APPLIED MICROBIOLOGY, Sept. 1971, p. 446–450

Vol. 22, No. 3
Printed in U.S.A.

Efficacy and Safety of Topical Lysostaphin Treatment of Persistent Nasal Carriage of *Staphylococcus aureus*

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Received for publication 18 June 1971

The efficacy of lysostaphin nasal spray and Neosporin ointment (Burroughs Wellcome & Co.) in altering nasal carriage of *Staphylococcus aureus* was studied with persistent carriers in an institution for mentally retarded children and adults. Treatment for 5 days with either agent significantly reduced carriage rates. This effect persisted through the 5th day after therapy with lysostaphin but not with Neosporin. By the 11th day after therapy, carriage rates in the treatment and control groups were not significantly different. Except for a single immediate wheal and flair skin test reaction, no other evidence of adverse reactions to topical lysostaphin was detected. No consistent changes in hemagglutination-inhibition titers to lysostaphin were observed after therapy. Lysostaphin appears to be slightly more effective than conventional topical antimicrobial therapy in reducing nasal carriage of staphylococci in this rigorously defined population of persistent carriers.

Lysostaphin, an enzyme that rapidly lyses the cell walls of *Staphylococcus aureus* (11, 13, 14), is effective in the treatment of established staphylococcal infections in experimental animals (5, 6, 12). Its action is thought to be due to a peptidase that cleaves cell wall polyglycine bridges and is found almost exclusively in coagulase-positive staphylococci (3). Lysostaphin is, therefore, a uniquely specific agent. It is effective in vitro against *S. aureus* strains irrespective of their phage susceptibility or their resistance to conventional antibiotics including methicillin (4, 15) but has no effect on bacteria of other genera.

Persistent nasal carriers, especially in a hospital setting, are an important source of *S. aureus*. Conventional antibacterial therapy, whether topical or systemic, has not consistently eliminated *S. aureus* from the nasal passages of such carriers, but topical lysostaphin has produced promising results with small groups of normal adults (8, 9) and children (7). This paper describes a controlled study of the effect of lysostaphin nasal spray on a rigorously defined group of persistent *S. aureus* nasal carriers in an institution for the mentally retarded.

MATERIALS AND METHODS

At the Joseph H. Ladd School in Exeter, R.I., approximately 900 mentally retarded children and adults live in cottages and large dormitories. To discover the persistent nasal carriers of *S. aureus*, an initial group of 595 residents was cultured weekly for 6 weeks. A sterile swab was inserted into both anterior nares and then streaked onto Trypticase soy agar. Swabs from patients receiving lysostaphin were cultured on Trypticase soy agar containing 0.1% trypsin prepared from a solution of 1:250 trypsin sterilized by Seitz filtration (Difco). After overnight incubation at 37 C, the plates were held at room temperature for 24 hr to allow development of colonial pigmentation. Three pigmented colonies from each plate were tested for coagulase production by the slide method. If no pigmented colonies were present, a single nonpigmented colony was tested. A tube coagulase test was performed on all pigmented strains negative by the slide method. Strains positive by either method were designated *S. aureus*.

The 152 residents (26% of the initial group) who had *S. aureus* cultured from their anterior nares on at least five of the six weekly cultures were entered in an initial 10-day study after parental consent was obtained. Forty-six persons were treated with intranasal lysostaphin, 45 with lysostaphin plus neomycin, 45 with Neosporin ointment, and 16 with an intranasal placebo identical to the lysostaphin solution except for the absence of this agent. Significant and approximately equal decreases in carriage rates occurred among those treated with the various therapeutic agents, but a significant and inexplicable decrease in the carriage of *S. aureus* also occurred among those in the placebo group. The erroneous administration of

active agents to the placebo group was suspected initially, but an intensive analysis of the methods of administration revealed no evidence to support this hypothesis. Therefore, the results of this study were inconclusive and a new study was undertaken.

Each of the 152 study subjects then was cultured 47, 54, 68, and 75 days after completion of the initial study. Ninety-five subjects were still positive on three of the four follow-up cultures; these were regarded as persistent carriers and constituted the population of this study.

Persistent carriers were assigned to three study groups that contained equal proportions of persons from each residence, had similar age and sex distributions, and had equal proportions of individuals who had received the various agents in the initial study.

Group 1 (30 carriers) was treated with three intranasal sprays of lysostaphin into each nostril three times a day for 5 days. (Provided as an investigational new drug by Grey B. Kornegay, Mead-Johnson Research Center, in the form of powder which was reconstituted daily to give a 0.5% saline solution of lysostaphin.)

Group 2 (32 carriers) was treated with intranasal application of Neosporin ointment [containing polymyxin B sulfate (5,000 units/g), zinc bacitracin (400 units/g), and neomycin sulfate (5 mg/g), which was provided by Burroughs Wellcome and Co.] by swab into each nostril three times a day for 5 days.

