Serum Antibacterial Activity After Oral Suspensions and Capsules of Triacetyloleandomycin and Erythromycin Estolate

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Comparison of serum antibacterial activity against a beta-hemolytic streptococcus and a penicillin-resistant staphylococcus was made in a cross-over study in volunteers after ingestion of oral suspensions and capsules of triacetyloleandomycin and erythromycin estolate. Oral suspensions yielded earlier peak titers, but ultimate peak titers and duration of activity were similar to those observed after ingestion of capsules. Antibacterial activity of serum against both organisms was consistently greater with both erythromycin estolate preparations than with the triacetyloleandomycin preparations. These in vitro data were comparable to observations made previously in monkeys infected with the same organisms, although comparative clinical efficacy in monkeys did not reflect these implied therapeutic differences.

Comparison of therapeutic effects of different antibiotics in serious infections in humans under strictly controlled conditions is a difficult goal to attain. Thus, one frequently must resort to comparison of different agents on certain pathogens in vitro and in experimental infections in animals as well as studies of the comparative antibacterial activity (ABA) of the patient's serum after administration of the antibiotic.

Previous studies from this laboratory have demonstrated that triacetyloleandomycin is an effective agent in the therapy of streptococcal (20) and staphylococcal (19, 24) sepsis in monkeys. Although the organisms employed were more sensitive to erythromycin and higher ABA was observed after erythromycin administration, mortalities were significantly greater after erythromycin ethylsuccinate and greater, but not significantly so, after erythromycin estolate than after triacetyloleandomycin.

The dosage forms employed in the studies with monkeys were commercially available oral suspensions, and serum ABA was measured against the streptococcus and staphylococcus employed in these studies. For comparative purposes, the present study was conducted in humans, assessing the serum ABA after administration of oral suspensions and capsules of both triacetyloleandomycin and erythromycin estolate.

MATERIALS AND METHODS

Each of 10 medical students weighing 33 to 90 kg (mean, 74.5 kg) was given two 250-mg capsules of triacetyloleandomycin, with a convenient amount of water, after an overnight fast. Doses per kilogram ranged from 5.6 to 9.4 mg (mean, 6.9 mg/kg). Blood samples were obtained before and 1, 2, 4, and 8 hr after the single dose. No food was taken until after the 2-hr sample. The same 10 subjects subsequently were given 500 mg of triacetyloleandomycin oral suspension, two 250-mg capsules of erythromycin estolate, and 500 mg of erythromycin estolate oral suspension. All antibiotics were direct purchases from a local pharmacy. A 4-day interval between medications was employed to prevent carry-over of the preceding dose. The four phases of the study were identical in design except for the antibiotic and dosage form utilized. With all four preparations, doses are expressed in terms of oleandomycin and erythromycin base equivalents.

The test organisms used in measuring serum ABA were Staphylococcus aureus phage type 80/81 and Streptococcus hemolyticus group A (Stollerman T14 strain), the same strains used previously in this laboratory in studies of therapy of staphylococcal (19, 21, 22, 24) and streptococcal infections (20, 23) in monkeys. Tests were performed as described previously (14, 17). In brief, serial twofold dilutions of serum were prepared in 0.5-ml amounts of Trypticase Soy Broth (TSB; BBL). Each tube was inoculated with 0.05 ml of a 1:1,000 dilution, in TSB, of a 6-hr TSB culture of the staphylococcus or with the same volume of a 1:100 dilution, also in TSB, of a 7-hr TSB culture.
of the streptococcus. Tests were incubated at 37°C for 16 to 18 hr; inhibitory end points were read as the greatest serum dilution showing no growth on visual examination of the tubes in a good light.

Minimal inhibitory concentrations (MIC) of oleandomycin and erythromycin bases for the staphylococcus used in measuring serum ABA were 0.31 and 0.078 μg/ml, respectively; the MIC values for the streptococcus were 0.112 and 0.008 μg/ml respectively.

