Penetration of Lincomycin, Penicillin, and Tetracycline into Serum and Bone* (33494)

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Although lincomycin has been highly recommended for use in the treatment of osteomyelitis on the basis of its apparent ability to penetrate into bone (1, 2), there has been only one comprehensive study reported in which this property was measured quantitatively (3). In that study a comparison was made between lincomycin, tetracycline, and erythromycin after oral administration to rats. The concentration of antibiotic in the serum and in the femur was assayed by the paper disc method utilizing *Bacillus cereus*. Lincomycin produced higher serum and bone concentrations than the other two antibiotics. However, the serum concentrations, and thus the bone levels, could be affected by differences in chymal binding and intestinal absorption of the drugs. Therefore, in the present investigation, the penetration of lincomycin, penicillin, and tetracycline into serum, femur, and mandible was compared after intramuscular injection. Moreover, the more accurate serial tube dilution method was used with *Staphylococcus aureus* as the test agent. Although this organism is not as sensitive as *B. cereus* to these antibiotics, it is a type against which they are frequently employed. The response, therefore, is a more reliable measure of clinical effectiveness.

Materials and Methods. Initially, 10 sensitivity tests were carried out on each antibiotic.[1] The assays were performed as described by Schaub and Foley (4). The dilution ranges of the antibiotics were as follows: 0.19–100 μg/ml for lincomycin, 0.11–100 μg/ml for tetracycline, and 0.0006–12 μg/ml for penicillin G. Overnight broth cultures of the organism were diluted to 1:1000 in nutrient broth, and 0.1 ml of the inoculum was added to each dilution tube. The tubes were incubated at 37° for 18 hr and the minimum concentration of antibiotic which inhibited growth of the organism was determined by gross inspection. The results of these tests demonstrated the minimum inhibitory concentration (MIC) to be 1.26 ± 0.33 μg/ml for lincomycin, 0.27 ± 0.01 μg/ml for tetracycline and 0.024 ± 0.000 μg/ml for penicillin.

Thirty-six female albino rats, weighing between 250 and 350 g were used for testing each antibiotic. The rats were injected intramuscularly in the hind leg with a 50-mg/kg dose of the drug. Nine animals were sacrificed from each group at intervals of 30 min, 1, 2, and 3 hr after the injection. The animals were given an anesthetic dose of sodium pentobarbital and subsequently killed by exsanguination through cardiac puncture. The blood from the animals of each time period was pooled and allowed to coagulate. After centrifugation, a 2-ml aliquot of the serum was taken for analysis.

The femurs and mandibles were dissected from the animals and freed of soft tissues under aseptic conditions. The femurs were split lengthwise and the marrow was removed. The teeth and condylar cartilages were removed from the mandibles. The 18 femurs from each time period were pooled and ground in a Culatti bone grinder through a grid with 0.7-mm holes. The same process was carried out with the mandibles. Bone powders from the rats receiving penicillin or lincomycin were suspended in 2.5 ml of sterile de-ionized water. In preliminary tests, these antibiotics proved to be 100% extractable by this method. Bone powders from the tetracycline injected rats were suspended in

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[1] The antibiotics for this study were generously supplied by the Upjohn Company, Kalamazoo, Michigan.

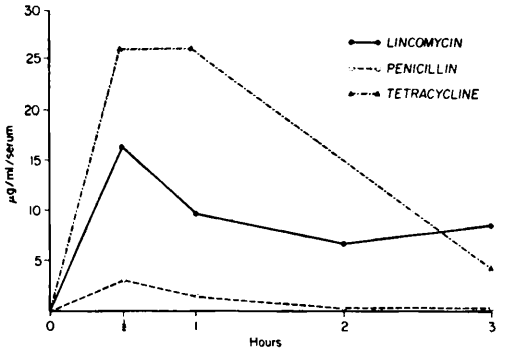

FIG. 1. Concentration of antibiotics in serum.

2.5 ml of sterile, pH 6.2, phosphate buffer because a previous study (3) reported more successful recovery with this solution. The suspensions were placed in a mechanical shaker for 1 hr at room temperature and then were centrifuged for 10 min at 2000 rpm. The supernatants and sera were assayed for antibiotic activity by the previously described serial tube dilution method (4). The concentration of antibiotic in the bone powders was determined as follows:

$$\frac{2^y \times \text{MIC} \times 2.5\text{ ml}}{\text{g of wet bone powder}} = \mu\text{g/g of bone.}$$

For the serum, the following formula was used:

$$2^y \times \text{MIC} = \mu\text{g/ml of serum.}$$

In each instance, y equals the number of tubes in which growth was inhibited.When no growth inhibition was noted, the antibiotic concentration was considered as zero. However, the true level could have been at any point between the MIC and zero.

