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# In Vivo Effects of Josamycin, Erythromycin, and Placebo Therapy on Nasal Carriage of *Staphylococcus aureus*

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Healthy nasal carriers of *Staphylococcus aureus* were randomly assigned to one of three treatment regimens: josamycin (1.5 g/day), erythromycin stearate (1.0 g/day), or placebo, each administered orally for 7 days. Quantitative nasal cultures were obtained from each subject at intervals before, during, and after treatment. All 25 placebo-treated subjects had positive nasal cultures for *S. aureus* at all culture intervals. Both josamycin and erythromycin were equally effective in reducing the carrier rates and in decreasing the total numbers of *S. aureus* isolated from subjects with positive cultures during treatment. No increase in in vitro antibiotic resistance was detected in isolates obtained after therapy. Both antibiotics were well tolerated, and toxicity was not encountered with either drug.

Josamycin is a new macrolide antibiotic produced by *Streptomyces narboneosis* var. *josamyceticus*. Although josamycin is still an investigational preparation in the United States, it has been used clinically in Japan since 1967 (4). Macrolide antibiotics, especially erythromycin, have been useful as an alternative to semisynthetic penicillins in the treatment of infections caused by *Staphylococcus aureus*. As new macrolide antibiotics are introduced, it is important to determine their relative antistaphylococcal activity, in addition to evaluating toxicity and the incidence of side effects (2).

This study compared the effects of oral therapy with josamycin, erythromycin stearate, and a placebo on *S. aureus* cultured from the nose of healthy carriers. Serial quantitative nasal cultures were obtained to evaluate the effects of therapy on carrier rates and on the number of organisms isolated. In vitro susceptibility of isolates obtained before and after therapy was compared to determine whether resistance was induced by in vivo exposure to either of the antibiotics.

## MATERIALS AND METHODS

Subjects for this study were healthy students and staff at the Baylor College of Medicine. Single quantitative nasal cultures were performed on 414 subjects, of which 103 contained *S. aureus*. From this group, a total of 75 *S. aureus* nasal carriers volunteered for the experimental portion of the study after two additional pretreatment nasal cultures obtained 1 week apart had verified the continued presence of pigmented, coagulase-positive staphylococci. The design of the study was explained to each subject and, before the subject was enrolled, written informed consent was obtained on a form approved by the Baylor Institutional Review Board for Human Research.

In selecting an antibiotic dose, preliminary information was available from Endo Laboratories, Inc., Garden City, N.Y., indicating that comparable blood levels were obtained with divided total daily doses of 1.5 g of josamycin and 1.0 g of erythromycin stearate. Consequently, the dose of josamycin chosen for therapy was higher than that of erythromycin stearate, in an attempt to maintain bioequivalence.

Volunteers were randomly assigned to one of three treatment regimens: 1.5 g of josamycin per day, 1.0 g of erythromycin stearate per day, or placebo. All therapy was administered orally in four divided doses for 7 days. Josamycin was supplied by Yamanouchi Pharmaceutical Company, Tokyo, Japan, as a round, orange, film-coated tablet containing 250 mg of josamycin. Erythromycin stearate was obtained from Abbot Laboratories as a round, pink, film-coated tablet bearing the Abbott Co. logo and containing 250 mg of erythromycin stearate. The placebo was identical in appearance to the josamycin tablet and contained microcrystalline cellulose. Because the two antibiotics were not identical in appearance, and because of the higher dose of josamycin than erythromycin, subjects treated with josamycin received alternating doses of two josamycin tablets or one josamycin tablet and one josamycin placebo, to provide a total daily dose of 1.5 g. Subjects treated with placebo received two josamycin placebo tablets every 6 h. Subjects treated with erythromycin stearate received one erythromycin stearate tablet and one josamycin placebo every 6 h. Medication was prepackaged and coded by Endo Laboratories, and supplied in individual-dose packages. The code was unknown to the investigators

but was accessible in case identification of the treatment regimen of a particular volunteer was required.

