Microbiological Activities of Lysostaphin and Penicillins Against Bacteriophage 80/81 Strains of *Staphylococcus aureus*

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ABSTRACT

ZYGMUNT, WALTER A. (Mead Johnson Research Center, Evansville, Ind.), EDWARD F. HARRISON, AND HENRY P. BROWDER. Microbiological activities of lysostaphin and penicillins against bacteriophage 80/81 strains of *Staphylococcus aureus*. Appl. Microbiol. **13**:491–493. 1965.—Using 20 clinical isolates of *S. aureus* (all bacteriophage 80/81 type), we found that lysostaphin inhibits the growth of all cultures at concentrations significantly lower than those observed with any of eight penicillins, a penicillin-like compound (cephalothin), or fusidic acid (a steroid antibiotic). All test cultures were shown to be resistant to penicillin G, ampicillin, and propicillin. Of the remaining penicillins (all penicillinase-insensitive), oxacillin, nafcillin, cloxacillin, and cephalothin were approximately equal in antimicrobial activity. Ancillin was slightly less active, and methicillin was even lower in potency. Cultures varied more widely in susceptibility to fusidic acid. None of the clinical isolates tested was found to be resistant to lysostaphin.

Recent reports on the discovery and antistaphylococcal specificity of lysostaphin (Schindler and Schuhardt, 1964), on its purification (Shindler and Schuhardt, 1965), on its utility against a large number of clinical strains of *Staphylococcus aureus* both in vitro (Schindler and Schuhardt, 1964; Cropp and Harrison, 1964; Harrison and Cropp, 1965) and in vivo (Schuhardt and Schindler, 1964), and on its enzymatic mode of action (Browder et al., Biochem. Biophys. Res. Commun., *in press*) have delineated some of the major features of this unique antibiotic. In contrast to the penicillins, which exert their effect by suppressing cell-wall synthesis, lysostaphin is effective by virtue of its ability to cleave the staphylococcal cell wall, without distinguishing between staphylococcal cells that produce penicillinase and those that do not, or among staphylococcal cells of different phage susceptibility.

In view of the prominence of penicillins in the chemotherapy of staphylococcal infections, it seemed pertinent to extend our earlier comparisons of lysostaphin with benzylpenicillin, ampicillin, and dimethoxypenicillin (Harrison and Cropp, 1965) to a larger group of the newer penicillins and, further, to include fusidic acid and cephalothin. For the broader comparisons

reported in this paper, we have selected 20 clinical isolates of *S. aureus* from the highly virulent bacteriophage 80/81 group.

MATERIALS AND METHODS

Bacterial strains. Twenty clinical isolates of *S. aureus*, all bacteriophage 80/81 type, were kindly supplied by W. R. Cole, Department of Surgery, Wohl Hospital, St. Louis, Mo. All phage typing was performed by Vera Gray of the same department.

Sensitivity tests. Minimal inhibitory concentrations (MIC) for each antibiotic were determined by conventional twofold serial dilution techniques in a liquid medium. An inoculum level approximating 10^6 viable cells per milliliter of medium was used.

Eleven antibiotics, including nine penicillins or penicillin-like compounds, were studied. They were obtained from the following suppliers: penicillin G (benzylpenicillin), E. R. Squibb & Sons, New York, N.Y.; ampicillin (α-aminobenzylpenicillin, sodium) Bristol Laboratories, Syracuse, N.Y.; propicillin (α-phenoxypropylpenicillin), Eli Lilly & Co., Indianapolis, Ind.; methicillin (dimethoxyphenylpenicillin, sodium), Bristol Laboratories; cephalothin [7-(thiophene-2-acetamido)cephalosporanic acid], Eli Lilly & Co.; ancillin (2-biphenylyl penicillin, sodium), Smith Kline & French Laboratories, Philadelphia, Pa.; cloxacillin (3 - o - chlorophenyl - 5 - methyl - 4 -

TABLE 1. *In vitro susceptibility of 20 bacteriophage 80/81 strains of Staphylococcus aureus to lysostaphin and other antibiotics*

