Arch. Pharm. Res. Vol. 16, No. 4, pp. 259-264, 1993

Effects of Radioprotectors on DNA Repair Capacity of Tumor Cells

Choonmi Kim and Mikyung Kim

College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea

(Received May 10, 1993)

Three cell lines, CHO, L929 and B16 which are non-tumorigenic, tumorigenic and cancer cells, respectively, were first tested for their survival in the presence of radioprotective ginseng protein fraction(GPF). The influence of three radioprotectors-GPF, cysteamine, and 1-Methyl-2-bis[(2-methylthio)vinyl] quinolinium iodide (MVQI) on DNA repair capacity of UV damaged cells was also investigated by measuring ^{3}H-thymidine incorporation of PUVA treated cells. In cell survival test, the GPF showed higher cytotoxicity in L929 and B16 than in CHO cells. However, the degree of cell killing was not high enough to consider it as an antitumorigenic agent. Variable results were obtained in the effects on DNA repair capacity depending on the protectors and cell lines used. In pretreatment, the presence of GPF and MVQI brought about a significant increase in the capacity in both CHO and B16 cells. However, in L929, the enhancing effect was not shown. In all three cell lines, cysteamine showed lower repair capacity than control, suggesting the primary damage reduction to occur, rather than repair enhancement. In posttreatment, GPF and MVQI resulted in stronger enhancing effects in L929 and B16 cells, while it was weaker in CHO cells. Here also cysteamine showed a very little or no increase in the capacity in all three cell lines. These results demonstrate that GPF has mild cytotoxicity in tumorigenic cells and that GPF and MVQI enhance DNA repair capacity of UV damaged cells, whether they are tumorigenic or not. On the other hand, cysteamine shows only damage reduction effect. Cells of different genetic origin seem to give different responses to the modifier and different modifiers may possibly work by different mechanisms.

Key words: Radioprotectors, Cell survival, UV damage, DNA repair capacity, PUVA treatment, ^{3}H-thymidine incorporation

INTRODUCTION

Radioprotective ginseng protein fraction(GPF) has been reported to reduce high frequencies of sister chromatid exchanges (Kim and Choi, 1988; Kim and Chung, 1990) and chromosome aberrations (Kim and Park, 1988) induced by UV irradiation. This suggested that GPF might have enhancing effects in repair processes of UV damaged DNA. However, there has been no direct evidence to prove this hypothesis. Therefore, DNA repair capacity of UV damaged CHO-K1 cells was measured in the absence or presence of GPF (Kim and Choi, 1992). As a result it was clearly demonstrated that the repair capacity of damaged cells was significantly increased. The data implies that the radioprotective activity of GPF can be attained from the enhancement of repair capacity of damaged DNA (Riklis *et al.*, 1988). The question was whether this effect also occurs in tumorigenic or cancer cells since radiotherapy is being widely used for cancer patients. If the protector which is aimed to protect normal cells also shows protective activity in cancer cells, it may result in reducing the effect of radiation therapy accordingly. Therefore, the study of radioprotectors such as GPF, cysteamine (Murray *et al.*, 1988) or MVQI(Foye *et al.*, 1987) in relation to their influences in DNA repair capacity was planned in this work. Three cell lines, CHO, L929 and B16, which are non-tumorigenic, tumorigenic and cancer cells, respectively, were chosen for the experiments and all cells were irradiated with UV light to induce damage in DNA molecules.

Correspondence to: Choonmi Kim, College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea

MATERIALS AND METHODS

Materials

Six year old Korean white ginseng was used for the purification of radioprotective GPF. Chemicals such as

Tris-(hydroxymethyl) aminomethane, carboxymethyl cellulose cation exchanger, Sephadex G-75, bovine serum albumin(BSA), N-2-hydroxyethyl piperazine N'-2-ethane sulfonic acid (HEPES), 4,5',8-Trimethylpsoralen (Trioxalene) and sodium pyruvate were purchased from Sigma Chemical Co. (St. Louis, Mo. USA). Eagle's minimum essential medium(EMEM), RPMI-1640 medium, fetal bovine serum(FBS), L-glutamine, nonessential amino acids, trypsin-EDTA, penicillin-streptomycin solution and Trypan Blue were the products of Gibco Lab. Life Technologies Inc. [Methyl-^{3}H]thymidine, [2-^{14}C]thymidine and Scint A (cocktail solution) were purchased from Packard Co..

