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Reviews

Laboratory assessment of antibacterial activity of zwitterionic 7-methoxyimino cephalosporins

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Zwitterionic 7-methoxyimino cephalosporins (cefpirome, cefepime, cefclidin, DQ2556, FK037 and SCE2787) possess a variable substitution at C3 which contains a quarternary nitrogen. These cephalosporins display low affinities for Class I β-lactamase and rapid penetration through the outer membrane of Gram-negative bacilli, so that an increased number of periplasmic β-lactam molecules interact with PBP's per unit of time. As a consequence, the new zitterionic compounds remain active against some, but not all, ceftazidime-resistant Enterobacteriaceae producing high levels of Class I β-lactamase or Bush type 2b β-lactamases. Antipseudomonas activities are generally similar to that of ceftazidime except that cefclidin is more active. The new zwitterionic compounds, especially cefpirome and FK037, express greater antistaphylococcal potency than does ceftazidime. A variety of animal models including meningitis and endocarditis have confirmed the potential of these compounds *in-vivo*. On the basis of structural and antibacterial characteristics, the expression 'forth generation' is acceptable to describe the zwitterionic 7-methoxyimino cephalosporins.

Introduction

During recent years, cephalosporins which possess an aminothiazolyl-oxyimino moiety, such as cefotaxime, ceftriaxone and ceftazidime (the 'third generation' cephalosporins), have been used extensively. The emergence of bacterial strains which are resistant to these compounds has, however, caused some concern about their use in certain circumstances. Resistance in Gram-negative bacteria is principally associated with the production of hydrolysing enzymes, particularly Class 1 chromosomal β-lactamases and plasmid encoded, extended spectrum β-lactamases. Many bacteria which synthesise these enzymes also possess an impermeable outer membrane. A new class of 7-methoxyimino cephalosporins, which are zwitterions has been developed in order to circumvent these potential mechanisms of resistance. This new class includes cefpirome, cefepime, cefclidin (previously E1040), DQ2556 and FK037 (Figure 1) (Jones *et al.*, 1990, 1991*b*; Pucci *et al.*,

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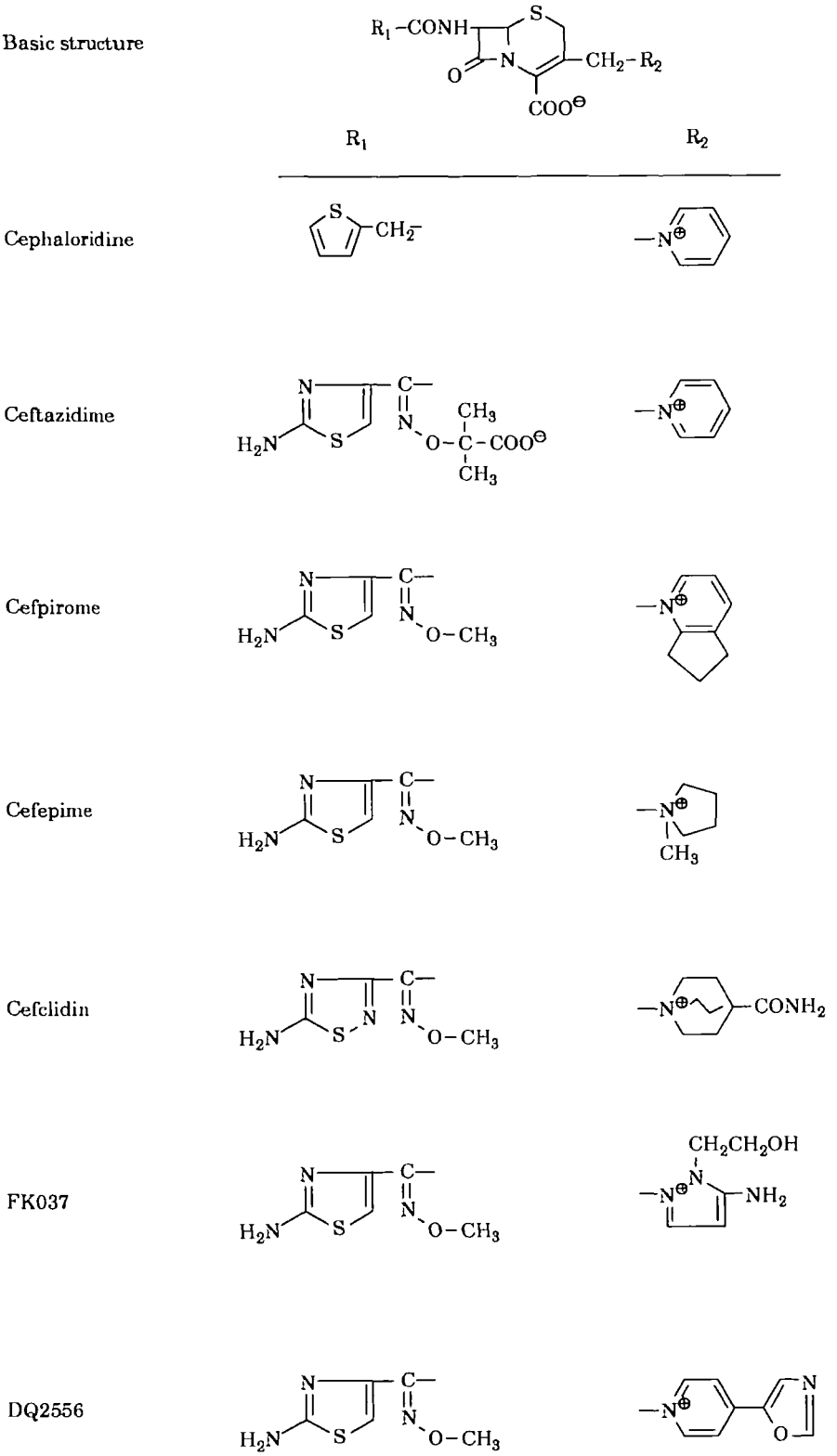

