Dual-action antibiotic hybrids

*J Antimicrob Chemother* 1994; 33: 197-200

It is a beguiling prospect to produce an antibiotic that attacks bacteria with two entirely different modes of action combined in one molecule. Such a concept has been explored for some time, and is now gradually coming to fruition, mainly by the development of hybrids of fluoroquinolones and β-lactam antibiotics.

Like many innovations in the antibiotic field, this enterprise has its roots largely in work carried out many years ago in academia. The seminal finding by Sabath, Jago & Abraham (1965) was that certain side chains of cephalosporins in position 3 were spontaneously dislodged when the β-lactam ring was hydrolysed (either chemically or by β-lactamase). The type of substituent involved in this mechanism (termed, appropriately, a 'leaving group'), is one that readily accepts electrons. In this way, free acetate appears during the hydrolysis of cephalosporin C or cephalothin, and pyridine from cephaloridine (Figure 1). This mechanism underlies the working of the β-lactamase-detecting cephalosporin PADAC (pyridinium-2-azo-ρ-dimethylamino chromophore) (Jorgensen, Crawford & Alexander, 1982), the leaving group being in this case a bright yellow chromophore. Such chemistry does not apply to cephalosporins whose 3-side chain is not an electron acceptor, for example cephalexin, cefaclor and Nitrocefin.

An early practical application of this reaction was the synthesis of cephalosporin MCO in the Glaxo laboratories (Greenwood & O'Grady, 1976; O'Callaghan, Sykes & Staniforth, 1976). This, the original dual action antibiotic, has a 3-side chain consisting of 2-mercaptopyridine-N-oxide; it is active against a wide range of bacteria in its own right, binding to PBPs in the usual way. When a β-lactamase producing strain is encountered, the 3-side chain is ejected, generating the anti-septic pyrithione ('Omadine') that is lethal against the cephalosporin-resistant strain. Unfortunately, pyrithione is too toxic for cephalosporin MCO to be useful as a systemic agent.

The next developments were the reports by Mobashery & Johnston (1986, 1987) that a compound consisting of desacetylcephalothin linked at the 3 position to β-chloro-λ-alanine, in the presence of β-lactamase, prevented the action of alanine racemase. This latter enzyme, involved in the early stages of bacterial cell wall biosynthesis, was inhibited by β-chloroalanine ejected from the cephalosporin as the result of the action of the β-lactamase. Work at Bristol-Myers Squibb (Rao, Fung-Tomc & Desiderio, 1993) has resulted in prodrugs of carbapenems made with β-chloro-L-alanine (Figure 2). The latter moiety increases the chemical stability of the molecule, facilitates oral absorption, and sets up a dual action potentiality. However, the mechanism of duality in this case differs from that outlined above, as here the β-chloroalanine moiety is detached without the β-lactam ring being broken; this is a consequence of the substitution being at the 4 position.

In a fashion that is analogous to the behaviour of the cephalosporins, penems also eliminate a leaving group substituent in the 2 position (note the differing numbering systems for penem and carbapenem nuclei) when the β-lactam ring is broken (Perrone et al., 1986), as...
shown in Figure 3. Antimicrobial compounds that have been linked thus to the penem nucleus, and are ejected by this mechanism, include nitrofurantoin, clioquinol ('Enterovioform'), cycloserine, oxolinic acid and fluoroquinolones; these compounds have been studied by the Farmitalia Carlo Erba group (Perrone et al., 1992). Adducts in which a penem and a fluoroquinolone are linked by a carbamate or ester bond (see below) have been investigated in more detail (Corraz et al., 1992; Perrone et al., 1992). They appear to be much more stable than their cephalosporin counterparts (see below), with chemical half-lives of between 65 and 80 h. Both in vitro and in vivo they display penem-like activity (e.g. binding to PBPs, and being active against anaerobes and fluoroquinolone-resistant Staphylococcus aureus) and fluoroquinolone-like activity (e.g. against Pseudomonas aeruginosa).