Before enrollment in the study, a preliminary history and physical examination was performed, and all subjects were found to be healthy and free of significant acute or chronic medical problems. Blood and urine samples were obtained twice before and 1 day after completion of therapy, to monitor clinical laboratory tests appropriate to detect possible adverse effects of the drug on hepatic, renal, or hematological function. Standard 12-lead electrocardiograms were obtained before therapy and on day 7 of therapy on all subjects.

A diary of side effects and complaints was maintained by each subject during the 7 days of medication and for 2 weeks after completion of therapy. A diary of drug administration was recorded in relation to meals and other activities during the 7 days of therapy.

Serial quantitative nasal cultures included at least two pretreatment cultures, two cultures during therapy, and three post-treatment cultures, which spanned the interval from 1 day to 4 weeks after completion of therapy. Of the planned cultures, 100% were obtained. *S. aureus* nasal carriers were defined as those subjects whose pretreatment nasal cultures consistently yielded pigmented, coagulase-positive staphylococci. Normal rabbit plasma (Difco) was employed in the coagulase tube test.

Three colonies of coagulase-positive staphylococci were selected and stored from the immediate pretreatment cultures from each subject. From post-treatment cultures of subjects who reacquired staphylococci, or who continued to have staphylococci cultured from the nose, three colonies were also obtained for in vitro testing. Pretreatment and post-treatment *S. aureus* isolates were tested for antibiotic susceptibility by the Kirby-Bauer disk susceptibility method, using antibiotic disks purchased from Baltimore Biological Laboratory (BBL). Strains were considered susceptible to erythromycin if a zone diameter of 18 mm or greater was measured around the 15-$\mu$g disk. Three different potencies of josamycin disks were employed (2- 15-, and 30-$\mu$g/disk). Strains were considered susceptible if the zone around the 15-$\mu$g josamycin disk was 18 mm or greater.

Phage typing of selected strains was performed by the State Public Health Laboratory in Austin, Tex.

Quantitative nasal cultures were performed as previously reported (8), using serial 10-fold dilutions of materials swabbed from the anterior part of the nose diluted in Trypticase soy broth (BBL). In addition to plating the cultures on Trypticase soy agar (where *S. aureus* was recognized by pigment production), aliquots were plated on Vogel and Johnson agar (BBL), on which coagulase-positive staphylococci formed characteristic black colonies. The use of Vogel and Johnson medium allowed quantitation of staphylococci when other organisms overgrew the Trypticase soy agar plates and provided more certainty in collecting isolates when this occurred.

Of the 75 volunteers originally enrolled in the study, 1 withdrew before therapy, and 3 were dropped from the study due to clinical illness or abnormal laboratory values detected before medication. Complete data was obtained from 73 treatment courses in 71 volunteers. Two volunteers were treated twice in courses 6 months apart because of incomplete or questionably abnormal clinical laboratory data from the initial course of therapy. One subject (treated with erythromycin) had base-line serum glutamic oxalacetic transaminase values of 21 units on two separate pretreatment tests, whereas a higher value of 37 units (normal range: <40 units) was obtained after 1 week of therapy. The other subject had a moderate decrease in hemoglobin level (16.3 to 14.6 g/100 ml), hematocrit (46.5 to 41.9%), and erythrocyte count ($5.2 \times 10^6$ to $4.7 \times 10^6$/mm$^3$) after 7 days of josamycin therapy. It is doubtful that these alterations represented adverse reactions to either erythromycin or josamycin. However, to be certain, both subjects were retreated, and neither subject had any abnormality after another 7-day course of therapy with the same drug.