Figure Zwittenonic cephalosporins

Table I. Factors influential in β-lactam activity against a β-lactamase hyperproducer *E. cloacae* strain

Antibiotic	Outer membrane permeability parameter P (nm/s)	β-Lactamase kinetic constants K_m (μm)	V_{max} (pmol/mg/s)	Antibiotic concentration in periplasm at the MIC (nM)
Cephaloridine	960	140	1650000	>2180
Cefotaxime	5.6	0.2	55	13
Cefpirome	37	140	250	200
Cefepime	29	180	216	250

Adapted from (Bellido *et al.*, 1991).

1991). Other products, such as cefozoprane (SCE2787), belong to the same class but the available data are preliminary.

Chemical structure and mode of action

The five new compounds shown in the Figure resemble third generation cephalosporins in possessing a cephem ring with an oxyimino moiety in the β-orientation at the carbon 7 position. All the compounds possess an aminothiazolyl group at C7 as in the third generation cephalosporins, apart from cefclidin with the closely related aminothiadiazolyl system. The main structural feature of the new compounds is a variable substitution at C3 which contains a quaternary nitrogen. This group is intrinsically positively charged in both acidic and alkaline conditions, and with the negatively charged carboxyl group makes these molecules zwitterions at the pH range encountered *in vivo*.

Several factors influence the activity of a β-lactam molecule against-Gram negative bacteria: the permeability of the outer bacterial membrane; the affinity for β-lactamase, which can be estimated by determining the K_m; the activity of the β-lactamase, as described by V_{max}; and the affinity for target proteins, i.e. the penicillin binding proteins (PBP). A new class of zwitterionic 7-methoxyimino cephalosporins have recently been developed which are active against Enterobacteriaceae that are resistant to third generation cephalosporins. The enhanced activities of cefpirome, cefepime and cefclidin against Class 1 β-lactamase hyper-producers are associated with low affinities (i.e. relatively high K_m values) for these enzymes (Table I) (Kobayashi *et al.*, 1986; Phelps *et al.*, 1986; Hiraoka *et al.*, 1988; Nikaido, Liu & Rosenberg, 1990; Bellido, Pechère & Hancock, 1991). DQ2556 appears to differ slightly in this respect since its K_m values for β-lactamases produced by *Citrobacter freundii* and *Enterobacter cloacae* are only two or three-fold higher than those of ceftazidime (Fujimoto *et al.*, 1990). In addition, the new zwitterionic 7-methoxyimino cephalosporins are highly resistant to hydrolysis by Class 1 β-lactamase, even though the V_{max} values are somewhat higher than those observed with third generation cephalosporins (Table I).

