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Effects of Radioprotective Ginseng Protein on UV induced Sister Chromatid Exchanges

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Abstract□To elucidate the reaction mechanism of ginseng protein on its antiradiation activity, its effects were studied on sister chromatid exchanges(SCE) induced by UV irradiation in CHO-Kl cells. When cells were irradiated with 254 nm UV light at the dose of 0 to 80 erg/mm^2, the frequencies of SCE were increased more than two fold. However, when radioprotective ginseng protein was added to the cells before and after UV irradiation, SCE frequencies were decreased significantly at all UV doses in both cases with no significant differences. As the amount of ginseng protein was varied from 100 to 500 μg/m*l*, with UV irradiation at 60 erg/mm^2, SCE frequencies dropped sharply at the first two concentrations and then reached a sort of plateau in both cases of pre-and post-treatment. When the ginseng protein was treated alone without UV irradiation, there were no changes in SCE frequencies no matter when the protein was added. These results suggest that the ginseng protein could reduce DNA damages, which may play an important role in the reaction mechanism of radioprotective activity of the protein.

Keywords□radioprotective ginseng protein, UV irradiation, pyrimidine dimer, sister chromatid exchange, excision repair, post-replication repair.

Anticancer and antiradiation effects are two of the most important pharmacological effects of ginseng components in these days of concern at worldwide dispersion of radioactive materials and increasing trend of cancer incidence.

There have been reports showing the inhibitory effects of ginseng extracts on the growth of tumor cells such as Ehrlich ascites[1] and Sarcoma 180 cells.[2] Clinically cancer patients were reported to have prolonged their survival periods by the administration of prostisol, purified from ginseng.[3] These patients showed increasing synthesis of RNA, protein and fatty acids, and red blood cell counts were also increased. Similar results were presented by Oura *et al.*[4,5] in mice, in that the ginseng extracts increased the mitosis of bone marrow and red blood cells, and the synthesis of serum albumin, γ-globulin, DNA, RNA, and proteins. An important finding following these reports was that the components showing the anticancer effects were present in the nonsaponin fraction.

Since 1980, antiradiation effect of partially purified ginseng protein has been reported by Yonezawa *et al.*[6,7] on X-ray irradiated mouse and by Takeda[8] on X-ray irradiated mouse, rat and guinea pig. Marked increase of survival rates by the protein was also reported on γ-ray irradiated mouse.[9] The protein was reported to inhibit catalase activity,[10] stabilize the DNA molecule by raising the transition midpoint (Tm) and form precipitates with nucleoproteins.[11] Molecular weight of the protein was estimated by SDS-polyacrylamide gel electrophoresis[12] and high performance liquid chromatography.[13]

The present study was aimed to find out the reaction mechanism of the ginseng protein on its antiradiation activity. Damage by UV light on the DNA molecule is known to lead to the formation of cyclobutylyl pyrimidine dimer[14] and the dimer, in turn, is known to cause sister chromatid exchange,[15,16] chromosome aberration,[17] tumor formation[18] and necrosis.[19] SCE reflects a symmetrical exchange at one locus between sister chromatids without alteration of overall chromosome morphology. It occurs spontaneously by the base analog, BrdU,[20,21] but the frequency increases significantly by X-ray,[22], UV light,[23] DNA damaging chemical mutagens and carcinogens.[24,25,26] Therefore it is regarded as a sensitive indicator of induced DNA damage.

In this experiment, CHO-K1 cells were exposed to UV light to induce high frequencies of SCE and

radioprotective ginseng protein was added before and after UV irradiation to see the influence on the SCE formation.

