Radioprotective Action of SH Compounds

III. Effects of Aminoethyliosothiuronium Br.HBr, Cysteamine and 2-Mercaptopropionylglycine Against Mitotic Delay Induced By X-Rays.

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INTRODUCTION

In the previous paper of this series on the radioprotection of SH compounds,[1)2)] the author reported that the decreased body weight of mice by X-irradiation recovered faster by the treatment with SH compounds, and that the survival rate of the rodents treated with SH compounds also increased. The protective action of these SH compounds against X-irradiation was studied at cellular level for the explanation of the mechanism of these effects. Glutathione (GSH) acts as to reduce the mitotic delay of Ehrlich ascites tumor cells induced by X-irradiation, and to eliminate the mitotic inhibition. It is known that GSH has a different radioprotective action against mitotic delay depending on the different stage of cell cycle.

In this report the author describes the protective effects of various SH compounds (2-Mercaptopropionylglycine : MPG, cysteamine and Aminoethylisothiouronium Br. HBr : AET) on the mitotic delay of Ehrlich ascites tumor cells induced by X-irradiation. The most interesting point to be emphasized the question whether radioprotection of various SH comounds on the mitotic delay shows a similar cytological action of GSH or not. The answer to this question would be seem to give an idea to understand the mechanism of radioprotection of SH compounds.

MATERIALS AND METHODS

1) *Animals* : Female mice of dd strain, with body weight of 20 to 25 gram and age of 8 to 10 weeks were used. Similar experiments with 4 mice in one group were repeated twice.

2) *Irradiation* : Mice bearing tumor were placed in the box of acrylite set on 10-cm thick acrylite board and irradiated under the condition of maximum back scatter. Physical factors of radiation were as follows : 200 kVp X-rays, 17.8 mA,

1.0 mm Cu+1.0 mm Al filter, H.V.L. 1.55 mm Cu, F.S.D. of 50 cm, and dose rate of 30 R/minute. For the measurement of the dose, Victoreen R-meter 602 type chamber was employed. Doses of 200 and 400 rads and R-Rad calculation index of 0.95 were used.

3) *Mitotic index*: Ehrlich ascites tumor cells were transplanted into the peritoneal cavity of mice (2 million cells/mouse). Seven days later irradiation was carried out and then the cells were taken out at intervals of 0, 3, 6, 9, 12, 15, 18, 21 and 24 hours. The cells were fixed with methanol and stained by Giemsa. In each experiment, 2,000 cells per animal were examined for determination the mitotic index. The mitotic index at each interval of observation was expressed as the number in 1,000 cells by using 4 mice.

4) *SH compounds*: SH compounds used in this experiment were 0.5 mg of MPG, 2 or 4 mg of cysteamine, and 3 or 6 mg of AET. Each of SH compounds was dissolved in 0.2 ml saline solution and injected into peritoneal cavity during the period between 15 and 20 minutes before irradiation.

RESULTS

1) *Effect of MPG.* As shown Fig. 1, mitotic figures began to disappear immeditely after irradiation of 200 rads until 3 hours when they disappeared completely.

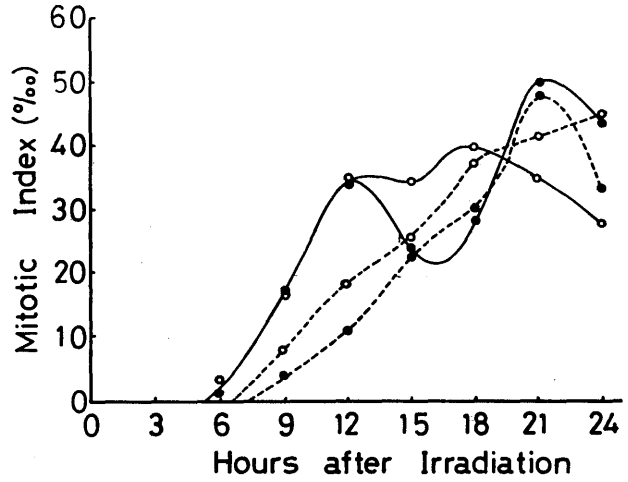

Fig. 1. Effects of MPG on the mitotic frequency of Ehrlich ascites tumor cells after 200 rads and 400 rads irradiation.

●——● , 200 rads only.

o——o , MPG (0.5mg/mouse)+200 rads.

●-----● , 400 rads only.

o-----o , MPG (0.5 mg/mouse)+400 rads.

After 6 hours a few mitotic cells appeared and the mitotic value recovered to the preirradiation value at 12 hours. Thereafter the values oscillated slightly. In the MPG-treated group, the recovery of mitotic figures appearing 6 hours after irradiation was similar to the group exposured to 200 rads (control), and the recovery rate was also identical with that of the control up to 12 hours. At 12 hours and later, however, the mitotic activity of MPG-treated cells was higher than that of control.