Overall, the new zwitterionic cephalosporins appear more stable to Class 1 β-lactamases as a result of the low affinities of these antibiotics for the enzymes and the reduced activities of the enzymes. Although the K_m values are similar for cephaloridin and the newer zwitterionic compounds, the much lower V_{max} values

associated with the latter cephalosporins account for their superior ability to resist hydrolysis. Other studies have shown that the zwitterionic 7-methoxyimino cephalosporins are susceptible to hydrolysis by β-lactamases derived from *Bacteroides fragilis*, or by the oxyimino cephalosporinases from *Stenotrophomonas maltophila*, *Proteus vulgaris* and *Burkholderia* (*Pseudomonas*) *cepacia* (Kobayashi *et al.*, 1986; Fujimoto *et al.*, 1990).

Studies on the permeability of bacterial outer membranes to the new zwitterionic cephalosporins have provided some interesting information. Results from proteoliposome assays initially indicated that cefpirome, cefepime and cefclidin penetrate *Escherichia coli* and *E. cloacae* several times faster than cefotaxime and ceftazidime (Nikaido *et al.*, 1990). A novel HPLC-based method expanded this observation to intact cells (Bellido *et al.*, 1991). These studies demonstrated that, although the new zwitterionic cephalosporins are slightly larger molecules than the third generation cephalosporins, cefpirome and cefepime penetrated the outer membrane of *E. cloacae* between five and seven times more rapidly than cefotaxime and ceftriaxone. This is probably due to the presence of the positive moiety in these antibiotics and the selectivity of the OmpF porins for cations as compared with anions (Benz, Schmid & Hancock, 1985). Experiments involving an OmpF-deficient mutant strain have shown that a significant proportion of DQ2556 enters *E. coli* via the OmpF porin (Fujimoto *et al.*, 1990; Nikaido *et al.*, 1990).

Cephaloridine (a first generation cephalosporin) is also a dipolar ionic molecule with a quaternary nitrogen at the C3 position with no net charge (Figure). It penetrates bacterial membranes even more rapidly than the new zwitterionic cephalosporins (Bellido *et al.*, 1991). There are two possible reasons for this speed of penetration: the shape and small size of the cephaloridine molecule and the absence of a methoxyimino moiety at the C7 position, which is a structural feature that decreases the rate of penetration of monoanionic cephalosporins (Yoshimura & Nikaido, 1985). By contrast, ceftazidime, which has a positive charge at the C3 position but a net negative charge, penetrates the outer membrane much more slowly. This is probably because this anionic compound is retarded by the interior, negative, Donnan potential of the bacterium (Sen, Halleman & Nikaido, 1988).

The rapid penetration through bacterial membranes and weak affinity for β-lactamases of new zwitterionic cephalosporins mean that an increased number of periplasmic β-lactam molecules are available to interact with their target proteins per unit time. The targets for the β-lactams in Gram-negative bacteria are PBPs located in the cytoplasmic membrane. Based on estimates of the minimal periplasmic antibiotic concentration required to inhibit cell wall synthesis, cefpirome and cefepime have a 10–20-fold lower affinity for *E. cloacae* PBPs than cefotaxime and ceftriaxone (Bellido *et al.*, 1991). The major target site for the zwitterionic 7-methoxyimino cephalosporins in *E. coli* is PBP3. The IC_{50}s for the five compounds have been calculated at $\leqslant 0.5$ mg/L; moderate affinities were also calculated for PBP1 (Watanabe *et al.*, 1988; Fujimoto *et al.*, 1990; Pucci *et al.*, 1991; Piddock, Traynor & Griggs, 1992; Tanaka, Otsuki & Nishino, 1992). In addition, cefepime was shown to have a 20-fold lower IC_{50} value for *E. coli* PBP2 compared with corresponding values for cefpirome and cefclidin (Pucci *et al.*, 1991).

Cefpirome, cefepime and cefclidin induced the production of filaments in *E. coli* exposed to concentrations close to the MIC (Watanabe *et al.*, 1988; Pucci *et al.*, 1991). Cefepime also generated bleb formation along the filaments when present at a

concentration 10-fold higher than the MIC (Watanabe *et al.*, 1988; Pucci *et al.*, 1991). With respect to *Pseudomonas aeruginosa*, the affinities of cefpirome, cefepime, cefclidin and DQ2556 were high, moderate and poor for PBP3, PBP1 and PBP2, respectively (Watanabe *et al.*, 1988; Fujimoto *et al.*, 1990; Pucci *et al.*, 1991). Cefpirome had the highest affinity for PBP2, PBP1 and PBB3 isolated from methicillin-susceptible *Staphylococcus aureus* (MSSA); DQ2556 exhibited the next highest affinity (Fujimoto *et al.*, 1990). Cefpirome and FK037 bound PBP1 and PBP2 from methicillin-resistant *S. aureus* (MRSA) and showed limited affinities (FK037 more than cefpirome) for PBP2a, which correlates with their antibacterial activities (Piacentini *et al.*, 1992; Piddock *et al.*, 1992). However, PBP2a was not saturated despite exposure to 64 mg/L of cefpirome (Piddock *et al.*, 1992).