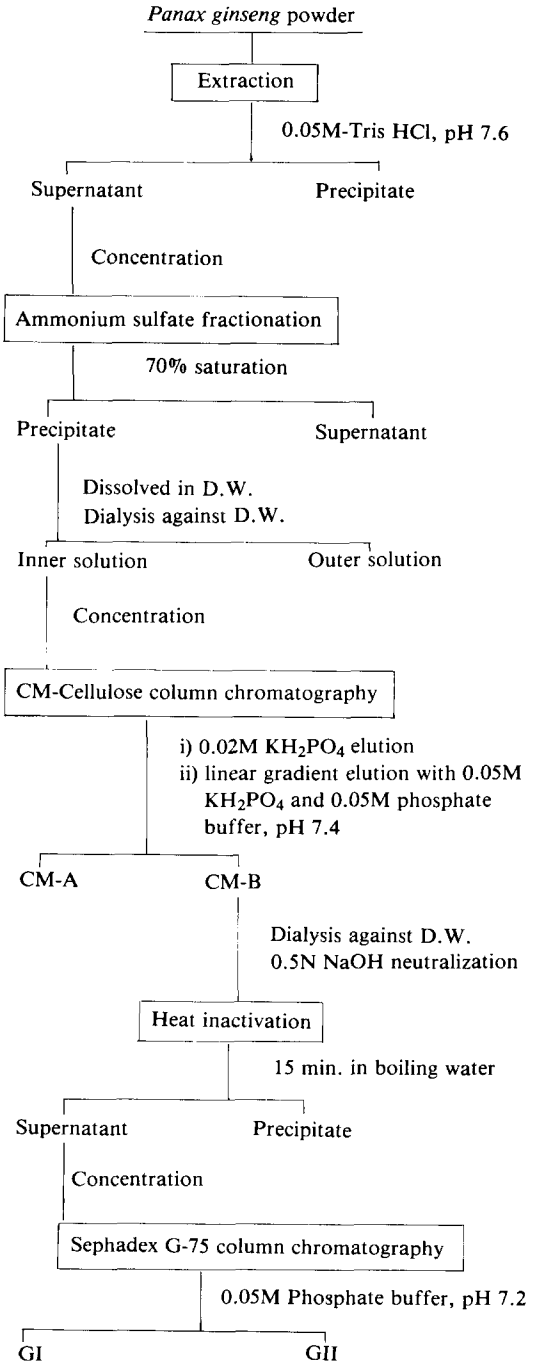

Fig. 1. Isolation and Purification of Ginseng Proteins.

EXPERIMENTAL METHODS

Materials

Six year-old white ginseng was used for purification. CM-Cellulose, Sephadex G-75, bovine serum albumin (BSA), bromodeoxyuridine (BrdU) and HEPES buffer were purchased from Sigma Co., Eagle's minimum essential medium (EMEM), fetal bovine serum (FBS), penicillin G, streptomycin, trypsin-EDTA, Dulbecco's phosphate buffered saline (PBS) and Colcemid were from Gibco Co., and Giemsa stain was the product of BDH Co.

Purification of ginseng proteins

Purification 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 shown in Fig. 1.[7,27] Total amount of ginseng used for purification was over 1200g.

Cell culture

Chinese hamster ovary cells (CHO-K1) were cultured in growth medium consisting of EMEM supplemented with 10% FBS, penicillin G and streptomycin, 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 plastic petridishes were irradiated with 254 nm UV light at an incident dose rate of 2.6 erg/mm^2/sec,determined by a radiometer. 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.

Treatments of ginseng protein

Ginseng protein fraction, GI, which has been reported to have radioprotective effect,[9] was dissolved in distilled water and filtered with 0.22 μ m membrane filter. Serum free medium was added to make the final concentrations of the protein to treat cells for 24 hrs before and after UV irradiation.

Sister chromatid exchanges

CHO-K1 cells in logarithmic growth phase were

exposed to UV light alone or with the treatments of ginseng protein. BrdU (10 μM) was then added and cells were incubated for 24 to 26 hrs in the dark to prevent photolysis. Cultures were harvested after 2 hr-exposure to Colcemid (a final conc. of 0.08 μg/m*l*) by shaking the dishes to dislodge the loosely attached mitotic cells. Collected cells were treated with 0.05M-KCl and fixed in methanol-acetic acid (3:1). Differential staining of sister chromatids were carried out by the modified method of Perry and Wolff.[28] Air dried preparations were stained with Hoechst 33258 and restained with 4% giemsa solution. Over 50 metaphases were observed in each experiment and the same experiment was repeated three times.

