4-Quinolones and the SOS response

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Summary. The SOS DNA repair system is induced in bacteria treated with 4-quinolones. However, whether the response exacerbates or repairs the damage caused by these drugs is still unclear. The recA13 and the recB21 mutations impair recombination repair and render bacteria unable to induce the SOS response when treated with nalidixic acid or other agents that affect DNA synthesis. However, UV treatment induces the SOS response in recB21 mutants but not in recA13 mutants. Both these mutants are hypersensitive to nalidixic acid and, therefore, either recombination repair or SOS repair would appear to repair DNA damage caused by the drug. However, since the lexA3 mutation (which also renders bacteria incapable of inducing the SOS response without affecting recombination repair) had no effect on the susceptibility of bacteria to nalidixic acid, the SOS response neither contributes to nor repairs DNA damage caused by the drug. Consequently, it would seem that the hypersensitivity of the recA13 and recB21 mutants to nalidixic acid is due to their deficiency in recombination repair. This view was confirmed by testing a recA430 mutant that is recombination-repair proficient but SOS repair-deficient and finding it to be no more sensitive to nalidixic acid than its parent. Thus it would appear that, although induced by nalidixic acid treatment, the SOS DNA repair system does not play any role in bacterial responses to the damage caused by the drug. In contrast, the recombination repair system does repair damage caused by nalidixic acid.

Introduction

The SOS DNA repair system is one of the responses which assists bacteria to recover from DNA damage. The response is controlled by two regulatory proteins, the recA and lexA gene products. These proteins govern transcription of a subordinate group of genes called the SOS genes. The expression of these genes, which code for products such as DNA repair enzymes and cell-cycle inhibitors, leads to a series of individual activities that together make up the SOS response (Walker, 1984). The basic organisation and regulation of the response is only partially understood at a molecular level. The lexA protein acts as a repressor that binds to similar operator sequences in each SOS gene and inhibits their transcription (Walker, 1984). The recA protein, on the other hand, functions as an inducer either directly, by cleaving the lexA repressor by a proteolytic mechanism, which is acquired by the recA gene product when it interacts with an unknown product that is formed as a result of certain types of DNA damage (Little and Mount, 1982), or indirectly by the so modified recA protein acting as an allosteric effector in the autocatalytic digestion of the lexA protein (Little, 1984; Sililiat et al. 1986). The SOS response so induced is transient because DNA repair, by definition, will progressively remove the inducing stimulus so that concentration of the modified recA protein will decline and, consequently, lexA repressor levels will rise, so causing the normal state of repression of the SOS genes to resume.

Treatment of bacteria with the 4-quinolones leads to induction of the SOS response (Gudas and Pardee, 1976; Phillips et al., 1987; Piddock and Wise, 1987). It is thought that this is due to the 4-quinolones deranging DNA replication (Goss et al. 1965; Crumplin et al. 1984; Benbrook and Miller, 1986). The role of the SOS response in bacteria treated with the 4-quinolones is still unclear. Phillips et al. (1987) have suggested that one of the SOS genes may code for the unknown protein which has been postulated by Dietz et al. (1966) and Smith (1984) to cause the lethal action of the 4-
quino-lones. This is because the concentrations of the 4-quino-lones that maximally induce the SOS response correlate well with the most bactericidal concentrations of these drugs (Phillips et al., 1987). On the other hand, Drlica (1984) has suggested that the SOS response may play a role in protecting bacteria from damage caused by these drugs because mutations that render bacteria unable to induce the SOS response increase bacterial sensitivity to nalidixic acid (McDaniel et al., 1978). Therefore, the role of the SOS response in bacteria treated with 4-quino-lones was investigated by observing the effect of recA, recB and lexA mutations on the sensitivity of Escherichia coli to nalidixic acid 50 µg/ml in nutrient broth.

Nalidixic acid was chosen for this investigation for two reasons. Firstly, nalidixic acid possesses only a single mechanism of action (termed mechanism A) which is possessed by all 4-quino-lones and is considered to be the basic mechanism of action of these drugs (Smith, 1984). Mechanism A requires the prior synthesis of an unknown protein before bacterial death can occur. This concept follows from the finding that protein and RNA synthesis are prerequisites for the activity of mechanism A (Dietz et al., 1966; Smith, 1984) but are not required for the second bactericidal mechanism (termed mechanism B) which is exerted by some 4-quino-lones (Ratcliffe and Smith, 1984). Secondly, more is understood about the conditions required for the induction of the SOS response by nalidixic acid than for other 4-quino-lones. For the SOS responses to be induced by treatment of bacteria with nalidixic acid but not by UV treatment, it is known that bacteria must have functional recB and recC genes (Chaudhury and Smith, 1985). These two genes code for exonuclease V (exo V) and it has been suggested that the action of exo V on DNA damaged by nalidixic acid may provide the unknown product that interacts with the recA protein so leading to lexA repressor cleavage and induction of the SOS response (Walker, 1984; Bailone et al., 1985). Hence, inactivation of the recB or recC genes as well as the recA gene will render bacteria incapable of inducing the SOS response when treated with nalidixic acid.

