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The protective effects of cysteamine against ionizing radiation in mouse fibroblasts growing *in vitro**

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THE radioprotective action of cysteamine (β-mercaptoethylamine, MEA) has been widely confirmed, primarily in intact animals,[1] bacteria,[2] and mammalian cells *in vitro*.[3, 4] Therkelsen,[5] however, reported little or no protection of 1·5 mM MEA against X-ray damage of L-strain fibroblasts in tissue culture. Vos and associates,[3, 4] on the other hand, have reported significant degrees of protection (dose-reduction factors of up to approximately 4) for human kidney cells, grown *in vitro*, irradiated in the presence of MEA, and tested by the ability of treated cells to form clones. The present experiments, using the parameter of population growth of L-strain mouse fibroblasts after irradiation in the presence of different concentrations of the agent, were undertaken in an attempt to determine whether MEA exerts a radioprotective effect. Some additional studies were performed, dealing with the radioprotective effect of MEA at reduced oxygen tension.

METHODS

Mouse fibroblasts (Earle's 'L' cells) were grown at 37° as monolayer cultures in 8-oz prescription bottles containing 20 ml. of Eagle's culture medium.[6] The gas phase was 5% CO_2 in air. The use of 5% CO_2 in all gas mixtures served to fix the pH of the bicarbonate medium at a constant value of 7·15. Replicate cultures of about 10^5 cells per bottle were dispensed by an automatic, sterile pipet machine. On the following day, the bottles were removed from the incubator and the medium replaced with fresh medium or with the appropriate experimental medium containing MEA.† The cells remained in the experimental medium for 15 min prior to irradiation, and the period of irradiation ranged from 2–14 min depending on the dose of radiation to be administered. The culture medium was drained off after a total time of 30 min in the experimental medium (regardless of radiation dosage); the cells were washed twice with 10 ml of fresh medium; 20 ml of fresh medium was then added, and the bottles were returned to the incubator.

Cell counts were obtained in an electronic cell counter. Representative bottles were counted on day 1, after the manipulations described above, and final counts obtained 4 days later. Each count reported in this paper is the mean count of 3 replicate bottles.

Anoxic conditions were achieved by the following procedure: 1 hr before irradiation, the gas phase in the culture bottles was replaced with a mixture of 5% CO_2–95% N_2. Analysis of this gas mixture revealed an oxygen content of less than 50 ppm. One hour later, the medium was withdrawn through hypodermic needles and replaced with experimental media (with or without MEA); these media were previously freed of oxygen by bubbling 5% CO_2–95% N_2 through them for 90 min. The bottles were flushed once again with 5% CO_2–95% N_2, and the gas flow was continued throughout the irradiation (or sham-irradiation) period. Irradiation commenced 15 min after addition of the experimental media.

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† Cysteamine hydrochloride (Fluka AG) was supplied in vacuum-sealed ampoules. It was dissolved in water, sterilized by filtration, and added to culture medium immediately before use.

The media were then drained off, the cells washed with fresh normal medium as previously described, and gassed with 5% CO_2–95% air. Thus all bottles were under anoxic conditions a total of 90 min and exposed to MEA exactly 30 min.

Cells were X-irradiated in their culture bottles with the following radiation factors: 250 kV constant potential, added filtration of 0·25 mm Cu–1 mm Al, HVL of 1·10 mm Cu, focus-cell distance of 65 cm, exposure dose rate of 70·5 r/min. Bottles containing the cells in 20 ml of culture medium were placed in a horizontal position under the vertical beam of the X-ray machine and irradiated in groups of three with exposure doses of 150, 300, 600, and 1,000 r. All irradiations were carried out at room temperature. Sham-irradiated control cells were handled identically, except that the X-ray machine was not turned on.

RESULTS

Cysteamine toxicity

There was little or no toxicity after exposure of cells to 10 mM MEA for the test period of 30 min, while only a small growth-inhibitory effect was found at 30 mM MEA (treated groups averaged 92% of control growth). Groups of cells exposed to 50 mM MEA for 30 min averaged only 37% of control growth. The toxicity of MEA increased markedly with longer periods of exposure; thus, even 10 mM MEA was toxic if the period of exposure was lengthened to 1 hr. Based upon these findings, MEA concentrations of 3, 10, and 30 mM were chosen for use in irradiation experiments, and the exposure period was held strictly to 30 min.