In-vitro antibacterial activities

Gram-negative organisms

The effectiveness of β-lactams against Enterobacteriacea depends on the type of β-lactamase produced by the target strain. No clear benefits, as compared with ceftazidime, have been demonstrated *in vitro* for the zwitterionic 7-methoxyimino cephalosporins with respect to strains which do not produce Class 1 chromosomally mediated β-lactamase or extended spectrum β-lactamases, such as *E. coli*, indole-negative *Proteus* spp., *Klebsiella* spp. or *Salmonella* spp. However, cefepime and FK037 were active (MIC_{90} 8 and 4 mg/L, respectively) against a collection of ceftazidime-resistant *Klebsiella pneumoniae* ($MIC_{90} > 64$ mg/L). Such data indicate the greater activity of these antibiotics compared with ceftazidime, but also suggest that the newer compounds are subject to some degree of hydrolysis. Other species, including *Enterobacter* spp. indole-positive *Proteus* spp., *Providencia* spp., *Morganella* spp., *Citrobacter* spp. and *Serratia* spp. produce low levels of Class 1 β-lactamase, which may be significantly increased by induction or mutational derepression. These high β-lactamase producers are protected against the third generation cephalosporins but are susceptible to the zwitterionic 7-methoxyimino cephalosporins ($MIC_{90} < 8$ mg/L). The increased antibacterial potency of the latter compounds is illustrated for *E. cloacae* in Table II. In addition, cefpirome generates significant β-lactamase induction less frequently than ceftazidime in *E. cloacae* (Reeves, Bywater & Holt, 1993).

A proportion of Enterobacteriacea which produce high levels of Class 1 β-lactamase, however, are resistant to the zwitterionic 7-methoxyimino cephalosporins. Cefepime can select derepressed mutants *in vitro* and *in vivo* (Piddock & Griggs, 1991; Pechere & Vladoianu, 1992), but to a lesser extent than ceftazidime. Recent multicentre studies have indicated that 6% and 10% of *E. cloacae* clinical isolates in USA and Europe respectively, are resistant to cefpirome; approximately 30% of strains in both areas were found to be ceftazidime resistant (Jones *et al.*, 1991*b*; Verbist & International Study Group, 1993). The newer compounds have not yet been compared in the same study. Overall, cefpirome, cefepime and cefclidin seem to display similar activities against Enterobacteriacea which produce Class 1 β-lactamase, while FK037 and DQ2556 appear to be slightly less active than cefpirome or cefepime (Fujimoto *et al.*, 1990; Jones *et al.*, 1991*b*; Tanaka *et al.*, 1992; Fu *et al.*, 1993; Washington *et al.*, 1993).

The zwitterionic 7-methoxyimino cephalosporins are active against strains which synthesise the Bush type 2b β-lactamases (TEM-1, TEM-2, SHV-1), but, overall, resistance to cefepime, cefpirome, cefclidin and FK037 is enhanced in strains which

Table II. MIC_{90}s (mg/L) of ceftazidime and five C3-substituted methoxyimino cephalosporins against *E. cloacae*

Number of strains tested	Ceftazidime	Cefpirome	Cefepime	DQ-2556	FK-037	Cefclidin	Reference
127	64	2					(Reeves *et al.*, 1993)
236	>16	8					(Jones *et al.*, 1991)
105	50	6.25		25			(Fujimoto *et al.*, 1990)
36	2	0.5	0.5				(King *et al.*, 1990)
27	1.56	0.2	0.1	0.39		0.2	(Tanaka *et al.*, 1992)
20	>16	0.5				1	(Jones *et al.*, 1991)
20	128	1			8		(Fu *et al.*, 1993)
322	>16		4		16		(Washington *et al.*, 1993)
46	64		8		8		(Neu *et al.*, 1993)
60	100					3.13	(Watanabe *et al.*, 1988)

Table III. MIC_{90}s (mg/L) of ceftazidime and five C3-substituted methoxyimino cephalosporins against *P. aeruginosa*