Antibiotic	Antibiotic concn (µg/ml)													
	>100	100	50	25	6.25	3.12	1.56	0.78	0.39	0.20	0.10	0.05	0.025	0.012
Penicillin G	20*													
Ampicillin	20													
Propicillin	14	3	2	1										
Fusidic acid					3	3	4	8	2					
Methicillin						20								
Ancillin								20						
Cephalothin								16	4					
Cloxacillin								5	15					
Nafcillin								1	19					
Oxacillin									13	7				
Lysostaphin (γ)									3	2	8	4	2	1
Lysostaphin (ε)										2	3	8	4	3

* Number of strains susceptible to minimal antibiotic concentrations.

isoxazolyl penicillin), Ayerst Laboratories, Rouses Point, N.Y.; nafcillin [6-(2-ethoxy-1-naphthamido) penicillin], Wyeth Laboratories, Philadelphia, Pa.; oxacillin (5-methyl-3-phenyl-4-isoxazolyl penicillin, sodium), Bristol Laboratories; fusidic acid (a steroid antibiotic supplied as a sodium salt), Leo Pharmaceutical Products, Ballerup, Denmark; lysostaphin (low molecular weight protein, Mead Johnson & Co.).

Two lysostaphin preparations (γ and ε) denoting different degrees of purification were employed in these studies. On the basis of biological activity, the ε preparation (250 units per mg) is about 65% more potent than the γ preparation (150 units per mg). Both preparations assayed about 95% protein by the method of Lowry et al. (1951) when lysozyme was used as a standard. In both cases, however, the major protein component was the antibiotic.

The following diluents were used for the antibiotics: pH 6.0, 0.1 M phosphate (cephalothin and all penicillins); pH 7.5, 0.05 M tris(hydroxymethyl)aminomethane in 0.145 M sodium chloride (lysostaphin); and distilled water (fusidic acid; solution adjusted to pH 7.2 after dissolution of the antibiotic). Stock solutions of the antibiotics in their respective buffers were sterilized by ultraviolet irradiation for 5 min. No impairment in microbiological activity was evident with this sterilization method. Only freshly prepared solutions of antibiotics were used.

Tests for penicillinase production were done according to the Haight and Finland's (1952) modification of the Gots method, with *Sarcina lutea* as the indicator organism. Tests were performed on each of the clinical isolates with each antibiotic used individually.

RESULTS AND DISCUSSION

Table 1 shows the MIC values observed against the 20 strains of *S. aureus* tested. All of the strains are resistant to penicillin G, ampicillin,

and propicillin and, hence, are penicillinase producers. The Haight and Finland tests for penicillinase production verified this conclusion. The main advantage of propicillin is associated with its enhanced activity over benzylpenicillin against certain gram-negative bacteria. Klein and Finland (1963) reported, however, that neither of these penicillins has any significant biological activity against penicillinase-producing staphylococci.

The efficacy of the penicillinase-insensitive penicillins in inhibiting the growth of these cultures varied within a 15-fold range in concentrations, 0.20 to 3.12 µg/ml. Oxacillin, nafcillin, and cloxacillin are equivalent in biological activity, and the isolates studied show median MIC values approximating 0.39 µg/ml. Relative to them, cephalothin and ancillin are only about one-half, and methicillin only about one-eighth, as potent. In the case of the penicillins and cephalothin, with the exception of propicillin, all of the isolates have MIC values within a twofold range in antibiotic concentration. On the other hand, these cultures vary in susceptibility to fusidic acid over a 16-fold range in concentration (Table 1).

A comparison of the in vitro efficacy of lysostaphin and the ten other antibiotics tested shows lysostaphin to be the most effective antibiotic in the series. Lysostaphin (γ) completely inhibits 75% of the cultures at antibiotic concentrations not exceeding 0.10 µg/ml. The more highly purified ε preparation inhibits 75% of the cultures at 0.05 µg/ml or less. In contrast, with the best of the penicillins tested (oxacillin), only 35% of the cultures show complete inhibition at 0.20 µg/ml. When the antibiotics are compared on an equivalent weight basis, lysostaphin is at least four to eight times more potent than the recently synthesized penicillins. When these com-

parisons are made on a molar basis (assuming a molecular weight of 30,000 for the enzyme, lysostaphin), the above ratios increase further by an additional factor of about 75 (resulting in a final ratio of 300- to 600-fold in favor of lysostaphin).

Additional studies are in progress to delineate more fully the role of this antibiotic in staphylococcal disease.

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