Session III
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Comparative *in vitro* Activity of Cefuroxime and its Interactions with Aminoglycoside Antibiotics

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Patients with severe sepsis in hospital, particularly those whose natural defences against infection are impaired, may die before the results of laboratory tests become available. As the commonest causes of such infections are *Escherichia coli*, *Pseudomonas æruginosa*, *Klebsiella* and *Staphylococcus aureus* and as mixed infections are not uncommon (Schimpff *et al.* 1978), therapy with a broad spectrum combination of antibiotics is frequently commenced as soon as appropriate specimens have been taken for culture.

Combinations of an aminoglycoside with 1 or 2 β-lactam antibiotics have been most widely used as initial empirical therapy (Schimpff *et al.* 1978) because of the breadth of cover such combinations offer against both Gram positive and Gram negative bacteria and because of the possibilities of synergism between the compounds. When culture and sensitivity results become known, treatment is often continued with one antibiotic alone, usually an aminoglycoside in the United Kingdom, but more usually a cephalosporin, particularly cephalothin, in other parts of the world.

The present study was undertaken to determine the activities of a number of aminoglycoside and β-lactam antibiotics, alone and in combination, against a selection of clinical isolates of Gram negative bacilli in an attempt to identify useful agents and their combinations for the treatment of severe Gram negative sepsis.

MATERIALS AND METHODS

Micro-organisms

One hundred and fifty-seven clinical isolates of the following Gram negative bacilli were tested: *Pseudomonas æruginosa* (43) *Klebsiella* (39), *Escherichia coli* (26), *Proteus mirabilis* (25), other *Proteus* (16), *Serratia liquefaciens* (12) and *Serratia marcescens* (6).

Antibiotics

The antibiotics used were sisomicin, gentamicin, tobramycin, amikacin, carbenicillin, cephalothin, cefazolin, cefuroxime and cefamandole.

Minimal Inhibitory Concentrations

MICs were determined by serial dilutions of the antibiotics in Mueller-Hinton broth in microtitre trays. The inoculum was standardized to give a final concentration of 10^4–10^5 colony forming units (CFU)/ml. The trays were incubated at 37°C for 18 h.

Test for Synergism

A modification of the chequer board method was used (Berenbaum 1977) to test for synergy between the aminoglycoside and the β-lactam compounds. The two antibiotics in each combina-

Table 1

Cumulative percentage of 43 strains of *Pseudomonas* inhibited by different antimicrobial agents

Antimicrobial agent	*MIC (μg/ml)*												
	0.063	*0.125*	*0.25*	*0.5*	*1.0*	*2.0*	*4.0*	*8.0*	*16*	*32*	*64*	*128*	*256*
Amikacin		—	9.3	27.9	72.1[a]	76.7	88.4[b]	88.4[b]	100.0				
Sisomicin	2.3	55.8	67.4	72.1	83.7	86.0	93.0[b]	95.3	95.3	95.3	100.0		
Gentamicin	2.3	41.9	60.5[a]	62.8	72.1	81.4	83.7[b]	93.0	95.3	95.3	95.3	100.0	
Tobramycin		58.1[a]	67.4	76.7	88.4	90.7	93.0[b]	95.3	100.0				
Carbenicillin		—	—	—	—	—	—	—	7.0	62.8[a]	83.7	88.4[b]	90.7
Cephalothin		—	—	—	—	—	—	—	—	—	—	2.3	2.3
Cefazolin		—	—	—	—	—	—	—	—	—	—	—	2.3
Cefuroxime		—	—	—	—	—	—	—	—	—	2.3	2.3	53.5
Cefamandole		—	—	—	—	—	—	—	—	—	—	—	2.3

[a] = median MIC; [b] = % 'clinically' susceptible

Table 2

Cumulative percentage of 39 strains of *Klebsiella* inhibited by different antimicrobial agents

