Radioprotective Action of SH Compounds

II. Effect of Glutathione on the Mitotic Delay Induced by X-Ray.

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INTRODUCTION

The effect of various SH compounds, especially MEA[1], AET[2], as chemical protectors for the biological action of radiation have been reported. As reconfirmed in the previous paper,[3] the administration of these SH compounds before irradiation has been found to alleviate the lethal effect of radiation[4)5)6)] or subsequent tissue injury, not only *in vivo*[7)8)9)] but also *in vitro*.[10)] To evaluate the mechanism of this protective effect of SH compounds, many studies[11)12)13)14)] are being conducted from the aspects of chemical and biological properties of the SH compounds and some hypothesis is advanced on the mechanism of the protective action of the SH compounds.[15)16)] The SH containing molecule is now considered to be one of the targets of radiation. Administration of SH compounds causes an increase in the SH content in the cells and an intimate relationship is found between the increase in the amount of -SH in the cells and the radioprotective action.[10)17)18)]

When cells undergo mitosis, -SH or -S-S- plays an important role on the formation of spindle fibers and cell membrane.[19)] Such mitosis is inhibited as the result of irradiation. This phenomenon is caused even with a low dose of X-ray and the mechanism of this action have been analyzed by the use of metabolic inhibitors and other methods.[20)21)22)]

Among SH compounds, glutathione (GSH) is one of the compounds found most abundantly in the living organism, and will participate in the mechanism of various biological metabolisms. Thus, it is to be expected that the compound would exert radioprotective action on the delay of mitosis.

Therefore, to elucidate the protective action at cellular level, the author studied the action of GSH on the delay of mitosis induced by irradiation.

MATERIALS AND METHODS

1) *Animals*: Female mice of dd strain, with body weight of 20-25 gram and age of 8 weeks were used. With 4 mice in a group similar experiments were repeated twice.

2) *Irradiation*: Mice bearing tumor were placed in a box of acrylite set on 10 cm acrylite board and irradiated under condition of maximum back scatter. Physical factors of radiation were as follows: 200 kVp X-rays, 17.8 mA, 1.0mm Cu+1.0mm Al filter, H.V.L. 1.55 mm Cu, F.S.D. 50 cm, and dose rate 30 R/min. Doses of 100, 200, 400 and 600 rads were used. R-Rad calculation index of 0.95 was used. For the measurement of the dose, Victoreen R-meter 602 type chamber was employed.

3) *Mitotic index*: Ehrlich ascites tumor cells were transplanted into the peritoneal cavity of mice (2 million/mouse), harvested 7 days after transplantation and stained by Giemsa. In each experiment, 2,000 cells per animal were examined for determinating the mitotic index. The mitotic index at a certain period of observation was expressed as the number in 1,000 cells by using 4 animals.

4) *SH compound*: Reduced type glutathione dissolved in 0.2 ml of saline solution was injected in various doses intraperitoneally at 30 minutes before irradiation.

RESULTS

1) *Changes of mitotic index of Ehrlich ascites tumor cells, depending on the dose of irradiation.* Fig. 1 shows the changes in mitotic index of Ehrlich ascites

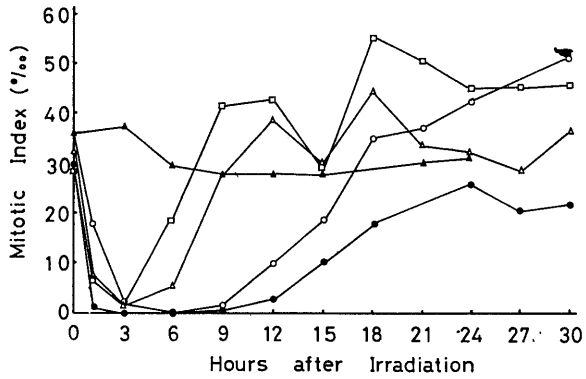
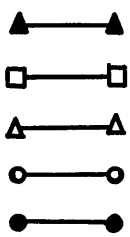


