The reacquisition of staphylococci by treated carriers: A demonstration of bacterial interference

R. RUSSELL MARTIN and ARTHUR WHITE *Indianapolis, Ind.*

Three randomly selected groups of nasal carriers of *Staphylococcus aureus* were treated topically with a placebo, lysostaphin, or gentamicin. Gentamicin markedly reduced carrier rates for coagulase-positive staphylococci, coagulase-negative staphylococci, and diphtheroids. Lysostaphin reduced carrier rates only for coagulase-positive staphylococci. Carriers with large numbers of coagulase-negative staphlococci and diptheroids before treatment reacquired coagulase-positive staphylococci significantly less frequently after lysostaphin treatment than after gentamicin treatment. Carriers with small numbers of coagulase-negative staphylococci and diptheroids before treatment reacquired coagulase-positive staphylococci more rapidly and at similar rates in both lysostaphin and gentamicin-treated patients. Thus, the selective in vivo activity of lysostaphin permitted demonstration of the role of bacterial interference in patients with large numbers of residual diptheroids and coagulase-negative staphylococci. Maximal effectiveness in treating carriers required both selective lysis of coagulase-positive staphylococci and the presence of large numbers of other bacteria before treatment.

Lysostaphin is an enzyme isolated from a nonpathogenic strain of staphylococcus which selectively lyses polyglycine bridges found almost exclusively in cell walls of coagulase-positive staphylococci.[1-3] Since similar linkages are rarely present in cell walls of other microorganisms, lysostaphin is highly selective in its activity against coagulase-positive staphylococci in vitro[4, 5] and in experimental animals.[6, 7]

In previous studies, lysostaphin was highly specific in its activity against *S. aureus* in nasal carriers.[8] During treatment with topical lysostaphin, coagulase-positive staphylococci were quickly and selectively cleared from nasal cultures

From the Departments of Medicine, Medical College of Georgia and Indiana University School of Medicine.
Supported by Grants AI 06813, AI 07902, TI 343, and TI 267 from the United States Public Health Service and by a grant from Mead Johnson & Co.
Received for publication Nov. 3, 1967.
Accepted for publication Jan. 15, 1967.

Table I. Frequency of positive cultures of *S. albus*, diphtheroids, and *S. aureus*

Time of treatment	*S. albus* (% positive)			*Diptheroids* (% positive)		
	Placebo	*Lysostaphin*	*Gentamicin*	*Placebo*	*Lysostaphin*	*Gentamicin*
Before	100	100	100	100	100	100
During 1-4 days	100	100	45	100	100	46
5-7 days	100	100	55	100	100	50
After 1 day	100	100	55	100	100	54
1 week	100	100	96	100	100	100
1 month	100	100	100	100	100	100
2 months	100	100	100	100	100	100
4 months	100	100	100	100	100	100

with only slight effect on coagulase-negative staphylococci and diphtheroids. Subjects who had larger numbers of coagulase-negative staphylococci and diphtheroids remaining in the nose after treatment reacquired coagulase-positive staphylococci significantly less frequently than subjects with smaller numbers of residual organisms.

In the present study, three randomly selected groups of nasal carriers of *S. aureus* were treated topically with a placebo, lysostaphin, or gentamicin. Gentamicin markedly reduced carrier rates for coagulase-positive staphylococci, coagulase-negative staphylococci, and diptheroids. Lysostaphin reduced carrier rates only for coagulase-positive staphylococci. Selective lysis by lysostaphin was more effective than less specific treatment with gentamicin only in patients with large numbers of other bacteria in nasal cultures before treatment.