Number of strains tested	Ceftazidime	Cefpirome	Cefepime	DQ-2556	FK-037	Cefclidin	Reference
35	4	8	8				(King *et al.*, 1990)
1012	≥32	≥32					(Verbist & International Study Group, 1993)
43	3.13	6.25	3.13	12.5		0.78	(Tanaka *et al.*, 1992)
61	64	32	16		32		(Fu *et al.*, 1993)
101[a]	6.25	12.5		25			(Fujimoto *et al.*, 1990)
26[b]	100	100		>100			(Fujimoto *et al.*, 1990)
334	50	100	25			6.25	(Watanabe *et al.*, 1992)
30	4	8				1	(Jones *et al.*, 1991)
651	>16		16		32		(Washington *et al.*, 1993)
334	50	100	25			6.25	(Watanabe *et al.*, 1992)

[a]Ceftazidime susceptible isolates.
[b]Ceftazidime resistant isolates.

produce extended spectrum β-lactamases; MIC < 8 mg/L have been recorded in most cases, i.e. lower than those of ceftazidime (Jacoby & Carreras, 1990; Jones *et al.*, 1991*a*; Neu, Chin & Huang, 1993). MICs of the newer compounds for clinical isolates are higher than those determined with corresponding wild type strains, which suggest some degree of hydrolysis. More active enzymes, such as TEM-9, SHV-2, SHV-3 or SHV-4, exist, however, and these do render bacteria resistant to the zwitterionic 7-methoxyimino cephalosporins (Jacoby & Carreras, 1990).

Cefpirome is slightly less active than ceftazidime against *P. aeurginosa* (Table III). Multicentre studies have demonstrated that the proportion of isolates resistant to ceftazidime and cefpirome in Europe was 24% and 31%, respectively; corresponding data in USA were 10% and 18%, respectively (Jones *et al.*, 1991; Verbist & International Study Group, 1993). In a study of a collection of *P. aeruginosa* with well characterised resistance mechanisms (non β-lactamase-mediated, production of Class 1 β-lactamase, or plasmid-mediated β-lactamases) the MICs of cefpirome and ceftazidime were similar (Gargalianos *et al.*, 1988). Transconjugant studies demonstrated that LCR-1 and PSE-2 enzymes protected *P. aeruginosa* against cefpirome, but PSE-1, PSE-3, PSE-4, TEM-2 and OXA-6 enzymes did not (Gargalianos *et al.*, 1988). An OXA-1-type penicillinase, which hydrolysed cefclidin, was detected in a resistant *P. aeruginosa* strain (Watanabe, Hiruma & Katsu, 1992). Most ceftazidime-resistant strains of *P. aeruginosa* examined in one study were resistant to cefepine and cefpirome, but only 4% were resistant to cefclidin (Watanabe *et al.*, 1992). Cefclidin is the most active anti-*P. aeruginosa* compound listed in Table III, followed by cefepime, cefpirome, DQ2556 and FK037. A mutational OmpD deficiency in isogenic *P. aeruginosa* strains did not affect the MICs of cefepime and cefpirome (J. C. Pechère, unpublished observations).

Tobramycin acted synergistically with cefpirome, cefepime, cefclidin and ceftazidime against 22–30% of *P. aeruginosa* isolates (Chin & Neu, 1989). A majority of *Burkholderia cepacia* isolates should be considered as being resistant (MIC > 8 mg/L) to the zwitterionic 7-methoxyimino cephalosporins (and to ceftazidime), despite the existence of some susceptible strains (Watanabe *et al.*, 1988; Fujimoto *et al.*, 1990; Fu *et al.*, 1993). Most *S. maltophilia* isolates are also resistant to the new compounds but several isolates are susceptible to ceftazidime (Watanabe *et al.*, 1988; Fujimoto *et al.*, 1990; Jones *et al.*, 1991*a*; Fu *et al.*, 1993; Reeves *et al.*, 1993; Verbist & International Study Group, 1993; Washington *et al.*, 1993). Ceftazidime and the zwitterionic 7-methoxyimino cephalosporins are equally active against *Acinetobacter* spp. (Watanabe *et al.*, 1988; Fujimoto *et al.*, 1990; Jones *et al.*, 1991*a*, *b*; Tanaka *et al.*, 1992; Fu *et al.*, 1993; Washington *et al.*, 1993).

