NOVOBIOCIN KILLS BACTERIA BY A MECHANISM UNRELATED TO 4-QUINOLONE LETHALITY

N.T. Ratcliffe, J.T. Smith. The Microbiology Section, Department of Pharmaceutics, The School of Pharmacy, London WC1N 1AX.

When Escherichia coli are incubated in increasing concentrations of 4-quinolone antibacterials in nutrient broth it is found that as the concentration is increased above their minimum inhibitory concentrations (m.i.c.'s) the drugs are progressively more bactericidal up to a concentration we term the most bactericidal concentration. Further increases beyond the most bactericidal concentration paradoxically result in progressively less bacterial kill (Ratcliffe and Smith, 1984). The ratio between the most bactericidal concentration and the m.i.c. for ten 4-quinolone antibacterials was found to be 43, ranging from 30 to 75, and the killing of E.coli by the drugs can be antagonized by rifampicin or by chloramphenicol addition (Smith, 1984).

The target site of the 4-quinolone antibacterials is believed to be the A subunit of DNA gyrase, while novobiocin is thought to act on the B subunit of the same enzyme (Cozzarelli, 1980). In this study the killing of bacteria by novobiocin was compared with that by the 4-quinolone antibacterial, norfloxacin. The organism used was Staphylococcus aureus E3T because E.coli are not sensitive to novobiocin.

When the Staphylococcus aureus was incubated in increasing concentrations of novobiocin in nutrient broth a similar phenomenon to that seen with E.coli treated with 4-quinolones occurred because the drug exhibited a single most bactericidal concentration of 150 μg/ml and concentrations greater or smaller than this caused less bacterial death. However, the m.i.c. of novobiocin against the coccus was only 0.075 μg/ml so the ratio between its most bactericidal concentration and its m.i.c. is 2000, which greatly exceeds the ratios obtained with the 4-quinolone antibacterials against E.coli.

In case the phenomenon was bacterium-related rather than drug-related the response of the coccus to norfloxacin was studied. It was found that its most bactericidal concentration was 15 μg/ml and its m.i.c. was 0.3 μg/ml. The ratio between these two values is hence 50 which falls within the range seen with E.coli and the 4-quinolones. Thus the very high ratio observed between the most bactericidal concentration and the m.i.c. of novobiocin with the coccus is a drug-specific phenomenon.

As a result of this difference between novobiocin and the 4-quinolones being observed, the effect of rifampicin and chloramphenicol addition on the bactericidal activities of novobiocin and norfloxacin was investigated. As expected the results with norfloxocin and the coccus were similar to those seen with E.coli and the 4-quinolones, because it was found that either rifampicin or chloramphenicol antagonized its bactericidal activity. Hence for norfloxacin to exert its lethal effect on E.coli or Staph. aureus a protein or proteins arising from de novo RNA synthesis followed by protein synthesis must be produced. However, the results with novobiocin were most unexpected. With Staph. aureus, while chloramphenicol addition antagonized the activity of novobiocin, rifampicin addition actually enhanced the lethality of this gyrase inhibitor. Thus for novobiocin to exert its lethal effect de novo protein synthesis is required but de novo RNA synthesis is not required. These findings reveal that a major difference exists between the mechanism of kill caused by novobiocin and that exerted by the 4-quinolones.

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