Fig. 1. Changes in the mitotic index after 100 rads, 200 rads, 400 rads and 600 rads X-irradiation. The mitotic inhibtion was released at 3, 6, 9 and 12 hours after 100, 200, 400 and 600 rads, respectively.



tumor cells after irradiation with 100, 200, 400 and 600 rads. The control values (without irradiation) were found to lie between 3.0 and 4.0 percent. Even within a short period after the irradiation with 100 rads, number of the mitotic cells immediately decreased and the mitotic cells disappeared at 3 hours after irradiation. Thereafter, however, the mitotic figure reappeared and the number of the mitotic cells also increased gradually. Subsequently 9–12 hours after irradiation, the number increased so fast that it overlapped that of control. But the value did not stay constant until 30 hours after irradiation, in the meantime the value at a certain period being about 5.0 percent. By the irradiation with 200 rads, the decrease in the mitotic index was more rapid than with 100 rads. In this instance, mitotic figure also disappeared completely at 3 hours after irradiation. Six hours later, a small number of mitotic pictures was observed, finally recovering to the control value at 9 to 12 hours after irradiation. By 400 rads, such an effect was further enhanced and no mitosis was observed until 9 hours. As 12 hours after irradiation, mitotic cells reappeared and the value reached that of the control group at 18 hours. Thereafter the value was not stable but increased over that of control. With 600 rads, even one hour after irradiation mitosis was observable and the state did not recover until 9 hours. At 12 hours after irradiation a few mitotic pictures were counted, and the number gradually increased with lapse of time. But even after 24 hours, the value did not reach the control value.

From these data it can be summerized as follows: By increasing the radiation doses from 100 to 200, 400 and 600 rads, the rate of disappearance of mitotic figures increased, and it required a longer time for the reappearance of mitosis.

Fig. 2 shows the relationship between the doses of irradiation and the time of mitotic delay. The interval from irradiation to the appearance of mitotic figures, was obtained from Fig. 1, 3, 4, 5 and 6. When the mitotic delay time was plotted on two dimensional logarithmic paper against the radiation dose effect curve

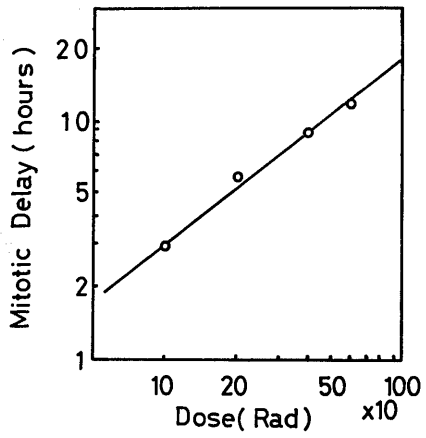


Fig. 2. Effects of X-irradiation on extent of mitotic inhibition. Scale are logarithmic.

was rectilinear. No effect of GSH was shown for mitotic delay time induced by X-irradiation: No changes in dose effect curve were observable by GSH administration.

2) *Effects of GSH on change of mitotic index after X-irradiation.* The changes of mitotic index induced by the treatment with GSH are shown in Figs. 3, 4, 5, and 6 after irradiation with 100, 200, 400 and 600 rads respectively. An increased mitotic index by GSH is observed at 12 hours after administration as shown in Fig. 3 without irradiation. The rate of disappearance of mitosis after irradiation with 100 and 200 rads was rather reduced by the GSH. However, no effect of GSH was observed on the mitotic delay time and on its recovery rate of mitosis, but the mitotic index became higher than that of non-GSH treated group approximately 12 hours after irradiation (Fig. 4). With exposure of 400 rads, the rate of disappearance of mitotic index in the group treated with GSH decreased as observable in the case of 100 or 200 rads (Fig. 5). The appearance of mitosis began at 12 hours after irradiation with 400 rads. The recovery of mitotic index in the group treated with GSH was much quicker than that of the non-treated group in the same period. As shown in Fig. 6, the mitotic index scarcely differed from that of the nontreated group at 12 hours after irradiation with 600 rads. The recovery of the mitotic index thereafter was stimulated by GSH treatment. During the recovery phase, the rate of reappearance of mitosis in the group treated with GSH is faster than in the non-treated group at 12 hours after irradiation of with either 400 or 600 rads. But as noted already, the administration of GSH caused a rise of mitotic activity at 12 hours after administration (Fig. 3). Thus, the relationship between such an effect of GSH facilitating mitosis and the irradiation time was evaluated in the following experiments.

