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Cephapirin: In Vitro Antibacterial Spectrum

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Cephapirin, a new semisynthetic cephalosporin derivative, was found to have an antibacterial spectrum similar to that of cephalothin. *Staphylococcus aureus* was inhibited by cephapirin concentrations of 0.09 to 12.5 μg/ml. *S. epidermidis*, *S. viridans*, *S. pyogenes*, and *Diplococcus pneumonia* isolates were inhibited by less than 1 μg/ml. The *Enterococcus* required a concentration of 25 μg of antibiotic per ml for inhibition. Approximately 65% of *Escherichia coli*, and all *Klebsiella*, indole-negative *Proteus*, and *Salmonella* strains tested were inhibited by the drug. *Serratia*, *Pseudomonas*, indole-positive *Proteus*, and *Erwinia* strains were highly resistant. Inoculum size was not an important factor in determining the level of sensitivity of *S. aureus* to cephapirin. The antibiotic does not appear to be significantly bound to serum protein. In vitro development of resistance to the drug was demonstrated with two isolates of *S. aureus*.

Cephapirin {7-[D-(4-pyridylthio)-acetamide]-cephalosporanic acid} is a new semisynthetic cephalosporin derivative with an antibacterial spectrum similar to that of cephalothin. Preliminary findings suggest that this agent may cause less phlebitis than cephalothin during intravenous administration. (Basic Data Brochure, Cephapirin sodium (BL-P1322), Department of Medical Research, Bristol Laboratories, Syracuse, N. Y., 1970). The purpose of this study was to determine the in vitro antibacterial activity of the drug in broth and in serum, and to study the development of resistance after repeated exposure of bacteria to the drug.

MATERIALS AND METHODS

Freshly isolated strains of bacteria from clinical specimens were obtained from the diagnostic bacteriology laboratory of The Mount Sinai Hospital. A 0.5-ml amount of a 10^{-4} dilution of overnight growth of the organisms was used as an inoculum. Trypticase soy broth (BBL) was used as growth medium for all organisms except *Diplococcus pneumonia* for which Todd-Hewitt broth was used.

The inoculum size varied from 10^4 to 10^5 organisms per ml. Cephapirin was diluted in distilled water and sterilized by passage through a 0.45-μm Nalgene filter. Cephapirin was freshly prepared for each experiment or was stored at −70 C and used within 1 week of preparation.

Minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of drug were determined with the standard twofold dilution method by using Trypticase soy broth (BBL). The MIC was defined as the lowest concentration of antibiotic in which no organisms could be seen after incubation for 18 hr at 37 C. The MBC (except for staphylococci) was defined as the lowest concentration of antibiotic from which wire loop subcultures onto agar plates showed no growth at 18 hr. The MBC for staphylococci was defined as <10 colonies per streak on the 18-hr agar plates (*see* below). After inoculation, all cultures were incubated at 37 C and examined for visible growth at 18 hr. Subcultures of clear tubes were then made on Trypticase soy agar plates with the use of a wire loop and were incubated overnight at 37 for determination of MBC.

The effect of inoculum size on the MIC was determined by inoculating 100-fold dilutions of the overnight growth of the organisms. The number of organisms was determined by colony counting with the standard pour-plate method.

Eight strains of *Staphylococcus aureus* were used to study the in vitro development of resistance after repeated exposure to the drug. For 16 days, subcultures of the cloudy tube with the highest concentration of antibiotic were incubated overnight and were again inoculated into another series of antibiotic-containing tubes.

The effect of serum on antibacterial activity of the drug was determined by diluting both the cephapirin and the bacteria in 100% human serum.

