The role of the SOS response in bacteria exposed to zidovudine or trimethoprim

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Summary. Trimethoprim was more potent than zidovudine as an inducer of the SOS response in *Escherichia coli*. The level of induction by each compound initially increased with rising drug concentration and then fell; this effect was less marked with zidovudine than with trimethoprim. The SOS response did not appear to be involved in the inhibition of bacterial multiplication as the MICs of trimethoprim or zidovudine for *recA430* and *lexA3* mutants, which are unable to induce the SOS response, were identical to the MICs for the parent strains. However, the bactericidal activity of each compound against strains deficient in the SOS response was reduced. This suggests that induction of the DNA repair system contributes to the bactericidal activity of the drugs.

Introduction

The SOS response, which assists bacteria to repair damage to DNA, is controlled by two regulatory proteins, the *recA* and *lexA* gene products. These proteins govern transcription of a subordinate group of genes coding for DNA repair enzymes and cell division inhibitors, the individual activities of which make up the SOS response.¹ The *lexA* gene product functions as a repressor binding to similar operator sequences on the SOS genes, while the *recA* gene product provides a direct or indirect signal for induction of the SOS response.¹ Treatment of bacteria with antibacterial agents such as the 4-quinolones, which damage DNA, leads to the induction of this DNA repair pathway.^{1–3}

One consequence of induction of the SOS response is bacterial filamentation as one of the SOS genes, the *sfiA* gene, codes for a protein that binds to the *sfiB* (*ftsZ*) gene product, which is essential for septation.^{4–7} After the SOS response is switched off, the *sfiA* gene product is degraded by the *lon* (*capR*) protease and bacterial cell division resumes.^{7,8}

Trimethoprim and zidovudine are synthetic compounds which inhibit bacterial DNA synthesis.^{9–11} Trimethoprim prevents incorporation of thymine into bacterial DNA by inhibition of dihydrofolate reductase.⁹ Zidovudine is a thymidine analogue in which the 3' hydroxy group is replaced by an azido group; zidovudine is incorporated into bacterial DNA and the azido group makes subsequent 5'–3' phosphodiester linkages impossible.¹⁰ Bacteria exposed to each of these compounds undergo filamentation,^{9–11} suggesting that they may induce the SOS response. To

investigate this, β -galactosidase activity was measured in a strain of *Escherichia coli* possessing a deletion of the *lac* operon and a *sfiA::flacZ* operon fusion. The susceptibility to trimethoprim and zidovudine of bacteria deficient in the SOS response was also determined to establish whether the SOS DNA repair system was involved in the antibacterial activity of these drugs.

Materials and methods

Antibacterial agents

Trimethoprim lactate and zidovudine (Wellcome) were dissolved in sterile distilled water.

Bacterial strains

Six *E. coli* strains were used in this investigation; *E. coli* GC4415⁴ *thr leu his pyrD trp::MuC⁺ lac malB galK rpsL srl-300::tnlO sfia::MudI (aplac)* (obtained from Professor I. Phillips) and a zidovudine-resistant mutant of this strain, which was deficient in thymidine kinase; *E. coli* AB1157, F[–] *thr-1 leu-6 proA2 his4 thi-1 argE3 lacY1 galK2 ara-14 xyl-5 mtl-1 tsx-33 strA31 sup-37*; *E. coli* AB2494, as strain AB1157 but also *metb-1 pps-31 lexA3*; *E. coli* SC1656, *thr leu thi proA argE3 ilv^{ts} galK sup-37 strA31 sfiB srlC300*; *E. coli* IC1657, as strain SC1656, but also *recA430* (obtained from Professor J. T. Smith).

Induction of the SOS response

This was measured as previously described¹² with the following modification: Nutrient Broth (Oxoid)

was used instead of LB Broth as the growth medium for zidovudine tests whereas Iso-Sensitest Broth (Oxoid) was used for trimethoprim.

SOS induction was expressed as β -galactosidase activity in Miller Units¹² derived by use of the formula:

$$\frac{1000 (OD_{420} - 1.75 \times OD_{550})}{t \times v \times OD_{600}}$$

where OD_{420} and OD_{550} were the optical densities at the relevant wavelength after t min; v was the reaction volume; and OD_{600} was the initial OD at 600 nm.

Minimum inhibitory concentrations (MICs)

MICs were measured on nutrient agar for zidovudine and on Iso-Sensitest agar for trimethoprim with the arithmetic dilution scheme previously described.¹³ An inoculum of 10^4 cfu per spot was used and the MIC was taken as the lowest concentration that inhibited visible growth after overnight incubation at 37°C.

Bactericidal activity

The bactericidal activities of zidovudine in nutrient broth and trimethoprim in Iso-Sensitest broth were measured by viable counting on nutrient agar as previously described.¹⁴

Results

Each of the test strains, with the exception of the zidovudine-resistant mutant of *E. coli* GC4415 (MIC of zidovudine > 500 mg/L), was inhibited by trimethoprim or zidovudine at a concentration of 0.1 mg/L.