3) *Effects of administration time of GSH before irradiation.* The administration of 30mg/animal of GSH was given intraperitoneally 30 minutes, 3 and 6 hours before irradiation with 400 rads. Fig. 7 shows the changes of mitotic index following the administration of GSH. In the group where the drug was administered 30 minutes before irradiation, the GSH administration resulted in an accelerated recovery, mitotic index of 2.1 percent, at 12 hours after irradiation. When the GSH administration was carried out 3 hours before irradiation, the results were similar to those in the group irradiated with 400 rads without GSH treatment (control) until 9 hours after irradiation. After 12 hours, the value of 1.7 percent was obtained, and it is observed to be a slower recovery than in the group administered 30 minutes before irradiation, but faster than in the control group. In the group administered 6 hours before irradiation, an increase of mitotic cells by GSH already appeared to some extent at the time of irradiation, but a sudden disappearance of mitosis was shown after irradiation. At 12 hours following the irradiation, mitotic index became 1.4 percent. The recovery was slower than in the group irradiated 30 minutes or 3 hours after administration, but faster than in the control group.

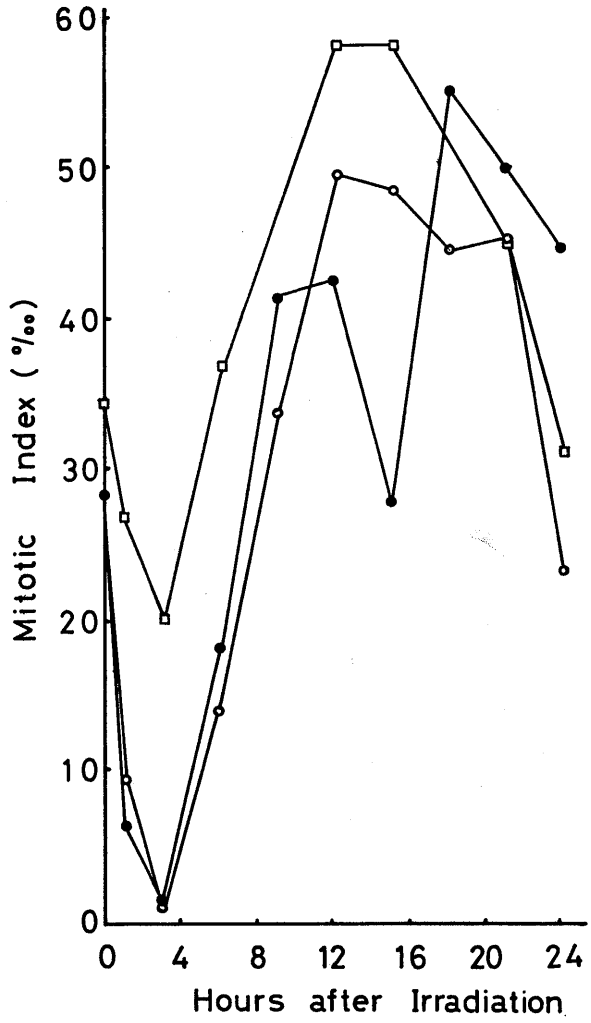


Fig. 3. Effects of glutathione (30 mg/mouse) on the recovery phase of radiation-induced mitotic delay. The GSH was added at 30 minutes before irradiation, and tumor-bearing mice were irradiated with 100 rad of X-rays, and then the mitotic index was determined at indicated periods.