When subculturing the clear tubes with wire loops onto agar plates to determine the MBC for staphylococci, a small number of organisms was always found to be present in the subcultures. If these colonies were picked, allowed to grow overnight, and retested against cephapirin, they did not maintain their resistance. Furthermore, when the same strain was tested more than once, the occurrence of these relatively resistant colonies was not constant. This phenomenon was observed in 76% of the Pen-R and MR isolates of *S. aureus*, but in only 47% of the Pen-S strains and 40% of *S. epidermidis*. (*S. aureus* is defined as Pen-S

when inhibited by a 1-μg disc, as Pen-R when not inhibited by a 10-μg disc, and as Pen MR when inhibited by a 10-μg disc but not by the 1-μg disc.) Isolates that contained these relatively resistant colonies were plated on cephapirin-containing agar plates to look for the possibility of small colony variants as described by Bulger (2). No significant difference in colony morphology was observed after 48 hr of incubation.

Since the original inoculum varied from 10^4 to 10^5, the finding of 10 colonies or less would suggest that 90 to 99% of the introduced organisms were killed. We therefore define the MBC for *Staphylococcus* as 90 to 99% killing of the inoculum.

RESULTS

Bacterial susceptibility. All gram-positive organisms tested, except for two strains each of *S. aureus* and *S. epidermidis* and all enterococci, were sensitive to 3.1 μg or less of cephapirin per ml (Table 1). Ninety-seven per cent of all strains of *S. aureus*, whether Pen-S or Pen-R, were inhibited by 1.5 μg or less of cephapirin per ml. The MBC for staphylococci was 3.1 μg/ml for 91% of strains. All strains of *Streptococcus viridans*, *S. pyogenes*, and *D. pneumonia* were killed by <1 μg of cephapirin per ml. All but one strain of

TABLE 1. *In vitro activity of cephapirin against gram-positive cocci*

Cephapirin (μg/ml)	Pen-S *Staphylococcus aureus*[a]		Pen-R *S. aureus*		Pen-MR *S. aureus*		*S. epidermidis*		*Streptococcus viridans*		*S. pyogenes*		*Diplococcus pneumoniae*		*Enterococcus*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<0.045	0[b]		0				1				6	3	6			
0.09	2		0		1	0	4	1	4	2	3	5	1	7		
0.18	12	4	11	6	2	1	2	3	1	1	2	2	0			
0.37	3	11	12	14	3	5	1	3	3	4	3	3	1	1		
0.75	2	2	3	2	2	1		1		1	1	2				
1.5	2	2	1	2		1										
3.1	0	1	1	1												
6.2		1													1	
12.5			1	3												1
25					1										11	5
50				2												
100							1	1								6
>100						1										
Total no. tested	21		29		9		8		8		15		8		12	

[a] MIC, minimal inhibitory concentration; MBC, minimal bactericidal concentration.
[b] Number of strains susceptible at that concentration.

TABLE 2. *In vitro activity of cephapirin against gram-negative bacilli*

Cephapirin (μg/ml)	*Escherichia coli*		*Klebsiella*		*Aerobacter aerogenes*		*Proteus* (indole negative)		*Proteus* (indole positive)		*Serratia*		*Salmonella*		*Pseudomonas*		*Erwinia*	
	MIC[a]	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
3.1	0[b]		6	4									2					
6.2	8	3	6	5			3	1					2					
12.5	8	6	2	4			7	2						3			2	
25	4	6		1	1			6						1			1	1
50	5	3			0			1									1	2
100	1	4			1													1
>100	1	5			3	5			2	2	3	3	1	1	3	3	2	2
Total no. tested	27		14		5		10		2		3		5		3		6	

[a] MIC, minimal inhibitory concentration; MBC, minimal bactericidal concentration.
[b] Number of strains susceptible at that concentration.

Enterococcus required >25 μg of cephapirin per ml for inhibition and killing.