Inhibitory concentrations of trimethoprim and zidovudine induced the SOS response in *E. coli* GC4415 after exposure for 1 h (fig. 1). The level of induction initially increased with rising drug concentration and then fell. The concentration at which maximum induction of the SOS response was achieved was about 5 mg/L with each drug. The induction of the SOS response caused by exposure to zidovudine was greater than that caused by exposure to trimethoprim, and the decline observed after the peak concentration was reached was more marked with trimethoprim than with zidovudine.

When thymidine 50 mg/L was added to Iso-Sensitest broth the SOS response was no longer induced by trimethoprim at concentrations up to 100 mg/L. Zidovudine did not induce the SOS response in a zidovudine-resistant derivative of *E. coli* GC4415 which was deficient in thymidine kinase activity (fig. 1). A linear increase in the induction of the SOS response was observed over a 3-h period of exposure to zidovudine or trimethoprim 5 mg/L; the level of induction achieved by trimethoprim was weaker than that observed with zidovudine (fig. 2).

Fig. 1. Induction of SOS response in *E. coli* GC4415 exposed to trimethoprim □—□, trimethoprim + thymidine 50 mg/L ■—■ or zidovudine ○—○, and in a zidovudine resistant derivative of *E. coli* GC4415 exposed to zidovudine ●—●.

Fig. 2. Induction of the SOS response in *E. coli* GC4415 exposed for 3 h at 37°C to trimethoprim 5 mg/L ●—●, zidovudine 5 mg/L ○—○, or no drug x—x.

After exposure for 3 h to inhibitory concentrations of trimethoprim or zidovudine, the bactericidal activity of either drug against a *lexA3* mutant (unable to induce the SOS response because its *lexA* protein is resistant to proteolysis catalysed by the *recA* gene product) was reduced in comparison to the effect on the parent strain AB1157 at all concentrations tested (fig. 3). Similarly, trimethoprim and zidovudine were less bactericidal against a *recA430* mutant than against the parent strain SC1656 (fig. 4).

Discussion

The finding that inhibitory concentrations of zidovudine and trimethoprim induced the SOS response in *E. coli* is consistent with the observations that these compounds inhibit bacterial DNA synthesis (an event associated with SOS induction¹) and cause filamentation.^{9,11} Blockage of the replication fork by these drugs may trigger the DNA repair system, because the inhibition of dihydrofolate reductase by trimethoprim causes thymine starvation and stalling of the replication fork.⁹ In keeping with this the SOS response was no longer induced by trimethoprim in the presence of thymidine which antagonises the effect of trimethoprim by relieving the thymine starvation effect of the drug. Similarly zidovudine, which after phosphorylation by thymidine kinase causes DNA chain termination to occur,¹⁰ did not induce the SOS response in a zidovudine-resistant derivative of *E. coli*

GC4415 lacking thymidine kinase activity. This mutant possesses an intact SOS repair system which is inducible by nalidixic acid (unpublished results). The fluctuation in the level of induction of the SOS response with rising drug concentration has also been observed with the 4-quinolones.^{2,3} Zidovudine was found to be a stronger inducer of the SOS response than trimethoprim, but both drugs appeared to be weaker inducers than the 4-quinolones² (also C. S. Lewin, unpublished results).

The SOS response does not appear to play a role in the events leading to the inhibition of bacterial multiplication by either zidovudine or trimethoprim, as an inability to induce the DNA repair pathway had no effect on MICs of the drugs. However, induction of the SOS response seems to contribute to the lethality of the compounds as their bactericidal activities against *E. coli* strains unable to induce the SOS response were decreased. Hence, rather than repairing the damage caused by trimethoprim or zidovudine, the induction of the SOS response appears to contribute to their bactericidal activity; however, this is not the only event involved in the lethality of trimethoprim or zidovudine as the drugs were still bactericidal against these mutant strains.

Interestingly, induction of the SOS response by nalidixic acid does not seem to contribute to the lethality of that drug.^{14,15} Thus, although trimethoprim, zidovudine and nalidixic acid are similar in that

Fig. 3. Survival of *E. coli* strain AB1157 (●—●) and AB2494 (○—○) after exposure at 37°C for 3 h to (A) trimethoprim in Iso-Sensitest broth or (B) zidovudine in nutrient broth.

Fig. 4. Survival of *E. coli* strains SC1656 (●—●) and IC1657 (○—○) after exposure at 37°C for 3 h to (A) trimethoprim in Iso-Sensitest broth or (B) zidovudine in nutrient broth.

they inhibit DNA synthesis, induce the SOS response¹⁻³ and require protein synthesis for their lethality,^{9, 11, 16} the lethal consequences of damage to bacterial DNA by the 4-quinolone appear to be different. This may be linked to the observation that the bactericidal activity of the 4-quinolones is greater than those of trimethoprim or zidovudine (C. S. Lewin, unpublished results).

It has been suggested that drugs which induce the SOS response are potential bacterial mutagens^{2, 17} because DNA repair is prone to error and mutations may be introduced into the bacterial chromosome.

The possibility that zidovudine might act as a bacterial mutagen should be investigated because patients suffering from the Acquired Immune Deficiency Syndrome are susceptible to opportunist bacterial infections. A worrying scenario will emerge if exposure of bacteria to zidovudine increases the frequency at which bacteria are able to develop resistance to antibiotics.

We thank the Scottish Home and Health Department for financial support.

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