RESULTS

Purification of ginseng proteins

Chromatogram of Sephadex G-75 column chromatography, which was the last step of the purification procedure, is shown in Fig. 2. Two fractions, GI and GII were obtained and relative yields of proteins in each fraction were 1.9 and 0.32% (w/w) respectively when compared with the amount of total proteins in crude extract. GI fraction has been reported to have antiradiation effect and was tested in the SCE experiments.

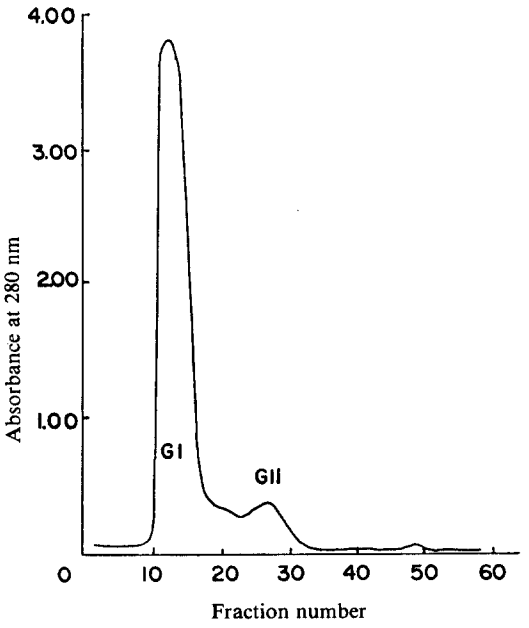

Fig. 2. Sephadex G-75 column chromatography of CM-B fraction.
The column was eluted with 0.05M phosphate buffer, pH 7.2. Each fraction of 5 m*l* was collected at a flow rate of 15 m*l*/hr.

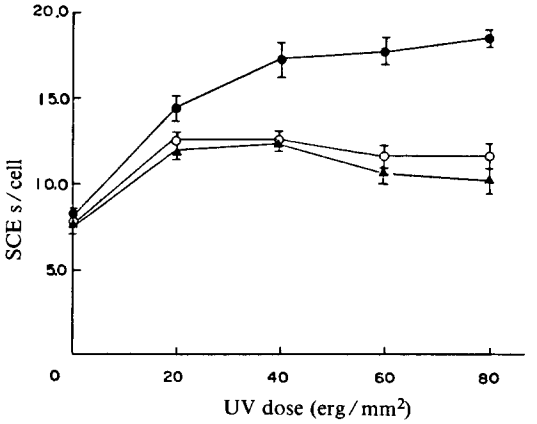

Fig. 3. Dose-response curves of sister chromatid exchanges against UV light.
Here, ● – ●: SCE of cells exposed to UV alone, ○ – ○ : SCE of cells post-treated with ginseng protein (final conc. 500 μg/m*l*), ▲ – ▲: SCE of cells pre-treated with ginseng protein (final conc. 500 μg/m*l*).

Sister chromatid exchanges

SCE frequencies in CHO-K1 cells, when UV light at the doses of 0, 20, 40, 60, 80 erg/mm^2 was itradiated, are shown in Fig. 3 as 8.17 ± 0.10, 14.55 ± 0.63, 17.38 ± 1.27, 17.80 ± 0.76 and 18.57 ± 0.46, respectively. As the dose of UV light increases, the frequencies of SCE also increase. Under the same experimental conditions, ginseng protein at the dose of 500 μg/m*l* was added before and after UV irradiation. Results are also shown in Fig. 3 as 7.67 ± 0.52, 11.97 ± 0.30, 12.43 ± 0.48, 10.69 ± 0.87, 10.24 ± 0.76, respectively, in the case of pre-treatments and 7.83 ± 0.19, 12.83 ± 0.12, 12.55 ± 0.35, 11.54 ± 0.56 and 11.77 ± 0.56, respectively, in the case of post-treatments. Here, ginseng protein lowered SCE frequencies markedly ($P < 0.01$) at each UV dose in both cases. However, pre- and post-treatment of the protein made no differences in SCE formation.

