CALORIMETRIC INVESTIGATIONS OF ESCHERICHIA COLI TREATED WITH CIPROFLOXACIN AND NALIDIXIC ACID

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Calorimetry has been extensively used to study the mechanisms of action of antimicrobials (Beezer, 1980). Changes of heat output caused by antimicrobial agents acting on bacterial metabolism can provide information on the rapidity of the onset of action of such drugs. Furthermore the actual shape of the observed heat output-time curve helps to elucidate the mechanism of drug action. Nalidixic acid (NAL) and ciprofloxacin (CIP) were studied at their most bactericidal concentrations, which are 90 and 1.5µg/ml, respectively (Smith, 1984). In addition the effect of RNA synthesis inhibition was tested by adding rifampicin (RIF) to a concentration of 160 µg/ml. The heat output of about 10^7 E.coli per ml in nutrient broth with and without the 4-quinolones and rifampicin was monitored in an adiabatic batch titration calorimeter. Each heat output-time curve was normalised according to the number of organisms present at the start of each experiment then characterised by the initial rate of heat output, the total heat output and the time at which heat output ceased. From replicate determinations the limits of error on the data in Table 1 are ±10%; arising from errors involved in viable count determinations.

Table 1. Calorimetric data for Escherichia coli

Drug addition	Initial heat output rate (µW/ml)	Total heat output (per cent)	Time to cessation of heat output (hours)
None	33.3	100	11.0
NAL	15.6	13	4.5
CIP	11.0	5.2	2.5
RIF	14.3	8.8	7.5
NAL+RIF	9.0	6.6	9.0
CIP+RIF	9.0	4.0	4.0

It can be seen that NAL and CIP decreased the initial rate of heat production, the total heat output and caused a premature termination of heat output. It has been proposed (Smith, 1984) that CIP possesses two bactericidal mechanisms while NAL possesses only one such mechanism. In agreement CIP caused a significantly greater reduction of the initial heat output rate, the total heat output and caused an even more rapid cessation of heat output than NAL. RIF alone caused a decreased heat production but heat production continued for much longer than with CIP or NAL since this concentration of RIF is bacteriostatic rather than bactericidal (Smith, 1984). When either NAL or CIP were combined with RIF,a greater decrease in the inital rates of heat production and total heat outputs occurred than that seen with CIP or NAL alone. However, RIF had a much more pronounced effect on the action of NAL than on that of CIP. Indeed the heat output from bacteria treated with CIP alone was less than that produced by bacteria treated with NAL and RIF together. Thus the activity of NAL was markedly influenced by the addition of RIF whereas that of CIP was scarcely affected, as reflected in the shapes of the heat output-time curves. This further supports the hypothesis (Smith, 1984) that ciprofloxacin possesses a second rifampicin-resistant antibacterial mechanism of action that is not possessed by nalidixic acid.

Beezer, A.E. (1980), Biological Microcalorimetry, Academic Press, London.
Smith, J.T. (1984), The Pharmaceutical Journal, 233: 299-305.