## RESULTS

Of the 25 subjects treated with placebo, *S. aureus* was isolated from every nasal culture obtained during pretreatment screening and during the 36 days encompassing therapy and post-treatment follow-up (Fig. 1). This documents that the criteria employed in defining *S. aureus* nasal carriers were effective in identifying a true carrier state. Both josamycin (used to treat 22 subjects) and erythromycin (used in 26 subjects) were equally effective in reducing the incidence of positive nasal cultures for *S. aureus* obtained during therapy. When carrier rates for *S. aureus* were compared 1 day or 1 week after completion of therapy, rates were not significantly different for josamycin- or erythromycin-treated subjects. However, from cultures taken 4 weeks after completion of therapy, 24 of 26 erythromycin-treated subjects

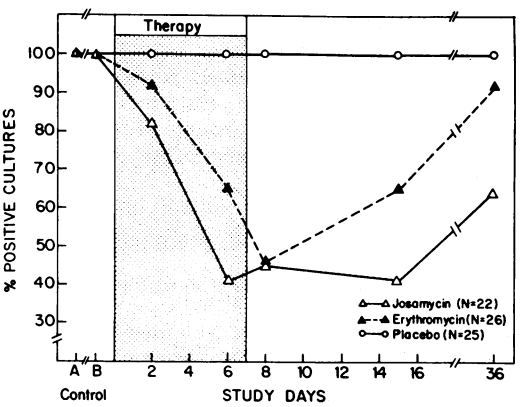


FIG. 1. *Incidence of positive cultures for S. aureus before, during, and after treatment with josamycin, erythromycin, and placebo.*

were once again carriers of *S. aureus* in the nose, whereas only 14 of 22 josamycin-treated subjects were nasal carriers ($\chi^2$, $P < 0.05$).

The mean numbers of *S. aureus* isolated from pretreatment quantitative nasal cultures were very similar from subjects treated with placebo or with erythromycin. The 22 josamycin-treated subjects had slightly lower pretreatment numbers of *S. aureus*, a difference attributable to the presence in the other two groups of several individuals with very high colony counts (Fig. 2). Reproducibility of the quantitative nasal culture method is illustrated by the consistency of the mean numbers obtained from the placebo-treated group, which remained throughout within 0.5 log of the control quantities. In cultures from subjects treated with either of the active antibiotics, over 99% reduction occurred in the numbers of *S. aureus* isolated from cultures that remained positive, and post-therapy numbers of bacteria were similar.

In addition to quantitation of *S. aureus* isolates, the total numbers of all other aerobic bacteria swabbed from the nose were also determined. Colony counts for these organisms were combined, without differentiation as to genera and species. During the 7 days of therapy, and for the 4 weeks after therapy, the total counts of aerobic bacteria isolated from subjects in each of the three treatment regimens ranged within 0.5 log of the control counts for each group. This is in contrast to previous observations of the effects of broad-spectrum, topical therapy with gentamicin in which suppression of the total aerobic nasal flora paralleled the decrease in staphylococci (8).

These pretreatment *S. aureus* colonies from each subject were tested for susceptibility to erythromycin and to josamycin. Post-treatment isolates from all subjects who reacquired or continued to carry *S. aureus* were also tested for susceptibility to both antibiotics. Few of the strains isolated from this group of young, healthy, ambulatory nasal carriers were resistant to either antibiotic. Of the more than 200 pretreatment isolates tested, no josamycin-resistant strains were found, and only three subjects (approximately 4% of the individuals tested) carried erythromycin-resistant strains. None of these three subjects was treated with erythromycin. One subject was treated with placebo, and the same strain (resistant to erythromycin, phage type 85) was consistently isolated. The other two subjects were treated with josamycin. One of these carried phage type 94, 96 before and after therapy. The other (subject no. 160) was a nasal carrier of phage type 52, 52A, 80, 81 on pretreatment culture. Cultures obtained on days 6, 8, and 15 of the study were negative for *S. aureus*. On day 36 (the final day of culture), a new strain of *S. aureus* (phage type 6) was isolated that was susceptible to erythromycin.