Materials and methods

Antibacterial preparation

Nalidixic acid (Sterling-Winthrop) was dissolved in 0.5 M NaOH (0.2 ml/mg) before being diluted to its appropriate concentration in sterile distilled water.

Escherichia coli strains

Seven strains of E. coli were used: AB1157, F- thr-1 leu-6 proA2 his4 thi-1 argE3 lacY1 galK2 ara-14 xyl-5 mtl-1 tsx-33 strA31 sup-37; AB2463, as AB1157 but also recA13; AB2470: as AB1157 but also recB21; IC5495, as AB1157 but also recA13 recB21; AB2494, as AB1157 but also meth-1 pps-31 lexA3; IC1656, thr leu thi proA argE3 ilvG sup-37 strA31 sfiB sr1C300; IC1657, as SC1656 but also recA430.

Determination of antibacterial effects of nalidixic acid

Nalidixic acid causes filamentation of bacteria (Goss et al., 1964) (which increases the turbidity of liquid cultures) so bacterial killing by the 4-quino-lones cannot be judged by measurements such as light scattering or optical density (Smith, 1984). Hence, rates of kill of bacteria by nalidixic acid were studied by viable counting.

Nutrient broth (Nutrient Broth No. 2, Oxoid) was inoculated with bacteria and then incubated overnight at 37°C to provide inocula for studies with nalidixic acid.

Sterile double strength nutrient broth was dispensed in 5-ml volumes in sterile 1 oz bottles. Aqueous nalidixic acid and sterile distilled water were added to give a final volume of 9.8 ml. The bottles were incubated at 37°C for at least 15 min and then 0.2 ml of the overnight culture, which contained approximately 2.5 x 10^8 cfu/ml, was added at time zero to complete the reaction mixtures. Hence, the viable count at time zero was c.5 x 10^6 cfu/ml. At 30-min intervals, 0.1-ml samples from each reaction mixture were decimally diluted in sterile nutrient broth. A 0.1-ml sample of each dilution was then spread on to a nutrient agar plate. The plates were incubated overnight at 37°C and the colonies were counted. When the effect of a range of concentrations of nalidixic acid on bacterial survival was estimated, 2.5 ml of double strength nutrient broth was placed in sterile 1 oz bottles. Aqueous nalidixic acid and distilled water were added to give a final volume of 4.9 ml (the concentrations of nalidixic acid used were based on the following geometric progression: 1.5, 3, 5, 9, and 15); 0-1 ml of the overnight culture was then added and the reaction mixtures were incubated at 37°C for a single period of 3 h. A 0-1-ml sample from each reaction mixture was then decimally diluted in sterile nutrient broth and a 0-1-ml sample of each dilution was spread on to a nutrient agar plate. The plates were incubated overnight at 37°C and the colonies were counted. All experiments were performed at least four times and the results presented are typical data for any one experimental series.

Results

In agreement with the results of McDaniel et al. (1978), a recA13 mutant was found to be hypersensitive to nalidixic acid 50 µg/ml when compared to its parent strain (fig. 1). Similarly, a recB21 mutant
was also found to be hypersensitive to nalidixic acid 50 µg/ml when compared to its parent strain (fig. 1). Hence, at first sight, it would seem that since these two mutants lack the SOS response and are hypersensitive to nalidixic acid, then the SOS response cannot contribute to the lethality of nalidixic acid as had been suggested (Phillips et al., 1987) but might play a role in protecting bacteria from damage caused by the drug (Drlica, 1984). However, when the effect of nalidixic acid on a *lexA*3 mutant (which is recombination-proficient but lacks the SOS response because its *lexA* protein is resistant to proteolysis catalysed by the modified *recA* gene product) was investigated, the results were most surprising. The sensitivity of the *lexA*3 mutant to nalidixic acid 50 µg/ml was found to be similar to that of its parent strain (fig. 2). As this mutant also lacks the SOS response, the SOS response does not appear to be involved in protecting bacteria from the damage caused by nalidixic acid. It would seem that, although induced by nalidixic acid treatment, the SOS response neither contributes to nor protects bacteria from damage caused by the drug.

The sensitivity of these mutants to nalidixic acid has so far been investigated at only a single drug concentration. Therefore, the sensitivity of mutants in nutrient broth containing nalidixic acid at concentrations of 0.15 to 1500 µg/ml was investigated. It can be seen in fig. 3 that the *recAI3* mutant was hypersensitive to nalidixic acid as compared to its parent strain at all concentrations tested. On the other hand, no significant difference between the sensitivity of the *lexA*3 mutant and the parent strain was observed throughout the same range of concentrations (fig. 3). This supports the conclusion that, despite being induced by nalidixic acid, the SOS response does not play any role in repairing the damage to the bacteria caused by the drug at any concentration.

