# Inhibition by Chloroquine of UV Repair in *E. coli* B[1] (34614)

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The occurrence of mutation repair in cells of higher organisms (1–3) as well as bacteria (4–6) suggests that such repair is of general biological importance. The so-called dark repair or excision repair is of greatest interest because of the wide range of mutagenic agents to which it is directed. These include alkylating agents, UV light, X-ray, and certain antibiotics. The repair process involves at least four steps: single-strand chain opening, excision of the involved region in the DNA, reinsertion of appropriate bases using the complementary strand as template, and rejoining of the DNA backbone. In addition, there is present in some organisms a specific enzyme which catalyzes the photolysis of UV-induced thymine dimers (7). Inhibitors of mutation repair should be useful in differentiating the individual steps in repair, and in evaluating the biological role of the overall process.

The present studies show inhibition of excision repair by chloroquine, a 4-aminoquinoline antimalarial drug which has been studied extensively for its ability to interact with DNA (8–10), and inhibit the DNA-primed synthesis of DNA (11). Furthermore, chloroquine can inhibit bacteriophage DNA synthesis without stopping replication of the bacterial host (12). Since one of the steps in excision repair involves limited resynthesis of a short segment of a strand of the DNA helix, it was of interest, therefore, to examine the effects of chloroquine on repair.

*Materials and Methods.* These experiments employed a wild strain of *E. coli* B obtained from Dr. E. E. Evans in this institution and maintained on minimal media in this laboratory. For irradiation a fresh overnight culture was diluted 100-fold in sterile saline and irradiated under the conditions shown for each experiment using a Will Scientific Company germicidal light filtered to emit light at 253 m$\mu$. Media employed were nutrient broth (Difco), 2% agar in nutrient broth, and minimal salts medium supplemented with glucose. The effects of irradiation were determined by direct count of surviving colonies after plating on media as indicated. The drug was not included in the irradiation medium because of possible light-screening effects.

*Results and Discussion.* The inclusion of chloroquine in the plating medium after UV irradiation resulted in a significant decrease in the number of viable cells at drug concentrations where there was no effect on unirradiated cells. These results are expressed in Fig. 1 as a semi-log plot of surviving fraction vs. UV dose in time. In a plot of this type, the radioresistance characteristic of repair is reflected in a shoulder or nonlinear region at low UV doses. As shown, this shoulder region was eliminated by the presence of chloroquine.

"Dark repair" or "liquid holding" repair may also be used as a measure of the excision repair process. Thus, cells show an increase in viability when held in non-nutrient media for an interval after irradiation. The effect of chloroquine on this postirradiation repair in *E. coli* B cells is shown in Table I. Thus, chloroquine at $10^{-3}$ $M$ inhibited the recovery from UV damage exhibited by holding irradiated cells in saline suspension in the dark at 37° for 1 hr, as would be expected from an effect on excision repair.

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TABLE I. Effect of Chloroquine on "Dark Repair" in *E. coli* B Cells.[a]

| Chloroquine | Increase in colony survival after 1 hr |
|---|---|
| 0 | 307% |
| $1 \times 10^{-3} M$ | 158% |

[a] Cells were diluted 100× in sterile saline and UV irradiated for 5 sec as described in the text. After irradiation, the cell suspension was divided into two portions to one of which chloroquine was added. Aliquots were diluted and plated on nutrient agar and after 1 hr in the dark at 37°.

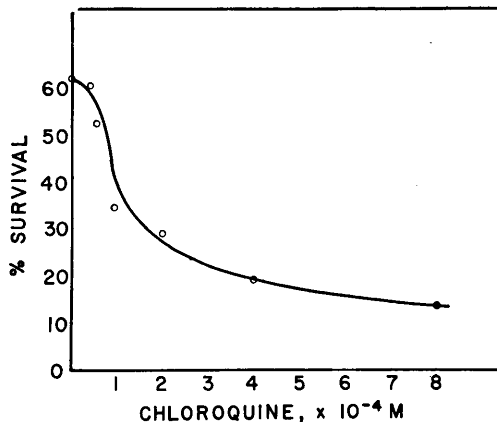


FIG. 2. Effect of varying chloroquine concentration on *E. coli* B survival after UV irradiation for 30 sec. Experiment performed as in Fig. 1.

The dose-response curve for chloroquine inhibition of repair was determined by including various concentrations of the drug in the plating medium after a standard period of irradiation. As shown in Fig. 2, drug concentrations as low as $10^{-4}$ *M* interfered with colony survival after irradiation.

The excision repair process apparently provides the mechanism for the repair of lesions in DNA resulting from X-irradiation and alkylation, as well as UV light. The experiments presented here show that the 4-aminoquinoline antimalarial, chloroquine, is an effective inhibitor of "dark repair" of ultraviolet light damage in *E. coli* B. The similarity may be noted between the properties of this aminoquinoline and the aminoacridines reported previously to inhibit repair (14). Since chloroquine is effective at rather low and nontoxic concentrations, it may provide a useful tool for exploring the biological role of repair, particularly in relation to such questions as carcinogenesis and resistance by tumors to chemotherapy; and in identifying the individual steps in the repair process, particularly when combined with a study of mutants with altered susceptibility to radiation.

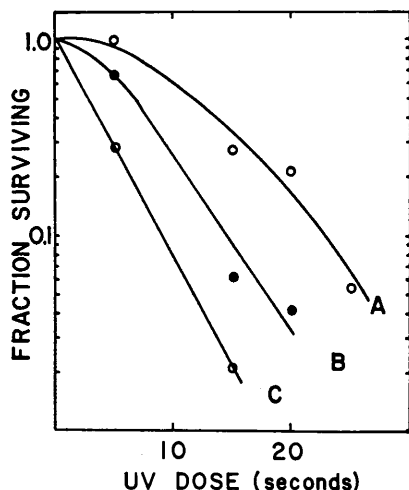


FIG. 1. Effect of chloroquine on *E. coli* B survival after UV irradiation. Experiment performed as described in text with plating on minimal agar with and without added drug: curve A, control; curve B, $5 \times 10^{-4} M$; and C, $1 \times 10^{-3} M$ chloroquine.

Recent experiments in this laboratory have shown that the sensitivity of certain tumors to alkylating agents and X-ray may be enhanced substantially by the administration of chloroquine or caffeine, another agent known to inhibit repair (15).

*Summary*. The 4-aminoquinoline, chloroquine, has been shown to decrease survival of *E. coli* B when placed in the plating medium after UV irradiation; and to inhibit "dark repair" or "liquid holding repair" of UV damage in cell suspensions.

This and similar drugs should prove useful in elucidating the mechanism and biological role of mutation repair.

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