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Effect of Temocillin in Combination with Other β-Lactam Antibiotics

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The antibacterial interactions of temocillin with other β-lactams were tested by checkerboard combination in Mueller-Hinton agar against 146 strains of members of the family *Enterobacteriaceae*, 35 *Pseudomonas aeruginosa* strains, and 35 *Staphylococcus aureus* strains. Most combinations showed a moderate degree of synergism. In only one *Klebsiella* strain was minor antagonism observed between temocillin and ampicillin.

Temocillin is a new β-lactamase-stable penicillin with good activity against gram-negative bacteria and a spectrum largely limited to members of the family *Enterobacteriaceae*, *Haemophilus influenzae*, and *Neisseria* spp. (3, 7, 8). Temocillin is protected against β-lactamases by a 6-α-methoxy group in the nucleus and inhibits strains resistant to ampicillin, ticarcillin, cefazolin, cefuroxime, and cefoxitin (3, 7, 8). Temocillin has been shown to be very stable in vitro against both chromosomal- and plasmid-mediated β-lactamases (3, 7). Jules and Neu (3), however, reported antagonism between temocillin and cefazolin or ticarcillin against different bacterial species. Antagonism between β-lactam antibiotics, due to induction of β-lactamases, has been observed mainly between cefoxitin and third generation cephalosporins and

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TABLE 1. Effect of temocillin in combination with other β-lactams based on the FIC_{min}

Drug combination[a] and species	Total no. of isolates	No. of isolates showing indicated reaction[b]				
		A	I	PS	S	ND
TIC + TEMO						
P. aeruginosa	35		8	9	7	11
S. marcescens	16			12	4	
Enterobacter sp.	20		5	12	3	
P. vulgaris	10		7	2	1	
M. morganii	9		3	5	1	
Providencia spp.	17		10	4	3	
AMP + TEMO						
E. coli	21		10	5	6	
P. mirabilis	20		7	13		
Klebsiella sp.	33	1	15	13	4	
Flucloxacillin + TEMO						
S. aureus[c]	24		1	19	4	
S. aureus[d]	11		1	2	2	6
CTX + TEMO						
P. aeruginosa	35		8	15	12	
S. marcescens	16		10	2	4	
Enterobacter sp.	20		6	7	7	
P. vulgaris	10		7	1	2	
M. morganii	9		4	4	1	
Providencia spp.	17		10	5	2	
CZL + TEMO						
E. coli	21			8	13	
P. mirabilis	20		5	15		
Klebsiella sp.	33		13	14	6	
S. aureus[c]	24			6	18	
S. aureus[d]	11		1	2	2	6

[a] TIC, Ticarcillin; TEMO, temocillin; AMP, ampicillin; CTX, cefotaxime; CZL, cefazolin.
[b] A, Antagonism; I, indifference; PS, partial synergism; S, synergism; ND, not determinable.
[c] Methicillin-susceptible strains.
[d] Methicillin-resistant strains.

TABLE 2. Effect of temocillin in combination with other β-lactams based on the FIC_{max}

Drug combination[a] and species	Total no. of isolates	No. of isolates showing indicated reaction[b]				
		A	I	PS	S	ND
TIC + TEMO						
P. aeruginosa	35	2	20	1	1	11
S. marcescens	16	1	14	1		
Enterobacter sp.	20	2	13	4	1	
P. vulgaris	10		10			
M. morganii	9		8		1	
Providencia spp.	17		15	1	1	
AMP + TEMO						
E. coli	21		16	4	1	
P. mirabilis	20		11	9		
Klebsiella sp.	33	2	24	6	1	
Flucloxacillin + TEMO						
S. aureus[c]	24		3	18	3	
S. aureus[d]	11		3	1	1	6
CTX + TEMO						
P. aeruginosa	35		23	12		
S. marcescens	16		12	2	2	
Enterobacter sp.	20	2	10	2	6	
P. vulgaris	10		9		1	
M. morganii	9	4	4	1		
Providencia spp.	17	2	12	2	1	
CZL + TEMO						
E. coli	21	1	9	7	4	
P. mirabilis	20	4	13	3		
Klebsiella sp.	33	1	16	14	2	
S. aureus[c]	20		9	13	2	
S. aureus[d]	11		2	3		6

[a] TIC, Ticarcillin; TEMO, temocillin; AMP, ampicillin; CTX, cefotaxime; CZL, cefazolin.
[b] A, Antagonism; I, indifference; PS, partial synergism; S, synergism; ND, not determinable.
[c] Methicillin-sensitive strains.
[d] Methicillin-resistant strains.

between cefoxitin and ureidopenicillins (1, 4–6). A rather high frequency of antagonism (10 of 45 strains) between cefotaxime and cefoperazone has been reported by Fu and Neu (2). Occasional antagonism involving moxalactam, ceftizoxime, and carbenicillin has also been observed (4, 5).