Purification of ginseng protein fraction

Radioprotective GPF was isolated and purified from Korean white ginseng by the procedures of Tris-HCl buffer (pH 7.6) extraction, 70% ammonium sulfate fractionation, CM-cellulose column chromatography, heat inactivation and Sephadex G-75 column chromatography as described before (Kim and Hwang, 1986).

Cell culture

Three cell lines, CHO-K1 as a non-tumorigenic, L929 as a tumorigenic, and B16 as a cancer cell line, were chosen for the experiments. CHO-K1 cells were incubated in EMEM containing 10% FBS and antibiotics at 37℃ in a humidified 5% CO_2 incubator. L929 and B16 cells were cultured in RPMI-1640 medium containing 5% FBS, L-glutamine (2 mM), β-mercaptoethanol (5×10^{-5} M), Na-pyruvate, non-essential amino acids (0.1 mM) and antibiotics. pH of culture medium was adjusted to 7.2-7.4 using 10 mM HEPES buffer and sodium bicarbonate.

Doubling time of cells

Cells of each line at the number of $1\text{-}3\times10^4$ cells/ml were plated in 60 mm petri dishes and trypsinized at every 12 or 24 hr interval to stain with Trypan Blue. Unstained live cells were counted using Hemocytometer and growth curves were drawn on semilogarithmic paper. The results were compared with reference data (Colowick and Kaplan, 1979).

Cell survival test

In 24 well plates, $3\text{-}5\times10^4$ cells/ml were plated and incubated for 24-48 hrs to reach exponential growth phase. Culture medium was exchanged with the one that contained radioprotective GPF at the dose of 100 to 500 μg/ml, measured as the amount of protein in the fraction. After 24 or 48 hr incubation, cells were stained with 0.4% Trypan Blue and live cells were counted using Hemocytometer. Cell survival rates were calculated by dividing the number of viable cells in samples with that of control.

Determination of DNA repair capacity

The number of cells growing exponentially in each well of 96 well plates was checked first by incubating for 24 hours in a medium containing 0.01 μCi ^{14}C-thymidine. Cells were then treated with 2×10^{-5} M trimethylpsoralen plus near UV light (300-400 nm) to block normal semiconservative replication of DNA (PUVA treatment) (Heimer *et al.*, 1977; Heimer and Riklis, 1979; Heimer *et al.*, 1983; Riklis, 1983; Riklis *et al.*, 1988). Experiments were carried out in two different sets; pretreated and posttreated, for each modifier tested. Three modifiers, GPF, cysteamine and MVQI were chosen for the study. For pretreated set, cells were incubated for 3 hrs in a medium containing one of the three modifiers, followed by the addition of 5 μCi/ml ^{3}H-thymidine and immediate irradiation of short wave UV light (254 nm). For posttreated set, cells were irradiated with short wave UV light first and then incubated for 90 min in a medium containing a modifier and ^{3}H-thymidine.

To measure the amount of ^{3}H-thymidine incorporated into DNA in the process of repair synthesis, cells in each well were harvested on a glass fiber filter using Microharvester and the amounts of ^{14}C and ^{3}H were counted using Liquid Scintillation Counter. By calculating the ratio of ^{3}H over ^{14}C, the possible error that can come from the difference in initial number of cells existed in each well was eliminated. DNA repair capacity number was obtained when the ^{3}H/^{14}C ratio of samples at each dose point was divided by the ratio of control.

RESULTS

Purification of ginseng protein fraction

Relative yield of protein contained in the fraction was about 1.7% of the total protein present in the crude extract.

Doubling time of cells

Growth curves of the three cell lines are shown in Fig. 1. Doubling times of CHO-K1 and B16 cells were 12-14 hrs, while that of L929 cells was about 20-22 hrs. These results agree well with those presented by others (Hsie *et al.*, 1981), indicating that the cells are in healthy and normal growing state (Fisher and Chun, 1968).