Group 3 (33 carriers) received no therapy.

Nasal swabs from all carriers were cultured 1, 5, 11, and 24 days after the completion of the therapy phase. Susceptibility of pre- and post-treatment *S. aureus* isolates to lysostaphin was determined by a tube dilution technique, and susceptibility to other antimicrobial agents was evaluated by the disc method of Bauer et al. (1). Isolates were phage-typed by standard methods at routine test dose (RTD). Those strains which could not be phage-typed at RTD were tested at 1,000 RTD and at 100 RTD after heat treatment (10).

The criteria for classifying alterations in phage types of *S. aureus* carried were as follows.

Persistence. Persistence was determined by the presence of the phage type on at least one of the three cultures obtained within the 3 weeks preceding therapy and at least once after therapy.

Elimination. Elimination was determined by the presence of the phage type on two cultures before therapy, including at least once in the 3 weeks immediately before treatment, and absence on all follow-up cultures.

Acquisition. Acquisition was determined by the absence of the phage type on all cultures in the 3 weeks before therapy but presence on at least two cultures after treatment.

Occasional strains that did not meet these criteria were regarded as transient nasal flora and were not included in the analysis.

Three studies were performed to assess the immunological consequences of lysostaphin therapy. (i) All treated individuals, including 10 who had received lysostaphin therapy in both studies, were observed for local reactions to intranasal application of the therapeutic agents. (ii) Nineteen individuals who had received a single 10-day course of intranasal lysostaphin and 14 individuals with no known exposure to lysostaphin were skin-tested 76 days after completion of therapy by intradermal injection of 0.1 cc (0.1 mg) of lysostaphin solution (especially provided for interdermal injection) in one arm and 0.1 cc of sterile saline in the other arm. Skin test sites were observed for erythema and induration 30 min, 24 hr, and 48 hr after injection. (iii) Lysostaphin hemagglutination-inhibition titers were assayed in 35 lysostaphin-treated residents and 19 unexposed individuals before and 18 and 75 days after therapy. The assays were performed on samples coded to obscure their identity by Walter A. Zygmunt, Mead-Johnson Research Center. Members of the original placebo group did not participate in the skin test or serological studies.

RESULTS

Bacteriology. Figure 1 depicts the percentage of persistent carriers harboring *S. aureus* in their anterior nares before and after therapy. On the first day after completion of therapy, only 40% of the lysostaphin-treated group and 63% of those receiving Neosporin carried *S. aureus*. Both of these values are significantly lower than the 97% carrier rate among controls ($P < 0.01$). The difference between the lysostaphin and Neosporin groups is not significant. However, 5 days after therapy only 60% of the lysostaphin-treated individuals carried *S. aureus*, whereas 94% of the Neosporin group and 97% of controls were again carriers. At this point, the carriage rate among the lysostaphin-treated individuals was significantly below that for both the Neosporin and control groups ($P < 0.05$). By the 11th and 24th days after therapy, no significant differences existed among the groups.

Only 36% of the lysostaphin group was positive on all post-treatment cultures, as opposed to 64% of the Neosporin group and 91% of the controls. The lysostaphin group differs significantly from the controls ($P < 0.0005$) but not from the Neosporin group which, in turn, is not significantly different from the control group.

In Table 1, the persistence, elimination, and acquisition of phage types in the three treatment groups are compared. Of the phage types present before therapy, 29% were eliminated after lysostaphin therapy, 24% were eliminated after Neosporin, and 6% were eliminated in the control group. The elimination of phage types during lysostaphin therapy was significantly greater than that which occurred in the control group ($P < 0.01$). However, the elimination of phage types after Neosporin did not differ significantly from that of the lysostaphin or control groups. Acquisition of new phage types occurred with similar frequency in all three groups.

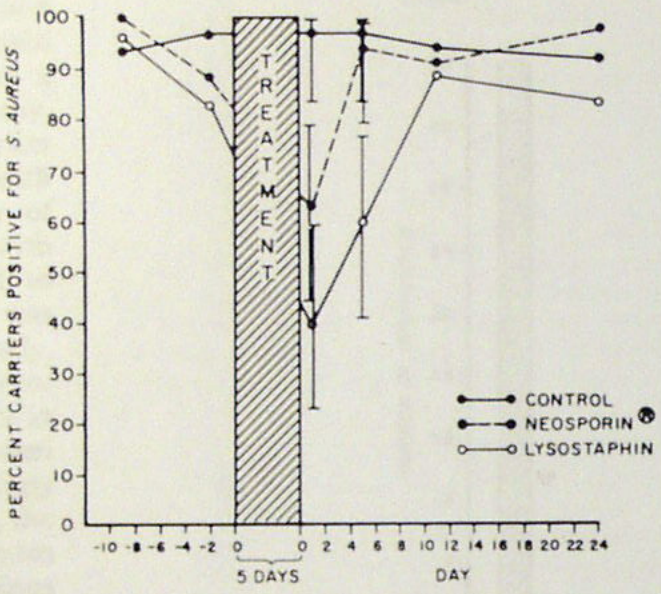

FIG. 1. *Effect of treatment on the recovery of Staphylococcus aureus from persistent carriers.*

No lysostaphin-resistant strains were detected either before or after therapy. The greatest minimum inhibitory concentration recorded was 1.6 μg/ml. Likewise, no strains resistant to either neomycin or bacitracin were detected.

