Photochemistry and Photobiology, 1977, Vol. 25, pp. 209–210. Pergamon Press. Printed in Great Britain

RESEARCH NOTE

EFFECT OF QUINACRINE ON SURVIVAL OF ULTRAVIOLET-IRRADIATED *uvr*, *rec*, *lex* AND *pol* MUTANTS OF *ESCHERICHIA COLI* K-12

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(*Received* 20 *May* 1976; *accepted* 22 *July* 1976)

INTRODUCTION

The availability of mutants deficient in specific repair systems allows the selective testing of the mechanism of action of suspected repair-inhibitor drugs. The present report describes the characteristics of quinacrine as a post-irradiation sensitizer of *E. coli* K-12 to UV radiation. Quinacrine (Atabrine) is an aminoacridine derivative chemically related to acriflavine. It is an intercalating agent that binds strongly to DNA (Kurnick and Radcliffe, 1962; O'Brien *et al.*, 1966); it inhibits DNA metabolism (Ciak and Hahn, 1967; Van Dyke and Lantz, 1970), and uncouples oxidative phosphorylation (Loomis and Lipmann, 1948). Quinacrine potentiates the killing of X-irradiated *E. coli* K-12 cells by preventing the *rec* gene-controlled repair (Type III; Town *et al.*, 1973) of single-chain breaks in DNA (Fuks and Smith, 1971). In the following experiments we have investigated the potentiation of UV-induced killing of some *uvr*, *rec*, *pol*, and *lex* mutants of *E. coli* K-12 by a 60 min post-irradiation treatment with quinacrine.

MATERIALS AND METHODS

Bacterial strains. The strains used were multiple auxotrophs of *E. coli* K-12. These included wild-type (AB2497), *recA13* (AB2487), *uvrB5* (AB2499), *polA1* (JG138), *lexA101* (DY95) (described in Van der Schueren and Smith, 1974), *recB21* (JC5743), *recA56 uvrB5* (SR72) (described in Kapp and Smith, 1970), *polA1 lexA101* (DY101) and *lexA101 uvrB5* (DY146) (described in Youngs and Smith, 1973). The experimental procedures and media have been described (Ganesan and Smith, 1970; Kapp and Smith, 1970). (Note: *lexA101* denotes the *exrA* mutation derived from *E. coli* B_{s-1}).

Irradiation. Ultraviolet radiation (254 nm) was delivered by an unfiltered 25-W General Electric germicidal lamp positioned 54 cm above the surface of a platform shaker. The fluence rate at this distance was $12\ J\ m^{-2}\ s^{-1}$. A perforated grill was used to adjust the fluence rate to $0.43\ J\ m^{-2}\ s^{-1}$ (for fluences below $10\ J\ m^{-2}$).

Potentiation of UV-induced killing by quinacrine. Overnight stationary-phase cultures were diluted 1:50 in fresh supplemented minimal medium (SMM) (Ganesan and Smith, 1970), and incubated in a Gyrotory water bath (New Brunswick Scientific) at 37°C to exponential growth phase ($\sim 10^8$ cells/mℓ). The bacteria were then centrifuged in a Sorvall centrifuge at 3000 rev/min for 5 min, resuspended in DTM buffer (SMM devoid of organic components), and 10 mℓ samples were irradiated in open Petri dishes on a shaker. Immediately after irradiation, the cells were diluted to $\sim 5 \times 10^7$ cells/mℓ, supplemented with glucose, amino acids, thymine, and thiamine to reconstitute the SMM growth medium, and, when indicated, with quinacrine at 75 μg/mℓ, and reincubated at 37°C in a Gyrotory water bath for 60 min. The cells were diluted in double-distilled water, and plated on SMM agar. Colonies were counted after 48–72 h of incubation at 37°C. Solutions of quinacrine-HCl (Sigma Chemical Co.) were made up immediately prior to their use.

RESULTS AND DISCUSSION

The addition of quinacrine to the post-irradiation incubation medium of wild-type cells enhances the UV-induced killing effect. This enhancement developed gradually during the first 60 min of post-irradiation (Fig. 1). Further incubation did not increase the sensitization but resulted in additional killing of the unirradiated control cells.

The degree of sensitization produced by quinacrine was critically dependent on the timing of the addition of the drug after irradiation. With a 20 min delay before adding the quinacrine, about 90% of the sensitization effect was lost. After a 60 min post-irradiation delay, there was almost a total loss of potentiation of killing by quinacrine (data not shown). This indicates that the recovery phenomenon in wild-type cells that is sensitive to quinacrine is completed within about 60 min after a UV fluence of $100\ J\ m^{-2}$.