After an exposure of 400 rads, mitotic inhibition lasted for 9 hours, then the mitotic index increased gradually, and returned to control values at 18 hours. Peak mitotic activity, 4.81 per cent, was observed at 21 hours. In the group treated with MPG before irradiation with 400 rads, the recovery of the mitotic cells occurred 9 hours after irradiation, but the mitotic index was higher than that of 400 rads (Fig. 1).

2) *Effects of cysteamine.*

a) *At the dose of 2 mg/animal.* Administration of cysteamine had no effect on the delay time of mitosis after X-irradiation with 200 rads, and the recovery

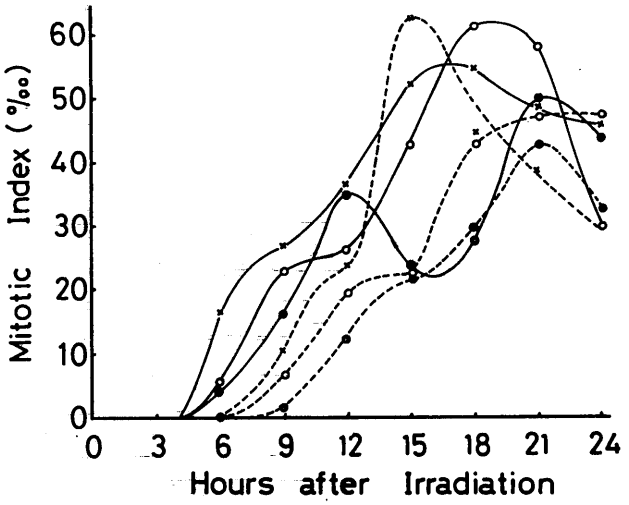

Fig. 2. Effects of cysteamine on the mitotic frequency of Ehrlich ascites tumor cells after 200 rads and 400 rads irradiation.

●——● , 200 rads only.

○——○ , cystemaine, (2 mg/mouse)+200 rads.

x——x , cysteamine (4 mg/mouse)+200 rads.

●-----● , 400 rads only.

○-----○ , cysteamine (2 mg/mouse)+400 rads.

x-----x , cysteamine (4 mg/mouse)+400 rads.

of mitosis occurred at 6 hours after irradiation. Subsequently mitosis gradually increased, and reached a first smaller peak of mitotic frequency at 9 hours. A second larger peak was observed at 18 hours. The mitotic index of the first peak was 2.31 per cent, and that of the second peak was 6.16 per cent.

After the irradiation with 400 rads, the mitotic frequency is shown by the ascending curve in Fig. 2, which recovered faster in the group treated with cysteamine. At 12 hours after irradiation with 400 rads, the mitotic index was 1.93 per cent with treated animals and 1.09 per cent with non-treated (Fig. 2).

b) *At the dose of 4 mg/animal.* By increasing the amount of cysteamine administration (4 mg) the recovery rate of mitotic index after irradiation was further accelerated as shown in Fig. 2; the mitotic index to be 1.58 and 2.63 per cent at 6 and 9 hours after irradiation with 200 rads, respectively. These recovery curves of mitotic index increased markedly up to 18 hours. The delay time of mitosis was slightly effective by the cysteamine treatment. The recovery rate of mitosis, however, was more rapid in the administration of cysteamine (Fig. 2).

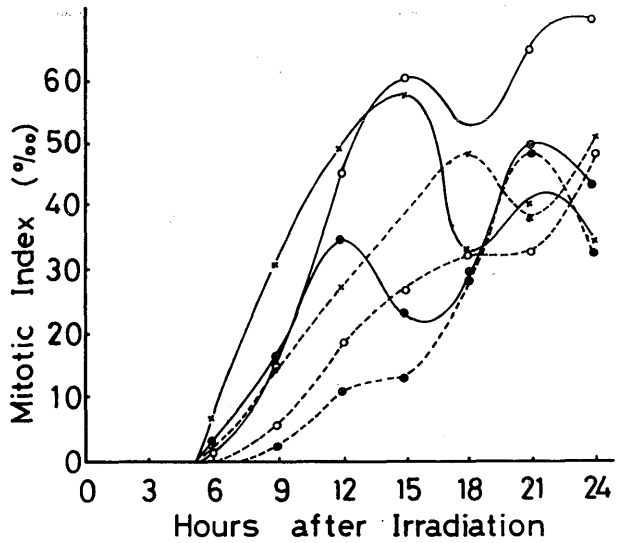

Fig. 3. Effects of AET on the mitotic frequency of Ehrlich ascites tumor cells after 200 rads and 400 rads irradiation.

●——● , 200 rads only.

o——o , AET (3 mg/mouse)+200 rads.

x——x , AET (6 mg/mouse)+200 rads.

●- - - -● , 400 rads only.

o- - - -o , AET (3 mg/mouse)+400 rads.

x- - - -x , AET (6 mg/mouse)+400 rads.

