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Mechanisms of the Radioprotective Activity of Ginseng Protein Fraction

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Introduction

There are several mechanisms proposed for radioprotection. The first and one of the earliest is the induction of biochemical or chemical hypoxia.[1] The second involves hydroxyl radical scavenging and hydrogen atom donation to repair mainly DNA radical formed by radiation. This mechanism known as chemical repair brings about the reduction of initial damage to DNA. The third mechanism is by the augmentation of DNA repair either through gene amplifications or enzyme induction. Enhancement of the existing DNA repair system through particular substances is another possibility to increase radioprotectivity. The fourth mechanism is manipulation of the antioxidizing system and the fifth method is enhancement of cell proliferation, particularly targeted to marrow stem cells.

Of these mechanisms, the classical thiol protectors are known to exhibit their effects mostly by hydroxyl radical scavenging and free radical repair by hydrogen atom donation.[2] However, since only a fraction of total damage can be reduced by this chemical repair, biological repair of DNA molecule is of particular importance in improving radioprotection. Therefore, it is desired to develop radioprotective compounds that have the capacity to decrease the amount of damage formed in DNA as well as the capacity to increase biochemical repair of already damaged DNA.

To elucidate the mechanism of the radioprotective ginseng protein fraction obtained from Korean white ginseng, it was postulated that the fraction might reduce initial damage to DNA by radiation[3] or increase the DNA repair capacity of the cell.[4]

Based on this hypothesis, we examined the effects of the radioprotective ginseng protein fraction on the formation of sister chromatid exchanges (SCE)[5,6] and DNA repair capacity.[7]

Purification of Ginseng Protein Fraction

Radioprotective ginseng protein fraction was purified from Korean white ginseng by Tris-HCl buffer extraction, 70% ammonium sulfate fractionation, CM-cellulose column chromatography, heat inactivation and Sephadex G-75 column chromatography.[8] Three fractions were obtained from the last column and the first two fractions were tested for the activity because the third fraction was extremely small.

Activity Assay for Radioprotection

The activity was tested on ICR mouse of 6 weeks old female, whole body irradiated with γ-rays. Twenty four hours before or immediately after the irradiation, ginseng protein in physiological saline was injected intraperitoneally into mice. Mice injected with saline alone with or without γ-ray irradiation were served as controls. As the result mice injected with saline alone without irradiation survived 100%, while, irradiated mice injected with saline died all. However, 30-day survival rates of mice treated with the first fraction (GI) were 45%, regardless of the time when the fraction was added. This data demonstrates strong protective effect of GI fraction against radiation. On the other hand, the second fraction (GII) did not show such a significant increase of 30-day survival rates.

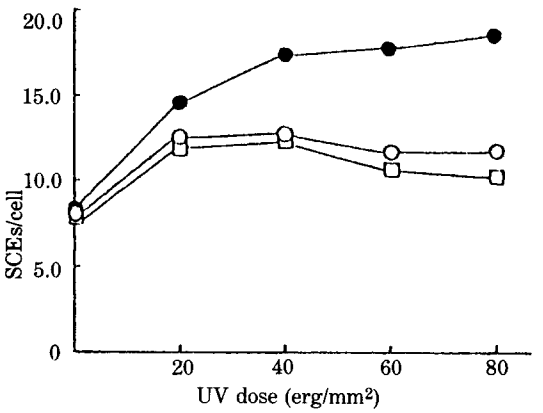

Fig. 1. Effects of ginseng protein on UV induced sister chromatid exchanges, Here, ● - UV alone; ○ - post-treated with 500μg protein/m*l*; □ - pre-treated with 500μg protein/m*l*.

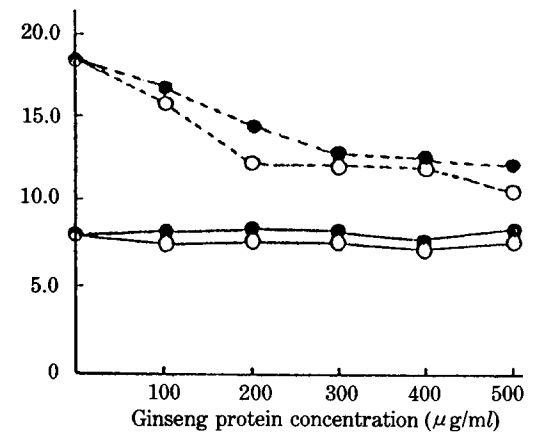

Fig. 2. Dose-response curves of sister chromatid exchanges against ginseng protein concentrations, with or wothout UV irradiation. Here, ●--- SCE of cells treated with ginseng protein after UV irradiation at 60 erg/mm^2, ○ --- SCE of cells treated with ginseng protein before UV irradiation at 60 erg/mm^2; ●- SCE of cells treated with ginseng protein and BrdU at the same time; ○ - SCE of cells treated with ginseng protein before BrdU treatment.