When the amount of ginseng protein was varied from 0 to 500 μg/m*l* and the UV dose was fixed at 60 erg/mm^2, the results are shown in Fig. 4. Corresponding to the ginseng doses of 0, 100, 200, 300, 400 and 500 μg/m*l*, the SCE frequencies were shown as 18.57 ± 0.46, 16.05 ± 0.30, 12.19 ± 0.60, 12.09 ± 0.17, 12.02 ± 0.26 and 10.69 ± 0.87, respectively, in the case of pre-treatments and $18.57 \pm$

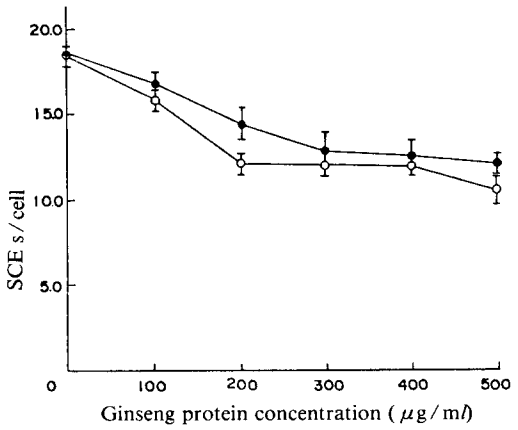

Fig. 4. Dose-response curves of sister chromatid exchanges against ginseng protein concentrations, with UV irradiation.

Here, ● – ● : SCE of cells treated with ginseng protein after UV irradiation at 60 erg/mm^2, ○ – ○ : SCE of cells treated with ginseng protein before UV irradiation at 60 erg/mm^2.

0.46, 16.80 ± 0.63, 14.53 ± 1.00, 13.00 ± 1.31, 12.62 ± 1.03 and 12.01 ± 0.73, respectively, in the case of post-treatments. The decrease of SCE was sharp up to 200 μg/ml ($P<0.05$) and then became slow as the dose increased. At the dose of 200 μg/ml, pre-and post-treatments showed different SCE frequencies but no other doses showed such a difference.

To see if ginseng itself does exert any effects on SCE formation, SCE frequencies were measured while varing ginseng doses as 0, 100, 200, 300, 400 and 500 μg/ml but without UV irradiation. The results are shown in Fig. 5 as 8.17 ± 0.10, 7.58 ± 0.25, 7.98 ± 0.07, 7.93 ± 0.17, 7.55 ± 0.21 and 7.67 ± 0.52, respectively, when the protein was added before BudR treatment and 8.17 ± 0.10, 8.18 ± 0.15, 8.21 ± 0.28, 8.23 ± 0.25, 7.60 ± 0.34 and 7.83 ± 0.19, respectively, when the protein and BrdU were added at the same time. At all protein doses, SCE frequencies were the same as the control. In other words, ginseng protein itself did not change SCE frequencies in CHO-K1 cells.

DISCUSSION

The DNA molecule is known to be the major target of radiation and other chemical mutagens and the primary damage induced by UV is the formation of pyrimidine dimers between the two adjacent thymine residues. Once dimers are formed in a DNA molecule, various repair processes are induced to repair the damage.

