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Inhibition by Nalidixic Acid of Post-uv Survival of *Escherichia coli* (39190)[2]

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Introduction. Cells have the capacity to repair a variety of lesions produced in DNA by physical and chemical agents. At least three repair processes occur and may be studied conveniently following damage (from uv light, for example). These include: (i) excision repair (repair replication) in which single-strand segments containing pyrimidine dimers or other lesions are excised from helical DNA followed by local resynthesis and closing of the gap by a DNA ligase (1-3); (ii) postreplicative repair in which unrepaired dimers remaining at replication simply result in gaps in daughter strands, which are filled in postreplicatively by localized resynthesis or by recombination (4); and (iii) reinitiation repair in which an arrested replicating fork, presumably at a dimer site, is reinitiated by a repair process specific for the replicative part of the cell cycle (5). It has also been suggested that mouse L cells have an S phase specific mode of repair (6). In addition, microorganisms can repair pyrimidine dimers by a photoreactivation process, which is particularly useful in mechanistic studies of uv damage and repair (7).

Elucidation of the mechanisms involved in semiconservative DNA synthesis and the various types of DNA repair could be facilitated greatly by the identification of specific inhibitors. Nalidixic acid (NA) was examined as one such possible inhibitor, and the results are the subject of this communication. This antibacterial agent was reported previously to inhibit reversibly DNA synthesis and replication *in vivo* (8, 9), but did not inhibit DNA polymerase I (10, 11), endonuclease I, exonuclease I, II, and III from *E. coli* (11); nor the polynucleotide ligase and methyltransferase-induced T_4 phage (11). Also, in the concentration range of 10^{-4} *M* it inhibited both semiconservative and "repair" synthesis in *E. coli* following extensive uv irradiation as shown by CsCl density gradient profiles of the DNA products (12). Recently, it was reported that such concentrations of the drug prevented semiconservative DNA synthesis in toluenized *E. coli* cells without interfering with repair synthesis provoked by uv light (13). A variety of other agents such as acridines (14, 15) and amino quinolines (16) which, in contrast to NA, bind to DNA (17, 18) have been shown to inhibit both repair and the DNA dependent polymerase (19, 20). The comparative effects of NA on post-uv survival were, therefore, of considerable interest. The present experiments with *E. coli* B showed that survival following uv irradiation was decreased substantially by plating on agar medium containing 5×10^{-7} *M* NA while survival of unirradiated controls was unaffected. This effect on uv survival was obtained with drug concentrations about 1000-fold less than those required to prevent completely bacterial replication.

Materials and methods. E. coli B was supplied by Dr. Tom Feary of this institution. Dr. Roy Curtiss provided cultures of B_{s-1}, B/r, TAU-bar, and Ts-7, the temperature-sensitive ligase mutant derived by Dr. C. Pauling from TAU-bar (21). *E. coli* B, B_{s-1}, and B/r were grown overnight in nutrient broth (Difco) and diluted in 0.9% saline to a cell denity of about 5×10^6/ml. *E. coli,* TAU-bar, and Ts-7 were grown overnight in tryptone broth, diluted 1:50 in fresh medium, and grown for 4 hr with shaking to midlog phase followed by dilution in saline as above. Irradiation was carried out in open petri dishes with shaking at room temperature under a germicidal lamp (Arthur H. Thomas) at a filtered wavelength of 253 nm. For most experiments the uv dose

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rate was 7.5 ergs/mm²/sec as measured by a Blak-Ray short-wave uv meter (UV Products, Inc.). The total dose for each experiment is given in the appropriate figure of legend.

Following irradiation, serial dilutions were made for plating on solid media with or without added nalidixic acid as indicated in the legends. Photoreactivation of the freshly spread plates was done at room temperature with four photoflood lamps at a distance of 18 in. Liquid holding recovery was followed by plating samples of an irradiated cell suspension in buffered saline after various periods in the dark at 37°.

Results. Survival of *E. coli* B following uv irradiation was reduced substantially when plating was done on media containing nalidixic acid. Figure 1 illustrates this effect for nalidixic acid at 5×10^{-7} *M* in nutrient agar following irradiation of the organisms at various dose levels. The same drug effect was also observed when post-uv growth was in minimal agar. The dose-effect curve for the drug, illustrated in Fig. 2 with a constant uv dose, showed an effect on survival at concentrations as low as 1×10^{-7} M. This effectiveness contrasts sharply with the much higher concentrations reported to inhibit DNA replication (7, 8).