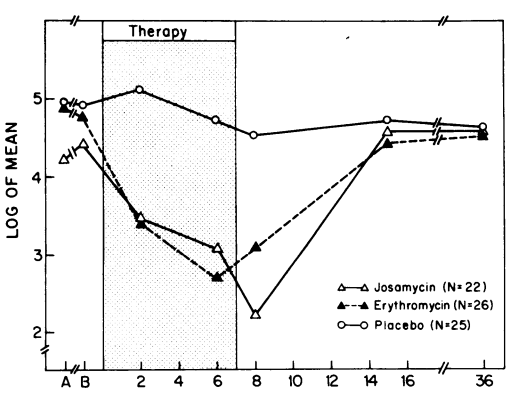


FIG. 2. *Log of mean of colony counts of S. aureus in experiment described in Fig. 1.*

No course of therapy was discontinued because of side effects or toxicities. No laboratory abnormalities definitely attributable to therapy with either antibiotic were documented during these studies. Electrocardiograms done on day 7 of therapy showed no changes. After analysis of the symptom diaries, the incidence of diarrhea, loose stools, or increased number of stools was not significantly greater with josamycin (7 of 22) or erythromycin (9 of 26) than with placebo (5 of 25).

## DISCUSSION

Previous studies employing quantitative nasal cultures have shown that antibiotics modify the nasal flora and that results of quantitative nasal cultures provide a reliable indication of in vivo efficacy of an antistaphylococcal compound (2, 3, 9). When staphylococci are suppressed in the nose by therapy, this effect typically persists only as long as the antimicrobial agent is administered or until an antibiotic-resistant strain colonizes the nose. Usually, when the antistaphylococcal agent is discontinued, the carrier state reappears within a short time (2, 8). Thus, it is not usually rewarding to try to eradicate the nasal carrier state for staphylococci by topical or systemic therapy, even of fairly long duration. Temporary suppression of *S. aureus* in the nose may, however, be of clinical benefit by reducing the reservoir from which these organisms seed the skin and cause clinical disease (1, 8).

The present study was designed to determine whether josamycin is effective against *S. au-*

*reus* in the nose and to compare the in vivo activity of this investigational antibiotic to that of a similar clinically established antibiotic, erythromycin. The results indicate that josamycin and erythromycin are comparable both in reducing nasal carrier rates and in decreasing the numbers of staphylococci cultured during therapy from subjects who remain nasal carriers.

The question of biological equivalence of josamycin and erythromycin is difficult to establish from the present data, since a higher daily dose of josamycin than of erythromycin was selected for treating the staphylococcal carriers. When the study was designed, the decision to use a higher dose of josamycin than erythromycin was based on preliminary bioequivalence information, which indicated that similar blood levels were obtained from oral administration of 1.5 g of josamycin and 1.0 g of erythromycin stearate daily. Subsequently, it has been documented that higher josamycin levels may be obtained after multiple doses, and that these antibiotics may be biologically equivalent (6). Consequently, the amount of antistaphylococcal activity achieved by josamycin may have been higher than that obtained by the dose of erythromycin.

Studies of the acquisition of antibiotic resistance by staphylococci after in vitro exposure to antibiotics have suggested that josamycin is less likely to induce resistance than erythromycin (4). Testing of pre- and post-therapy isolates in the current study failed to identify the development of resistance to either antibiotic after the 7 days of oral therapy. The low incidence of resistance in pretreatment cultures (0% to josamycin and 4% to erythromycin) are similar to reports from other laboratories in the United States in which staphylococci have been tested for in vitro susceptibility (5, 7).

The percentage of carriers who reacquired staphylococci after 1 week of oral therapy was similar for josamycin- or erythromycin-treated subjects until the final culture, 36 days after initiation of therapy, when carrier rates for the josamycin-treated group were significantly lower. The reason for this difference is not readily apparent, since it is difficult to relate the effects of the 1 week of therapy to changes in the carrier rates 4 weeks later.

From subjects whose cultures were positive for *S. aureus*, lower numbers of staphylococci were isolated 1 day after completion of therapy from josamycin-treated subjects than from those who received erythromycin. This may reflect lower initial staphylococcal counts in the subjects treated with josamycin. Alternatively, the effectiveness of josamycin in the nose may have been greater than that of erythromycin 24 h after therapy. In a small group of subjects, josamycin has been documented to be present in saliva and in lacrimal secretions in a concentration higher than those usually obtained with erythromycin (6).

In this study, josamycin was found to have low toxicity, few side effects, and good in vivo effectiveness against nasal *S. aureus*.

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