Cysteamine Rapidly Decreases Mitotic Cells in Random Culture of Hela S-3 Cells

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(Received April 1, 1983)

Radioprotector/Cysteamine/Mitosis/HeLa

Mitotic cells disappeared nearly completely within 60 min after the addition of 30 mM cysteamine in HeLa S-3 cell culture, while the control culture contained mitotic cells with the frequency of 3.4%. Inhibition of mitosis became gradually irreversible when the treatment was extended over 60 min, but cytolysis occurred much more slowly. Ethanolamine exhibited a similar but much less significant effect.

INTRODUCTION

In addition to the well-known radioprotective action, aminothiol derivatives such as cysteamine (2-mercaptoethylamine) and WR-2721 (S-2[3-aminopropylamino]ethylphosphorothioic acid) are known to have an oncostatic action. Chemical modification of either amino or thiol group of cysteamine results in marked reduction of the oncostatic activity of the compound as well as its radioprotective activity. The length of the carbon chain between the two groups also is implicated in both activities of the compound. It has thus been assumed that '2-point attachment' is requisite for the actions of cysteamine, although it is unclear what component of the cells is the principal target of its binding. Regardless of whether its effect is direct or indirect, it is clear that the two actions of cysteamine are both related to the cell-division machinery. In this regard, it has been reported that cysteamine inhibits DNA synthesis by increasing intracellular cyclic AMP concentration, and that an inhibitor of cyclic AMP phosphodiesterase such as 1-methyl-isobutyl xanthine increases intracellular cyclic AMP concentration and shows a radioprotective effect. In view of current revival of interest in radioprotective substances, we wish to report here that cysteamine rapidly decreases mitotic cells in the random culture of HeLa S-3 cells.
Cells
HeLa S-3 cells have been maintained in culture in Ham’s F-10 medium containing 10% calf serum and 0.5% heart infusion broth, while they were occasionally stored in liquid nitrogen by a conventional method with use of 10% dimethylsulfoxide. The cells were harvested from confluent cultures by trypsinization, washed by centrifugation with the culture medium, and re-plated in 60-mm plastic petri dishes (Falcon) in 4-ml (120,000 cells) portions as a single-cell suspension. The cells were then incubated for 18 hours at 37°C under humidified air containing 5% CO₂ before the treatment with the chemicals as described below.

Chemicals
Cysteamine hydrochloride (Sigma) was dissolved in various concentrations in the culture medium. The solution was made to pH 7.4 and sterilized by Millipore membrane. The medium of the above-described cell cultures was changed to the fresh experimental medium, and incubation was continued further for various periods of time. Ethanolamine (2-aminoethanol) also was obtained from Sigma and used in the same manner as in the case of cysteamine. Control cultures received the medium which was processed as above without either chemicals.

Mitotic index
After the treatment with the chemicals, the cells were exposed to 0.5% sodium citrate solution for 2 min and fixed with a 1:3 mixture of glacial acetic acid and absolute ethanol for 10 min. The cells were then stained with 1% Orcein solution in 50% aqueous acetic acid. Mitotic cells were counted under a microscope, and their frequency was expressed in percentages of total cell number counted. Four different areas of each petri dish were examined and 500–1000 cells were counted in each area.

Cell proliferation rate
In a separate experiment, the cells were plated as a single-cell suspension in 35-mm plastic petri dishes in the population of 300/dish and incubated as above in 2 ml/dish of the medium with or without the chemicals. The number of cells was counted microscopically in a room of 37°C, while location of the cells or cell-clusters was identified by the grid drawn on the bottom of the dish.

RESULTS

Figure 1 shows the effect of cysteamine and ethanolamine on the frequency of mitotic cells in the random cultures of HeLa S-3 cells. Mitotic index was
decreased from the control level of 3.4 ± 0.2% (average of 16 areas in 4 dishes ± S.E.M.), e.g., to 1.4 ± 0.1% in 15 min after the administration of 30 mM cysteamine to the cells (statistically significant at the probability of less than 0.01). It took 1—2 hours for 5 mM cysteamine to decrease mitotic index in a same magnitude as that caused by 15-min’s treatment with 30 mM cysteamine, while the mitotic index was decreased furthermore in these cultures by longer incubations. In the cultures containing 0.5 mM cysteamine, however, the mitotic index recovered nearly to the normal level after a temporal decrease.