Antimicrobial agent	*MIC (μg/ml)*												
	0.063	*0.125*	*0.25*	*0.5*	*1.0*	*2.0*	*4.0*	*8.0*	*16*	*32*	*64*	*128*	*256*
Amikacin	—	—	—	20.5	48.7	84.6[a]	97.4	100.0					
Sisomicin		30.8	64.1	69.2[b]	69.2	69.2	69.2[b]	74.4	92.3	97.4	100.0		
Gentamicin		12.8	56.4[a]	69.2[b]	69.2	69.2	69.2[b]	69.2	69.2	94.9	97.4	100.0	
Tobramycin		15.4	43.6	64.1[a]	71.8[b]	71.8	71.8[b]	71.8	71.8	79.5	94.9	97.4	
Carbenicillin		—	—	—	—	2.6	5.1	5.1	5.1	5.1	5.1	12.8	30.8
Cephalothin		—	—	—	—	2.6	15.4	25.5	38.5	46.2[b]	48.7	59.0[a]	64.1
Cefazolin		—	—	—	5.1	25.7	33.4	38.5	41.1	48.7[b]	51.3[a]	59.0	64.1
Cefuroxime		—	—	—	7.7	46.2	71.8[a]	87.2	92.3[b]	92.3[b]	94.9	94.9	97.4
Cefamandole		—	7.7	15.4	28.2	38.5	41.0	41.0	41.0	46.2[b]	53.8[a]	56.4	66.7

[a] = median MIC; [b] = % 'clinically' susceptible

Table 3

Cumulative percentage of 31 strains of *Proteus* inhibited by different antimicrobial agents

Antimicrobial agent	*MIC (μg/ml)*												
	0.063	*0.125*	*0.25*	*0.5*	*1.0*	*2.0*	*4.0*	*8.0*	*16*	*32*	*64*	*128*	*256*
Amikacin		—	—	—	—	9.7	48.4	83.9[ab]	96.8	96.8	100.0		
Sisomicin		3.2	6.5	12.9	45.2	64.5[a]	87.1[b]	87.1	90.3	93.5	93.5	100.0	
Gentamicin		—	6.5	16.1	38.7	67.7[a]	87.1[b]	90.3	90.3	90.3	93.5	100.0	
Tobramycin		—	3.2	9.7	32.3	61.3[a]	80.6[b]	87.1	93.5	93.5	93.5	96.8	100.0
Carbenicillin		—	9.7	9.7	22.6	41.9	77.4[a]	90.3[b]	90.3	90.3	90.3	90.3[b]	90.3
Cephalothin		—	—	—	—	6.5	67.7[a]	77.4[b]	77.4	77.4[b]	77.4	77.4	80.6
Cefazolin		—	—	—	—	6.5	41.9	77.4[ab]	77.4	77.4[b]	77.4	77.4	80.6
Cefuroxime		—	—	3.2	25.8	48.4	77.4[a]	83.9	83.9	87.1[b]	90.3	93.5	93.5
Cefamandole		—	—	6.5	22.6	38.7	74.2[a]	77.4[b]	77.4	77.4[b]	80.6	80.6	83.9

[a] = median MIC; [b] = % 'clinically' susceptible

Table 4

Cumulative percentage of 26 strains of *E. coli* inhibited by different antimicrobial agents

Antimicrobial agent	*MIC (μg/ml)*												
	0.063	*0.125*	*0.25*	*0.5*	*1.0*	*2.0*	*4.0*	*8.0*	*16*	*32*	*64*	*128*	*256*
Amikacin		—	—	—	7.7	30.8	73.1[a]	88.5	100.0				
Sisomicin		11.5	42.3	76.9[a]	96.2[b]	96.2	96.2[b]	96.2	96.2	96.2	100.0		
Gentamicin		3.8	34.6	69.2[a]	88.5	96.2[b]	96.2[b]	96.2	96.2	96.2	96.2	100.0	
Tobramycin		3.8	19.2	50.0[a]	80.8	96.2[b]	96.2[b]	96.2	96.2	96.2	96.2	96.2	
Carbenicillin		—	—	3.8	15.4	23.1	57.7[a]	73.1[b]	73.1	73.1	73.1	73.1[b]	76.9
Cephalothin		—	—	—	3.8	3.8	11.5	23.1	57.7[a]	73.1[b]	88.5	92.3	96.2
Cefazolin		—	3.8	3.8	11.5	42.3	76.9[a]	92.3[b]	92.3[b]	92.3[b]	92.3	96.2	96.2
Cefuroxime		—	3.8	7.7	15.4	50.0[a]	88.5	100.0[b]					
Cefamandole		—	30.8	46.2	57.7[a]	69.2	84.6	92.3[b]	92.3	92.3[b]	92.3	92.3	92.3

[a] = median MIC; [b] = % 'clinically' susceptible

Table 5

Cumulative percentage of 18 strains of *Serratia* inhibited by different antimicrobial agents