GSH only

100 rads

GSH + 100 rads

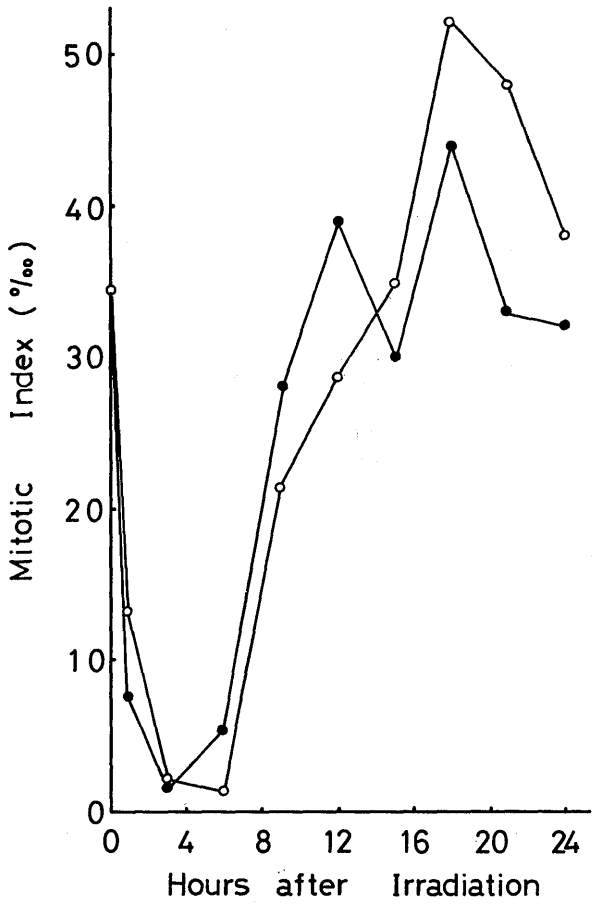


Fig. 4. Effects of glutathione (30 mg/mouse) on the recovery phase of radiation-induced mitotic delay. The GSH was added at 30 minutes before irradiation, and tumor-bearing mice were irradiated with 200 rad, and then the mitotic index was determined at indicated periods.

●——● 200 rads

○——○ GSH+200 rads

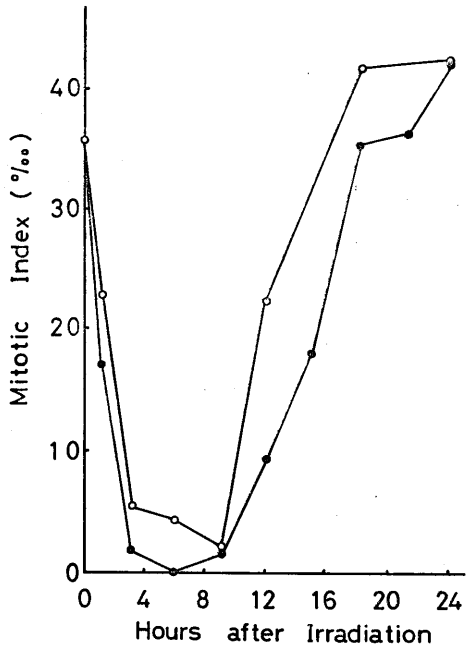


Fig. 5. Effects of glutathione (30 mg/mouse) on the recovery phase of radiation-indiuced mitotic delay. TheGSH was added at 30 minutes before irradiation, and tumor-bearing mice were irradiated with 400 rad.