Further support for this conclusion is provided by investigations with a *recA13recB21* double mutant. Such a mutant was found to be even more sensitive to nalidixic acid 50 µg/ml than either of the single *recA13* or *recB21* mutants (fig. 1). If the hypersensitivity to nalidixic acid of the *recA13*, *recB21* and *recA13recB21* mutants was due to their inability to induce the SOS response, then no difference would be expected in their sensitivity to nalidixic acid because either mutation alone should abolish the induction of the SOS response by the drug. Hence it seems that the hypersensitivity of these three mutants to nalidixic acid is not due to SOS repair but to their recombination repair defects. This view is strengthened by the double mutant being more susceptible than either single mutant to nalidixic acid because the double mutant has a greater deficiency in recombination repair than either single mutant. Furthermore, there is no requirement for the *lexA* gene in recombination...
repair (Petersen et al., 1988) and we find that the *lexA* mutation does not alter bacterial sensitivity to nalidixic acid.

When a *recA430* mutant, which is recombination repair proficient but is unable to induce the SOS response (Walker, 1984; Blanco et al. 1936), was investigated it was found to be no more sensitive to nalidixic acid 50 μg/ml than its parent strain (fig. 4). This finding confirms that the hypersensitivity to nalidixic acid treatment conferred by the *recA13* and *recB21* mutations is due to the role of these genes in recombination repair rather than to their role in SOS repair. Hence, it would appear that *recBC*-dependent recombination repair does play a role in protecting bacteria from the damage caused by nalidixic acid but, on the other hand, the SOS response, while being induced by the drug, neither contributes to, nor repairs, the damage it causes.

**Discussion**

Since mutations in the *recA* and the *recB* genes which render bacteria incapable of inducing the SOS response were found to increase the sensitivity of bacteria to nalidixic acid, in agreement with other results (McDaniel et al., 1978), the SOS response does not seem to contribute to the lethality of the drug as had been suggested previously (Phillips et al., 1987; Chow et al., 1988). Neither would the SOS response seem to be involved in protecting bacteria from the damage caused by nalidixic acid as the *lexA3* mutation which also renders bacteria unable to induce the SOS response had no effect on the sensitivity of bacteria to nalidixic acid. Therefore it would seem that, despite its induction, the SOS response is neither involved in protecting bacteria from the damage caused by the drug nor does it contribute to the lethality of the drug. Hence, as error-prone SOS repair does not seem to repair the type of DNA damage caused by nalidixic acid then such damage should not be expected to lead to mutations as has been suggested previously (Phillips, 1987).

The hypersensitivity to nalidixic acid of the *recA13* and *recB21* mutants appears to be due to their recombination repair defects since a *recA430* mutant, which is SOS-deficient but recombination repair-proficient, was found to be no more sensitive than its parent to the drug. Hence *recBC*-dependent recombination repair is involved in repairing damage to the bacterial chromosome caused by nalidixic acid. Another DNA repair pathway which is also independent of the SOS response, the *recF*-pathway (Peterson et al., 1988), also seems to be implicated in the repair of nalidixic acid damage as a *recF* mutation has also been shown to increase
bacterial susceptibility to the drug (McDaniel et al., 1978).

These conclusions would seem to apply, at least in part, to all 4-quinolones since they all exhibit mechanism A (Smith, 1984). Hence the correlation between the 4-quinolone concentration at which maximum induction of the SOS response occurs and the most bactericidal concentration of the drug does not appear to be due to one of the SOS gene-products being the unknown protein that causes bacterial death. As Phillips et al. (1987) measured the ability of the 4-quinolones to induce the SOS response by following the amount of protein synthesised from a sfiA-lacZ fusion gene, their correlation may simply have been caused by the 4-quinolones increasingly inhibiting RNA synthesis at concentrations greater than their most bactericidal concentrations (Crumplin and Smith, 1975). The induction of the SOS response, though coinciding with the most bactericidal concentration of a 4-quinolone, probably stems from the increasing antagonism of RNA synthesis by 4-quinolones at high concentrations.

A further conclusion can be drawn from these investigations on the sensitivity of recA, recB and lexA mutants to nalidixic acid. Although the SOS response is induced by nalidixic acid (Gudas and Pardee, 1976), it was found not to play a role in the bacterial response to the drug. In other words, a response that is induced upon treatment of bacteria with nalidixic acid seems to have no effect on bacterial survival. Hence it is not sufficient to show the induction of a gene by a 4-quinolone to conclude that the gene is involved in the bacterial response to the drug. Before such a conclusion can be drawn it is necessary to show that inactivating the gene has an affect on the susceptibility of bacteria treated with the 4-quinolone. A possible explanation for the induction of a set of genes that are not directly involved in the bacterial response to the 4-quinolones could result from the general effects of supercoiling on transcription (Smith, 1981) because reduced chromosomal supercoiling caused by the 4-quinolones is known to induce some genes while others are repressed.

A preliminary report of this investigation was presented at the 27th Interscience Conference on Antimicrobial Agents and Chemotherapy (Lewin CS, Ratcliffe NT, Smith JT 1987 Nalidixic acid and the SOS response. Abstract no. 474, p 179).

REFERENCES


