Mutation Research, 94 (1982) 393–396
Elsevier Biomedical Press

Effects of rifampin on the lethality and the mutation frequency of ultraviolet-irradiated Chinese hamster V79 cells

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(Received 3 November 1980)
(Revision received 26 September 1981)
(Accepted 12 October 1981)

The possible existence of the inducible error-prone functions, analogous to 'SOS functions' in bacteria (Radman, 1975; Witkin, 1976; Hanawalt et al., 1979), is reported for mammalian cells induced by DNA-damaging agents such as UV radiation or chemical carcinogens in a system of virus reactivation (Hanawalt et al., 1979; Sarasin and Hanawalt, 1978; Lytle et al., 1978; DasGupta and Summers, 1978; Sarasin and Benoit, 1980). In the case of mammalian cells, however, the question whether these inducible functions operate on cellular recovery itself is still obscure. A tickle dose of UV radiation administered 24 h before a killing dose of the radiation increased the survival of CHO cells (Troll et al., 1978), but no increase in ultraviolet-induced mutation frequency was produced by pre-exposure to X-rays in CHO cells (Cleaver, 1978).

The antibiotic rifampin, a derivative of rifamycin SV, is a specific inhibitor of the formation of protein X (Satta et al., 1979), the *recA* gene product in *Escherichia coli* (McEntee, 1977); this inhibition of inducible repair is manifested by decreased survival (Pollard and Achey, 1975). It is of interest to examine the effects of rifampin on ultraviolet-irradiated mammalian cells to know whether there is a rifampin-sensitive process in mammalian cells as in *Escherichia coli*.

In this communication, we report that a non-toxic concentration of rifampin enhanced the lethality of ultraviolet-irradiated Chinese hamster V79 cells, but had no effect on the ultraviolet-induced mutation frequency in these cells.

Chinese hamster V79 cells were grown in Eagle's minimal essential medium (MEM) supplemented with 5% fetal bovine serum under 5% CO_2 at 100% humidity. Under routine conditions their doubling time was 11 h and the plating efficiency was 82.5 ± 11.8. Rifampin was dissolved in dimethyl sulfoxide at a concentration of 50 mg/ml and diluted in complete medium before use.

Colony survival was estimated as follows. Exponentially growing cells were trypsinized with 0.1% trypsin solution. Appropriate numbers of cells (to give 50–200

0027-5107/82/0000-0000/$02.75 Elsevier Biomedical Press

colonies) were plated in 60-mm petri dishes and incubated for 4–5 h to allow the cells to attach. Attached cells were washed with Dulbecco's phosphate-buffered saline (PBS) once, exposed to ultraviolet radiation (predominantly 254 nm) from a germicidal lamp at a dose rate of 0.46–0.88 $J/m^2/sec$, and cultured in 5 ml of fresh pre-warmed medium with or without 100 μg of rifampin per ml. After 24 h incubation at 37°C, rifampin-containing medium was washed away and replaced by normal medium. 7 days later (a total of 8 days after ultraviolet irradiation), colonies were fixed with 10% formalin in PBS, stained with 0.5% methylene blue and counted.

A replating method (Van Zeeland and Simons, 1976; Van Zeeland, 1978) was used for the detection of induced mutation frequency. 1×10^5 cells were plated in 100-mm petri dishes and cultured for 16–20 h. Cells were irradiated by ultraviolet radiation and treated with rifampin (100 μg/ml) for 24 h as described above. 2 and 5 days after ultraviolet irradiation, cells were replated to keep them growing exponentially. 8 days after ultraviolet irradiation, 0.9×10^5 cells were plated in 100-mm dishes (8 dishes per point) and grown in the presence of 6-thioguanine (10 μg/ml). After 8–10 days' incubation, 6-thioguanine-resistant colonies were fixed, stained and counted as described above. A small aliquot (100–200 cells per 60-mm dish) was plated and grown in normal medium for determination of the cloning efficiency. The mutation frequencies were calculated by correcting the observed frequencies of mutants by the corresponding cloning efficiencies.