Effects on Sister Chromatid Exchanges

CHO-K1 cells in logarithmic growth phase were irradiated with UV light or treated with MMS. Ginseng protein fraction was added to cells 24 hours before or immediately after the treatment. Chromosome preparations were made by air drying technique and differential staining of sister chromatids were carried out by the modified method of Perrry and Wolff.[9]

When the cells were irradiated with increasing dose of UV light, the frequencies of SCE were increased markedly. However, when the ginseng protein was added (final conc. of 500 μg/m*l*) to the cells, before or after UV irradiation, high frequencies induced by UV light was decreased significantly (Fig. 1). When ginseng-protein concentration was increased at a UV dose of 60 erg/mm^2, SCE frequencies were reduced considerably. On the other hand, cells with no UV light showed almost constant and normal number of SCEs throughout the ginseng concentrations (Fig. 2). This implies that the ginseng itself does not play any role in SCE formation.

Since methylmethanesulfonate (MMS) has been reported to show X-ray mimetic effect,[10,11] MMS was used instead of ionizing radiation to see the effect of ginseng protein fraction on SCE formation. When MMS concentration was increased from 10^{-5} to 10^{-3}M, the SCE was increased many fold. However, the addition of the ginseng lowered the frequency significantly as shown in Fig. 3. Ginseng dose-response of MMS treated cells is depicted in Fig. 4 at two different MMS concentrations. At higher dose of MMS, the addition of ginseng protein reduced the frequencies significantly, more in the case of pre-treatment. At lower dose of MMS SCEs were also decreased but the effects were smaller.

Effects on DNA Repair Capacity

DNA repair was determined by the incorporation of ^{3}H-thymidine into DNA whose semiconservative synthesis has been inhibited by treatment with trimethylpsoralen + near UV light (365 nm).[12-14] Cells in logarithmic growth phase were incubated in a medium containing ^{14}C-thymidine and then the medium was replaced by PBS containing trimethylpsoralen. The cells were exposed to near UV, followed by incubation in a regular medium to allow for residual semiconservative DNA synthesis

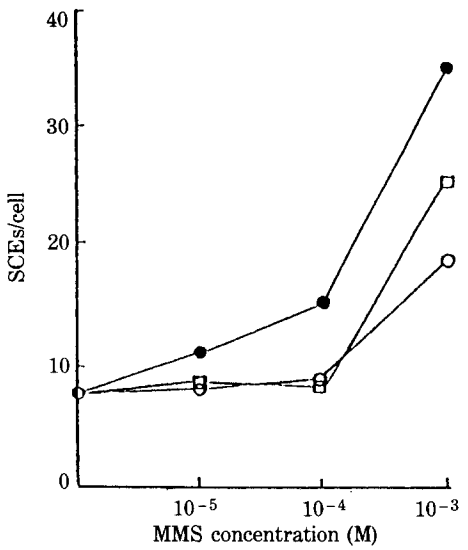

Fig. 3. Effects of ginseng protein fraction on MMS induced sister chromatid exchanges. Here, ● – MMS alone; □– post-treated with 500μg protein/ m*l*; ○– pre-treated with 500μg protein/m*l*.

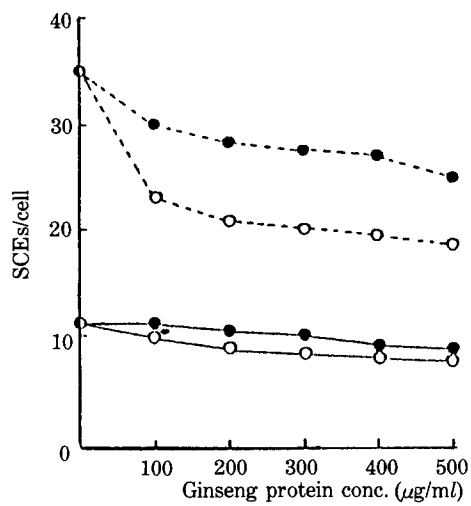

Fig. 4. Dose-response curves of sister chromatid exchanges against ginseng protein concentration. Here, ○ pre-treated cells; ● post-treated cells; ----10^{-3} M MMS; —10^{-5} M MMS.

to complete. To induce DNA damage, exposure to UV light (254 nm) was performed in a medium containing ^{3}H-thymidine. The results were expressed as repair capacity numbers, obtained by dividing the number of counts at each dose point by the number of counts at control zero dose, thus eliminating

