates were 79.2 and 11.5%, respectively, in control and 85.4 and 24.0%, respectively, in ginseng protein (100 μg / m*l*) treated cells (Fig. 2). Here again, the survival rate was significantly increased ($p < 0.05$) in ginseng treated cells. Ginseng concentrations above 300 μg/m*l* were also tested, but the survival rates were found to be rather lower than the control.

The results imply that UV and ginseng doses at which cells could still survive, the ginseng protein reduces the damage done by UV light, resulting in the increase of cell survival. However, at higher UV and ginseng doses, the damage reducing effect of ginseng protein appears to be cancelled out by the cytotoxic effect of both UV and ginseng protein. It has been found that ginseng protein itself exerts mild cytotoxic effect at the concentration above 300 μg/m*l*.[24] The effect of the ginseng seems to be a little stronger in post-treatment but the reason could not be elucidated from the data in this experiment.

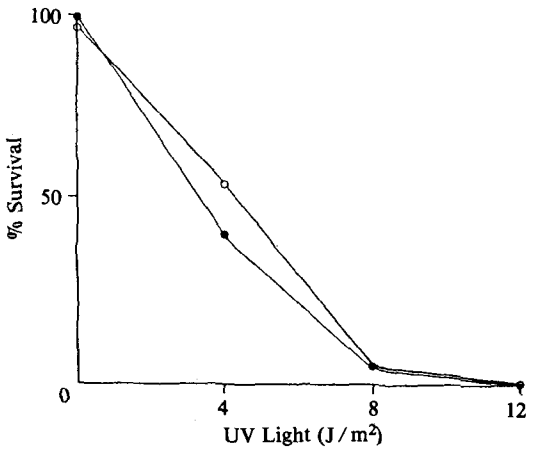

Fig. 1. Relative survival of UV irradiated CHO-K1 cells, pre-treated with ginseng protein.
Here, -●-●-: Control, -○-○-: Ginseng treated at 100 μg/m*l*.

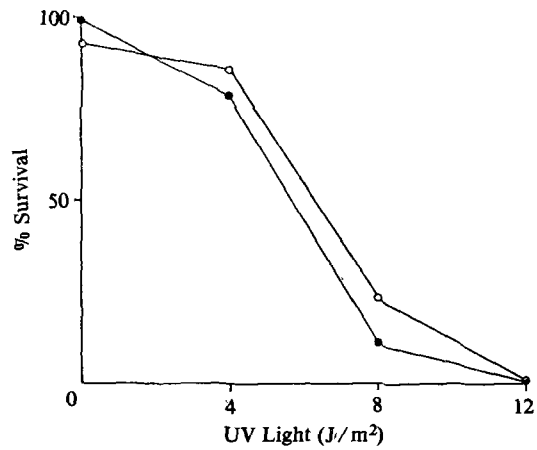

Fig. 2. Relative survival of UV irradiated CHO-K1 cells, post-treated with ginseng protein.
Here, -●-●-: Control, -○-○-: Ginseng treated at 100 μg/m*l*.

Chromosome aberration (CA)

The effect of ginseng protein on the UV induced CA frequencies is shown in Fig. 3. When UV light alone at the dose of 0, 10, 20, 30 J/m^2 was irradiated to CHO-K1 cells, the CA frequencies were 4.33 ± 1.70, 34.33 ± 2.49, 57.67 ± 2.49 and 93.00 ± 6.98 per 100 cells, respectively, presenting a marked rise ($p < 0.01$) upon the increment of UV dose. Addition of the ginseng protein at 800 μg / m*l* under the same conditions lowered the frequen-

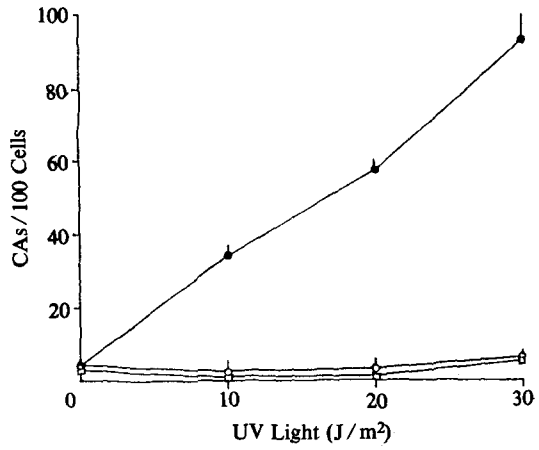

Fig. 3. Effects of ginseng protein on UV induced chromosome aberrations in CHO-K1 cells.
Here, -●-●-: UV alone, -○-○-: Post-treated with ginseng at 800 μg/m*l*. -□-□-: Pre-treated with ginseng at 800 μg/m*l*.

cies significantly ($p < 0.01$) to 3.33 ± 0.47, 0.33 ± 0.47, 1.00 ± 0.82, 5.67 ± 1.70, respectively, in the case of pre-treatment and 3.67 ± 1.70, 2.67 ± 2.49, 3.33 ± 2.49, and 6.33 ± 1.25, respectively, in post-treatment. The CA frequencies after ginseng treatment were almost invariable regardless of the UV dose difference and showed no significant difference between pre- and post-treatment.

