LYSOSTAPHIN THERAPY IN MICE INFECTED WITH *STAPHYLOCOCCUS AUREUS*

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A new bacteriolytic agent for staphylococci, designated lysostaphin (Ln) has been described by Schindler and Schuhardt (Proc. Natl. Acad Sci. U.S. **51**:414, 1964). The unit of Ln was defined as the amount required to bring about a 50% reduction in the turbidity of a standard suspension of *Staphylococcus aureus* FDA 209P in 10 min at 37 C. The Ln used in these studies was produced, partially purified, and concentrated by methods to be described (Schindler and Schuhardt, *in press*). The protein content of Ln, as determined by the method of Lowry et al. (J. Biol. Chem. **193**:265, 1951), was 3.7 mg/ml or 29.1 μg per unit. The concentrated Ln was kept at −20 C. Just prior to use, it was thawed, assayed, and diluted to the desired unitage in 0.05 M tris-(hydroxymethyl)aminomethane-HCl buffer at pH 7.4 containing 0.145 M NaCl.

The animals used in these experiments were 2- and 3-month-old C3H mice of 20 g average weight, obtained from the Clayton Biochemical Institute, The University of Texas. A culture of *S. aureus* Sv (Smith) was obtained from F. C. Kelly, of Oklahoma University. After passage through C3H mice, the intraperitoneal (ip) average infectious dose (ID_{50}) of saline-washed cells of this culture was established. For therapeutic studies, 5 ID_{50} of these cells were suspended in 0.145 M NaCl and injected ip in 0.1-ml amounts. More than 90% of the untreated control mice receiving this infectious dose died in 8 to 24 hr.

All therapeutic studies were conducted on test and control groups of four or more mice each. Therapy consisted of a single injection of Ln, either ip or subcutaneously (sc). Preliminary therapy experiments indicated that 10 units of Ln by either route of injection cured all mice, when the ip injections were given at times ranging from 2 hr before to 4 hr after infection. Sc therapy was administered 15 min or 1 hr after infection. Additional early tests reduced the 100% curative dose for 2-hr postinfection ip therapy to less than 1 unit. Cure in these early studies was established both by survival for 30 days and by the fact that all mice tested after 7 days of survival yielded negative peritoneal and visceral cultures. Thereafter, normal survivors after 14 days were considered cured.

TABLE 1. *Single injection TD_{50} of lysostaphin in mice*

Lysostaphin dose		No. surviving/no. tested	
Units	Route*	24 hr	14 days
0.1	ip	4/4	4/4
0.05	ip	4/4	3†/4
0.02	ip	4/4	4/4
0.01	ip	3/4	3/4
0.005	ip	0/4	—
0.5	sc	4/8	4/8
None	—	1‡/8	1‡/8

* Abbreviations: ip, intraperitoneal; sc, subcutaneous.

† Mechanical injury to head appeared to be the cause of death of one mouse 5 days after treatment.

‡ It is possible that the total infecting dose was not administered ip in this animal.

TABLE 2. *LD_{50} of lysostaphin given intraperitoneally in mice*

Single dose of lysostaphin (units injected)	No. surviving after 30 days / No. tested
10	28*/28
20	12*/14
30	2/4

* Included a number of infected mice.

Table 1 gives the results of the final experiment to determine the average therapeutic dose (TD_{50}) of this Ln preparation. The ip therapy was administered 2 hr, and the sc therapy 1 hr, after infection in these animals.

Table 2 summarizes the average lethal dose (LD_{50}) evidence for the relative nontoxicity of this

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Ln preparation when administered as a single ip dose to mice. Due to a shortage of supply, the sc LD_{50} of this Ln was not determined.

The ip TD_{50} of 0.01 unit of this Ln represents a total protein TD_{50} of 14.55 μg/kg. The ip LD_{50} of 30 units represents a total protein LD_{50} of 43.65 mg/kg. We have no way of knowing at present what percentage of the toxic effects was due to specific Ln as compared to that due to possible nonstaphylolytic impurities. However, the ip therapeutic index of 3,000 for this product encourages us to hope that Ln may prove to be a valuable therapeutic agent in staphylococcal infections in man and animals.