Lysostaphin in Experimental Renal Infections

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ABSTRACT

By use of a renal staphylococcal infection model in mice, single intravenous doses of lysostaphin ranging from 1.56 to 50 mg/kg were effective in: (i) controlling the staphylococcal population of kidneys, (ii) reducing the mortality rate, and (iii) clearing high numbers of kidneys of infection. Semisynthetic penicillins and other antistaphylococcal antibiotics given in the same manner did not have significant activity. Only by the administration of a long-acting, depot form of penicillin (Bicillin) could results comparable to those seen with lysostaphin be obtained. The results of this study suggest that lysostaphin may be useful in staphylococcal septicemias in preventing the establishment of new foci of infection.

Lysostaphin is an extracellular bacterial enzyme with highly specific antistaphylococcal activity primarily against coagulase-positive strains. Its in vitro antistaphylococcal activity has been studied extensively (2, 10). Subsequently, comparisons were made in vivo with other antistaphylococcal antibiotics and semisynthetic penicillins (4, 12, 13). All of these investigations substantiate the fact that lysostaphin is an extremely potent antistaphylococcal antibiotic which offers a novel and potentially useful approach to chemotherapy.

Schuhardt and Schindler (11) demonstrated that lysostaphin is effective in vivo against experimental staphylococcal peritonitis when given by either the intraperitoneal or subcutaneous routes. This observation was confirmed in our laboratory. Using other in vivo models, Harrison and Cropp (Can. J. Microbiol., in press) found that lysostaphin is capable of reducing the edema produced by a localized staphylococcal infection in the mouse leg, and that topical application of the antibiotic reduces the number of viable staphylococci in experimental rabbit ear infections.

Because of the high chemotherapeutic potency of this antibiotic in each of the experimental infection models that were tried, the studies reported here were undertaken to establish the chemotherapeutic potential of lysostaphin in staphylococcal renal abscesses produced experimentally in mice. The infection model used is similar to certain chronic staphylococcal abscesses seen in man which currently are not entirely amenable to therapy with clinically available antibiotics.

Materials and Methods

Test animals. Male albino mice (Swiss-Webster strain) ranging from 25 to 30 g in body weight were used. The mice were caged in groups of five animals each and received food and water ad lib.

Infection model. Staphylococcus aureus Giorgio strain, kindly supplied by R. M. McCune, Jr., Cornell University Medical School, was used to produce the renal abscesses. A 24-hr culture grown in Trypticase Soy Broth (BBL) at 37°C was centrifuged, washed once, and resuspended to its original volume in physiological saline. The suspension was diluted with saline so that each mouse received approximately 10^9 viable staphylococci when 0.5 ml of the inoculum was injected into the tail vein. At 1 hr after infection, the kidneys contained approximately 10^4 viable staphylococci per milliliter as determined by standard plate counts. A total of 40 mice were infected with each drug tested, so that groups of 10 mice could be withdrawn from the test and examined at intervals of 4, 11, 18, and 21 days after infection and medication.

The chemotherapeutic effects of the antistaphylococcal agents were determined by use of procedures previously reported (6, 7, 8). Each agent was administered as a single intravenous dose (in milligrams per kilogram) 1 hr after infection except for Bicillin (benzathine penicillin G), which was given as a single intramuscular dose. At the time intervals designated, groups of 10 mice were withdrawn from the test, and the kidneys were examined for viable staphylococci by use of two different methods. Three or four animals from each group were killed, and their kidneys were removed. Separate homogenates were prepared from the kidneys of each animal in sterile buffer. The total staphylococcal populations of the kidneys were expressed as numbers of viable cells per milliliter of kidney as described by McCune and Tomsett (9). The volume of the kidney was taken into account in
considered cloxacillin and results. Colony counts were made after 2 days of incubation at 37°C. The data obtained from the counts were used to calculate the mean number of viable staphylococci per milliliter of kidney. The mean of the logarithms of the kidney staphylococcal populations was plotted for each drug as a function of time in days.

The kidneys from the animals remaining in each group were examined qualitatively for the presence of viable staphylococci. The kidneys were removed aseptically and bisected with sterile scissors. A streak plate was made by passing the inner surface of the kidney over the surface of a Mannitol Salt Agar plate several times. After incubation, the plates were examined for colonies of staphylococci. Kidneys that failed to produce typical staphylococcal colonies were considered to be cleared of the infection. Since the data on cleared kidneys obtained by homogenate plate counts were in general agreement with that obtained by the qualitative streak plate method, both sets of data were included in the final tabulation of results.

Antibiotics. The following antibiotics were used: kanamycin, oxacillin, methicillin, and ampicillin (Bristol Laboratories, Syracuse, N.Y.); vancomycin and propicillin (Eli Lilly & Co., Indianapolis, Ind.); cloxacillin and dicloxacillin (Ayerst Laboratories, Rouses Point, N.Y.); nafcillin and Bicillin—mixture of benzathine, procaine, and potassium penicillin G (Wyeth Laboratories, Philadelphia, Pa.); lincomycin (The Upjohn Co., Kalamazoo, Mich.); penicillin G, potassium salt (USP) (Nutritional Biochemicals Corp., Cleveland, Ohio); cephaloridine (Glaxo Laboratories, Greenford, Middlesex, England); and lysostaphin (Mead Johnson & Co., Evansville, Ind.).