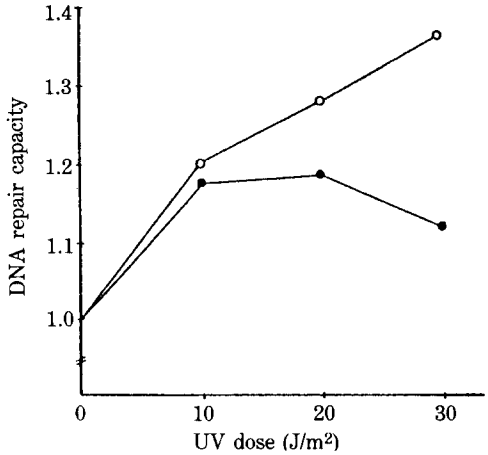

Fig. 5. Effect of ginseng protein on DNA repair capacity of CHO-K1 cells irradiated with UV light in the presence of ^{3}H-thymidine.
Ginseng protein was added to the cells 3 hrs prior to exposure and was removed before UV irradiation. Here, ● – control cells; ○– cells with 500μg/m*l* ginseng protein

variations between experiments due to the use of different specific activity thymidine solutions.

The shape and extent of the effect is demonstrated in Fig. 5. The response of ginseng protein fraction at the concentration of 500 μg/m*l* brought about a significant increase in repair capacity as compared to the control cells. Logically, such an increase could reflect the dual action of reducing the amount of damage and increasing the repair of DNA. When bovine serum albumin was added to cells for comparison, there were no increase in repair capacity, suggesting that the effect is specific to ginseng protein fraction (Fig. 6).

Ginseng protein clearly plays a role in the DNA repair process as shown in Fig. 7. When the concentration of ginseng protein was increased from 100 to 500 μg/m*l*, the repair capacity was increased markedly and more when ^{3}H-thymidine was present during exposure. This effect may be due to the fast repair induced by ^{3}H-thymidine during UV irradiation.

Conclusions

The DNA molecule is known to be the major

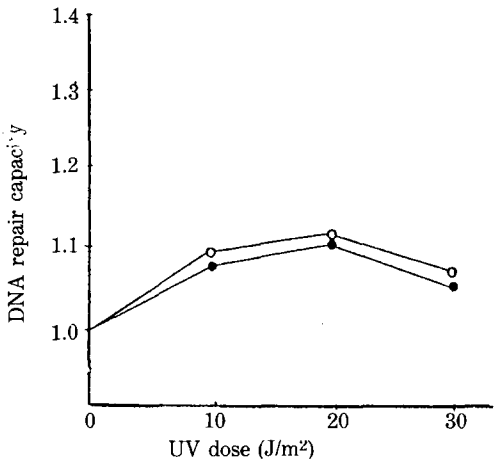

Fig. 6. Effect of BSA on DNA repair capacity of CHO-K1 cells irradiated with UV light in the presence of ^{3}H-thymidine.
BSA was added to the cells 3 hrs prior to exposure and was removed before UV irradiation. ● - control cells; ○ - cells with 500μg/m*l* BSA

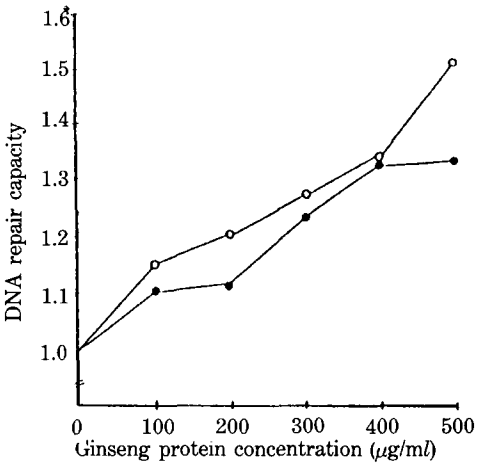

Fig. 7. Dose-response of ginseng protein on DNA repair capacity.
Cells were irradiated with UV light at the dose of 30 J/m^2. Ginseng protein was added to the cells 3 hrs prior to exposure.
Here, ○ - UV was irradiated in the presence of ^{3}H-thymidine; ● - UV was irradiated in the absence of ^{3}H-thymidine

target of radiation and the primary damage induced by UV light is the formation of pyrimidine dimers between the two adjacent thymine residues.[15] On the other hand, MMS mainly forms methylated base, resulting in base modification.[16,17] Cellular repair processes such as excision repair seem to play a major role in repair of these lesions. Wolff *et al.*[18] and Cleaver[19] 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.

The results that the radioprotective ginseng protein fraction reduces high frequencies of SCE induced by UV or MMS implies that the active component might reduce the amount of unrepaired damaged DNA, in other words, increase DNA repair capacity of damaged cells. To elucidate this assumption, DNA repair capacity was measured by the incorporation of labelled thymidine into DNA in trimethylpsoralen + near UV (PUVA) treated cells. The results show significant increase of DNA repair capacity by the addition of ginseng protein fraction. The potential mechanisms by which this component express its protective effects may be numerous, however, it was concluded that one of mechanisms is clearly by acting as an enhancer of DNA repair, expressed in increased repair synthesis.

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