Cell survival rates

As shown in Fig. 2, survival rate of CHO-K1 cells after 24 hr incubation was decreased about 20% in the presence of GPF at the dose of 500 μg/ml, imply-

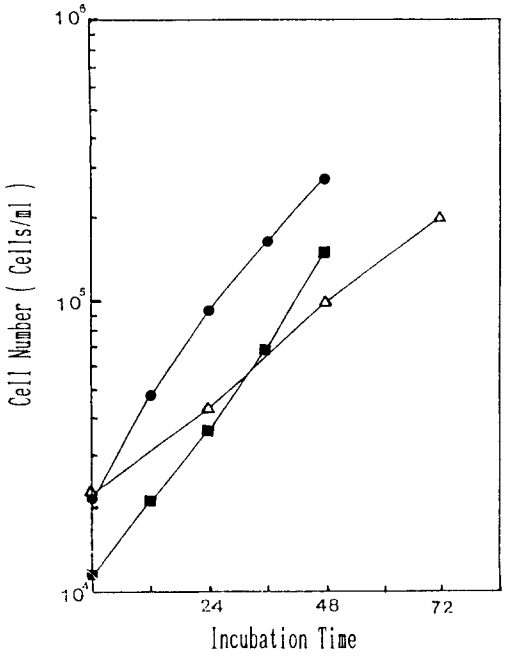

Fig. 1. Growth curves of CHO-K1, L929 and B16 cells.
● CHO-K1; △ L929; ■ B16.

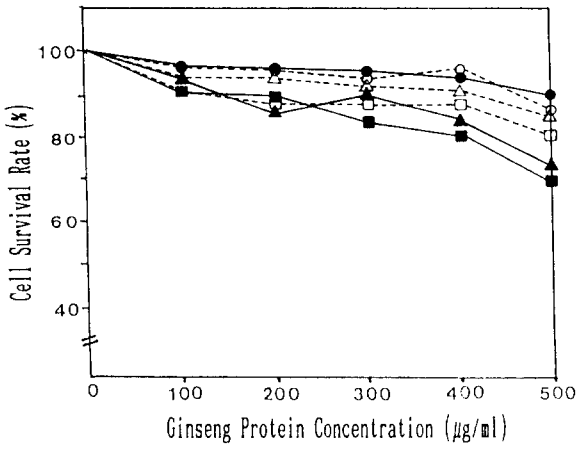

Fig. 2. Percent survival of cells incubated for 24 and 48 hrs in different concentrations of ginseng protein fraction. Here, open symbols: 24 hr; closed symbols: 48hr incubation.
○● CHO-K1; △▲ L929; □■ B16.

ing mild cytotoxicity of the fraction. This result is agreeable with the data presented by Kim & Yoon (Kim and Yoon, 1988), who tested the survival rates by different method. In L929, the result was similar to that of CHO-K1 cells, even though it was a little lower. However, in B16 cells, the survival rate was somewhat lower than those of the other two cell lines. After 48 hr incubation, the reduction in survival rates was appeared to be even larger in both L929 and B16 cells, especially when higher doses of GPF were treated. Since the similar effect was not seen in non-tumorigenic

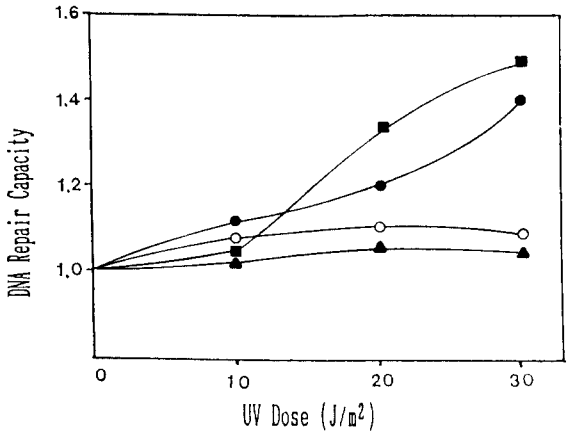

Fig. 3. DNA repair capacity of UV irradiated CHO-K1 cells, pre-treated with three different radioprotectors. Each modifier was added to cells 3 hrs prior to exposure and removed just before irradiation.
○ Control; ● Ginseng; ▲ Cysteamine; ■ MVQI.

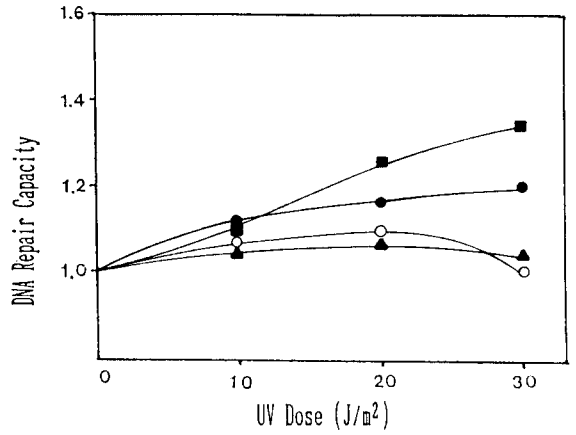

Fig. 4. DNA repair capacity of UV irradiated CHO-K1 cells, post-treated with three different radioprotectors. Each modifier was added to cells immediately after exposure to UV light.
○ Control; ● Ginseng; ▲ Cysteamine; ■ MVQI.