RESULTS

Triacetyloleandomycin and erythromycin estolate both were absorbed more rapidly when given as an oral suspension than when given in capsule form (Fig. 1 and 2). Serum staphylococcal ABA at 1 hr was significantly higher \( (P < 0.01) \) in subjects given triacetyloleandomycin oral suspension than in the same subjects receiving capsules (Table 1). Peak antistaphylococcal activity was noted at 1 hr in 8 of 10 subjects given oral suspension and at 2 hr in the remaining two, whereas 3, 5, and 2 of 10 exhibited peak titers at 1, 2, and 4 hr, respectively, after taking capsules. In addition, triacetyloleandomycin in oral suspension was absorbed more uniformly than was triacetyloleandomycin in capsules. For example, all 10 subjects given oral suspension showed serum staphylococcal ABA at dilutions of 1:4 or 1:8 at 1 hr, whereas titers of <1:2, 1:2, 1:4, and 1:8 were observed at this time in 2, 3, 2, and 3 of 10 subjects, respectively, given capsules. Geometric mean antistaphylococcal titers were not significantly different in the two groups at 2 hr, although titers were slightly higher in subjects receiving oral suspension (Fig. 1). At 4 hr, the reverse was true; titers were slightly higher, but not significantly so, in those given capsules. At 8 hr, titers had declined to 1:2 or 1:4 in both groups.

Similar results were obtained when sera from subjects given triacetyloleandomycin capsules and oral suspension were compared by using the streptococcus as the test organism (Fig. 2). Serum streptococcal ABA was significantly higher \( (P < 0.01) \) at 1 hr in those given oral suspension than in those given capsules (Table 1). In addition, peak antistreptococcal titers were observed at 1 hr in 9 of 10 in the former group, whereas 4, 4, and 2 of 10 given capsules exhibited peak titers at 1, 2, and 4 hr, respectively. Anti-streptococcal titers were similar in the two groups at 4 and 8 hr.

Erythromycin estolate was also absorbed more rapidly when given as oral suspension (Fig. 1); antistaphylococcal titers were significantly higher \( (P = 0.05) \) at 1 hr than in subjects given capsules. However, titers in the latter group continued to rise rapidly after the 1st hr so that geometric means at 4 and 8 hr were slightly higher, but not significantly so, than in the group receiving oral suspension. Use of the streptococcus as the test organism yielded similar results (Fig. 2).