Methods

Subjects in this study were medical students, house officers, and faculty of the Medical College of Georgia. All subjects had at least three consecutive positive cultures for coagulase-positive staphylococci during the 2 weeks before treatment. Subjects were assigned by random selection to be treated with a placebo solution,* lysostaphin solution,* or gentamicin ointment. The lysostaphin solution contained 0.5 per cent lysostaphin in a citrate buffer. The placebo contained buffer without lysostaphin and gentamicin was dispensed at a concentration of 1 mg. of gentamicin base per gram of petrolatum ointment. Treatment was applied three times a day for 7 days. Quantitative nasal cultures[9] were obtained on the first to the fourth day of treatment, fifth to the seventh day of treatment, the first day after treatment, at weekly intervals for one month, and monthly for 4 months after therapy. Over 98 per cent of the planned cultures were obtained from all subjects during treatment and for the first 2 months after therapy. Ninety-five per cent of the cultures were obtained 3 months after therapy was discontinued, and 89 per cent of the cultures were obtained 4 months after therapy was completed. A single technician performed the quantitative cultures, and the treatment regimens were known only after the study was completed.

Propagation of phages and phage typing were by methods described previously.[10]

*Supplied by Mead Johnson Research Center, Evansville, Ind.

isolated from subjects treated with placebo, lysostaphin, or gentamicin

S. aureus (% positive)			*S. aureus* (carrier rates compared with placebo)	
Placebo	*Lysostaphin*	*Gentamicin*	*Lysostaphin* (χ^2: *P*)	*Gentamicin* (χ^2: *P*)
100	100	100		
100	9	16		
100	9	14	< 0.001	< 0.001
100	24	24	< 0.001	< 0.001
96	43	52	< 0.001	< 0.01
96	52	81	< 0.01	> 0.1
95	62	86	< 0.02	> 0.1
91	63	74	< 0.05	> 0.1

Results

Three randomly selected groups of persistent *S. aureus* nasal carriers were treated with intranasal lysostaphin, intranasal gentamicin, or a placebo solution. There were no significant differences in the three groups in the numbers of coagulase-positive staphylococci, coagulase-negative staphylococci, or diptheroids isolated from the nose before therapy.

In the patients treated with the placebo solution, the carrier rates for coagulase-positive staphylococci, coagulase-negative staphylococci, and diphtheroids were 90 per cent or greater before, during, and after therapy (Table I).

During therapy with lysostaphin, the carrier rates for coagulase-positive staphylococci fell to 9 per cent. Following treatment with lysostaphin, the carrier rates for coagulase-positive staphylococci gradually rose, but they remained significantly lower than the carrier rates for the placebo group during the 4 month observation period. The carrier rates for coagulase-negative staphylococci and for diphtheroids were 100 per cent before therapy and did not change during or after treatment with intranasal lysostaphin.

The carrier rates for all three types of bacteria decreased during treatment with gentamicin. The carrier rates for coagulase-positive staphylococci rose more slowly, but the rates one month after discontinuation of therapy were not significantly different either from the carrier rates before therapy or from the carrier rates for patients treated with placebo solution.

The numbers of *S. aureus, S. albus,* and diphtheroids in positive cultures did not change significantly during or after placebo therapy from the numbers isolated before therapy (Table II).

The numbers of coagulase-positive staphylococci isolated from positive cultures of patients treated with lysostaphin fell from a mean log of 5.2 before therapy to a mean log of 3.2 5 to 7 days after therapy was started. The numbers of staphylococci isolated from the positive cultures rapidly returned to pretreatment levels one week after discontinuation of therapy. There was a slight de-

Table II. Numbers of *S. albus*, diphtheroids, and *S. aureus* isolated from positive cultures of subjects treated with placebo, lysostaphin, or gentamicin

Time of treatment	*S. albus*, log of mean of positive cultures			*Diphtheroids*, log of mean of positive cultures			*S. aureus*, log of mean of positive cultures		
	Placebo	*Lysostaphin*	*Gentamicin*	*Placebo*	*Lysostaphin*	*Gentamicin*	*Placebo*	*Lysostaphin*	*Gentamicin*
Before	5.0	5.1	5.1	4.8	5.6	5.2	5.0	5.2	5.1
During 1-4 days	5.2	4.5	3.7	4.8	5.1	2.6	4.8	3.0	2.0
5-7 days	5.1	4.5	3.4	4.9	5.1	3.6	4.8	3.2	2.1
After 1 day	5.3	4.5	4.5	5.0	5.3	4.0	4.7	3.0	4.3
1 week	5.3	4.9	5.2	4.9	5.6	5.6	5.4	4.9	4.5
1 month	4.8	4.9	5.2	4.6	5.8	5.3	4.5	5.2	5.1
2 months	5.1	5.1	5.0	5.0	5.8	4.9	4.6	5.0	5.5
4 months	5.2	5.6	5.2	5.3	5.3	5.2	4.2	5.8	5.1