In view of the limited spectrum of temocillin, a combination of this drug with other β-lactams is very likely in the empiric therapy of severe infections, and possible antagonism would be a serious drawback. Therefore, we examined more extensively the effect of temocillin on the activity of other β-lactam antibiotics, and vice versa, against different strains of species of members of the family *Enterobacteriaceae* and against *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains.

The antibiotics were chosen in view of their potential activity against each bacterial species and were tested alone and in combination with temocillin. Temocillin, ampicillin, and cefazolin were tested against *Escherichia coli, Proteus mirabilis*, and *Klebsiella* sp.; temocillin, ticarcillin, and cefotaxime were tested against *Serratia marcescens, Enterobacter* sp., *Proteus vulgaris, Morganella morganii, Providencia stuartii, Providencia rettgeri*, and *P. aeruginosa*; and temocillin, flucloxacillin, and cefazolin were tested against *S. aureus*. Within each species a selection was made for strains with different degrees of susceptibility or resistance to the individual drugs to be tested in combination with temocillin. The influence of temocillin on the minimal inhibitory concentration (MIC) of other β-lactam antibiotics, and vice versa, was determined by the agar dilution method in Mueller-Hinton agar, with an inoculum of ca. 10^5 CFU per

TABLE 3. Antagonism between temocillin and other β-lactams

Combination drug[a] and isolate	MIC (μg/ml) of		Results based on FIC_{max}				Results based on FIC_{min}			
	Combination drug	TEMO[b]	MIC (μg/ml) of combination drug/TEMO	FIC_{max}		ΣFIC	MIC (μg/ml) of combination drug/TEMO	FIC_{min}		ΣFIC
				Combination drug	TEMO			Combination drug	TEMO	
TIC										
S. marcescens 82/18	2	8	4/2	2	0.25	2.250	0.5/4	0.25	0.5	0.750
Enterobacter sp.										
82/17	64	16	128/1	2	0.062	2.062	0.25/8	0.004	0.5	0.504
79/48	64	8	128/1	2	0.125	2.125	32/4	0.5	0.5	1.000
P. aeruginosa										
77/01	32	256	64/32	2	0.125	2.125	16/128	0.5	0.5	1.000
77/31	512	>256	1,024/32	2	0.062	2.062	512/64	1	0.125	1.125
CTX										
M. morganii										
82/11	2	2	0.004/4	0.002	2	2.002	0.12/1	0.062	0.5	0.562
82/14	0.12	2	0.004/4	0.031	2	2.031	0.015/1	0.125	0.5	0.625
82/23	0.06	1	0.004/2	0.062	2	2.062	0.015/1	0.25	1	1.250
82/26	2	2	0.004/4	0.002	2	2.002	0.5/1	0.25	0.5	0.750
P. stuartii										
82/6	2	2	0.004/4	0.002	2	2.002	0.5/1	0.25	0.5	0.750
82/8	0.03	1	0.004/2	0.125	2	2.125	0.015/1	0.5	0.5	1.000
Enterobacter sp.										
79/06	32	8	64/2	2	0.25	2.250	32/1	1	0.125	1.125
79/48	16	8	32/1	2	0.125	2.125	4/4	0.25	0.5	0.750
CZL										
Klebsiella sp. 82/37	8	2	0.25/4	0.031	2	2.031	0.5/2	0.062	1	1.062
P. mirabilis										
82/03	8	1	0.25/2	0.031	2	2.031	0.25/1	0.031	1	1.031
82/04	8	1	0.25/2	0.031	2	2.031	0.25/1	0.031	1	1.031
82/06	8	2	0.25/4	0.031	2	2.031	2/1	0.25	0.5	0.750
82/20	8	1	0.25/2	0.031	2	2.031	0.5/1	0.062	1	1.062
E. coli 80/08	64	8	0.25/16	0.004	2	2.004	16/2	0.25	0.25	0.500
AMP										
Klebsiella sp.										
82/15	>1,024	2	512/4	0.25	2	2.250	4/4	0.002	2	2.002
82/38	>1,024	2	4/4	0.002	2	2.002	32/2	0.016	2	1.016

[a] Drug used in combination with temocillin. TIC, Ticarcillin; CTX, cefotaxime; CZL, cefazolin; AMP, ampicillin.
[b] TEMO, Temocillin.

spot delivered by a multipoint inoculator.