Effect of cysteamine on radiation sensitivity

The results of experiments using three different concentrations of MEA with cells irradiated over a dose range of 0–1,000 r are shown in Fig. 1. The data for the two highest concentrations of

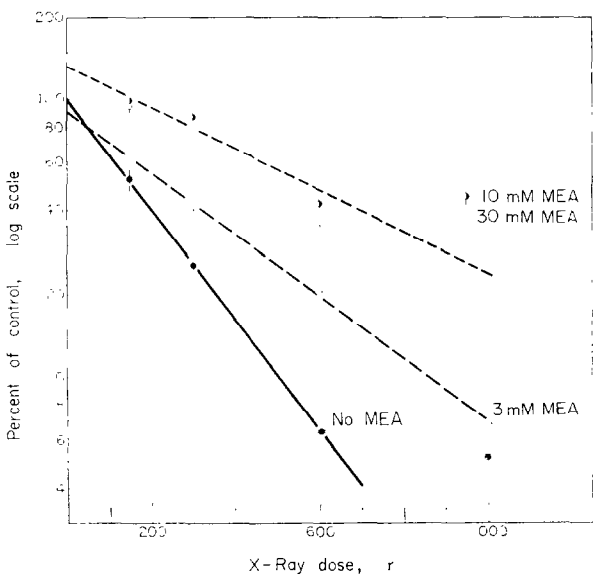

FIG. 1. Radioprotective effect of cysteamine hydrochloride. Replicate cultures in 8-oz prescription bottles were treated with MEA for a total time of 30 min, and irradiated at 150, 300, 600, and 1,000 r as described in the text. Cell counts were determined at the time of irradiation and 4 days later. Each point represents the mean and standard error of a group of 3 bottles, expressed as the per cent of control growth (sham-irradiated). The curve of radiosensitivity in the absence of MEA is derived from three separate experiments, and thus each point on this curve represents the mean of 9 bottles. Cysteamine itself, under the specified conditions, had no detectable effect on cellular growth.

MEA were combined, since there were no significant differences between these two groups. The point at 1,000 r for the controls was not included for the calculation of regression coefficients in view of the fact that the next lower dose, 600 r, reduced the count at day 5 almost to the initial cell count determined on day 1.

The dose-reducing factors (DRF)* were: for 3 mM, DRF = 1·80; and for 10 and 30 mM, DRF = 2·73.

Effect of cysteamine on cells irradiated under anoxic conditions

Control cultures subjected to the anoxic conditions described above (5% CO_2–95% N_2) showed no significant toxicity from the 90-min period of anoxia, with or without added MEA (5 and 10 mM). If, however, anoxic conditions (no MEA) were continued longer than about 2·5 hr, there was significant subsequent growth inhibition. Cells irradiated in oxygen in the presence of two different concentrations of MEA (Fig. 2) exhibited dose reduction of a magnitude similar to the data presented