A series of compounds analogous to the penem-fluoroquinolones described above has been constructed from the carbapenem nucleus in the laboratories of Hoffman La Roche (Corraz et al., 1992). They are less chemically stable than the penems (a typical half-life was 5 h), but again can show both carbapenem-like and fluoroquinolone-like activity. An added bonus was that one adduct showed increased stability to renal dehydropeptidase.

These studies have also been extended to the penicillins: linking norfloxacin to 6 APA via an amidino bond (as in mecillinam) has been briefly reported (Roberts et al., 1990) to produce a dual-action antibiotic of high potency.

The most attention, judging by the volume of literature, has been paid to the construction and properties of dual-action hybrids between cephalosporins and fluoroquinolones. The situation is made complicated in that various cephalosporins may be linked, always via their 3 side chain, to different points of any fluoroquinolone (Figure 4). Thus, a very large number of different hybrid antibiotics can be synthesized following this pattern. A selective approach has been taken, in that the Roche group has concentrated on variously-linked adducts of desacetylcefotaxime with ciprofloxacin or fleroxacin (e.g. Georgopapadakou & Bertasso, 1993), while workers at Procter & Gamble (Demuth et al., 1991) chose a single method of joining desacytylephalothin (via an ester bond) to a range of quinolones, including ciprofloxacin. At least one of the compounds described, Ro-23-9424 (the ester-linked fleroxacin/desacetylcephalothin adduct), is reported to have proceeded to clinical trial (Corraz et al., 1992).

In ester-linked compounds, the 3-carboxyl group of the fluoroquinolone is joined to the cephalosporin nucleus at the latter’s 3 position. In the carbamates and tertiary amines, it is the distal nitrogen of the fluoroquinolone’s piperazine ring (the 4’ position) that is involved in binding to C-3 of the cephalosporin ring (Figure 5).

These different types of linking have important consequences in defining the properties of the various adducts. For a quinolone to be microbiologically active, its 3-carboxyl group must be free; hence, the ester-linked compounds (in which the substitution is through the 3-carboxyl), while intact, can act only as cephalosporins (Georgopapadakou & Bertasso, 1993). Quinolone activity is generated by one of three mechanisms: firstly, in the presence of an active β-lactamase; secondly, when the compound undergoes spontaneous hydrolysis (esters Ro 23-9424 and Ro 24-6392 have chemical half-lives of 3 h at pH 7 and 37°C) and thirdly, when the PBPs are acylated
in the course of the known mode of action of cephalosporins.

Thus, cephalosporin-fluoroquinolone esters act as cephalosporins and as prodrugs for fluoroquinolones (Georgopapadakou et al., 1989; Georgopapadakou & Bertasso, 1993). With Ro 23-9424, filamentation of Escherichia coli was observed after 1 h, but no nucleoid segregation, indicating binding to PBP3 but not the SOS response; after 2 h, irregularly spaced nucleoids appeared, showing that quinolone release had occurred. Ro 23-9424 was found (Beskid et al., 1989; Jones, Barry & Thornberry, 1989) to be active against a higher proportion of pathogens than either of its separate components (cefotaxime and floxacin). P. aeruginosa, enterococci and Bacteroides fragilis were not susceptible. Ro 23-9424 was active against strains of E. coli that were resistant to desacetylcefotaxime and to floxacin (Pace, Bertasso & Georgopapadakou, 1991), despite the fact that it has difficulty transversing the outer membrane (perhaps due to its high molecular weight, 732).

In carbamate and tertiary amine adducts, the 3-carboxyl of the quinolone component is free, so the intact molecules are capable of acting as quinolones. These compounds are considerably more stable than the corresponding esters, having chemical half-lives of 10 h (Ro 24-4383) and 120 h (Ro 24-8138), respectively. Their quinolone-like activity in vitro (causing filamentation and nucleoid segregation) was more pronounced than their cephalosporin-like action, but the latter was found when quinolone-resistant strains were challenged. Thus, carbamates and amines display dual activity in their intact molecules. The cefotaxime/ciprofloxacin carbamate Ro 24-4383, while the most active in vitro of the entire series investigated by Roche (Beskid et al., 1991), was inferior to the esters in models of animal infection (Christenson et al., 1990).