Antimicrobial agent	*MIC (μg/ml)*												
	0.063	*0.125*	*0.25*	*0.5*	*1.0*	*2.0*	*4.0*	*8.0*	*16*	*32*	*64*	*128*	*256*
Amikacin		—	—	11.1	50.0[a]	94.4	94.4	100.0[b]					
Sisomicin		33.3	61.1[a]	66.7	72.2	72.2	83.3[b]	83.3	88.9	100.0			
Gentamicin		22.2	61.1[a]	66.7	72.2[b]	72.2	72.2[b]	83.3	83.3	94.4	94.4	100.0	
Tobramycin		11.1	44.4	61.1[a]	66.7[b]	66.7	66.7[b]	72.2	77.8	77.8	83.3	94.4	
Carbenicillin		—	—	—	—	16.7	61.1[a]	61.1	66.7[b]	66.7	66.7	66.7[b]	66.7
Cephalothin		—	—	—	—	—	—	—	—	5.6[b]	11.1	22.2	44.4
Cefazolin		—	—	—	—	—	—	—	5.6[b]	5.6[b]	5.6	27.8	44.4
Cefuroxime		—	—	—	—	11.1	55.6[a]	61.1	72.2	77.8[b]	77.8	77.8	77.8
Cefamandole		—	—	—	—	16.7	44.4	55.6[a]	61.1[b]	61.1[b]	66.7	72.2	77.8

[a] = median MIC; [b] = % 'clinically' susceptible

Table 6

Geometric mean FIC index (ΣFIC) for sisomicin

SISOMICIN with	*E. coli 26*[a]	*Klebsiella 39*[a]	*Proteus 31*[a]	*Serratia 18*[a]	*P. ærugino 43*[a]
Carbenicillin	1.52 (20)	1.41 (12)	0.54 (28)	1.00 (11)	1.086 (42)
Cephalothin	0.53 (26)	0.45 (25)	0.64 (23)	0.46 (8)	—
Cefazolin	0.69 (24)	0.59 (28)	0.61 (25)	0.32 (8)	—
Cefuroxime	0.97 (26)	0.91 (39)	0.5 (28)	0.78 (14)	—
Cefamandole	0.92 (25)	0.81 (26)	0.32 (26)	0.58 (14)	—

[a] = total number of strains tested for MICs
Figure in brackets is number of strains susceptible to both compounds in pair at ⩽ 256 μg/ml
Significant synergy is defined as ΣFIC < 0.6

Table 7

Geometric mean FIC index (ΣFIC) for gentamicin

GENTAMICIN with	*E.coli 26*[a]	*Klebsiella 39*[a]	*Proteus 31*[a]	*Serratia 18*[a]	*P.æruginos 43*[a]
Carbenicillin	1.46 (20)	1.74 (5)	0.66 (38)	1.07 (11)	0.94 (42)
Cephalothin	0.79 (24)	0.45 (25)	0.56 (25)	0.58 (9)	—
Cefazolin	0.97 (25)	0.54 (25)	0.62 (26)	0.18 (8)	—
Cefuroxime	0.69 (26)	0191 (38)	0.47 (28)	1.00 (14)	—
Cefamandole	1.18 (25)	0.73 (24)	0.36 (26)	0.67 (14)	

[a] = total number of strains tested for MICs
Figure in brackets is number of strains susceptible to both compounds in pair at ⩽ 256 μg/ml
Significant synergy is defined as ΣFIC < 0.6

tion were mixed in the proportion of their MICs for each bacterial strain. Four MICs of each aminoglycoside were mixed with 4 MICs of each β-lactam antibiotic in Mueller-Hinton broth to give a final sum of the fractions of the inhibitory concentrations of 8 (FIC Index = ΣFIC = 8). The mixtures were serially diluted in Mueller-Hinton broth in microtitre trays to give a range of ΣFICs from 8 to 0.063. The trays were inoculated and incubated in the same way as for MIC determination. Significant synergy was arbitrarily defined as FIC Index (ΣFIC) < 0.6 which is equivalent to reducing the concentration of each agent in the mixture to about 25% of its MIC for the particular organism under test. Significant antagonism was defined as FIC Index > 1.5.

Results

The MICs of the aminoglycosides and β-lactams for *P. æruginosa* are shown in Table 1. The median MIC (concentration inhibiting 50% of strains; MIC_{50}) of amikacin was 1.0 mg/l and 88% of strains were susceptible at the clinically useful level of 8 mg/l. The median MICs of sisomicin and tobramycin were 0.125 mg/l and 93% of strains were susceptible to 4 mg/l. The median MIC of gentamicin was 0.25 mg/l and 84% of strains were susceptible at 4 mg/l. Of the β-lactams, only carbenicillin has useful activity against *P. æruginosa* with a median MIC of 32 mg/l and 88% of strains sensitive to 128 mg/l.