Table III. Comparison of the reacquisition of coagulase-positive staphylococci in subjects with larger or smaller numbers of coagulase-negative staphylococci and diphtheroids at the end of treatment with lysostaphin or gentamicin

Period following treatment	*Lysostaphin or gentamicin*			*Lysostaphin only*		
	Greater than 10^5 S. albus and diptheroids (10 subjects)	*Fewer than 10^5 S. albus and diptheroids (32 subjects)*	$\chi^2 : P$	*Greater than 10^5 S. albus and diptheroids (9 subjects)*	*Fewer than 10^5 S. albus and diptheroids (12 subjects)*	$\chi^2 : P$
1 day	1/10* (10)	9/32 (28)	> 0.1	0/9 (0)	5/12 (42)	< 0.05
1 week	1/10 (10)	19/32 (59)	< 0.01	1/9 (11)	10/12 (83)	< 0.01
1 month	4/10 (40)	26/32 (81)	< 0.01	3/9 (33)	11/12 (92)	< 0.01
2 months	4/10 (40)	27/32 (84)	< 0.01	3/9 (33)	10/12 (83)	< 0.05
4 months	3/ 8 (38)	23/30 (77)	< 0.05	2/7 (29)	9/11 (82)	> 0.05

*No. positive per No. cultures (per cent positive).

crease in numbers of *S. albus* and diphtheroids isolated from carriers of these strains during lysostaphin therapy, but control values also were reached during the first week after treatment.

During therapy with gentamicin, the numbers of coagulase-positive staphylococci, coagulase-negative staphylococci, and diphtheroids isolated from patients who remained carriers of these organisms fell rapidly. However, the numbers of *S. aureus, S. albus,* and diptheroids isolated from carriers did not differ from the numbers isolated from patients treated with placebo solution during the first week following treatment.

Nine subjects treated with lysostaphin but only one subject treated with gentamicin had more than 100,000 *S. albus* and diptheroids at the end of therapy; the remaining 32 subjects treated with these drugs had less than 100,000. The 10 subjects with larger numbers of residual organisms following

Table IV. Comparison of the reacquisition of coagulase-positive staphylococci following treatment with lysostaphin or gentamicin with the numbers of diphtheroids and *S. albus* isolated before therapy

Period following treatment	*Gentamicin*	*Lysostaphin (12 subjects)*	$\chi^2 : P$
Greater than $10^{5.2}$ S. albus and diphtheroids	(13 subjects)		
1 day	1/13* (6)	2/12 (17)	> 0.1
1 week	6/13 (53)	2/12 (17)	> 0.1
2 weeks	9/13 (69)	2/12 (17)	< 0.01
3 weeks	9/13 (69)	2/10 (20)	< 0.02
1 month	10/13 (77)	5/12 (42)	> 0.05
2 months	11/13 (83)	5/12 (42)	< 0.05
3 months	11/13 (88)	6/12 (50)	> 0.05
4 months	10/12 (83)	5/10 (50)	> 0.05
Less than $10^{5.2}$ S. albus and diphtheroids	(8 subjects)	(9 subjects)	
1 day	4/8 (50)	3/9 (33)	> 0.1
1 week	5/8 (63)	7/9 (78)	> 0.1
2 weeks	5/8 (63)	8/9 (89)	> 0.1
3 weeks	7/8 (88)	8/9 (89)	> 0.1
1 month	7/8 (88)	8/9 (89)	> 0.1
2 months	7/8 (88)	8/9 (89)	> 0.1
3 months	4/7 (57)	8/9 (89)	> 0.1
4 months	4/7 (57)	7/8 (88)	> 0.1

*No. positive per No. cultured (per cent positive).

treatment reacquired coagulase-positive staphylococci slowly and significantly less frequently than subjects with smaller numbers of remaining organisms (Table III). Similar results were obtained by comparing reacquisition of coagulase-positive staphylococci in patients treated only with lysostaphin.