The concentrations of the antibiotics alone and in the checkerboard combination were chosen in a range able to inhibit the most susceptible as well as the very resistant isolates. Against members of the *Enterobacteriaceae*, temocillin was used in a range of 1 to 64 μg/ml, and against *P. aeruginosa* and *S. aureus*, it was used in the range of 32 to 256 μg/ml. Cefotaxime was tested between 0.004 to 256 μg/ml against members of the *Enterobacteriaceae* and between 2 to 1,024 μg/ml against *P. aeruginosa*. Against members of the *Enterobacteriaceae*, the test range of ampicillin was 0.25 to 1,024 μg/ml, and that of cefazolin was 0.25 to 512 μg/ml. For tests against *S. aureus* the range for flucloxacillin and cefazolin was 0.06 to 64 μg/ml.

The fractional inhibition concentration (FIC = MIC in the combination/MIC alone) for each component and the sum of the FICs (ΣFIC) of each checkerboard combination was calculated, resulting in a maximal sum of FICs (FIC_{max}) and a minimal sum of FICs (FIC_{min}) for every combination against each isolate. Synergism was defined as $\Sigma FIC \leq 0.5$, partial synergism was defined as $0.5 < \Sigma FIC < 1$, indifference was defined as $\Sigma FIC = 1$ to 2, and antagonism was defined as $\Sigma FIC > 2$. With some isolates of *S. aureus* and *P. aeruginosa*, the MIC of one or both antibiotics in the combination was higher than the highest concentration tested, hence the ΣFIC was not determinable.

Synergism between two drugs was more pronounced when results were based on the FIC_{min} (Table 1). Most strains showed either partial synergism (41%) or synergism (24%) with any combination of temocillin and another β-lactam, 30% of the strains were indifferent, 5% were not determinable, and only one *Klebsiella* sp. strain showed antagonism. When the FIC_{max} was used, the results were somewhat different (Table 2). With all combinations tested, 59% of the strains were indifferent, 24% showed partial synergism, 7% showed synergism, and only 5% showed antagonism.

The complete results of the 21 strains demonstrating some degree of antagonism are shown in Table 3. In all instances the increase of the MIC in the combination was limited to one of the antibiotics at one concentration. Against 14 of the 21 strains the MIC of temocillin was increased when combined with either cefotaxime, cefazolin, or ampicillin. Against a further five strains, the MIC of ticarcillin was increased, and two *Enterobacter* sp. strains, which already had a low susceptibility to cefotaxime, showed a twofold increase of the MIC. However, against all of these strains, with the exception of *Klebsiella* sp. strain 82/15, the ΣFIC of the other checkerboard combinations showed either indifference or partial synergism.

In contrast to the observation of Jules and Neu (3), who found antagonism in 9 of 10 *S. aureus* strains with the combination of temocillin and cefazolin, we observed synergism in 18 of the 24 methicillin-susceptible strains and partial synergism in the remaining 6 strains. The mean FIC_{min} of these 24 strains was 0.425 (range 0.25 to 0.625), and the mean FIC_{min} of the 5 determinable methicillin-resistant isolates was 0.600.

From the results presented, it is apparent that temocillin is not a major inducer of β-lactamases, and that it has even less tendency to produce antagonistic effects than the other β-lactams used in the combinations. Indeed, in the relatively few instances in which some minor antagonism was observed, taking the FIC_{max} as a criterion, it was the MIC of temocillin that was adversely influenced by the presence of the other β-lactam in two-thirds of the cases. Furthermore most of the observed effects point to a moderate degree of synergism.

It is difficult to explain the disagreement between our results and those published by Jules and Neu (3), as we used the same checkerboard technique in agar. However, it was not clear from their paper at which concentrations the antagonism was observed nor on which criteria it was based.

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