In general, *Haemophilus influenzae* isolates are equally susceptible to all of these molecules even when the bacteria produce β-lactamase (Watanabe *et al.*, 1988; Fujimoto *et al.*, 1990; Tanaka *et al.*, 1992; Fu *et al.*, 1993; Reeves *et al.*, 1993). β-lactamase-negative strains which were less susceptible to cefuroxime, however, were also less susceptible to cefepime and cefpirome (James *et al.*, 1992). *Moraxella catarrhalis* is susceptible to the new zwitterionic 7-methoxyimino cephalosporins, but ceftazidime is more active against this species (Tanaka *et al.*, 1992; Fu *et al.*, 1993; Washington *et al.*, 1993).

Gram-positive organisms

The zwitterionic 7-methoxyimino cephalosporins possess interesting anti-staphylococcal activities (Rolston & Bodey, 1986; Chin *et al.*, 1991; Fu *et al.*, 1993). According to MIC_{90}

Table IV. MIC_{90}s (mg/L) of ceftazidime and five C3-substituted methoxyimino cephalosporins against *S. aureus*

Phenotype	Number of strains tested	Ceftazidime	Cefpirome	Cefepime	DQ-2556	FK-037	Cefclidin	Reference
Methicillin-susceptible	1035	16	1					(Verbist & International Study Group, 1993)
	186	0.5	1					(Reeves *et al.*, 1993)
	43	50	6.25	50	6.25		12.5	(Tanaka *et al.*, 1992)
	36	16	4	4		2		(Fu *et al.*, 1993)
	21	8	0.5				8	(Jones *et al.*, 1991)
	121	25	1.56		1.56			(Fujimoto *et al.*, 1990)
	31	8	1	1				(King *et al.*, 1990)
	576	8		2		1		(Washington *et al.*, 1993)
	90	16		4		2		(Neu *et al.*, 1993)
	51	25					12.5	(Watanabe *et al.*, 1988)
Methicillin-resistant	23	128	32					(Reeves *et al.*, 1993)
	36	>100	50	>100	50		>100	(Tanaka *et al.*, 1992)
	91	>100	100		50			(Fujimoto *et al.*, 1990)
	47	128	16	64		16		(Fu *et al.*, 1993)
	41	>64		>64		32		(Neu *et al.*, 1993)

values, they are more active than ceftazidime against *S. aureus* (Table IV) and coagulase-negative staphylococci. Although the results of direct comparative studies are not available, the activity of zwitterionic 7-methoxyimino cephalosporins against methicillin-susceptible isolates is probably similar to that of cephalothin (the most potent anti-staphylococcal cephalosporin). MIC_{90} of 2, 8 and 16 mg/L have been obtained for cefpirome, cefepime and E1040, respectively, against MSSA and methicillin-susceptible coagulase-negative staphylococci (Rolston & Bodey, 1986; Chin *et al.*, 1991). Some MRSA are susceptible to the new zwitterionic antibiotics: 30% of MRSA strains in a USA study had MICs of cefpirome <8 mg/L (Jones *et al.*, 1991*b*). By comparison with cefpirome, FK037 selected fewer highly resistant mutant strains of methicillin-resistant staphylococci, showed a higher affinity for PBP 2a, and had lower MICs (Watanabe *et al.*, 1992). Because of the relative unreliability of MICs of methicillin-resistant staphylococci, the practical value of these findings remain to be determined by appropriate clinical studies.

Enterococcus faecalis and *Enterococcus faecium* are resistant to cephalosporins. It is possible that the wide usage of these antibiotics accounts for the recent increases in the number of enterococci observed in many hospitals. Some of the zwitterionic 7-methoxyimino cephalosporins may differ in this respect since cefpirome, FK037, cefepime and DQ2556 (but not cefclidin) exhibit limited activity against some *E. faecalis* strains (Arai & Hayasi, 1990; Fujimoto *et al.*, 1990; Jones *et al.*, 1991*b*; Tanaka *et al.*, 1992; Fu *et al.*, 1993; Verbist & International Study Group, 1993; Washington *et al.*, 1993). The MICs of these antibiotics are relatively high and the practical value of these observations when treating *E. faecalis* infections is probably limited. It is hoped, however, that the use of the newer cephalosporins will be associated with fewer enterococcal super-infections. *E. faecium* must be considered to be totally resistant to this class of antibiotics.

The zwitterionic 7-methoxyimino cephalosporins are very active against most *Streptococcus* spp., including against those alpha-haemolytic streptococci which cause endocarditis (Wilcox *et al.*, 1993). *Streptococcus pneumoniae* is also very susceptible, but limited data suggest that increasing penicillin-resistance is associated with increasing MICs of the new zwitterionic 7-methoxyimino cephalosporins (Spangler, Jacobs & Appelbaum, 1994). The limited activity of these agents against *Listeria monocytogenes*, another species which is generally resistant to older cephalosporins, probably lacks clinical significance.

