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In-Vitro Activity of Lysostaphin

Bioassay in Serum

Dolores N. Jones, B.S.,* Edward L. Quinn, M.D.,** and Frank Cox, M.D.***

Lysostaphin, an antistaphylococcal agent more potent than penicillin,1 was shown to possess special properties in vitro that may make it a specially useful therapeutic agent.2 In order to arrive at rational dosage regimens, bioassay techniques for determining serum concentration and animal pharmacology studies are necessary. Experiments to evaluate a bioassay technique for lysostaphin in dog serum using the method of Tavormina (written communication, 1967) were designed and the results are the subject of this report.

Materials and Methods

Lysostaphin reference standard solution was supplied by Mead Johnson Research Laboratories, as were lyophilized preparations of Staphylococcus aureus, 209P (Preparation A and Preparation B).

Optical density determinations were carried out using a Coleman Model 9 Junior colorimeter with a Coleman 6-310 adapter to hold 12 x 75 mm culture tubes.

Lysostaphin reference standard solution (320 units/ml) was diluted with buffer (0.05 M Tris — 1.145 Na Cl pH 7.5) and stored at 5° C in vials containing 20 units/ml. This working standard was renewed weekly.

Using lyophilized Staphylococcus aureus 209P cells, a 3.0 mg/ml suspension was prepared in 0.05 M Tris — 1.145 M Na Cl buffer (pH 7.5). This suspension was freshly prepared for each assay, held at 5° C in ice, and used within four hours.

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One-half ml amounts of lysostaphin standard solution, serially diluted to contain 0, 0.03, 0.04, 0.08, 0.12, 0.16, 0.20, and 0.5 units, were added to the assay tubes in triplicate. One-half ml amounts of staphylococcal cell suspension and one-half ml aliquots of Tris Na Cl buffer solution were added to each tube. Base-line optical density readings were taken within two minutes of inoculation. The tubes were then incubated in a 37° C water-bath for exactly 10 minutes, at which time, final optical density readings were done. Optically matched tubes for turbidometric readings were not necessary since each tube served as its own control with per cent decreases in optical density calculated from readings at 0 and at 10 minutes. The per cent decreases of optical densities were calculated for each concentration of lysostaphin (the average of triplicate determinations being used). A standard reference curve was constructed on graph paper by plotting the per cent reduction in optical density on the vertical and the concentration of lysostaphin in the standard solutions on the horizontal axis. Since lysostaphin is only minimally bound to serum proteins, it was

**Figure 1**

Standard reference curve for the bioassay of lysostaphin. The variation related to different preparations of lyophilized *S. aureus* (209P) cells is shown.
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not necessary to construct the standard curve from determinations done in a serum diluent.

Serum samples to be assayed were prepared as follows: Using pooled normal dog serum, known quantities of lysostaphin standard solution were added to aliquots of serum to give final concentrations of 0.02, 0.1, 0.2, 0.8, 1.6, and 3.2 units ml. Samples were diluted to approximately 0.1 units with buffer solution, and added to assay tubes in triplicate. Lower concentrations were assayed undiluted. Additions of staphylococcal cells and buffer were made as above. Base-line optical density readings, incubation and final optical density readings were carried out in a similar manner. The per cent decrease in optical density was calculated and the lysostaphin concentration of each sample was read from the standard reference curve. This was multiplied by the reciprocal of the dilution to obtain the assayed lysostaphin concentration. The amount of lysostaphin added to the serum was compared to the assayed values.

![Figure 2](image-url)

Standard reference curve for the bioassay of lysostaphin. The reproducibility of the method when the same preparation of lyophilized *S. aureus* (209P) cells were employed is shown.

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Results

The Standard Reference Curve. As shown in Fig. 1, different lyophilized preparations of Staphylococcus aureus 209P cells varied in their susceptibility to lysostaphin. In one preparation (A), the reduction of optical density caused by the lysis of the staphylococcal cells ranged from 11% with 0.02 units of lysostaphin to 67% with 0.5 units. The other preparation (B) of the same organism was considerably less susceptible to lysostaphin. Reduction in optical density was only 6% with 0.02 units and only 50% with 0.5 units of lysostaphin. However, when the same lyophilized preparations were employed, variation in the susceptibility curves was not so marked. Furthermore, extended refrigerator storage of the unreconstituted lyophilized cells and the lysostaphin standard solutions did not affect the results. These findings are illustrated in Fig. 2. Variations in the reduction of optical density were less than 2% when any given points of the “A” cell preparation curves were compared. Similar results were seen when “B” cell preparation curves were compared. Although variation here was slightly greater than with the “A” cell preparation, differences were noted in optical density reductions of no more than 5%. These data indicate the importance of determining standard curve values for each different lyophilized staphylococcal cell preparation.

Bioassay of Lysostaphin in Dog Serum. When known amounts of lysostaphin were added to dog serum, the method of bioassay under study yielded concentration values similar to the amount of lysostaphin added to the serum (Table I).

<table>
<thead>
<tr>
<th>Concentration of lysostaphin added (μg/ml)</th>
<th>Concentration of lysostaphin assayed (μg/ml)</th>
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<tbody>
<tr>
<td>0.02</td>
<td>0.02</td>
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<tr>
<td>0.10</td>
<td>0.105</td>
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<td>3.78</td>
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Summary and Conclusion

A bioassay method for determining lysostaphin concentrations in dog serum, as described by Tavormina, was evaluated. The method yielded accurate and reproducible results and should provide a satisfactory procedure for bioassay determinations necessary in pharmacologic studies of this agent.

ACKNOWLEDGEMENT

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REFERENCES