●——● 400 rad only

○——○ GSH+400 rad

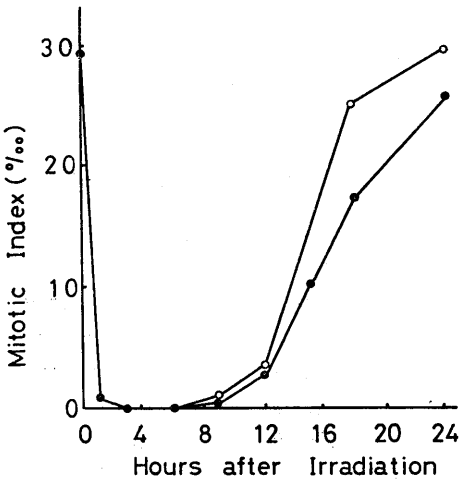


Fig. 6. Effects of glutathione (30 mg/mouse) on the recovery phase of radiation-induced mitotic delay. The GSH was added at 30 minutes before irradiation, and tumor-bearing mice were irradiated with 600 rad.

●——● 600 rad only

○——○ GSH+600 rad

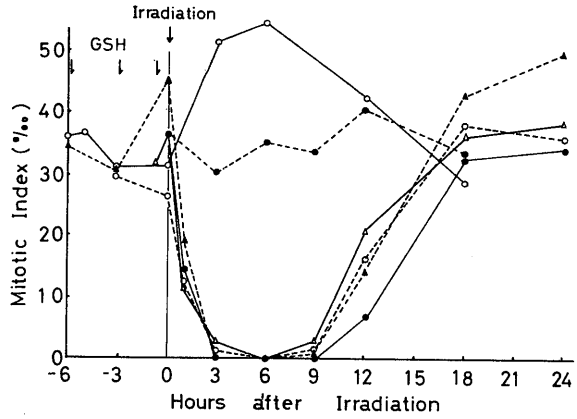


Fig. 7. Effects of glutathione (30 mg/mouse) on the recovery phase of radiation-induced mitotic delay. The GSH was added at 30 minutes, 3 hours and 6 hours before irradiation, and tumor-bearing mice were irradiated with 400 rads.

GSH only
400 rads only
without GSH, without irradiation
GSH treatment at 30 minutes before irradiation
GSH treatment at 3 hours before irradiation
GSH treatment at 6 hours before irradiation

4) *Effects of administration time of GSH after irradiation.* GSH (30 mg/animal) was administered intraperitoneally 30 minutes, 3, 6 and 9 hours after irradiation with 400 rads. Fig. 8 shows the effect of GSH treatment after irradiation on the changes in the mitotic index of the irradiated cells. In the curve in which the drug was administered 30 minutes after irradiation, the curve was almost the same as that of the group irradiated with 400 rads without GSH treatment (control). In the group in which the drug was administered 3 hours after irradiation, the recovery of mitotic index was further decreased; only 1.0 percent mitosis after 15 hours was observed. This result shows the inhibitory action of GSH on the recovery of mitosis. In the group in which the drug was administered 6 hours after irradiation, the effect remained similar to that in the group given the drug 3 hours after irradiation until 18 hours. The mitotic index thereafter increased and became 3.04 percent by 24 hours. In this instance, GSH treatment also inhibited the reappearance of mitosis. In the group where the drug was administered at 9 hours, some mitotic figures were seen at 12 hours, with a similar curve to that in the control group. However, GSH showed a slight inhibition on the recovery of mitosis.