Strict anaerobes

Among anaerobic bacteria, *B. fragilis* has generally been found to be resistant to the zwitterionic 7-methoxyimino cephalosporins, but the combination of cefpirome with tazobactam enhanced the antibactericidal activity of the former (Watanabe *et al.*, 1988; Fujimoto *et al.*, 1990; Jones *et al.*, 1990; Kato *et al.*, 1993; Neu *et al.*, 1993). MIC_{90}s of cefpirome and FK037 <1 mg/L have been determined for *Clostridium perfringens*, *Peptostreptococcus anaerobius* and *Peptostreptococcus asacharolyticus*, *Porphyromonas gingivalis*, *Mobiluncus* spp. and *Gardnerella vaginalis* (King, Boothman & Phillips, 1990; Limbert *et al.*, 1992; Kato *et al.*, 1993).

Table V. Penetration of antimicrobial agents into cerebrospinal fluid (CSF)*

Antibiotic	Percentage of serum concentration in CSF
Cefpirome	22
Cefepime	20
Ampicillin	12
Cefuroxime	9
Ceftriaxone	6
Cefoperazone	6
Cefotaxime	4
Penicillin	3
Cefamandole	2
Cephalothin	1

*Modified from Tauber *et al.* (1985).

Animal model studies

A number of studies have evaluated the in-vivo efficacy of new zwitterionic cephalosporins. Most of these have studied the efficacy of cefpirome or cefepime. Limited animal model data involving cefclidin, DQ2556 and FK037 have been published.

Meningitis

The penetration of C3 cephalosporins into cerebrospinal fluid (CSF) is good relative to other β-lactams. Cefpirome and cefepime treatment resulted in the highest percentage of serum concentration of antibiotic in CSF in rabbits (Taüber *et al.*, 1985, Table V). High CSF concentrations of an antimicrobial agent relative to the MIC, and especially to the MBC, of a microorganism are necessary for optimal efficacy in the treatment of meningitis. The concentration in the CSF of an antimicrobial agent should be at least 10–30-fold higher than the MBC to achieve maximum activity (Taüber *et al.*, 1985). As a result of their excellent penetration into the CSF, the new zwitterionic cephalosporins may represent a therapeutic advantage over other cephalosporins for the treatment of central nervous system infections.

Cefpirome was as effective as cefotaxime in the treatment of *S. pneumoniae* experimental meningitis (Taüber *et al.*, 1985). It was also highly effective therapy for experimental meningitis caused by *H. influenzae* or *E. coli* (Jafari *et al.*, 1991). Based on the potent in-vitro activity of the zwitterionic cephalosporins and the results of animal model studies, these drugs should be effective therapy for community-acquired meningitis (except for infections caused by *L. monocytogenes*) and for hospital-acquired meningitis due to susceptible strains of Enterobacteriacea. Additional studies are necessary to clarify the role of new zwitterionic cephalosporins for the therapy of pseudomonal meningitis.

Infective endocarditis

Cephalosporins have been studied extensively for the treatment of experimental endocarditis caused by staphylococci. First generation cephalosporins are considered to be approximately equivalent in efficacy to nafcillin or oxacillin *in vitro* against MSSA and in the therapy of experimental endocarditis caused by MSSA. Third generation

Table VI. Results of β-lactam treatment of *S. aureus* endocarditis in rabbits (Steckelberg *et al.*, 1993)

Treatment	number surviving/ number treated	number with sterile vegetations/number number surviving	Log_{10}cfu of staphylococci/g of vegetation (mean ± S.D.)	Rank performance In-vivo	In-vitro[a]
None		0/9	9.8 ± 2.2		
Ceftizoxime	15/15	0/15	7.2 ± 2.9^{b}	9	8
Cefotaxime	12/12	1/12	6.7 ± 3.7^{c}	8	6
Ceftriaxone	15/17	1/15	5.8 ± 3.0^{c}	7	7
Cefoperazone	14/17	1/14	4.9 ± 3.3^{d}	6	5
Cefuroxime	16/18	0/16	4.9 ± 3.5^{d}	5	4
Cefazolin	15/15	0/15	$4.0 \pm 2.6^{b,d}$	4	2
Cefpirome	16/17	3/16	$3.6 \pm 2.3^{b,c}$	3	3
Ceftazidime	9/12	2/9	$3.3 \pm 3.0^{b,c}$	2	9
Nafcillin	14/15	2/14	$2.3 \pm 1.6^{b,c,d}$	1	1