Ethanolamine in the concentration of 30 mM caused 50% decrease of mitotic cell frequency in 1 or 2 hours. The effect was approximately equivalent to the effect of 5 mM cysteamine. Like in the case of 0.5 mM cysteamine, the effect of 30 mM ethanolamine was abated during prolonged incubations.

Separately, it was shown that the cells restored normal mitotic activity when they were washed thoroughly after the treatment with 30 mM cysteamine.

![Graph showing the rapid decrease of the frequency of mitotic cells after the addition of either cysteamine or ethanolamine in the random culture of HeLa S-3 cells.](Fig. 1)
for 15 min or with 5 mM cysteamine for 60 min, whereas there occurred no restoration of mitosis when the cells were treated with either 5 mM or 30 mM cysteamine for a period as long as 4 or 8 hours.

Figure 2 shows that the doubling time was about 25 hours for the cells in normal culture, while the number of cells decreased exponentially with time in the cultures containing 10 mM cysteamine or 10 mM ethanolamine. After the incubation of 96 hours, about 70% of the cells were retained in the cysteamine-treated culture, while only 40% of the plated cells were retained in the culture treated by ethanolamine.

![Graph showing cell number decrease](image)

**Fig. 2.** Decrease of the number of cells as a function of time in the culture containing cysteamine or ethanolamine as compared with exponential increase of the cell number in the control normal culture. Each point in the figure represents the average of two petri dishes.

**DISCUSSION**

In a previous study, we showed that the radioprotective effect was achieved maximally within a few min after the administration of cysteamine to HeLa S-3 cells, while washing of the cells quickly abolished the radioprotective effect even when the cells were treated with 30 mM cysteamine for 30 min. We reconfirmed these observations in the present study (data not shown) and thus concluded that the cells have preserved the same property as that we ob-
served previously. The result suggests that cysteamine penetrates into the cells quite rapidly and does not bind to the cellular components covalently in such a short period of time.

In the present study, we have shown that the frequency of the mitotic cells decreases rapidly under the condition equivalent to that employed in the radiation protection experiment. The result does not necessarily suggest that the radioprotective action of the compound is dependent upon its cytostatic action. Ethanolamine is not radioprotective for HeLa S-3 cells as reported previously, although it is significantly cytostatic as reported here. Nevertheless, the results obtained by the radioprotection experiment and by the present study seem to suggest that the rapid cytostatic effect is caused by reversible binding of the compound onto certain components of the cells.

Radiation also causes a rapid decrease of mitotic cells (see a review, e.g., by Doida), while the cells regain the mitotic activity after a certain period of time depending upon the amount of the radiation dose. Thus, the cytostatic action of cysteamine observed in the present study resembles the mitosis inhibitory action of radiation.

It has long been known that cysteamine exhibits a paradoxical dose-response curve for its toxicity to cultured cells; namely, it is much more toxic in lower concentrations (0.1–2 mM) than in higher concentrations (10–50 mM). We showed previously that the paradoxical cytotoxicity of cysteamine can be ascribed to peroxide generated only by the low concentrations of cysteamine. The effect of cysteamine observed in the present study can not be explained by peroxide however, because it was more intensely produced in 30 mM than in 0.5 mM. Furthermore, the effect on the mitotic index developed more rapidly than the cell-killing effect of the compound in lower concentrations.

Long-term existence of cysteamine in concentrations as high as 5–30 mM in the cell culture caused persistent inhibition of mitosis (Fig. 1) and resulted in complete cessation of the increase of cell number (Fig. 2). The concentration of cysteamine mentioned above is comparable to that required for the radioprotective action of the compound, whereas the radioprotective action is exerted at the instant of radiation. Although it is unclear whether or not the rapid cytostatic action of cysteamine observed above is directly related to the radioprotective action of the compound, the result of the present study suggests that persistent existence of the compound in the body is rather deleterious to regenerating tissues.

ACKNOWLEDGEMENT

We thank Dr. Bun-ichi Tamaoki, Director of the Division of Pharmaceutical Science of NIRS for his general support to us.
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