Gram-negative bacteria were in general less susceptible to the drug (Table 2); 65% of *Escherichia coli* were inhibited by 25 μg/ml or less. The MBC for *E. coli* was <25 μg/ml in 50% of the strains tested. All *Klebsiella* species tested were sensitive to <12.5 μg of the drug. *Aerobacter aerogenes* was more resistant, requiring 25 μg or more for inhibition. Fifty per cent of strains tested required >100 μg/ml for inhibition. Indole-negative *Proteus* was inhibited and killed by 12.5 μg/ml or less, whereas the two strains of indole-positive *Proteus* were not inhibited by 100 μg of cephapirin per ml. Four of five strains of *Salmonella* were inhibited by 6.2 μg or less of cephapirin per ml. *Pseudomonas*, *Serratia*, and *Erwinia* were uniformly resistant to the drug.

Effect of inoculum size. The effect of inoculum size on MIC and MBC values for five strains of *E. coli* and six strains of *S. aureus* are shown in Tables 3 and 4. With the gram-negative organisms tested, an increase in inoculum size of 10^4 increased the MIC from 6.2 to >100 μg/ml. The MBC also increased correspondingly. In contrast, a 10^4 increase in inoculum size of *S. aureus* only increased the MIC twofold from a modal value of 0.18 to 0.37 μg/ml.

TABLE 3. *Effect of inoculum size on minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of Escherichia coli*

Strain no.	Inoculum size					
	10^3		10^5		10^7	
	MIC	MBC	MIC	MBC	MIC	MBC
1	6.2[a]	12.5	12.5	25	>100	>100
2	6.2	6.2	6.2	6.2	100	>100
3	6.2	6.2	6.2	12.5	100	>100
4	6.2	12.5	12.5	12.5	100	>100
5	6.2	6.2	12.5	12.5	>100	>100

[a] Values expressed as micrograms per milliliter.

TABLE 4. *Effect of inoculum size on minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of Staphylococcus aureus*

Strain no.	Inoculum size					
	10^3		10^5		10^7	
	MIC	MBC	MIC	MBC	MIC	MBC
1	0.37[a]	0.37	0.37	1.5	3.1	>6.2
2	0.18	0.37	0.18	0.37	0.37	>6.2
3	0.37	0.37	0.37	0.37	0.37	0.37
4	0.18	0.37	0.37	6.2	0.37	>6.2
5	0.18	0.18	0.18	0.18	0.37	3.1
6	0.18	0.18	0.37	>6.2	1.5	>6.2

[a] Values expressed as micrograms per milliliter.

TABLE 5. *Comparison of the effect of 100% human serum on minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of Staphylococcus aureus*

Strain	Cephapirin (μg/ml)			
	MIC		MBC	
	Serum	TSB[a]	Serum	TSB
1	0.18	0.18	0.37	0.18
2	0.18	0.37	0.75	0.37
3	0.18	0.37	3.0	0.37
4	0.37	0.37	3.0	0.37
5	0.37	0.18	0.75	0.37

[a] Trypticase soy broth.

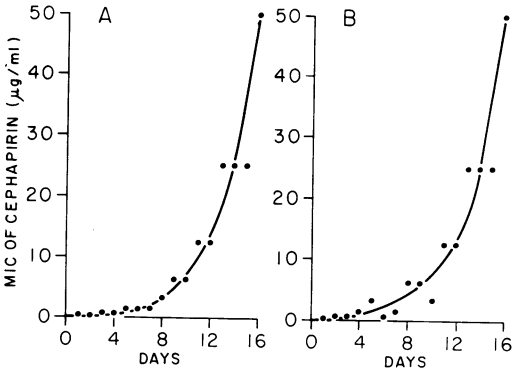

FIG. 1. *Changes in MIC values of two strains of S. aureus (1A and B) during daily subculture to increasing concentrations of cephapirin. For 16 days, the cloudy tubes with the highest antibiotic concentrations were subcultured and after overnight incubation were inoculated into another series of antibiotic-containing tubes.*

Effect of human serum. A comparison of the MIC and MBC values in human serum and in Trypticase soy broth for five strains of *S. aureus* is shown in Table 5. There was only a one tube dilution variance in all MIC values when differences occurred. This is within the expected error of the method used. In three of five strains, the MBC was also within one dilution in serum or Trypticase soy broth. However, in two strains the MBC was increased threefold using serum as the diluent.