Triacetyloleandomycin was absorbed more rapidly than erythromycin estolate, particularly
| Antibiotic and dosage form | Test organism | Time (hr) after dose* | Volunteer no. | Mean^b titer | Peaks |
|---------------------------|--------------|----------------------|---------------|--------------|-------|
|                           |              | 1 2 3 4 5 6 7 8 9 10 |               |              |       |
| TC^c                      | Staphylococcus | 1 4d 2 <2 <2 (8) 2 8 (8) (4) 2 |               | 3.2          | 8.8  2.1 |
|                           |              | 2 (8)* <2 4 8 (8) (16) 8 4 (8) |               | 6.5          |       |
|                           |              | 4 4 8 (16) (8) 4 8 4 4 8 8 |               | 6.4          |       |
|                           |              | 8 2 2 4 2 2 4 2 1 2 2 2 |               | 2.6          |       |
| TS                        | Staphylococcus | 1 4 (8) 8 (8) 8 (4) (8) 8 (8) (8) (8) (8) |               | 7.2          | 8.0  1.2 |
|                           |              | 2 (8) 8 8 (16) 8 8 8 8 8 |               | 8.0          |       |
|                           |              | 4 4 8 8 8 4 4 4 4 4 4 |               | 5.2          |       |
|                           |              | 8 2 2 4 2 2 4 2 2 2 2 |               | 2.6          |       |
| EC                        | Staphylococcus | 1 <2 16 <2 <2 16 4 32 <2 2 16 8 |               | 5.2          |       |
|                           |              | 2 16 (128) 16 64 (8) 64 16 16 (32) 8 |               | 22.4         |       |
|                           |              | 4 (64) 128 (32) (128) 8 (128) (32) (64) 32 (32) |               | 51.2         |       |
|                           |              | 8 32 32 16 16 4 64 16 16 8 16 16 |               | 17.6         |       |
| ES                        | Staphylococcus | 1 8 (64) 8 16 8 64 16 16 32 32 (32) |               | 22.4         | 48.0 2.6 |
|                           |              | 2 16 64 16 16 (16) (128) 32 (64) (64) 32 (32) |               | 35.2         |       |
|                           |              | 4 (32) 64 (32) (32) 16 64 (64) 64 64 32 |               | 44.8         |       |
|                           |              | 8 32 16 16 8 4 32 16 16 16 8 |               | 12.8         |       |
| TC^c                      | Streptococcus | 1 (16)d 2 <2 <2 (16) 2 8 (8) (4) 4 |               | 4.0          | 12.0 2.0 |
|                           |              | 2 16 (16) 2 4 8 (16) (16) 8 4 (8) |               | 7.6          |       |
|                           |              | 4 8 8 (16) (16) 8 8 8 4 4 4 |               | 7.6          |       |
|                           |              | 8 2 4 8 8 2 4 4 2 2 2 |               | 3.4          |       |
| TS                        | Streptococcus | 1 16 (16) (16) (16) (16) (16) (16) (8) (8) (8) (8) |               | 12.8         | 13.6 1.1 |
|                           |              | 2 (32) 16 16 16 16 8 16 16 8 8 8 |               | 13.6         |       |
|                           |              | 4 8 4 8 4 4 4 4 4 4 4 |               | 7.6          |       |
|                           |              | 8 3 4 4 4 2 4 4 2 2 2 |               | 3.6          |       |
| EC                        | Streptococcus | 1 4 64 4 128 (32) 256 4 8 (64) 32 |               | 25.6         | 230.4 3.0 |
|                           |              | 2 16 (512) 64 (512) 32 256 64 64 64 32 (32) |               | 89.6         |       |
|                           |              | 4 (256) 512 (256) (512) (256) (256) (256) (256) 64 (256) |               | 230.4        |       |
|                           |              | 8 64 128 64 128 8 512 128 128 32 64 |               | 83.2         |       |
| ES                        | Streptococcus | 1 64 (512) 32 128 32 (1,024) 128 (256) 64 128 128 128 |               | 128.0        | 307.2 2.3 |
|                           |              | 2 (128) 512 128 128 (128) (1,024) 256 256 256 (256) |               | 230.4        |       |
|                           |              | 4 128 256 (512) (256) (256) (512) (256) (256) 256 256 128 243.2 |               | 230.4        |       |
|                           |              | 8 64 128 256 64 128 16 256 128 64 64 32 |               | 83.2         |       |

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* All sera obtained before dose showed no activity at 1:2, the lowest dilution tested.

b Means of titers are geometric means; means of hours are arithmetic means.

c TC and TS = triacylcholeandomyin capsules and oral suspension. EC and ES = erythromycin estolate capsules and oral suspension.

d Reciprocal of serum dilution inhibitory for the staphylococcus or streptococcus in tube dilution (broth) test.

e Peak titers in parentheses.
when given as oral suspensions. For example, peak antistaphylococcal titers were observed at 1 hr in 8 of 10 subjects given triacetyloleandomycin oral suspension and at 2 hr in the remaining two (Table 1). In contrast, 8 of 10 and 2 of 10 given erythromycin estolate oral suspension exhibited peak antistaphylococcal titers at 2 or 4 hr and at 1 hr, respectively (Table 1). This difference was highly significant ($P < 0.01$). Similarly, peak antistaphylococcal titers were observed later in subjects given erythromycin estolate capsules than in those given triacetyloleandomycin capsules (Table 1). This difference was less significant ($P = 0.05$) than when peak titers after the two oral suspensions were compared ($P < 0.01$).

For closer scrutiny, the data on each individual in this study, in reference to ABA observed at different time intervals after each of the four preparations, are shown in Table 1.