The numbers of diphtheroids and of coagulase-negative staphylococci changed only slightly during therapy with lysostaphin. Therefore, the reacquisition of coagulase-positive staphylococci was also less rapid in those subjects who had large numbers of coagulase-negative staphylococci and diphtheroids before treatment than in those subjects who had small numbers of these organisms before treatment (Table IV). However, the numbers of *S. albus* and diphtheroids isolated from patients treated with gentamicin fell markedly during therapy, and there was no correlation between the numbers of *S. albus* and diphtheroids isolated before therapy and the reacquisition of coagulase-positive staphylococci after therapy. Therefore, in subjects with large numbers of coagulase-negative staphylococci and diphtheroids before therapy, coagulase-positive staphylococci were isolated less frequently following lysostaphin treatment than following gentamicin treatment. In subjects with small numbers of diphtheroids and coagulase-negative staphylococci isolated before therapy, there were no significant differences in the reacquisition of coagulase-positive staphylococci by these two groups of treated patients.

Pretreatment staphylococcal isolates from each subject were phage typed and compared with phage types of isolates from subjects who subsequently had positive cultures for *S. aureus*. Posttreatment isolates were of the same phage type as the initial strains, with the exception of isolates from one gentamicin treated subject.

Discussion

In the present studies, selective treatment of *S. aureus* nasal carriers with lysostaphin was compared with treatment with gentamicin, which is active in vivo against coagulase-positive staphylococci and other bacteria frequently isolated from nasal cultures, *S. albus,* and diphtheroids. Treatment with gentamicin reduced carrier rates for all three groups of organisms but treatment with lysostaphin reduced carrier rates only for coagulase-positive staphylococci.

The selective in vivo activity of lysostaphin permitted demonstration of the role of bacterial interference in subjects with large numbers of residual diphtheroids and coagulase-negative staphylococci. Carriers with large numbers of coagulase-negative staphylococci and diphtheroids before treatment reacquired coagulase-positive staphylococci significantly less frequently following lysostaphin treatment than following gentamicin treatment. However, carriers with small numbers of coagulase-negative staphylococci and diphtheroids before treatment reacquired coagulase-positive staphylococci more rapidly and at similar rates in subjects treated with lysostaphin and in subjects treated with gentamicin. Therefore, maximal effectiveness in treating carriers required both selective lysis of coagulase-positive staphylococci and the presence of large numbers of other bacteria in the nose before treatment.

Several studies have demonstrated that one bacterial strain can interfere with the growth of other strains. Nasal inoculation with a staphylococcal strain of low virulence prevented subsequent colonization with other coagulase-positive staphylococci.[11-15] Also inoculation of experimental burns with avirulent staphylococci prevented infection with more virulent staphylococci.[16] The phenomenon of bacterial interference is not limited to coagulase-positive staphylococci since studies by Ribble and Shinefield[17] and by McCabe[18] have demonstrated that all strains of coagulase-positive staphylococci and coagulase-negative staphylococci that were relatively avirulent for embryonated eggs afforded protection against subsequent challenge with virulent staphylococci, *Diplococcus pneumoniae, Salmonella typhimurium, Escherichia coli, Proteus mirabilis,* and influenze virus. In the present study, large numbers of other nasal bacteria at the end of therapy with lysostaphin prevented subsequent reacquisition of coagulase-positive staphylococci.

The mechanism by which one bacterial strain interferes with the growth of another has not been established. Studies in vitro have shown that depletion of nicotinamide by one bacterial strain will inhibit the growth of other strains.[19] However, in studies with embryonated eggs, nicotinamide did not prevent bacterial interference.[17] Bacterial interference required infection with viable organisms, [16, 17] and some of the interferring strains inhibited growth of the

challenge strains and protection could not be transferred by administration of sterile filtrates of the fluid in which the protective strain had grown.[16, 17]

Further studies of the mechanisms by which bacterial interference occurs in vitro, in experimental animals, and in nasal carriers should be important in controlling the reservoir of staphylococcal disease.