CHO-K1 cells, it might be concluded that the fraction showed stronger cytotoxicity on tumorigenic and cancer cells.

DNA Repair capacity

Three different cell lines irradiated with UV light were treated with each of three radioprotective modifiers and the effects were checked on DNA repair capacity of damaged cells. Fig. 3 and 4 present the results on CHO-K1 cells. In pretreated cells, GPF and MVQI gave almost no effect on DNA repair activity of the cell at low UV dose. However, the repair capacity was markedly increased at higher dose of UV.

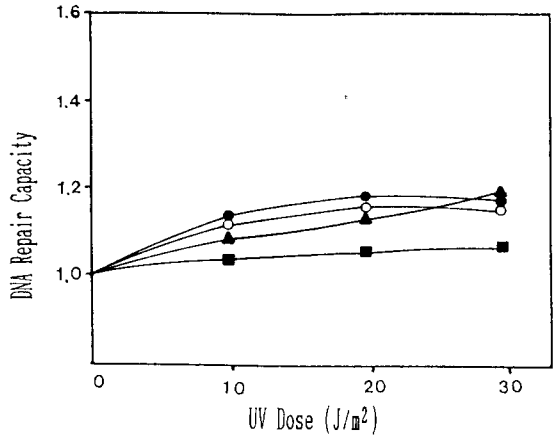

Fig. 5. Effects of three different radioprotectors on DNA repair capacity of UV irradiated L929 cells (pre-treatment). Each modifier was added to cells 3 hrs prior to exposure and removed just before irradiation.
○ Control; ● Ginseng; ▲ Cysteamine; ■ MVQI.

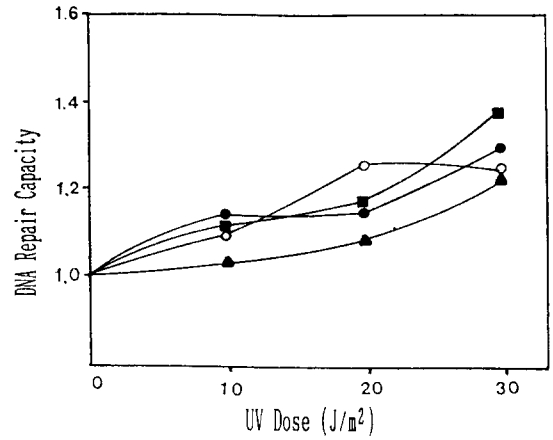

Fig. 7. DNA repair capacity of UV irradiated B16 cells, pretreated with three different radioprotectors. Each modifier was added to cells 3 hrs prior to exposure and removed just before irradiation.
○ Control; ● Ginseng; ▲ Cysteamine; ■ MVQI.

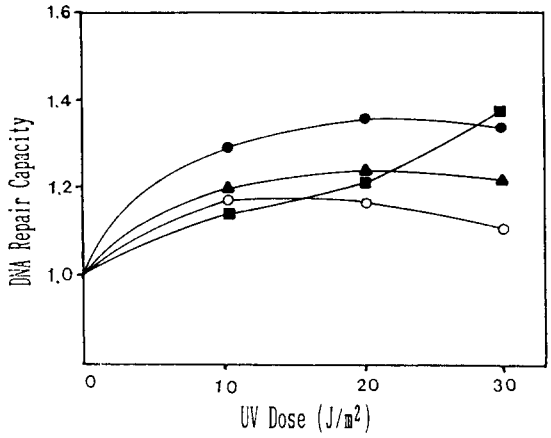

Fig. 6. Effects of three different radioprotectors on DNA repair capacity of UV irradiated L929 cells (post-treatment). Each modifier was added to cells immediately after exposure to UV light.
○ Control; ● Ginseng; ▲ Cysteamine; ■ MVQI.

On the other hand, cysteamine showed a little lower capacity number than that of the control. This data may imply that the radioprotective activity of cysteamine has no direct correlation to the repair capacity of damaged DNA and rather suggest that its protective action may come from damage reduction effect as described by Riklis (Riklis, 1983). In posttreated cells, similar results were obtained with each of three modifiers. The enhancing effects of GPF and MVQI were somewhat lower than those obtained from pretreated cells.