Since CA has been reported to be formed by misrepair of damaged DNA, the radioprotective ginseng protein may act at one or more of the steps of repair processes to reduce the amount of misrepaired DNA remained, which may in turn reduce the formation of CA.

To see the dose response of CA against ginseng protein, the CA frequencies were measured at different doses of UV, while changing the concentration of the ginseng protein as 200, 400, 600, 800 μg/ml. When ginseng protein was treated to the cells without UV irradiation, the CA frequencies were not significantly different from those of the control that had no UV and no ginseng treatment. It suggests that the ginseng protein itself does not have any effect in CA formation (Figs. 4 and 5).

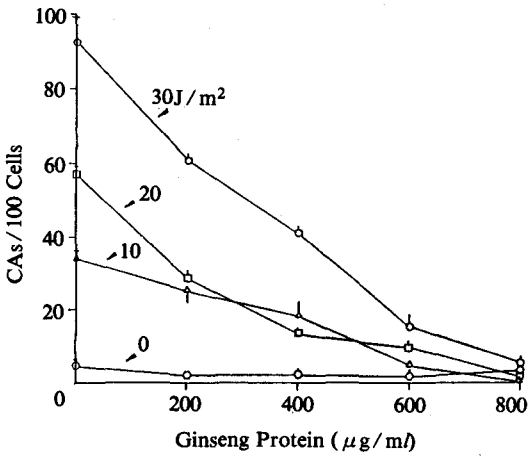

Fig. 4. Dose-response curves of chromosome aberrations against pre-treated ginseng protein concentration, at different UV doses.

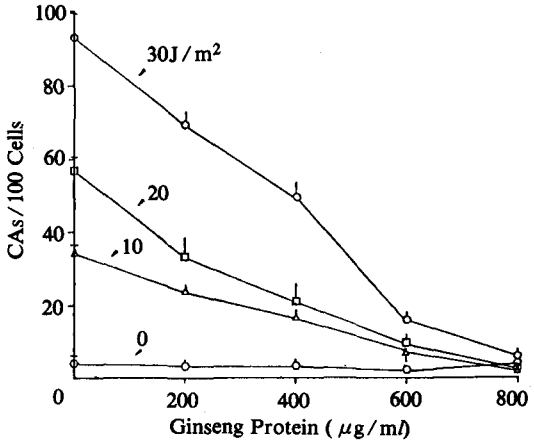

Fig. 5. Dose response curves of chromosome aberrations against post-treated ginseng protein concentration, at different UV doses.

At UV dose of 10 J/m^2, the frequencies were 25.33 ± 3.30, 18.33 ± 4.78, 5.00 ± 0.82, 0.33 ± 0.47, respectively, in pre-treatment and 24.00 ± 1.41, 16.67 ± 2.05, 7.33 ± 2.04, and 2.67 ± 2.49, respectively, in post-treatment. At 20 J/m^2, they were 28.00 ± 2.94, 13.33 ± 1.70, 9.33 ± 2.62 and 1.00 ± 0.82, respectively, in pre-treatment and 33.33 ± 5.44, 21.33 ± 4.78, 9.33 ± 0.94 and 3.33 ± 2.49, respectively, in post-treatment. At 30 J/m^2, they were 60.67 ± 1.70, 41.33 ± 1.70, 15.67 ± 3.68 and 5.67 ± 1.70, respectively, in pre-treatment and 69.67 ± 2.87, 49.33 ± 3.86, 16.00 ± 2.45 and 6.33 ± 1.25, respectively, in post-treatment (Figs. 4 and 5). The data showed the consistent reduction of CA frequencies as the dose of ginseng protein increases at all UV doses tested ($p < 0.01$). At 800 μg/ml and 30 J/m^2, the frequency reached down to 6.10% of the control Even though it was assumed that the time when the protein was treated could make some difference in its effect, the results in this study present no statistically significant differences in pre-and post-treatment. It seems to show that the mechanism(s) involved in this effect of the protein may not be affected by the time ginseng was added.