There are three repair processes identified in mammalian system, that is, photoreactivation, excision repair and post-replication repair. Photoreactivation[29] is specific for UV damage but occurs in most prokaryotes and lower eukaryotes. On the other hand, excision repair[30,31] occurs in a large range of organisms and can be divided into two pathways, *i.e.*, nucleotide excision and base excision repair depending on the DNA damage UV light was reported to induce the nucleotide excision repair which was characterized by an insertion of about 100 nucleotides per dimer. Post-replication repair was first demonstrated in UV irradiated *E. coli*[32] and later in mammalian cells[33,34] and requires post-irradiation replication. When damaged DNA is replicated, gaps may form in the daughter strands opposite to the pyrimidine dimer, which may later be mended by a recombinational process as in *E. coli*[35] or by *de novo* replication as in eukaryotic cells.[33,34] However, this process is considered to be error prone repair and caused by the presence of damaged bases on parental DNA strand.

UV light has been found to increase SCE.[36] The biological significance of SCE is not fully understood yet, however, it has been proved to be a highly sensitive indicator of induced DNA damage. Since SCE require a post-irradiation replication for their completion,[23] and it was depressed remarkably by a post-treatment with caf-

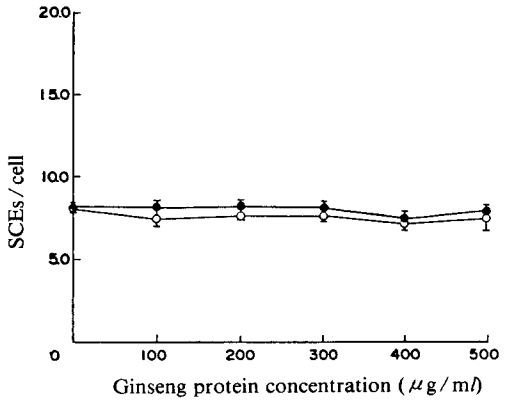

Fig. 5. Dose-response curves of sister chromatid exchanges against ginseng protein concentrations, without UV irradiation.

Here, ● – ● : SCE of cells treated with ginseng protein and BrdU at the same time, ○ – ○ : SCE of cells treated with ginseng protein before BrdU treatment.

feine, an inhibitor of post-replication repair, it was postulated that the mechanism involved in the UV induced SCE has correlation with a post-replication repair of DNA damage. On the other hand, Wolff *et al.*[37] and Cleaver[38] reported that SCE formation was proportional to the amount of DNA damage not repaired by excision repair, suggesting that unexcised remaining damage in DNA is responsible for the formation of SCE. Since CHO cell is known to have a poor ability to excise these dimers,[39] it is probable that the UV induced lesions may remain unexcised for a long time and contribute to the formation of SEC. However, it is not possible at the moment to point out precisely which of the repair processes are responsible for the SCE formation.

In this study, when radioprotective ginseng protein was added in CHO cells, SCE frequencies were not changed, indicating that the ginseng itself does not play any role to SCE formation. However, when high frequencies of SCE were induced by UV irradiation, the addition of the ginseng lowered the frequencies significantly. Considering that UV light blocks the DNA chain growth in its replication, thus leads to the SCE formation, it is possible that the ginseng counteracts the delay of chain growth by facilitating excision repair which minimizes inhibition of chain growth, possibly by cutting dimers ahead of the growing point.[40] It is also possible that the ginseng reduces DNA strand breaks by stabilizing the DNA molecule. There have been reports that radioprotective compounds reduce chromosome breaks and bridge formation induced by radiation[41] and stabilize the DNA molecule by elevating Tm to give resistance to heat denaturation.[42] The ginseng protein used in this study has been found to raise the Tm value of the DNA and form precipitates with nucleoproteins. Therefore, stabilization of DNA molecule to resist damage could be another possibility for the mechanism of antiradiation activity of the protein.

Since pre-and post-treatments of the ginseng showed no significant differences in its effects, further experiments should be carried out to determine the precise role of the protein in the excision repair and stabilization of the DNA in relation to the SCE formation.

CONCLUSION

Upon the addition of the radioprotective ginseng protein, the high frequencies of sister chromatid exchanges induced by UV irradiation were reduced significantly. This result suggests that the protein could reduce DNA damages that lead to the SCE formation.

ACKNOWLEDGEMENT

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