When L929 cells were treated with modifiers before UV irradiation, GPF and cysteamine showed almost the same results as the control, while MVQI gave even lower capacity (Fig. 5). However, when cells were treated by modifiers immediately after UV irradiation, DNA repair capacity was increased in all three cases. Enhancing effect was much higher in GPF and MVQI than cysteamine treated cells as shown in Fig. 6. These data present the possibility that, at least in L929, the repair enhancing activity of the modifiers can only be expressed when they are added to damaged cells, not in intact cells. In the case of cysteamine, even though it showed mild increase in the capacity, being different from all other cases, this data alone may not be enough to interprete it as having DNA repair enhancing effect.

Results for B16 cells show somewhat different from those of the other two cell lines. In pretreated cells, DNA repair capacity was increased by MVQI and GPF, while cysteamine showed lower repair activity than the control in all dose levels (Fig. 7). Fig. 8 demonstrates higher repair capacity shown by MVQI and GPF in posttreated cells. Cysteamine also showed lower capacity than the control just as in the case of pretreated cells. Here, MVQI presented the most prominent increase in the repair capacity among all the cases examined, while the enhancing effect of GPF was shown by its typical pattern as was before (Kim and Choi, 1992).

DISCUSSION

UV radiation, particularly short wavelength below 320 nm, has much in common with those of X- and γ-rays in its biological effects (Peak, M. J. and Peak, J. G., 1980). It can be absorbed by a variety of biological compounds and result in chemical modification of various biomolecules. Biological actions of UV ra-

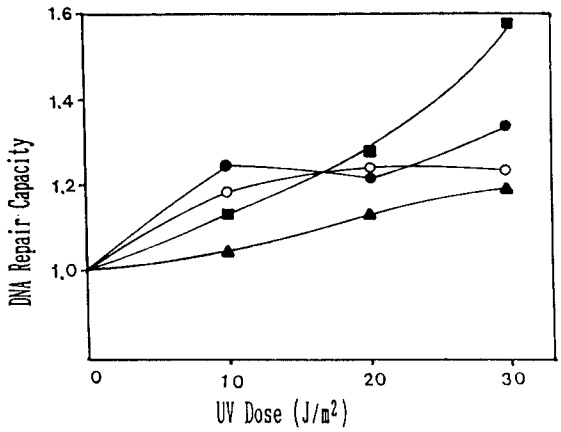

Fig. 8. DNA repair capacity of UV irradiated B16 cells, post-treated with three different radioprotectors. Each modifier was added to cells immediately after exposure to UV light. ○ Control; ● Ginseng; ▲ Cysteamine; ■ MVQI.

diation include changes in DNA, RNA, enzymes and synthesis of macromolecules. Damages in DNA can occure several forms such as base damage, single and double strand breaks and DNA cross links. One of the most prominent effect by UV irradiation is the induction in DNA of cyclobutane dimers at adjacent pyrimidine base sites. This dimers can be repaired by excision repair process before DNA replication process starts in the cell, thus avoiding mutation.

Radioprotectors which are protective against X-rays also protect cells from short wavelength UV radiation. If the protectors accelerate repair processes of damaged DNA, DNA repair capacity will increase. On the other hand, if the protectors only reduce primary damage formation and do not affect repair abilities of cells, DNA repair capacity will be even lower than the control because there will be less damage to repair. However, if both damage reduction and repair enhancement play their roles in the mechanism, the increment of repair capacity will be smaller in comparison with the case in which repair enhancement works alone.

When modifiers are treated before UV irradiation (pretreatment), the possibility of damage reduction to occur coexists with that of repair enhancement. Therefore, lower capacity number will be expected in this case. On the other hand, when modifiers are treated after UV damage is induced(posttreatment), only repair enhancement can be counted in the measurements of its DNA repair capacity. Hence the capacity number will be expected to be larger than the pretreated case. This was the case in B16 cells when GPF or MVQI was treated. However, the results were reverse in CHO-K1 cells. Moreover, L929 showed another pattern, which gave no increment of repair capacity in pretreated cells while significant increase in post-treated cells, even in the case of cysteamine treatment.