REFERENCES

1. Schindler, C. A., and Schuhardt, V. T.: Lysostaphin: A new bacteriolytic agent for the staphylococcus, Proc. Nat. Acad. Sc. 51: 414, 1964.
2. Browder, H. P., Zygmunt, W. A., Young, J. R., and Tavormina, P. A.: Lysostaphin: Enzymatic mode of action, Biochem. & Biophys. Res. Comm. 19: 383, 1965.
3. Strominger, J. L., and Ghuysen, E. F.: Mechanisms of enzymatic bacteriolysis, Science 156: 213, 1967.
4. Cropp, C. B., and Harrison, E. F.: The in vitro effect of lysostaphin on clinical isolates of *Staphylococcus aureus,* Canad. J. Microbiol. 10: 823, 1964.
5. Harrison, E. F., and Cropp, C. B.: Comparative in vitro activities of lysostaphin and other anti-staphylococcal antibiotics on clinical isolates of *Staphylococcus aureus,* Appl. Microbiol. 13: 212, 1965.
6. Harrison, E. F., and Zygmunt, W. A.: Lysostaphin in experimental renal infections, J. Bact. 93: 520, 1967.
7. Schaffner, W., Melly, M. A., and Koenig, M. G.: Lysostaphin: An enzymatic approach to staphylococcal disease. II. In vivo studies, Yale J. Biol. & Med. 39: 230, 1967.
8. Martin, R. R., and White, A.: The selective activity of lysostaphin in vivo, J. Lab. & Clin. Med. 70: 1, 1967.
9. White, A., Hemmerly, R., Martin, M. P., and Knight, V.: Studies on the origin of drug-resistant staphylococci in a mental hospital, Am. J. Med. 27: 26, 1959.
10. White, A. C., Foster, F., and Knight, V.: Propagation of phages in liquid medium, Antibiotics & Chemother. 9: 81, 1959.
11. Shinefield, H. R., Sutherland, J. M., Ribble, J. C., and Eichenwald, H. F.: Bacterial interference: Its effects on nursery-acquired infection with *Staphylococcus aureus.* II. The Ohio epidemic, Am. J. Dis. Child. 105: 655, 1963.
12. Shinefield, H. R., Boris, M., Ribble, J. C., Cale, E. F., and Eichenwald, H. F.: Bacterial interference: Its effect on nursery-acquired infection with *Staphylococcus aureus.* III. The Georgia epidemic, Am. J. Dis. Child. 105: 663, 1963.
13. Boris, M., Shinefield, H. R., Ribble, J. C., Eichenwald, H. F., Hauser, G. H., and Caraway, C. T.: Bacterial interference: Its effect on nursery-acquired infection with *Staphylococcus aureus.* IV. Louisiana epidemic, Am. J. Dis. Child. 105: 674, 1963.
14. Boris, M., Seller, T. F., Eichenwald, H. F., Ribble, J. C., and Shinefield, H. R.: Bacterial intereference, Am. J. Dis. Child. 108: 252, 1964.
15. Budd, M. A., Boring, J. R., and Brachman, P. S.: Antibiotic-induced modification of resistance to nasal colonization by *Staphylococcus aureus,* Antimicrob. Agents & Chemotherap. 1964: 681, 1965.
16. Bascom, F. A., and Wannamaker, L. W.: Bacterial interference in experimental burns, J. Exper. Med. 125: 319, 1967.
17. Ribble, J. C., and Shinefield, H. R.: Bacterial interference in chick embryos, J. Clin. Invest. 46: 446, 1967.
18. McCabe, W. R.: Bacterial interference induced in embryonated eggs by staphylococci, J. Clin. Invest. 46: 453, 1967.
19. Ribble, J. C.: A mechanism of bacterial interference in vitro, J. Immunol. 98: 716, 1967.