Effect of Cephalosporin C and Various Penicillin Derivatives on Staphylococcal Penicillinase and Penicillinase-Producing Staphylococci.* (26725)

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Cephalosporin C, an antibiotic which is chemically related to, but not identical with penicillin(1,2), has been shown to be active against penicillin-resistant staphylococci(3) and insensitive to the action of purified *Bacillus cereus* penicillinase(4). The unavailability of this compound prevented verification and extension of these reports by other laboratories. With cephalosporin C made available to us through the courtesy of Squibb Institute for Medical Research, we undertook a series of investigations to compare cephalosporin C with penicillin G and a variety of penicillin derivatives as to their effect upon penicillinase-producing staphylococci and staphylococcal penicillinase.

Materials and methods. A number of strains of staphylococci isolated from clinical infections were available. For comparative purposes, strains 46 S (sensitive to penicillin) and 46 R (penicillinase-producing) which were genetically related were frequently employed. Susceptibility to the various antibiotics was determined by means of standard 2-fold tube dilution methods. Penicillinase activity was assayed by means of the manometric method adapted for use with staphylococcal cells(5) from the original method of Henry and Housewright(6). Induction of staphylococcal penicillinase synthesis was accomplished by the methods described by Geronimus and Cohen(7). Cephalosporin C, phenoxyethylpenicillin, and 6-aminopenicillanic acid were provided by Dr. Ralph E. Bennett of Squibb Inst. for Med. Res., dimethoxyphenylpenicillin (6-(2,6-dimethoxybenzamido)penicillanic acid) by Bristol Laboratories, and penicillin G by Charles Pfizer and Co. Other reagents and chemicals were obtained from the usual commercial sources.

Results and discussion. Relative effectiveness of cephalosporin C against penicillin-resistant and penicillin-sensitive staphylococci. Table I shows that cephalosporin C is equally effective against both penicillin-resistant and penicillin-sensitive strains of staphylococci. Penicillin G, which inhibits sensitive strains at concentrations of less than 0.15 μg/ml, is ineffective against some resistant strains at concentrations in excess of 300 μg/ml. Cephalosporin C, while not nearly so effective as penicillin G against sensitive strains, is more effective than penicillin G against penicillin-resistant strains when heavy inocula are employed. As shown for strain 604 R, with penicillin G minimal inhibitory concentration increases to a considerable extent with increased inoculum size, while with cephalosporin C there is a change of only one tube in the dilution series.

Effect of combinations of penicillin G and cephalosporin C against penicillin-resistant staphylococci. Crawford and Abraham(3) provided evidence for synergistic action between cephalosporin C and penicillin G. To test this possibility, tube-dilution series of both penicillin G and cephalosporin C in

TABLE I. Relative Effectiveness of Penicillin G and Cephalosporin C against Penicillin-Sensitive and Penicillin-Resistant Staphylococci.

Agent tested	Hr incubation	Minimal inhibitory conc. (μg/ml) Strain 46 R*	46 S*	604 R†	604 R‡
Penicillin G	18	2.4	<.15	9.4	>300
	24	18.8	"	37.5	"
	48	37.5	"	150	"
Cephalosporin C	18	125	62.5	125	125
	24	"	"	"	250
	48	"	"	"	"

* R = resistant to penicillin G; S = sensitive to penicillin G.

† Inoculum for the series shown in first 3 columns was 0.1 ml of a 1:1000 dilution of the inoculum culture.

‡ Inoculum for the series shown in last column was 0.1 ml of a 1:10 dilution of the inoculum culture.

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TABLE II. Effect of Combinations of Penicillin G and Cephalosporin C against Penicillin-Resistant *Staphylococcus aureus* 46 R.

Agent tested	Agent added	Conc. added	Minimal inhibitory conc. (μg/ml) 24 hr	48 hr
Pen. G	None	—	75	>300
"	Ceph. C	100	.3	4.7
"	"	50	4.7	>300
"	"	10	4.7	>300
Ceph. C	None	—	62.5	125
"	Pen. G	100	.5	62.5
"	"	50	.5	"
"	"	10	15.6	"

which one agent was diluted while the other agent was maintained at a constant level were compared at several concentrations of each of the agents. As shown in Table II, 24-hour readings might lead one to conclude that the 2 agents act synergistically. However, minimal inhibitory concentration after 48 hours is within one to 2 tubes of that observed with either agent alone up to the point where the combined concentration of the 2 agents is in excess of the minimal inhibitory concentration for one agent alone. Viability end points taken at the 24-hour interval confirmed the fact that 48-hour visible end points were the more reliable. It appears that slight alterations in the end-points obtained with combinations of the 2 antibiotics are more likely a reflection of relative concentration of penicillin and inoculum size rather than as a result of any clear-cut synergistic action between the 2 agents. Crawford and Abraham(3) also indicated that the combined effect was less obvious with heavier inocula.

TABLE III. Effect of Staphylococcal Penicillinase on Cephalosporin C and Various Penicillin Derivatives.

Substrate (6×10^{-3} M)	μl CO_2/mg/30 min.*
Penicillin G	794
α-Phenoxyethylpenicillin	750
6-Aminopenicillanic acid	(0)
Dimethoxyphenylpenicillin	(0)
Cephalosporin C	(0)

* Using cells in which penicillinase activity had been induced with dimethoxyphenylpenicillin. Relative rates of hydrolysis of the various substrates were comparable with uninduced cells or following induction with other derivatives.