Table 2 shows the results obtained with *Klebsiella*. All were susceptible to amikacin at 8 mg/l; 69% to sisomicin and gentamicin, and 72% to tobramycin at 4 mg/l. With the cephalosporins at 32 mg/l, 46% were susceptible to cephalothin and cefamandole, 49% to cefazolin and 92% to cefuroxime.

Tables 3, 4 and 5 similarly show the MIC results obtained against *Proteus*, *E. coli* and *Serratia* respectively.

Tables 6–9 summarize the results of the synergy experiments. Amikacin is the aminoglycoside which synergizes best with the β-lactam compounds and it is the only one which does not produce an antagonistic mixture with carbenicillin. Cefuroxime combinations with all 4 aminoglycosides are either additive or synergic against *E. coli*, *Klebsiella* and *Serratia*. Against *Proteus*, cefuroxime shows significant synergy with the aminoglycosides and in combination with amikacin the FIC Index is reduced to 0.3. Overall, the cephalosporins and aminoglycosides interact well against the Gram negatives, and none of the combinations is antagonistic.

A number of our isolates of *Klebsiella* were multi-resistant, being sensitive only to amikacin, netilmicin and cefuroxime (Table 10). The other aminoglycosides and β-lactams had no useful activity against these strains. The MICs of cephradine and cefoxitin were also measured and were found to be 64 and 32 mg/l respectively. Cefuroxime was the only cephalosporin to show a

Table 8

Geometric mean FIC index (ΣFIC) for tobramycin

TOBRAMYCIN with	*E. coli* 26[a]	*Klebsiella* 39[a]	*Proteus* 31[a]	*Serratia* 18[a]	*P. æruginosa* 43[a]
Carbenicillin	1.46 (20)	2.30 (5)	0.62 (28)	1.28 (11)	1.16 (42)
Cephalothin	1.09 (25)	0.57 (25)	0.54 (26)	0.58 (9)	—
Cefazolin	1.03 (25)	0.62 (26)	0.69 (26)	0.25 (8)	—
Cefuroxime	0.97 (26)	0.90 (38)	0.48 (29)	1.05 (14)	—
Cefamandole	0.56 (25)	0.86 (24)	0.39 (26)	0.78 (14)	—

[a] = total number of strains tested for MICs
Figure in brackets is number of strains susceptible to both compounds in pair at < 256 μg/ml
Significant synergy is defined as ΣFIC ≤ 0.6

Table 9

Geometric mean FIC index (ΣFIC) for amikacin

AMIKACIN with	*E. coli* 26[a]	*Klebsiella* 39[a]	*Proteus* 31[a]	*Serratia* 18[a]	*P. æruginosa* 43[a]
Carbenicillin	0.62 (20)	0.57 (5)	0.38 (27)	0.78 (11)	0.73 (42)
Cephalothin	0.53 (25)	0.33 (25)	0.52 (22)	0.46 (9)	—
Cefazolin	0.46 (24)	0.42 (25)	0.73 (22)	0.27 (7)	—
Cefuroxime	0.65 (26)	0.61 (38)	0.30 (28)	0.42 (14)	—
Cefamandole	0.46 (25)	0.52 (23)	0.33 (26)	0.61 (14)	—

[a] = total number of strains tested for MICs
Figure in brackets is number of strains susceptible to both compounds in pair at < 256 μg/ml
Significant synergy is defined as ΣFIC < 0.6

Table 10

'Resistant' *Klebsiella*

	MIC (mg/litre)
Amikacin	0.5
Sisomicin	16
Netilmicin	0.125
Gentamicin	32
Tobramycin	64
Carbenicillin	> 256
Cephalothin	> 256
Cefazolin	> 256
Cefuroxime	8
Cefamandole	> 256
Cephradine	64
Cefoxitin	32

high degree of resistance to the β-lactamases of these strains and details of these will be reported elsewhere.

Discussion

Cefuroxime is clearly the most active of the cephalosporins against the strains of enterobacteria examined. Cefazolin and cefamandole have an activity similar to, and only slightly better than, cephalothin. Carbenicillin is the only β-lactam with useful activity against *P. æruginosa* and it is also extremely active against *Proteus*.