Post-treatment with of rifampin (100 μg/ml) for 24 h increased the lethality of ultraviolet-irradiated V79 cells as shown in Fig. 1. D_0 and n were 4.3 J/m^2 and 4.7 for non-treated cells, and 3.2 J/m^2 and 3.5 for rifampin-treated cells, respectively. The treatment with rifampin (100 μg/ml) alone for 24 h had little effect on the

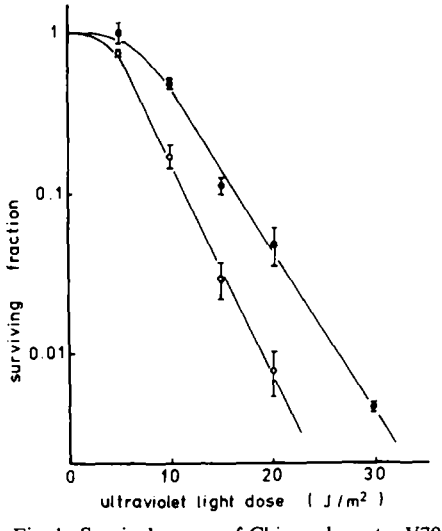


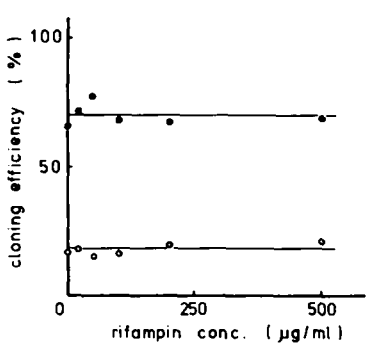
Fig. 1. Survival curves of Chinese hamster V79 cells irradiated by ultraviolet radiation. ●, in the absence of rifampin; ○, in the presence of rifampin (100 μg/ml) for 24 h immediately after ultraviolet irradiation.

lethality of unirradiated cells, as shown by the cloning efficiency of 97.1 ± 13.4% of the control. Treatment with rifampin at a concentration as low as 20 μg/ml for the whole period of incubation for developing colonies (8 days), or treatment with rifampin at a concentration higher than 200 μg/ml for 24 h decreased the colony-forming ability of unirradiated V79 cells to less than 50% of the control. No decrease in the survival of ultraviolet-irradiated V79 cells was produced by treatment with rifampin (up to 500 μg/ml) for 2 h before ultraviolet irradiation (Fig. 2).

These results indicate that rifampin might inhibit the inducible repair responsible for survival by a specific inhibition of the production of a protein similar to protein X in *Escherichia coli* even though rifampin is much less effective in inhibiting eukaryotic RNA polymerase (Wehrli et al., 1968; Meilhac et al., 1972), because rifampin has a specific property to inhibit protein X production that is separate from inhibition of RNA synthesis in *Escherichia coli* (Satta et al., 1979). As the enhancement of the survival of ultraviolet-irradiated herpes simplex virus by ultraviolet-irradiated host cells is inhibited by the addition of cycloheximide, an inhibitor of protein synthesis (Lytle and Goddard, 1979; DasGupta and Summers, 1978), we assumed that there may be a similar protein in mammalian cells.

Fig. 3 shows the effects of rifampin on the mutation frequency of V79 cells induced by various doses of ultraviolet radiation. The radiation increased the mutation frequency of V79 cells (continuous line). Post-treatment with rifampin (100 μg/ml) for 24 h immediately after ultraviolet irradiation, which increased its lethality to V79 cells (Fig. 1), did not change the ultraviolet-induced mutation frequency.

It is difficult to obtain complete inhibition of inducible repair, because longer treatment with rifampin or treatment with higher concentrations is toxic to unirradiated cells. Therefore, it is probable that the present experiments were done under conditions where inducible repair is partially inhibited. So one cannot eliminate the



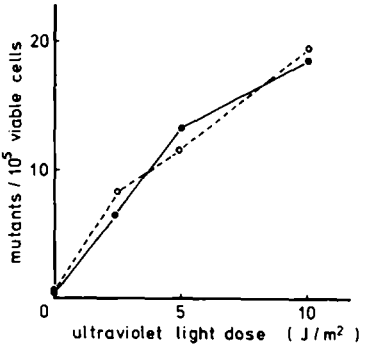


Fig. 2. Effect of pre-treatment with rifampin. Chinese hamster V79 cells were treated with various concentrations of rifampin for 2 h followed by ultraviolet irradiation after the removal of rifampin. ●, unirradiated control; ○, ultraviolet-irradiated (15.0 J/m^2).

Fig. 3. Effect of ultraviolet irradiation on the induction of mutation in Chinese hamster V79 cells. ●, without rifampin treatment; ○, treated with rifampin (100 μg/ml) for 24 h immediately after ultraviolet irradiation.

possibility that the cells are killed by the inhibition of inducible repair resulting in the decrease in mutation frequency and that this decrease might be as small as the experimental error. Although this possibility remains to be considered, the present results, together with the findings of Troll et al. (1978) and Cleaver (1978), suggest that there may be some inducible repair in mammalian cells which would act on the cellular recovery itself, but might not be error-prone.

I am grateful to Dr. Kazuo Yamamoto and Mr. Parmender Paul Mehta of our Department for fruitful discussions. This work was supported in part by grants from the Ministry of Education, Science and Culture.

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