Survival curves were obtained for the various radiation sensitive mutants of *E. coli* K-12, with and without a post-UV irradiation treatment with quinacrine. The results indicate that wild-type and *uvrB* (Fig. 2), and *polA* cells (data not shown) are sensitized by treatment with quinacrine. The *recA*, *recB*, *recA uvrB*, *lexA*, *lexA polA*, *recB uvrB* and *lexA uvrB* cells were

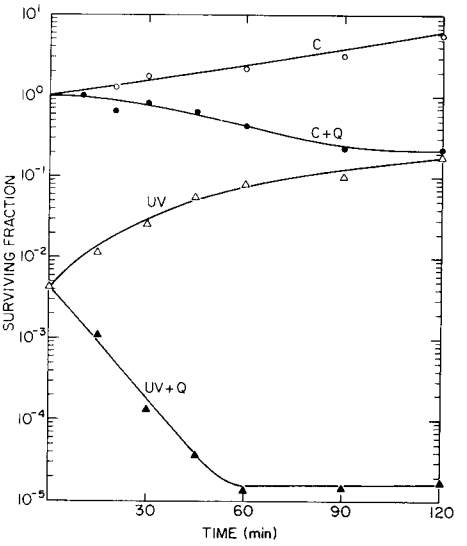

Figure 1. The kinetics of the potentiation of UV-induced killing by quinacrine. *E. coli* K-12 wild-type (AB2497) cells in exponential growth were UV irradiated (95 J m^{-2}) and then incubated at 37°C in SMM growth medium containing quinacrine (Q) at 75 μg/mℓ. Samples were taken for colony counts at various periods after irradiation. ○, Unirradiated cells; ●, unirradiated cells treated with quinacrine; △, irradiated cells; ▲, irradiated cells treated with quinacrine.

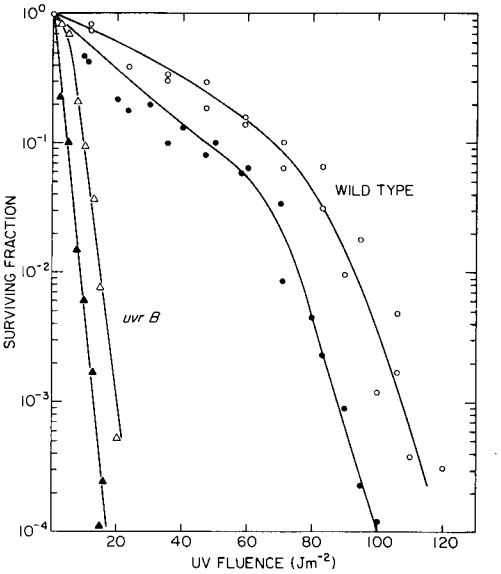

Figure 2. Survival curves for UV-irradiated cells of *E. coli* K-12 wild-type and *uvrB5* cells with and without a 60 min post-irradiation incubation in SMM growth medium containing quinacrine at 75 μg/mℓ. ○, wild-type (AB2497); ●, wild-type treated with quinacrine; △, *uvrB5* (AB2499); ▲, *uvrB5* treated with quinacrine. The results for *uvrB* are the average of two experiments; all of the data points for two experiments are shown for the wild-type strain.

not sensitized by treatment with quinacrine (data not shown). These results suggest that UV recovery functions controlled by *recA*, *recB* and *lexA* genes are inhibited by quinacrine.

Two repair processes that act on UV radiation-damaged DNA have been shown to be controlled by the *rec* and *exr* genes; (1) the medium dependent repair of DNA single-strand breaks produced enzymatically in parental strand DNA during excision repair (Youngs *et al.*, 1974), and (2) the post-replicational repair of gaps in daughter-strand DNA synthesized after UV irradiation (Youngs and Smith 1976). The fact that *uvrB* cells are sensitized by incubation with quinacrine, suggests that post-replicational repair is inhibited by this drug. The survival data do not allow a conclusion as to the possible inhibition of excision repair by quinacrine. However, preliminary data from alkaline sucrose gradients indicate that a 60 min post-irradiation treatment with quinacrine irreversibly blocked the closing of excision repair-induced DNA single-strand breaks (i.e. there was also no repair during 60 min of incubation after the removal of the quinacrine, in fact, extensive degradation occurred) in the wild-type strain after 20 or 40 J m^{-2} (data not shown).

In summary, survival data suggest that quinacrine inhibits a portion of post-replication repair, and preliminary gradient data indicate that quinacrine also inhibits a portion of excision repair. Because of the significant toxic effect, and excessive degradation of DNA produced by this drug, further mechanistic studies are probably not warranted.

Acknowledgements—This work was supported by U.S. Public Health Service research grant CA-02896 and research program project grant CA—10372 from the National Cancer Institute, DHEW.

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