Synergy could be demonstrated between cefuroxime and the 4 aminoglycoside antibiotics but there was considerable variation between species and even within species for any particular combination. Amikacin was the most synergistic of the aminoglycosides with all the β-lactam antibiotics and the only one to synergise consistently with carbenicillin. With the exception of carbenicillin, therefore, it would appear that the degree of synergy which a β-lactam plus aminoglycoside combination exhibits is determined more by the aminoglycoside than the β-lactam in the combination.

It would seem reasonable, nevertheless, that one should use data on synergy such as in Tables 6–9 only as a guide to the probability of a particular combination being synergistic against a particular microorganism, and as a pointer to which combinations to test in clinical situations where combination chemotherapy is indicated.

Synergy data cannot be used without taking account of the MICs of the agents in the combination against the infecting microorganism, as highly active compounds in additive combination (ΣFIC=1) can have a better *real* activity, at least *in vitro*, than synergistic combinations of less active compounds. *In vivo*, the levels of each antibiotic which can be achieved in blood and at the site of infection are obviously of paramount importance and serum antibacterial activity (measured by back titration) against the offending pathogen gives a good indication of the interaction of all these factors. However, knowing the serum levels (L) of the antibacterial agents (A and B) that one can expect and their MICs and FIC index (ΣFIC) against the infecting organism, the expected degree of serum anti-

Table 11

Expected serum antibacterial activity (MID)

Antibiotic agent	*Dose (mg/kg)*	*1 h level (mg/litre)*	*Expected MID*[a]			
			E. coli	*Proteus*	*Klebsiella*	*Serratia*
Amikacin	7.5	20	5	2.5	10	20
Sisomicin	1.0	4	8	2	16	16
Gentamicin	2.0	8	16	4	32	32
Tobramycin	2.0	8	8	4	16	16
Carbenicillin	15.0	25	6	6	—	6
Cephalothin	15.0	15	1	4	0.1	—
Cephalothin	45.0	150	9	37.5	1	—
Cefazolin	15.0	75	19	9	1	—
Cefuroxime	15.0	40	20	10	10	10
Cefamandole	15.0	25	25	6	0.4	3
Amikacin + Cefuroxime	7.5 +15.0	—	38.5	42	33	71
Gentamicin + Cefuroxime	2.0 +15.0		52	30	46	42

[a] MID $\geqslant$ 8 is desirable

bacterial activity (maximum inhibitory dilution; MID) can easily be predicted from the equation:

$$\text{MID} = \frac{\frac{L_A}{MIC_A} + \frac{L_B}{MIC_B}}{\Sigma FIC}$$

Thus, combinations of cefuroxime with amikacin and the other aminoglycosides can be expected to produce excellent serum antibacterial activity (Table 11), well in excess of the 8-fold dilution shown by Klastersky and his co-workers (1976) to be associated with good clinical response.

The expected MIDs with the compounds individually (L_A/MIC_A) are particularly interesting (Table 11). Gentamicin, sisomicin and tobramycin can be expected to produce better MIDs than amikacin. Cefuroxime can be expected to produce much better MIDs than cephalothin after the same 1 g dose, and against *E. coli* and *Klebsiella* better activity should be obtained with 1 g of cefuroxime than with 3 g of cephalothin (45 mg/kg). Similarly cefuroxime should be more active than cefamandole or cefazolin against *Proteus*, *Klebsiella* and *Serratia liquefaciens*. The expected MIDs for cefuroxime compare very favourably with those for the aminoglycosides and in this respect cefuroxime is more active than any of them against *E. coli* and *Proteus*.

Against the multi-resistant *Klebsiella* (Table 10) only amikacin, netilmicin and cefuroxime had any marked activity, although it was also sensitive to cefoxitin at 32 mg/l.

It would seem reasonable therefore to consider the use of cefuroxime in the treatment of many infections now treated with gentamicin, even if the aim is only to eliminate the risk of VIII cranial nerve toxicity. In such situations, the choice of cefuroxime would certainly be more logical than that of cephalothin, which is probably still the most widely used parenteral antibiotic in hospital practice, or cefazolin.

However, only extensive clinical experience can determine the real place of cefuroxime in our therapeutic armamentarium, whether cefuroxime in combination with aminoglycosides will be less toxic than the aminoglycoside plus cephalosporin combinations commonly used at present and whether cefuroxime alone will deal adequately with the majority of enterobacterial infections.

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