Effect of staphylococcal penicillinase on cephalosporin C and various penicillin derivatives. Penicillin G and phenoxyethyl-penicillin are hydrolyzed at approximately the same rates by staphylococcal penicillinase (Table III). Cephalosporin C, 6-amino-penicillanic acid, and dimethoxy-phenylpenicillin are acted upon slowly, if at all. Steinman(8) recently reported that 6-amino-penicillanic acid and dimethoxyphenylpenicillin are hydrolyzed at definite, but very low, rates by both *B. cereus* and *S. aureus* penicillinases. While rates of hydrolysis of these compounds are of such a low order of magnitude as to be of relatively little practical importance, it is probably incorrect to assume that they are not attacked by the enzyme. Under the conditions of this experiment, the absolute rates of hydrolysis of these compounds were not detectable.

TABLE IV. Effect of Cephalosporin C on Induction of Staphylococcal Penicillinase.

Inducer	Conc.	μl CO_2/mg/30 min.*
None	—	76
Penicillin G	1.78×10^{-3} M	360
Dimethoxyphenyl-penicillin	1.78×10^{-5} M	940
"	$.36 \times 10^{-5}$ M	1,070
"	1.78×10^{-6} M	967
Cephalosporin C	3.54×10^{-4} M	1,160
"	1.78×10^{-4} M	1,220
"	$.89 \times 10^{-4}$ M	1,015

* Using penicillin G (6×10^{-3} M) as substrate. The results shown represent induced levels observed using concentrations of the inducer near the optimal levels determined from preliminary experiments conducted over a much wider range of inducer concentrations.

Cephalosporin C as an inducer of penicillinase synthesis. Cephalosporin C was found to be an active inducer of penicillinase synthesis by staphylococcal cells. Table IV shows a comparison of penicillinase activity in cells induced with cephalosporin C, dimethoxyphenylpenicillin, and penicillin G. Although the induced levels may vary slightly from one experiment to another, cephalosporin C and dimethoxyphenylpenicillin appear to be approximately equal in their inducing ability and both compounds are definitely superior to penicillin G in this respect. Not shown are the effects of 6-amino-penicil-

TABLE V. Effect of Cephalosporin C on Staphylococcal Penicillinase Activity.

Inducer	Substrate*	μl CO_2/mg/30 min.
None	Penicillin G	43
"	Cephalosporin C	(0)
"	Pen. G + Ceph. C	31
Pen. G	Penicillin G	240
"	Cephalosporin C	(0)
"	Pen. G + Ceph. C	238
"	" + 5 × Ceph. C	181
Ceph. C	Penicillin G	871
"	Cephalosporin C	(0)
"	Pen. G + Ceph. C	833
"	" + 5 × Ceph. C	787

* Penicillin G and cephalosporin C were employed at 6×10^{-3} M or at 5 times this concentration where indicated.

lanic acid and phenoxyethylpenicillin which are intermediate between penicillin G and cephalosporin C in their ability to induce penicillinase synthesis. While levels of penicillinase activity following induction with cephalosporin C and dimethoxyphenylpenicillin are usually comparable, there is a definite difference in the concentration required for optimal induction. Our results with staphylococci compare favorably with those of Pollock(9) who found cephalosporin C to be an active inducer of *B. cereus* penicillinase and of Steinman(8) showing that dimethoxyphenylpenicillin induced both *B. cereus* and *S. aureus* penicillinase synthesis.

Effect of cephalosporin C on penicillinase activity. Variation in concentration of cephalosporin C from zero to as high as 1:1 molar ratios of cephalosporin C to penicillin G failed to elicit significant changes in the rate of penicillinase activity (Table V). At 5:1 molar ratios of cephalosporin C to penicillin G slight decreases in activity were observed. Abraham and Newton(4) reported that cephalosporin C competitively inhibited the soluble penicillinase of *B. cereus*. The affinity of the enzyme for cephalosporin C was indicated to be only about one-third that for penicillin G as evidenced by the requirement for relatively high concentrations of cephalosporin C to achieve significant inhibition. To the extent that relatively high concentrations of cephalosporin C are also required to inhibit staphylococcal penicillinase, our results are consistent with those obtained using the soluble penicillinase from *B. cereus*. In other experiments, preincubation of staphylococcal cells with cephalosporin C for periods of as long as 4 hours prior to addition of substrate failed to produce significant change in rate of penicillinase activity.

Summary and conclusions. 1. Cephalosporin C is equally inhibitory to penicillin-resistant and penicillin-sensitive staphylococci. However, against sensitive strains it is not nearly so effective an antibiotic as penicillin G. 2. Cephalosporin C and penicillin G apparently do not act synergistically in inhibiting the growth of penicillin-resistant staphylococci. 3. Cephalosporin C, dimethoxyphenylpenicillin and 6-aminopenicillanic acid are hydrolyzed slowly, if at all, by staphylococcal penicillinase. 4. Cephalosporin C is an active inducer of penicillinase synthesis in staphylococci, being comparable to dimethoxyphenylpenicillin in this respect but superior to penicillin G. 5. Cephalosporin C, at relatively high concentrations, does not significantly inhibit staphylococcal penicillinase.

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