Immunology. No local or systemic reactions were detected in those treated either once or twice with intranasal lysostaphin.

Skin test results with lysostaphin are presented in Table 2. Both erythema and induration were somewhat more frequent in patients previously exposed to lysostaphin than in the controls, but none of these differences is statistically significant. A wheal and flair reaction at 30 min occurred in one person who had been exposed previously to lysostaphin. This reaction subsided within 2 hr, and the patient experienced no delayed or systemic reactions.

Lysostaphin hemagglutination-inhibition titers in 54 individuals with no known exposure to lysostaphin were distributed in a biphasic manner (Fig. 2). Thirty-six (67%) had titers of less than 2, whereas 15 (28%) had titers of 16 or greater. The highest titer detected was 256. After 10 days of lysostaphin therapy, six of 35 persons (17%) had fourfold or greater increases in titer and three (9%) had similar decreases in titer. Among 19 individuals not treated with lysostaphin, five (26%) had fourfold or greater increases in titer and none had decreases of this magnitude. These differences are not significant.

DISCUSSION

When a persistent nasal carrier of *S. aureus* is implicated as the source from which these organisms are infecting others or himself, eradication of the carrier state becomes highly desirable. Because of the unpredictable and frequently inadequate response to conventional intranasal or systemic antibiotics, new therapeutic approaches, such as lysostaphin, are being investigated (7–9).

Intranasal lysostaphin spray significantly reduced the number of carriers of *S. aureus* in the mentally retarded, rigorously defined persistent carriers used as subjects in this study. This effect was apparent on the 2nd and 5th days after the completion of therapy but was not detectable 11 days after treatment was stopped. Carriage rates among the Neosporin group had returned to pretreatment levels by the 5th day after therapy; thus, the effect of lysostaphin is slightly more prolonged than that of Neosporin.

Martin and White (9) noted a 91% reduction in carriage of *S. aureus* after intranasal lysostaphin treatment of healthy medical personnel, with a partial effect persisting for 4 months after therapy. Harris et al. (7) rendered 10 pediatric carriers culture-negative for periods of 10 to 53 days with intranasal lysostaphin. The greater efficacy of lysostaphin in those two studies compared with the present investigation can be attributed to several factors. (i) The persistent carrier state was more rigorously defined in the current study. (ii) All individuals in the present study, institutionalized because of mental retardation, were in close contact possibly facilitating interpersonal transfer of strains. (iii) The duration of therapy was shorter than in the previous studies. Thus, the current investigation constituted a more rigorous test of therapeutic efficacy than did the previous studies.

Eradication of all *S. aureus* from the anterior nares is not necessarily the only criterion of successful treatment. In an epidemiologically important carrier, eradication of the specific phage type of *S. aureus* causing infections may be sufficient, even if the individual continues to carry other strains of *S. aureus*. In the present study, lysostaphin therapy was followed by a signifi-

TABLE 1. *Effect of treatment on carriage of S. aureus phage types*

Study group	No. in group	Pretherapy phage types			Acquired phage types
		Total[a]	Persistence	Elimination	
Lysostaphin	30	45	32 (71)[b]	13 (29)	6
Neosporin	32	46	35 (76)	11 (24)	6
Control	33	48	45 (94)	3 (6)	7

[a] Multiple phage types were carried by some individuals.

[b] Values in parentheses are expressed as percentages.

TABLE 2

Reaction	
Erythema	
Induration	

[a] Number of sites.

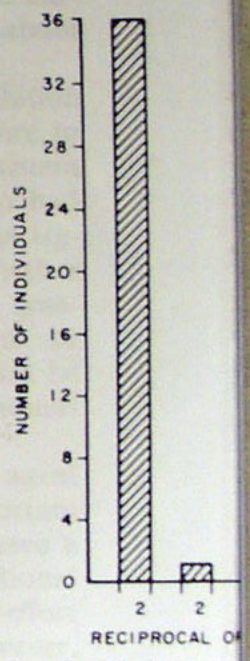

FIG. 2. *Lysosta tibody titers am therapy.*

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TABLE 2. *Skin test reactions to intradermal lysostaphin among lysostaphin-treated and control subjects*

Reaction	Time read	Lysostaphin treated			Controls		
		Subjects	Reacting[a]	Per cent	Subjects	Reacting[a]	Per cent
Erythema	30 min	19	2	11	14	0	0
	24 hr	19	12	63	14	5	36
	48 hr	19	4	21	13	2	15
Induration	30 min	19	2	11	14	1	7
	24 hr	19	6	32	14	1	7
	48 hr	19	2	11	13	0	0

[a] Number of subjects showing more than 5 mm difference between lysostaphin and saline skin test sites.