[a]Based on the MIC with an inoculum of 10^5 cfu/mL.
[b]$P \leq 0.05$ for ceftizoxime compared with cefazolin, cefpirome, ceftazidime or nafcillin.
[c]$P \leq 0.05$ for cefotaxime or ceftriaxone compared with cefpirome, ceftazidime or nafcillin.
[d]$P \leq 0.05$ for cefoperazone, cefuroxime or cefazolin compared with nafcillin.

cephalosporins are less active than cefazolin or nafcillin *in vitro* against MSSA (Donowitz & Mandel, 1990) but animal model studies have suggested that this activity may not correlate well with in-vivo efficacy (Baker & Fass, 1984; McColm, Ryan & Acred, 1984).

The efficacies of cefpirome, nafcillin and other cephalosporins for the treatment of MSSA experimental endocarditis were compared in rabbits (Table VI) (Steckelberg *et al.*, 1993). Cefpirome, ceftazidime, and nafcillin were equivalent in efficacy and were significantly more effective ($P < 0.05$) than cefuroxime, cefoperazone, ceftriaxone, cefotaxime, or ceftizoxime. There was no significant difference between the outcome of therapy with cefpirome or cefazolin. Previous studies have emphasised the discrepancy between the in-vitro and in-vivo activity of ceftazidime against MSSA (Baker & Fuss, 1984; McColm & Ryan, 1985). McColm & Ryan (1985) suggested that this discrepancy may be due to the good penetration of ceftazidime into cardiac vegetations. Lamb *et al.* (1993) compared the efficacy of cefepime therapy of MSSA experimental endocarditis with that of cefpirome, ceftazidime, vancomycin, and imipenem. Cefpirome was more active than cefepime at comparable dosages. Cefpirome and vancomycin were equivalent in efficacy and both were more effective than imipenem. Cefepime and ceftazidime exhibited similar efficacy. The results of these studies suggest that semisynthetic penicillins or first generation cephalosporins remain the β-lactams of choice for the treatment of serious infection caused by MSSA. Although ceftazidime has been effective in therapy of MSSA experimental endocarditis, we believe that, because of its high MIC_{90} value, this drug should not be used preferentially for the treatment of MSSA infection.

Among the new zwitterionic cephalosporins, cefpirome was active *in vivo* in a mouse thigh model of MRSA experimental infection (Eng *et al.*, 1989). Cefpirome therapy was superior to that of cephalothin and was similar in efficacy to vancomycin. In our laboratory, cefpirome and cefazolin were significantly more effective ($P < 0.05$) than no treatment of MRSA experimental endocarditis but these agents were significantly less effective ($P < 0.05$) than vancomycin (Tallan *et al.*, 1989).

Other animal model studies

Cefpirome was effective therapy for disseminated *P. aeruginosa* infection in leucopenic mice (Valdes *et al.*, 1990*a*). The addition of gentamicin or rifampin or of both antibiotics did not significantly improve survival as compared to that resulting from monotherapy with cefpirome. Additional animal model studies are necessary to clarify the role of new zwitterionic cephalosporins combined with other antimicrobial agents to prevent the emergence of resistance during therapy of Pseudomonas infection.

Klesel *et al.* (1984) reported that cefpirome was superior to cefotaxime, ceftazidime, cefuroxime or cefoperazone in the therapy of experimental enterococcal infection. In a separate study, cefpirome was more effective than ceftazidime in protecting mice with systemic mixed infection caused by *E. faecalis* and *E. coli* (Arai & Hayashi, 1990). Cefpirome was slightly more effective than therapy with ampicillin. However, the strain of *E. coli* used in the mixed infection was resistant to ampicillin in vitro.

Conclusion

Many investigators have used the term 'fourth generation' cephalosporins to describe the new zwitterionic cephalosporins (Neu, 1993; Sader & Jones, 1993). How valid is the use of this term? As shown in this review, this novel class of cephalosporins shares common structural features, a low affinity for class I β-lactamases, a rapid penetration through the outer membrane, and an extended antibacterial spectrum towards Enterobacteriaceae and some Gram-positive cocci. On this basis, and where the classification of cephalosporins into 'generations' seems acceptable the expression 'fourth generation' is appropriate for describing the zwitterionic 7-methoxyimino cephalosporins, especially if the on-going clinical studies confirm the promising in-vitro and animal data.

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