Development of resistance. Repeated exposure of eight strains of *S. aureus* to cephapirin resulted in the development of resistance to the drug in

TABLE 6. *Comparison of median minimal inhibitory concentrations (MIC) of cephalothin and cephapirin*

Organism	Cephapirin median MIC (μg/ml)[a]	Cephalothin median MIC (μg/ml)				
		Chang and Weinstein (3)	Griffith and Black (4)	Anderson and Petersdorf (1)	Walters et al. (9)	Herrell et al. (5)
Staphylococcus aureus Pen S + R		0.25			<1.0	
S. aureus Pen R	0.37		0.40			0.39
S. aureus Pen S	0.18		0.20			0.198
S. epidermidis	0.135					
S. viridans	0.135	0.175	0.10			0.09
S. pyogenes	0.09	0.75	<0.04		<1.0	<0.05
Diplococcus pneumonia	<0.045	0.05	0.20		<1.0	0.09
Enterococcus	25	10.0	37.5			>12.5
Escherichia coli	12.5	10.0	25	12.0	5–10	25
Indole-positive *Proteus*	>100	10.0	2	>100		25
Proteus mirabilis	12.5	2.5		8.0	5–10	12.5
Salmonella	6.2	5.0	2.0		1–5	1.56
Klebsiella	6.2		26.0	7.5	1–5	6.25
Aerobacter aerogenes	>100	>50	37.5			
Pseudomonas	>100	>50	>50	>100	>50	>100

[a] Inoculum size was, respectively: cephapirin, 10^4 dilution of overnight growth; Chang and Weinstein, 10^3 dilution of overnight growth; Griffith and Black, 5×10^6 organisms for the first eight organisms tested and 5×10^5 for the remaining seven tested; Anderson and Petersdorf, 10^4 organisms for the last seven organisms tested; Walters et al., "tube dilution method"; and Herrell et al., blood-agar streak plates, 5×10^5 to 5×10^6 organisms for the first eight organisms tested and 10^4 dilution of overnight growth for the remaining seven.

two strains (Fig. 1). The MIC for these two strains increased from 0.18 to 50 μg/ml in 2 weeks.

DISCUSSION

Cephapirin was found to be quite active in vitro against most gram-positive organisms with the exception of enterococci. Among the gram-negative organisms, most *E. coli*, *Klebsiella* and indole-negative *Proteus* were susceptible to concentrations of drug easily obtainable in man (2). These results compare favorably with the published in vitro antibacterial activity of cephalothin when the broth dilution method is used (Table 6). In general, cephapirin and cephalothin were found to be similarly active against all organisms tested (1, 3–5, 9). The cephalosporin derivatives appear to be inactive against *Aerobacter* and *Pseudomonas* species in vitro. In addition, we tested cephapirin against *Erwinia* and *Serratia* and found these organisms to be very resistant.

The antimicrobial activity of cephapirin was not appreciably affected by serum. With serum as the diluent, the median MIC values against staphylococci and streptococci were increased two- to fourfold with cephalothin (7), whereas there was only a one tube difference in the MIC values against five strains of *S. aureus* with cephapirin. Absence of a significant serum effect on the MIC has also been reported with cephalexin (8) and cephaloridine (6).

Inoculum size was more important with *E. coli* than with *S. aureus*. When the number of organisms was increased, the amount of drug necessary to inhibit the growth of *E. coli* also increased. Klein et al. (7) found a similar result with cephalothin. In contrast to cephaloridine (6), inoculum size was not as important with other gram-negative and with gram-positive organisms.

Exposure of *S. aureus* to progressively increasing concentrations of cephapirin led to step-wise development of resistance in only two of eight strains tested.

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