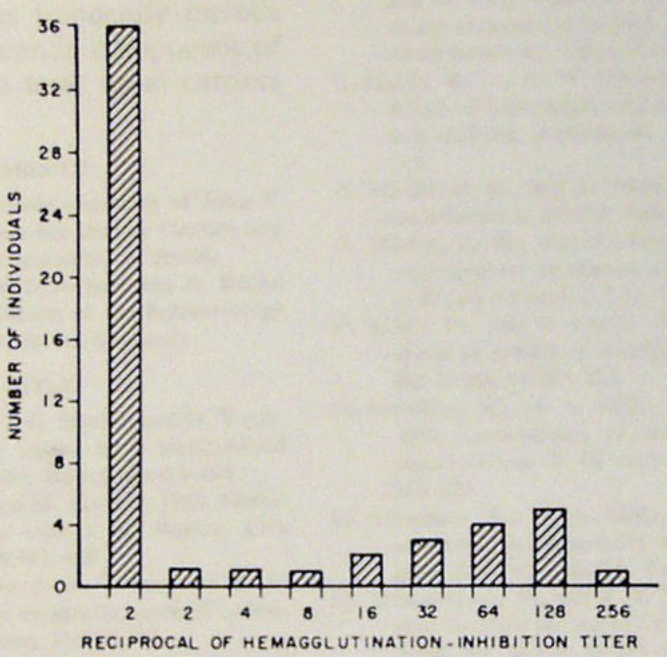

FIG. 2. *Lysostaphin hemagglutination-inhibition antibody titers among 54 persons before lysostaphin therapy.*

cantly greater elimination of specific phage types than occurred in the controls. In contrast, Martin and White (9) could not demonstrate a significant alteration in phage types among their lysostaphin-treated adults who again became positive for *S. aureus* after therapy.

An impressive feature of the present study is the rapidity with which treated individuals again became positive for *S. aureus*, either by reappearance of strains carried before therapy or by acquisition of new strains. Host and environmental factors presumably underlie this predisposition to *S. aureus* carriage and may have contributed to the observed difficulties in altering the truly persistent carrier state in this population.

The antigenicity of lysostaphin has been a major concern in the evaluation of this protein agent. Humans with no known exposure to lysostaphin have rarely had precipitin antibodies to lysostaphin (8), and hemagglutinating antibodies occasionally have developed in lysostaphin-treated persons (7). Previous studies have not encountered clinical manifestations of hypersensitivity in humans exposed to topical lysostaphin, but experience is thus far limited. In the present study, no local or systemic reactions were observed among the 111 individuals exposed to intranasal lysostaphin for the first time or among the 10 carriers who were rechallenged with intranasal lysostaphin. Skin tests performed in 19 individuals 3 months after a 10-day exposure to lysostaphin showed no significant difference from controls. One previously lysostaphin-treated carrier had a transient wheal and flair reaction about his skin test site.

Determination of hemagglutination-inhibition titers in individuals with no known exposure to lysostaphin revealed that 28% of the persistent carriers in this institutionalized population had titers of 16 or more. The titers did not rise significantly after therapy with lysostaphin. Whether hemagglutination-inhibition antibodies to lysostaphin correlated with skin test reactions or therapeutic response to lysostaphin could not be determined with the small numbers of individuals studied.

Lysostaphin may be a potentially useful agent for the treatment of epidemiologically important nasal carriers of *S. aureus*. It appears to have a slight advantage in efficacy over conventional antibiotics with respect to both duration of effect and elimination of specific phage-types. However, until greater clinical experience relating to the safety of this agent has been accumulated, it will not be possible to determine if the slight benefit outweighs the potential risk. The greatest clinical use of lysostaphin may develop in those areas of the world where methicillin-resistant staphylococci assume epidemiological importance. Such

strains tend to be multiply-resistant to antibiotics, and their resistance patterns frequently include neomycin (2) which is a common component of topical preparations used to treat nasal carriers of *S. aureus*.

ACKNOWLEDGMENTS

The authors acknowledge the valuable assistance of John V. Bennett and Helen Camp of the Center for Disease Control and Rita M. Adair of the Rhode Island Department of Health.

J. R. Caldwell, K. E. Quickel, W. Schaffner, and R. Selden were Epidemic Intelligence Service Officers of the Epidemiology Program, Center for Disease Control, during this study.

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