This is a continuing story: much ingenuity has gone into the construction and development of dual-action antibiotics, especially the cephalosporin/fluoroquinolone adducts. Will these 'quinoccephs' or 'cepoquins' come to have a useful clinical role, or are they just gimmicks, diverting resources from the search for truly new chemical entities? Only time and experience will tell, but so far the signs look favourable.

J. M. T. HAMILTON-MILLER
Department of Medical Microbiology,
Royal Free Hospital School of Medicine,
London NW3 2QG, UK

References


significant morbidity and may sometimes prove fatal. Eighteen percent of those with sepsis and septicaemia which do produce considerable patient discomfort, significantly increases the risk of wound dehiscence or incisional hernia, and prolongs both the duration and cost of hospitalization. Wound infection may also be associated with more serious complications including deep intra-abdominal sepsis and sepsicaemia which do produce significant morbidity and may sometimes prove fatal.

Postoperative wound infections affect at least 920,000 of the 23 million patients who undergo surgery each year in the USA (Wenzel, 1992). Eighteen percent of those with postoperative wound infections have disability lasting more than 6 months. The direct costs of the excess duration of hospitalization exceed $1.5 billion annually.

The principles of antibiotic prophylaxis are now well established. The choice of antibiotic depends on the pathogenic microorganisms likely to be present at the time of surgery, while the mode of administration should be chosen to achieve high tissue concentrations at this time, without provoking toxicity or precipitating the emergence of resistant organisms. In order to achieve high tissue concentrations antibiotics are usually administered parenterally.

Randomized prospective controlled trials undertaken in the late 1970s clearly demonstrated the value of prophylactic antibiotics in surgery. In most studies the wound infection rate fell from around 20% in the control group to less than 5% in the treated group. These findings have been widely accepted and absorbed into routine surgical practice (Haddock, Hansell & McArdle, 1988). Since then, a wide range of antibiotics have been evaluated but none has offered an obvious advantage. In a recent Dutch meta-analysis study, the results of 42 randomized, controlled trials with a total of 4129 patients were analysed (Meijer, Schmitz & Jeekel, 1990). In the control groups the overall wound infection rate was 15%; there was an overall advantage of 9% in favour of antibiotic prophylaxis. Comparison of wound infection rates in patients treated with 'first-' generation versus 'second-' or 'third-' generation cephalosporins (1128 patients in 11 trials), as well as single-dose versus multi-dose regimens (1226 patients in fifteen trials) did not show any significant difference. The authors, therefore, concluded that the choice of antibiotics should be governed by cost. It is for this reason that the newer fluoroquinolones, for example ciprofloxacin, are of interest.

Ciprofloxacin is a 4-fluoroquinolone antibacterial agent which acts by inhibiting DNA gyrase, a bacterial topoisomerase which is responsible for negative supercoiling of DNA within the bacterial cell (Zeiler & Grohe, 1984). It has an extended antibacterial spectrum and is highly active against both Gram-negative and Gram-positive bacteria (Felmingham et al., 1985). Previous studies have shown that when given parenterally, it is an effective prophylactic agent for patients undergoing biliary tract surgery (Kögl et al., 1989; Kujath, 1989). Of particular interest, however, is the fact that it achieves high con-

---

**Oral prophylaxis in biliary tract surgery**


Postoperative sepsis remains a major cause for concern for all surgeons undertaking gastrointestinal tract surgery. Any intra-abdominal procedure in which the integrity of the gastrointestinal tract is breached is likely to result in postoperative septic complications. Wound infection, though rarely fatal, causes considerable patient discomfort, significantly increases the risk of wound dehiscence or incisional hernia, and prolongs both the duration and cost of hospitalization. Wound infection may also be associated with more serious complications including deep intra-abdominal sepsis and sepsicaemia which do produce significant morbidity and may sometimes prove fatal.

Postoperative wound infections affect at least 920,000 of the 23 million patients who undergo surgery each year in the USA (Wenzel, 1992). Eighteen percent of those with...