**DISCUSSION**

Previous studies from this laboratory comparing triacetyloleandomycin and erythromycin in experimental streptococcal (20) and staphylococcal (19, 24) infections in monkeys employed commercially available oral suspensions of the antibiotics. Thus, one of the purposes of the present study was to compare the antibacterial effect of human serum against the same organisms after administration of oral suspensions of triacetyloleandomycin and erythromycin estolate. This cross-over study allowed the additional opportunity to compare the results obtained after ingestion of capsules at the same dose level. It is not the purpose of this paper to attempt to review the literature on comparative absorption and antibacterial activity of macrolide antibiotics. Such an attempt to assess the differences in agents, taking into account the differences in preparations, dose, methods of assay, time intervals of study, and other variables, would not necessarily contribute to the assessment of the role of the monkey as a biological model for therapy studies.

In the present study in man, oral suspensions of erythromycin estolate yielded greater antibacterial activity against the streptococcus and staphylococcus than did triacetyloleandomycin. After erythromycin estolate oral suspension, mean peak titers of antistreptococcal and antistaphylococcal activity of 1:307 and 1:48, respectively, were observed as compared to 1:14 and 1:8, respectively, after triacetyloleandomycin. The 500-mg dose in man represented approximately 7 mg/kg. In normal monkeys receiving a similar dose of 6.25 mg/kg, peak antistaphylococcal titers did not exceed 1:2 in all three animals studied after receiving triacetyloleandomycin, but only one of three showed detectable activity above 1:2, with a peak titer of 1:4 after the same dose of erythromycin estolate (24). After triacetyloleandomycin and erythromycin estolate doses of 12.5 mg/kg, peak titers were 1:4 to 1:8 and 1:2 to 1:8, respectively, and comparable 1:4 to 1:16 peak titers were observed after 25 mg/kg of either antibiotic. Thus, in contrast to man, in whom antistaphylococcal activity of serum was considerably greater after erythromycin estolate than after triacetyloleandomycin, similar levels were observed in normal monkeys. However, in sera from infected monkeys (24), antistaphylococcal activity was consistently higher after erythromycin estolate and the relationship was similar to that observed in normal humans. Greater antibacterial activity against the streptococcus was seen after erythromycin estolate than after triacetyloleandomycin in the sera of infected monkeys (20).

In essence then, comparative antibacterial activity in serum of infected monkeys more closely simulated that observed in normal humans, whereas differences in activity in normal monkeys receiving triacetyloleandomycin and erythromycin estolate were not remarkable. The obvious missing parameter is a controlled study of the sera of patients with severe sepsis receiving these agents; however, this is an unattainable goal. In studies of a less critical beta-streptococcal illness, however, Breese (1, 2) noted that the higher serum levels found in patients treated with erythromycin estolate than in patients treated with triacetyloleandomycin were not necessarily reflected in superior clinical results.

These studies do not suggest that antibacterial activity is not an important factor in assessing the potential efficacy of an antibiotic. They do, however, raise the question as to whether the clinical superiority of one agent over another can be extrapolated on comparative values. It would seem that once the therapeutic level is reached for a susceptible organism, satisfactory results can be expected. This level, as presently measured in terms of serum, usually does not necessarily predict what is going on in the tissues of the body.

The basic observations in this particular study comparing the two different preparations of triacetyloleandomycin and erythromycin estolate in humans follow the pattern one would expect after review and analysis of many excellent papers (3–13, 15, 16, 18) covering different facets of comparative studies of macrolide antibiotics. However, none of the previous studies compared all four preparations in a cross-over experiment. The present study demonstrated that oral suspensions showed earlier absorption, but peak levels and duration of demonstrable antibacterial ac-
tivity were similar when oral suspensions or capsules were administered. The study also demonstrated higher serum activity with erythromycin estolate, which could be accounted for, in part, by the greater sensitivity of the streptococcus and staphylococcus to this antibiotic.

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