The photoreversibility of the nalidixate-enhanced lethality was demonstrated in the experiments in Fig. 3, suggesting that the drug either interfered with pyrimidine dimer repair or the ability of the organism to survive the consequences of repaired or unrepaired dimers. Experiments on liquid-holding recovery from uv damage were done with *E. coli* B with and without 5×10^{-6} *M* nalidixate in the holding medium. As shown in Table I, the drug did not prevent LH recovery even at the ten-fold increase in concentration over the experiments in Fig. 1. In contrast to the lack of effect of the drug in the repair media, however, it was observed that nalidixate at 5×10^{-7} *M* in the growth medium following LH still resulted in a substantial decline in survival (Table II). In fact, the actual numbers of nonsurvivors due to the drug *increased* during the initial period of liquid holding. These results suggested either that nalidixate inhibited postreplicative repair or had its effect on a terminal event in repair or on a final restoration of the repaired sequence which is

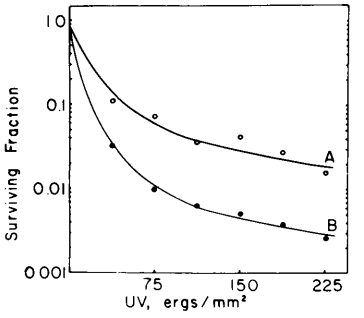

FIG. 1. Effects of 5×10^{7} nalidixic acid in the culture media on post-uv survival of *E. coli* B. Organisms were irradiated as in text, and aliquots were removed for colony counts at the doses shown and spread on nutrient agar with (curve B) and without (Curve A) drug.

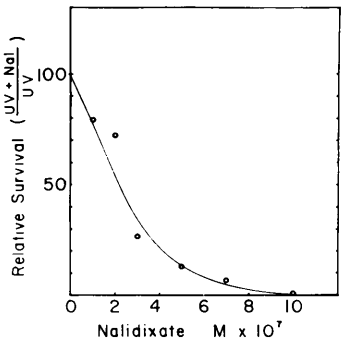

FIG. 2. Effect of nalidixic acid concentration on post-uv survival of *E. coli* B. Organisms received a dose of 150 ergs/mm² and were plated on nutrient agar containing the concentrations of drug shown.

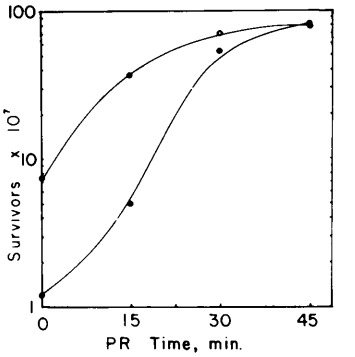

FIG. 3. Effects of photoreactivation on nalidixate inhibition of post-uv survival of *E. coli* B. Organisms received 37.5 ergs/mm² of uv irradiation, and following spreading on nutrient agar without (top curve) and with (bottom curve) drug, were photoreactivated for the times shown before incubation at 37°.

ordinarily completed at or after the next round of replication.

The sensitivity of an organism which ordinarily tolerates dimers rather well was studied by using B/r in experiments identical to those done with B. Similarly, B_{s-1} was used as an example of an organism which does no appreciable excision repair. Neither showed any decrease in postirradiation survival as a result of plating on 5×10^{-7} *M* nalidixate.

A mutant of *E. coli* with a temperature-sensitive ligase (Ts-7) derived from the parent strain TAU-bar provided an opportunity to study an organism in which the terminal step in excision repair is presumably rate-limiting. This organism, when grown initially for 2 hr at 40° following uv irradiation shows considerably less survival than at 25°. Experiments with the parent strain showed that its survival following uv was decreased by plating on nalidixate much like *E. coli* B, although a drug concentration 10 times higher was required. Figure 4 compares the effects of drug on the temperature-sensitive mutant (Ts-7) at 25° and when exposed to 40°, and shows that restricting the effectiveness of the ligase step did not produce any enhancement of the nalidixic acid effect. Additional experiments, reported elsewhere, however,

TABLE I. EFFECTS OF NALIDIXIC ACID ON LIQUID-HOLDING RECOVERY OF *E. coli* B.[a]

Time in liquid (hr)	Number of survivors/ml		
	−uv	+uv	
		Control	5×10^{-6} *M* nalidixate in LH
0	8.7×10^6	1.7×10^6	1.6×10^6
2	8.8×10^6	$6.9\ 2\ 10^6$	5.3×10^6

[a] Organisms were irradiated as described in the text at a dose of 37.5 ergs/mm² and nalidixate added to p of the cell suspension to a concentration of 5×10^{-6} *M*. Samples were removed for colony counts on nutrient agar before and after holding in the dark at 37° for 2 hr. An unirradiated sample of the original cell suspension was also held as a control for survival.