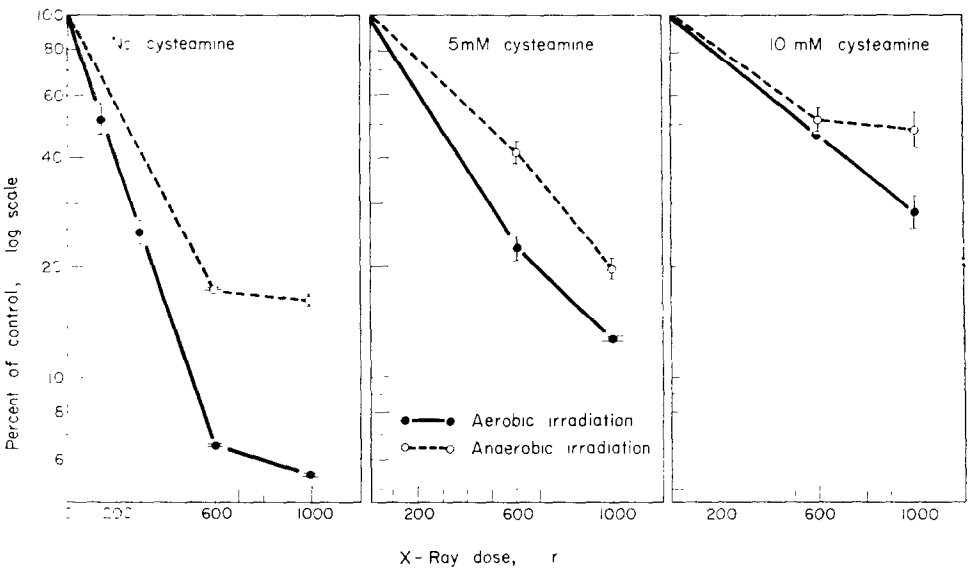

FIG. 2. Radioprotective effect of cysteamine hydrochloride under aerobic and anaerobic conditions. Replicate bottles were irradiated at 600 and 1,000 r under conditions of normal oxygenation (gas phase 5% CO_2–95% air) and anoxic conditions (gas phase 5% CO_2–95% N_2), and the radioprotective effect of MEA at 5 and 10 mM examined. Cell counts were determined at the time of irradiation and 4 days later. Each point represents the mean and standard error of a group of 3 separate bottles, expressed as the per cent of control growth in the absence of radiation. Under conditions specified in the text, neither the 90-min period of anoxia nor the 30-min period of exposure to MEA had any detectable effect on the growth of cells in the absence of radiation.

above. Cells irradiated under anoxic conditions showed less radiation effect than did cells tested under aerobic conditions; this was in addition to the radiation protection afforded by cysteamine. Thus, the radiation effect was reduced by substitution of nitrogen for air, or by the addition of MEA in the culture medium, and there was a still greater reduction when the cells were irradiated in the presence of MEA as well as nitrogen.

DISCUSSION

The present findings amply confirm the radioprotective action of cysteamine on a representative mammalian cell *in vitro*. Therkelsen,[5] however, using L cells grown and quantitated under similar

* DRF was computed by dividing the slope of the linear regression line (Fig. 1) of the untreated group by the slopes of the lines of the groups receiving the low and high concentrations of MEA.

conditions, did not report qualitatively comparable dose reduction. From a comparison of the exposure conditions in the two series of experiments, it appears most likely that the differences between Therkelsen's findings and those of the present study are a reflection of the relatively small amount of potentially protective agent present during irradiation in the former experiments. This is supported by our finding and that of Vos *et al.*[3] that the degree of protection is correlated with concentration of protective agent present during irradiation.

Although the parameter used to determine radioprotective effect differed in the experiments of the Dutch group,[3, 4] the sequence of steps immediately preceding and during irradiation was similar to that in the present study; and among the DRF's these workers reported were ~1·8 (4 mM MEA), ~3·3 (16 mM MEA), and ~3·8 (32 mM MEA). These findings are strikingly and almost quantitatively comparable with the present data.

It is conceivable that MEA exerts its radioprotective effect by depleting the cellular environment of oxygen. While it is true that in our experiments (Fig. 1), 10 mM MEA (0·2 mmole per bottle) protected the cells in the presence of about 2 mmoles oxygen (per bottle), the oxygen concentration in the medium in the immediate cellular environment might nevertheless have been sharply reduced. Our data relative to MEA protection during irradiation under anoxic conditions are limited, but it may be seen (Fig. 2) that a combination of anoxic conditions and MEA provided greater radioprotection than did either procedure alone. In confirmation of Vergroesen *et al.*,[4] and Kohn and Gunter,[7] this finding demonstrates that the radiation protection exerted by cysteamine probably cannot be ascribed to a condition of anoxia resulting from simple oxidation of the added chemical agent.

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Aminonucleoside of puromycin: Elimination of nephrotoxicity by acetylation of the aminoribose moiety

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Puromycin,[1, 2] its aminonucleoside (I, Fig. 1; hereafter abbreviated PA) as well as some benzylidine[3] and amino acid analogs[4] have been shown to possess antitumor activity against the mouse mammary adenocarcinoma and certain strains of mouse leukemia. Puromycin itself is less active in this respect than PA; indeed, its antitumor as well as its trypanocidal properties[5] may well reside in the aminonucleoside portion of the molecule Clinical trials against a spectrum of human tumors have been reported for both puromycin[6] and for PA,[7, 8] but were not furtner pursued in the absence of beneficial results, and especially with the appearance of nephrotoxic manifestations in the form of excessive