There have been many reports[25,26] about the relationships between the mechanism of CA formation and DNA repair processes. However, it is still not possible to indicate which of the repair processes are responsible for the CA formation, though, it seems obvious that the chromosome breaks and rejoining should occur to form CA. Therefore, it was postulated that the ginseng protein may show its radioprotective effect by reducing the DNA damage such as chromosome breaks or helping repair of damaged DNA at certain enzymatic steps.

LITERATURE CITED

1. Takeda, A., Yonezawa, M. and Katoh, N.: Restoration of radiation injury by ginseng. I. Responses of X-irradiated mice to ginseng extract. *J. Radiat. Res.* **22**, 323 (1981).
2. Takeda, A., Katoh, N. and Yonezawa, M.: Restoration of radiation injury by ginseng. III.

Radioprotective effect of thermostable fraction of ginseng extract on mice, rats and guinea gigs. *J. Radiat. Res.* **23,** 150 (1982).
3. Oura, H., Hiai, S., Odaka, Y. and Yokozawa, T.: Studies on the biochemical action of ginseng saponin. *J. Biochem.* **77,** 1057 (1975).
4. Yonezawa, M., Takeda, A. and Katoh, N.: *Proc. 3rd Inter. Ginseng Symp.* pp. 17-20 (1980).
5. Yonezawa, M., Takeda, A. and Katoh, N.: Restoration of radiation injury by ginseng. II. Some properties of radioprotective substances. *J. Radiat. Res.* **22,** 336 (1981).
6. Kim, C. and Han, G.S.: Radioprotective effects of ginseng proteins. *J. Pharm. Soc. Korea* **29,** 246 (1985).
7. Kim, C. and Chun, M.Y.: Interaction between radioprotective ginseng proteins and nucleic acid. *J. Korean Res. Inst. Better Liv.* **38,** 193 (1986).
8. Kim, C. and Choi, J.E.: Effects of radioprotective ginseng protein on UV induced sister chromatid exchanges. *Arch. Pharm. Res.* **11**(2), 93 (1988).
9. Puck, T.T. and Marcus, P.I.: *J. Exptl. Med.* **103,** 653 (1956).
10. Rauth, A.M.: *Radiation Res.* **31,** 121 (1967).
11. Maher, V.M., Birch, N., Otto, R. and McCormic, J.J.: *J. Natl. Cancer Inst.* **54,** 1287 (1975).
12. Haseltine, W.A.: *Cell* **33,** 13 (1983).
13. Reynolds, R.J., Natarajan, A.T. and Lohman, P.H.M.: Micrococcus Leteus UV-endonuclease sensitive sites and sister chromatid exchanges in Chinese hamster ovary cells. *Mut. Res.* **64,** 353 (1979).
14. Bender, M.A., Griggs, H.G. and Walker, P.L.: *Mut. Res.* **20,** 387 (1973).
15. Hart, R.W., Setlow, R.B. and Woodhead, A.D.: *Proc. Natl. Acad. Sci. USA.* **74**(12), 5574 (1977).
16. Rotman, R.H. and Seolow, R.B.: *Photo Chemistry and Photobiology.* **29,** 57 (1979).
17. Kato, H.: Photoreactivation of sister chromatid exchanges induced by ultraviolet irradiation. *Nature* **249,** 552 (1974).
18. Bender, M.A., Griggs, H.G. and Walker, P.L.: Mechanism of chromosomal aberration production. *Mut. Res.* **20,** 387 (1973).
19. Casarett, A.P.: *Radiation Biology,* Prentice Hall, pp. 152-155 (1968).
20. Kim, C. and Hwang, J.J.: Polyacrylamide gel electrophoresis on ginseng protein. *J. Pharm. Soc. Korea* **30,** 343 (1986).
21. Pattern, M.K. Jr.: Measurement of growth and viability of cells in culture. *Method in Enzymology* **58,** 141 (1979).
22. Bhayan, B.K., Loughan, B.E., Fraser, T.J. and Day, K.J.: Comparison of different methods of determining cell viability after exposure to cytotoxic compounds. *Exp. Cell Res.* **97,** 275 (1976).
23. Evans, H.J.: in *Progress in Genetic Toxicity* Elsevier/North Holland, Amsterdam, pp. 24-51 (1977).
24. Kim, C. and Yoon, S.R.: Cytotoxic effect of radioprotective ginseng protein fraction on CHO-KI cells. *J. Pharm. Soc. Korea,* 32(5), 11 (1988).
25. Evans, H.J. and Scott, D.: Influence of DNA synthesis on the production of chromatid aberrations by X-rays and maleic hydrazide in *Vicia faba. Genetics* **49,** 17 (1964).
26. Scott, D.: in *Chromosomes Today* Vol. 6, Elsevier/North Holland, Biomedical Press, pp. 391-401 (1977).