Similar effects of cysteamine in a large amount were observed when exposed to 400 rads as shown in Fig. 2. Therefore, the recovery rate of mitotic figures was accelerated markedly by cysteamine administration. However, no effect of cysteamine was observable on the delay time of mitosis.

3) *Effects of AET*

a) *At the dose of 3 mg/animal.* In the group treated with 3 mg AET the mitotic index showed no distinct difference from that of group irradiation alone (control) until 9 hours. Thereafter, Ehrlich ascites tumor cells treated with AET maintained subsequently a much higher mitotic frequency.

As shown in Fig. 3, in exposure to 400 rads, the recovery of the mitotic index was accelerated with administration AET of 3 mg. A peak mitotic index shifted from 21 to 18 hours after irradiation (Fig. 3).

b) *At the dose of 6 mg/animal.* With exposures to 200 rads, the mitotic index in the group treated with 6 mg AET was 0.33 per cent 6 hours after irradiation, and 3.09 per cent at 9 hours. These values were remarkably high as compared in radiation of 400 rads alone. A peak mitotic index of 5.75 per cent was seen at 15 hours after irradiation. The mitotic index of the group treated with 6 mg AET recovered faster than that of the group with 3 mg AET after irradiation with 200 rads (Fig. 3).

From these data it is concluded that by the increase in the concentration of AET, the recovery rate is accelerated and that the delay time of mitosis induced by X-irradiation shows a little change when the amounts of AET are changed.

DISCUSSION

In the previous paper[2)3)] the author found that GSH reduced the mitotic inhibition of X-rays on Ehrlich ascites tumor cells, and that the action of GSH was connected with certain stage of cell division cycle. In the present data, it was suggested that the radioprotective action of MPG, cysteamine and AET also interacts at a certain stage of cell divisiom cycle the same that of GSH; the delay time of mitosis induced by X-ray and the initial recovery of the mitotic inhibition on the tumor bearing mice were unaffected by MPG, cysteamine (2 mg), AET (3 mg) and GSH. However, the groups treated with SH compounds show a much higher mitotic frequency as compared with that of non-treated group at 12 hours after the irradiation with 200 rads and later. As shown in Fig. 2, two peaks of mitotic index observed with an exposure to 200 rads, shifted to the side of short period in figure with the administration 2 or 4 mg cysteamine. The shift of the second peak of the mitosis after irradiation was bigger than that of the first peak. Similar effect was observed by the irradiation with 400 rads. Quite Similar phenomena occurring in the treatment of cysteamine are observed also in the group treated with

AET of 3 or 6 mg.

As described already, on the basis of these data it may be suggested that in the presence of SH compounds at a low concentration, these drugs affected at the end point of G_1 or S stage in a cell division cycle against the mitotic delay induced by X-ray, and then SH compounds at higher concentration may act to the other stage (i.e. G_2) of mitotic cycle. These similar findings were observed by using L-5 cells in vitro.[4]

Radiation has a differential effect upon cell lethality during the cell cycle, follows then, the magnitude of mitotic delay depends upon the stage at which irradiation occurs in the cell generation cycle.[5][6] Concerning the effect of SH compound on the S stage of cell division cycle, Sinclair and co-worker reported that cysteamine was more effective in S stage for decreasing the division delay by using sychronized Chinese hamster cells.[7][8]

Sakai,[9] on the other hand, measured the amount of SH groups during the division cycle in sea urchin eggs and found a marked increase at a stage before mitosis. Furthermore, Sparks and Walker[10] reported that GSH content in cell increased with the administration of FUdR to prevent the DNA synthesis in L-cells. The cellular non-protein SH also increased with the treatment of SH compounds.[11] These data suggest that there are some relationship between DNA synthesis and SH content. From these evidences, it appeares that the dependence of protective effect of SH compound against mitotic delay upon cell cycle position, is related to the changes in physiological conditions during the cell cycle.

SUMMARY

Protection by SH compounds (MPG, cysteamine and AET) against the mitotic delay induced by X-irradiation in Ehrlich ascites tumor cells was studied. With exposure to 200 rads, the mitotic delay time and its recovery of the group treated with SH compounds (0.5 mg MPG, 2 mg cysteamine and 3 mg AET) were similar to those of non-treated group up to 12 hours after irradiation, but at 12 hours and later the mitotic index of the groups treated with SH compounds was at a much higher level. The recovery rate from the mitotic inhibition became faster by increasing the concentration of the SH compounds (4 mg cysteamine or 6 mg AET) injected. Similar effects of SH compound were observable in the case of 400-rad irradiation. Increasing the concentration of the SH compound (4 mg cysteamine or 6 mg AET) treated, the recovery of mitosis was accelerated remarkably. From these data, it is suggested that the SH compound has a differential protective effect on mitotic delay during the cell cycle.

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