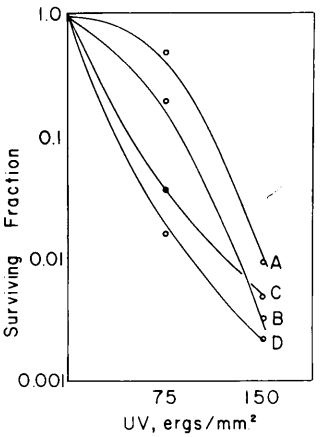

FIG. 4. Effects of nalidixate on post-uv survival of Ts-7 at 25 and 40°. Bacteria were grown to midlog phase at 25°, and after irradiation of diluted cell suspension to the extents shown aliquots were plated on tryptone broth agar. Half of the plates were incubated at 25° (curves A and B), and half were incubated at 40° for 2 hr and then continued at 25° (curves C and D). Curves B and D indicate those survivors on 5×10^{-6} *M* nalidixic acid. Curve D is corrected for a 75% decrease in viability due to nalidixate alone.

TABLE II. EFFECT OF LIQUID-HOLDING AND POSTHOLDING GROWTH WITH NALIDIXIC ACID ON THE POST-UV SURVIVAL OF *E. coli* B.[a]

LH time	−uv	+uv (37.5 ergs/mm²) Post-LH plating	
		Nutrient agar	Nutrient agar + 5×10^{-7} *M* NA
0	1.19×10^7	2.07×10^6	2.4×10^5 (1.83×10^6)
30 min	1.13×10^7	4.4×10^6	4.1×10^5 (4.0×10^6)
1 hr	1.21×10^7	6.7×10^6	5.6×10^6 (6.1×10^6)
2 hr	1.19×10^7	6.2×10^6	1.3×10^6 (4.9×10^6)
3 hr	1.19×10^7	5.6×10^6	9×10^5 (4.7×10^6)

[a] Organisms received a uv dose of 37.5 ergs/mm² followed by liquid holding in the dark at 37° for the times shown. Samples were plated on nutrient agar without and with nalidixate as shown. The numbers in parentheses indicate the numbers of "nonsurvivors" at each time due to nalidixate.

showed an interesting increase in thermal sensitivity due to the drug (22).

Discussion. These experiments have shown nalidixate to be a potent inhibitor of post-uv survival of *E. coli* B, TAU-bar, and Ts-7 when included in the plating medium. Strains B/r and B_{s-1}, which show increased resistance and sensitivity, respectively, to uv irradiation did not show the post-uv drug effect. The vulnerability to nalidixate could be abolished by photoreactivation, implicating pyrimidine dimers as the lesion involved in the drug effect. The finding that the increased post-uv survival resulting from liquid holding was not inhibited by inclusion of drug in the holding medium indicated that the drug effect is not a simple inhibition of excision repair. In fact, the additional finding that LH actually increased the number of nonsurvivors due to nalidixate suggested either that a postreplicative process is involved in repairing lesions not repairable during the holding phase or that LH repair is not completed until the next round of replication, thus expressing the drug-vulnerable step only on growth medium. The lack of drug effect on the limited post-uv survival of B_{s-1} suggested that the drug-vulnerable step depended on the excision repair system, and furthermore that the drug effect is not simply interfering with the organism's ability to tolerate persistent pyrimidine dimers. The drug resistance of B/r may mean that its uv resistance has resulted from a generous excess of the enzyme step(s) vulnerable to the drug.

The relationship between the present findings and previous reports that nalidixic acid prevented conversion of "small" to "large" DNA in *E. coli* after severe uv damage is not clear since the earlier experiments employed concentrations of the drug some 10^3 higher under conditions where there was no survival of the organisms (12). The present findings are also interesting in light of the recent report that post-uv repair synthesis was not inhibited by large concentrations of nalidixic acid (13).

These findings with nalidixic acid suggest that it may be an agent of considerable interest in elucidating the mechanisms and biological consequences of DNA repair.

Summary. Nalidixate inhibited the post-uv survival of *E. coli* B. TAU-bar and Ts-7, but not Bs-1 or B/r when included in the plating medium. Removal of the drug sensitivity by photoreactivation was consistent with pyrimidine dimers as the target for the effect. Nalidixate did not inhibit liquid-holding recovery from uv when included in the holding medium, but survival was inhibited if the drug was in the subsequent plating medium. In fact, there was an actual increase in the number of nonsurvivors due to the drug following a period of holding.

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