The lysostaphin used in this study was a lyophilized preparation which had a potency of 200 units of lytic activity per mg, and which was approximately 95% protein when assayed by the method of Lowry et al. (5) when lysozyme was used as a standard.

All drugs were dissolved in physiological saline except lysostaphin, which was dissolved in 0.05 M Tris(hydroxymethyl)aminomethane (Tris) in physiological saline, pH 7.5.

Sensitivity tests. The antibiotic sensitivity of the Staphylococcus aureus Giorgio, expressed as the minimal inhibitory concentration (MIC), was determined by the conventional twofold tube dilution method (3) with Trypticase Soy Broth. The level of inoculum used was 10⁶ organisms per milliliter.

RESULTS AND DISCUSSION

The MIC values for the test culture obtained in our laboratory (Table 1) agree with those reported by McCune (6) for oxacillin and methicillin, although the sensitivity to penicillin G was greater in our experiments (0.095 µg/ml compared with 1.6 to 3.1 µg/ml). The culture was virulent as evidenced by the overall high rate of kidney infection in the nonmedicated control mice (93%). The 3-week mortality rate in the nonmedicated groups ranged from 55 to 80% (60% average) in five different experiments. The bacterial census was adequate for evaluating the chemotherapeutic effect of the antistaphylococcal agents.

Single intravenous doses of lysostaphin, ranging from 1.56 to 50 mg/kg, produced significantly lower bacterial populations in the kidney (Fig. 1). Lysostaphin decreased the staphylococcal census by 5 log units as compared with the nonmedicated control kidneys. Similar observations have been made by W. Schaffiner, M. A. Melly, and M. G. Koenig (Clin. Res. 14:343, 1966) using a comparable test model. At 0.5 mg/kg, elevated bacterial counts were observed at 4 and 11 days; however, these and 21-day kidney populations were markedly decreased. Thus, a single dose of lysostaphin at this low level was apparently sufficient to control the staphylococcal population to the point where the normal host defense mechanisms could function.

High numbers of kidneys were cleared of the infection by the single-dose therapy of lysostaphin (Table 2). The percentage of kidneys cleared ranged from 46 to 85% for lysostaphin, in contrast to 7% for the nonmedicated control animals. Likewise, low mortality rates were obtained with all of the drug concentrations when lysostaphin was used for medication. The mortality rate of 55 to 80% for the nonmedicated control animals was lowered significantly by single-dose therapy of lysostaphin (0 to 15%).

Single intravenous doses of lysostaphin as low as 1.56 mg/kg demonstrated a significant therapeutic effect, whereas numerous semisynthetic penicillins and other antibiotics administered in a similar manner at levels up to 25 mg/kg were essentially inactive. Only by the administration of a long-acting, depot form of penicillin (Bacillin) Table 1. In vitro sensitivity of Staphylococcus aureus Giorgio to various antibiotics

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>MIC µg/ml</th>
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<tbody>
<tr>
<td>Lysostaphin</td>
<td>0.095</td>
</tr>
<tr>
<td>Propicillin</td>
<td>0.095</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>0.095</td>
</tr>
<tr>
<td>Cephaloridine</td>
<td>0.095</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0.19</td>
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<tr>
<td>Cloxacillin</td>
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<tr>
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<tr>
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<tr>
<td>Oxacillin</td>
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</tr>
<tr>
<td>Lincomycin</td>
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</tr>
<tr>
<td>Methicillin</td>
<td>3.12</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>6.25</td>
</tr>
</tbody>
</table>
Fig. 1. Chemotherapeutic effect of lysostaphin, semisynthetic penicillins, and antibiotics on the renal staphylococcal populations in infected mice after single-dose therapy.
could results comparable to those observed with lysostaphin be obtained.

Best clearance of the renal infections was demonstrated by penicillins having benzyl or phenoxy side chains (penicillin G, ampicillin, propicillin) of the several penicillins tested. Penicillin G produced a low mortality rate, but it was not impressive in the total clearance or bacterial-census evaluation. Cephaloridine, the only cephalosporin tested, produced good clearance of the infection, but the kidney bacterial populations remained elevated until the terminal plating. The remaining semisynthetic penicillins kanamycin, vancomycin, and lincomycin were essentially inactive.

This study indicates that lysostaphin may have a role in systemic therapy of certain chronic staphylococcal lesions and their accompanying septicemias. Lysostaphin, because of its enzymatic mode of action (1), has the advantage of bringing about a rapid decrease in the staphylococcal titers, whereas most conventional anti-staphylococcal antibiotics merely inhibit bacterial multiplication and, consequently, require much longer time periods to elicit marked decreases in staphylococcal counts in the blood. Lysostaphin has the added advantage of lysing coagulase-positive staphylococci readily regardless of their metabolic state, whereas all penicillin-like antibiotics inhibit only those bacteria which are actively multiplying and presumably building new cell wall structures.

From the data presented in this study, it appears that lysostaphin warrants further consideration as a potential anti-staphylococcal agent for systemic use alone or as an adjunct to therapy with the semisynthetic penicillins.

**Acknowledgments**

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**Literature Cited**