Difference between cells on the modification of radiation response has been reported to be shown especially when biological or biochemical parameters were involved (Riklis, E. *et al.*, 1988) as is in this case. And the time when modifiers are treated to cells also seems to give an important influence in expressing their repair enhancing effect. Detailed explanation for the difference in relation to their action machanism and genetic trait can not be presented here from the data obtained in this study. However it is obvious that the GPF and MVQI do increase DNA repair capacity of UV damaged CHO-K1, L929 and B16 cells, at somewhat different degrees and conditions. While, cysteamine did not affect the DNA repair capacity of all three cell lines tested in this study. Therefore, it can be concluded that radioprotective ginseng component and MVQI significantly enhance DNA repair capacity of tumor cells such as L929 and B16 as well as non-tumorigenic CHO-K1 cells.

ACKNOWLEDGEMENT

This work was supported by research fund from The Research Institute for Pharmaceutical Science of Ewha Womans University.

REFERENCES CITED

Colowick, S. P. and Kaplan, N. O. (Eds), *Methods in Enzymology,* Vol. 58, Academic Press, New York, 1979, p150.

Foye, W. O., Jones, R. W., Ghoshal, P. K. and Almassian, B., Antiradiation Compounds. 20. 1-Methylquinolinium(pyridinium)-2-dithioacetic acid derivatives. *J. Med. Chem.,* 30, 57-62 (1987).

Heimer, Y. M., Ben-Hur, E. and Riklis, E., Psoralen and near ultraviolet light; A probe for study of control of protein synthesis. *Nature,* 268, 170-171 (1977).

Heimer, Y. M. and Riklis, E., Inhibition by trioxalene plus near ultraviolet light of the induction of ornithine decarboxylase in Chinese hamster cells. *Biochem. J.,* 183, 179-180 (1979).

Heimer, Y.M., Kol, R., Shiloh, Y. and Riklis, E., Psoralen plus near-ultraviolet light; A possible new method for measuring DNA repair synthesis. *Radiat. Res.,* 95, 541-549 (1983).

Hsie, A. W., Casciano, D. A., Couch, D. B., Krahn, D. F., O'Neill, J. P. and Whitfield, B. L., The use of Chinese hamster ovary cells to quantify specific locus mutation and to determine mutagenicity of chemicals. *Mutat. Res.,* 86, 193 (1981).

Kim, C. and Choi, J. E., Effects of radioprotective ginseng protein on UV induced sister chromatid exchanges. *Arch. Pharm. Res.,* 11, 93-98 (1988).

Kim, C. and Choi, M. K., DNA repair enhancement

by radioprotective ginseng protein fraction. *Yakhak Hoeji,* 36, 449-454 (1992).

Kim, C. and Chung, S.L., Effects of ginseng saponin, BSA and cysteamine on sister chromatid exchanges. *J. Korean Res. Inst. Better Living,* 45, 193-198 (1990).

Kim, C. and Han, G. S., Radioprotective effects of ginseng proteins. *Yakhak Hoeji,* 29, 246-252 (1985).

Kim, C. and Hwang, J. J., Polyacrylamide gel electrophoresis on ginseng proteins. *Yakhak Hoeji,* 30, 343-347 (1986).

Kim, C. and Park, S. Y., Effects of ginseng protein on relative survival and chromosome aberration of UV irradiated cells. *Arch. Pharm. Res.,* 11, 225-229 (1988).

Kim, C. and Yoon, S. R., Cytotoxic effect of radioprotective ginseng protein fraction on CHO-K1 cells. *Yakhak Hoeji,* 32(5), 313-318 (1988).

Murray, D., Vanankeren, S. C., Milas, L. and Meyn, R. E., Radioprotective action of Aminothiols *in vitro* and *in vivo*: Comparison between effects on DNA damage and cell survival. *Pharm. Ther.,* 39, 151-153 (1988).

Peak, J. M. and Peak, J. G., Protection by glycerol against the biological actions of far UV light. *Radiat. Res.,* 83, 553-558 (1980).

Riklis, E., DNA repair as a probe of radiosensitivity and radioprotection: In Nygaard, O. F. and Simic, M. G. (Eds.), Radioprotectors and anticarcinogens, Academic Press. New York, 1983, pp. 363-380.

Riklis, E., Kol, R., Green, M., Prager, A., Marko, R. and Mintsberg, M., Increased radioprotection attained by DNA repair enhancement. *Pharm. Ther.,* 39, 311-322 (1988).

Yonezawa, M., Katoh, N. and Takeda, A., Some properties of the radioprotective substances. *J. Radiat. Res.,* 22, 336-343 (1981).