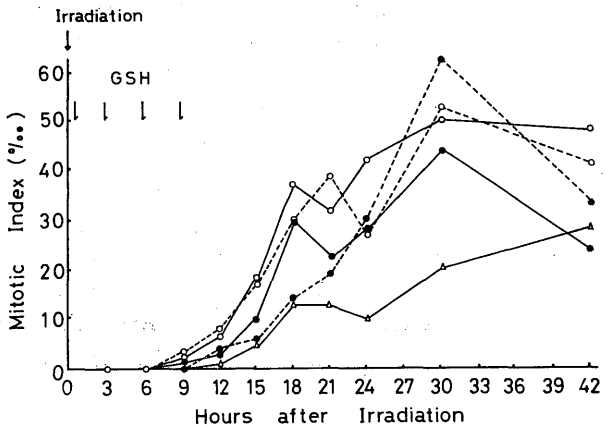


Fig. 8. Effects of glutathione (30 mg/mouse) on the recovery phase of radiation-induced mitotic delay. The tumor-bearing mice were irradiated with 400 rads (○———○; without GSH), and the GSH was added at 30 minutes (○·····○), 3 hours (△———△), 6 hours (●·····●) and 9 hours (●———●) after irradiation.

DISCUSSION

In this report the author aimed to clarify the protective action of GSH against mitotic delay induced by X-irradiation at cellular level. The results of this experiment show that GSH induced a slight decrease in mitotic index at 3 hours after administration and then a gradual increase in the index until 9–12 hours. The number of the index at this period was greater than that before administration of GSH. These patterns of the change in mitotic index was similar to those observed by X-irradiation. But the stimulation of the mitotic recovery by irradating with GSH treatment was observed only when the reagent was administered before the irradiation. These data suggest that the action of GSH on the mitosis is not equal to that of X-irradiation. Relative to the effect of SH compounds on the mitosis of *in vivo* cells, Maisin *et al.*[7)8)] and Maisin[9)] studied the effect of AET and 5-HT, radioprotective substances, on the recovery from disturbances of bone marrow and the intestinal tract caused by irradiation. The recovery of mitosis in each organ is markedly accelerated by the administration of radioprotective agents. The result of the present experiment shows that the delay-time of mitosis becomes longer rectilinearly as the dose of irradiation is increased.[22)] Hence it was expected that the recovery curve of mitosis would shift to the side of low dose in figure by the administration of radioprotective drugs, if the SH compound protects equally with all stages of cell division against mitotic inhibition caused by X-irradiation. But the data do not confirm the expected results. Therefore other unknown action of SH compound may exist against the mitotic cycle of the cells, e. g. an excess

amount of SH compound inhibits the DNA synthesis *in vitro* as reported by Sinclair,[23] the inhibition of DNA synthesis with FUdR treatment induces an increasing GSH content in the cell,[24] protective effect of SH compound against the inactivation of SH enzyme by the irradiation.[25]

One of the possible mechanisms may be suggested. As reported previously,[3] the protective action of SH compounds against radiation injury can be observed only when the SH compounds are present at the time of X-irradiation. These protective action of GSH are also confirmed in the case of mitotic delay induced by irradiation. These results suggest that GSH acts at a certain stage of cell cycle.[26][27] To the inhibitory action of GSH administered after irradiation it can be considered that this inhibition is the result of the additive action of GSH inhibition to the radiation injury.

SUMMARY

The radioprotective action of GSH was studied at the cytological level using the mitotic index of Ehrlich ascites tumor cells.

1. In the irradiation with 100 or 200 rads, the rate of recovery of mitotic index in the treated with 30 mg GSH was similar to that in the control group. But 12 hours after the irradiation and on, the mitotic index increased more than the value of the control.
2. With 400 or 600 rads, the recovery of the mitotic index was accelerated by the pretreatment with 30 mg GSH.
3. Irradiation with 400 rads 30 minutes, 3 hours and 6 hours after administration of 30 mg GSH resulted in the fastest recovery of mitotic index in the group where GSH was given 30 minutes before irradiation, followed by the group given GSH 6 hours previously.
4. Of the administration of GSH (30mg) at 30 minutes, 3 hours, 6 hours and 9 hours after irradiation with 400 rads the one that gave the most intense inhibition that 3 hours after irradiation. A slight inhibition was seen when administered at 9 hours after irradiation.
5. Treatment with GSH alone induced a slight decrease in the mitotic index and later a marked increase at 9 to 12 hours.

On the basis of these data, the mechanisms of the radioprotective action of GSH were discussed.

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