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Post-exposure filamentation without concomitant cell death of *Escherichia coli* exposed to ciprofloxacin in the absence of RNA or protein synthesis

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The 4-quinolone antibacterials are rapidly bactericidal against Gram negative rods such as *Escherichia coli*. The major binding sites for these drugs are DNA-bound bacterial toposiomerases, but the reason why such binding leads to death is subject to debate (Chen et al 1996). Quinolone exposure leads to induction of the 'SOS' response, resulting in DNA repair, inhibition of cell division and filamentation. This process requires RNA and protein synthesis. However, it is unclear whether SOS induction contributes overall to cell recovery or lethality. We are therefore investigating quinolone-induced filamentation of *E. coli* to determine if filamented cells are dead cells, or whether filamentation is necessary for repair.

E. coli strain AB1157 was grown overnight in Oxoid No. 2 nutrient broth. It was then diluted into nutrient broth at 37°C containing 0.02 mg L^{-1} ciprofloxacin (Cip) (4 x MIC, giving 2% survivors after 3 h exposure). Cultures were also set up containing Cip plus 20 mg L^{-1} chloramphenicol (Cm) to inhibit protein synthesis, or Cip plus 160 mg L^{-1} rifampicin to inhibit RNA synthesis. Washed cells were also resuspended in phosphate-buffered saline (PBS) containing Cip. Bacteria continue RNA and protein synthesis in PBS, but cannot divide. Following exposure for 3 h, cells were washed free of drug(s) and resuspended in drug-free nutrient broth at 37°C. Cell viability was determined at hourly intervals, and cell length measured by optical microscopy.

No further cell death occurred after any treatment once drug(s) had been removed and bacteria resuspended in drug-free broth. Mean cell length increased 15-fold during the 3 h exposure to Cip in nutrient broth, and remained approximately constant for 6 to 8 h after drug removal, during which time the viable count increased 100-fold. The proportion of cells similar in length to control, untreated cells increased significantly during this time. No increase in mean cell length occurred in cells exposed to Cip for 3 h in the presence of Cm or Rif, or exposed to Cip in PBS. Filamentation commenced 2 or 4 h after removal of Cip plus Cm or Cip plus Rif respectively, and 1 h after PBS exposure. The order of these delays correlated with the times taken for the respective cultures to recommence exponential growth after drug removal.

Piddock et al (1990) reported that Cip-induced filamentation is inhibited by the protein synthesis inhibitor Cm. Our data extends this to the RNA synthesis inhibitor Rif, confirming that quinolone-associated filamentation is an induced function, dependent on RNA and protein synthesis. Resumption of protein and RNA synthesis after Cip has been removed thus permits SOS induction and hence filamentation in response to pre-existing damage inflicted by the quinolone. Inhibition of filamentation in PBS results not from lack of RNA or protein synthesis to trigger SOS induction, but from insufficient nutrients in PBS for cell wall synthesis.

We have shown that Cip-induced filamentation is expressed during the post-exposure period once cells are allowed to resume RNA and protein synthesis or provided with sufficient nutrients for cell wall manufacture. The observation that lethality does not continue once drug(s) have been removed implies that filaments formed after drug removal are viable, indicating that such SOS-induced filamentation is not contributing to the lethal action of ciprofloxacin.

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