Results. The average bone and serum concentrations of the three antibiotics are shown in Figs. 1–3. Tetracycline gave the highest initial serum levels but fell below that of lincomycin at the 3-hr period (Fig. 1). Penicillin G consistently attained the lowest concentrations, being barely detectable at 3 hr. All 3 drugs reached their peak concentrations at 30 min.

In the femur, lincomycin produced higher levels than penicillin. However, while the peak concentration of lincomycin was present at 1 hr, that of penicillin was achieved at 30 min. No penicillin was detectable after the second hour whereas lincomycin was present during the entire test period (Fig. 2).

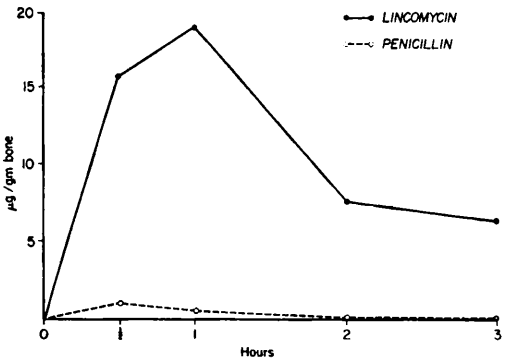

FIG. 2. Concentration of antibiotics in femoral bone powder. Values are expressed in terms of wet weight.

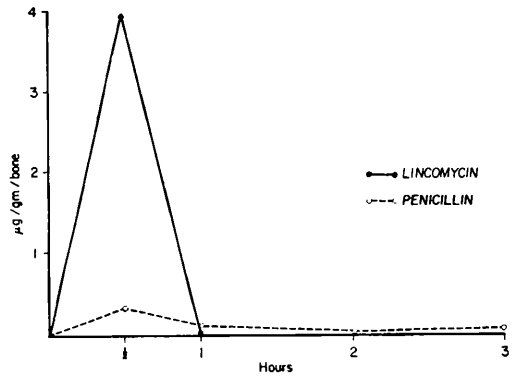

FIG. 3. Concentration of antibiotics in mandibular bone powder. Values are expressed in terms of wet weight.

Both lincomycin and penicillin reached peak levels in the mandible at the end of 30 min, but again lincomycin attained the highest concentration (Fig. 3). Lincomycin was not detectable after the first 0.5 hr but small amounts of penicillin were measured throughout the 3 hr of the study. Tetracycline was not detectable in either femur or mandible by the method employed.

Discussion. The results of these experiments clearly demonstrate that lincomycin produces greater bone concentrations than penicillin G. While this is partially a reflection of the dissimilar blood levels resulting from differences in absorption from the injec-

tion site, comparison of the blood–bone concentration ratios indicates that the greater ability of lincomycin to penetrate into bone is also a factor. Whether it is superior to tetracycline could not be determined because the method used in this study did not extract the strongly bound drug from the bone in concentrations high enough to be detected by the test organism. It was possible, however, to show its presence qualitatively by comparing the effect of femoral bone powders from normal and tetracycline-treated rats on an inoculated broth. The cultures were incubated for 18 hr, Gram stained, and viewed microscopically. Those which contained tetracycline bone powders showed a greatly diminished bacterial growth.

Bound tetracycline could be extracted and measured by lowering the pH of the extraction solution or decalcifying the bone with EDTA. However, this would provide an answer without clinical significance since it would still not be possible to determine what fraction of the total tetracycline was available in active form at any given time.

Although it is not possible to compare the bone levels of tetracycline attained in this study with those measured by Grady and Stern (3), one can compare the blood concentrations. Following oral administration they found the levels of lincomycin to be higher than those of tetracycline. However, in the present study, the situation after parenteral administration was reversed. This difference indicates that variations in chymal binding and absorbability from the intestinal tract do affect the blood levels and therefore the bone concentrations of the two antibiotics. It is probably this factor, rather than a greater ability to penetrate into bone, that accounts for the higher bone levels of lincomycin reported by Grady and Stern (3).

While lincomycin penetrated into the bone in greater concentrations than penicillin, this factor alone does not qualify it as a superior antibiotic. The efficiency of an antibiotic is dependent not only upon the amount of active drug penetrating into a desired area and the duration of its presence, but also upon the sensitivity of the organism to the antibiotic employed. Thus, while the penetrance of lincomycin is 8–10 times that of penicillin (Figs. 2, 3), if one were treating an osteomyelitis caused by *S. aureus*, it could theoretically require 53 times[2] more lincomycin than penicillin in the area to produce the same bactericidal effect.

Although lincomycin and penicillin penetrated into both femur and mandible, the demonstrated levels were comparatively higher in the femur (Figs. 2, 3). In all probability this resulted from the greater proportion of vascular cancellous bone in the former structure. This emphasizes another important factor, the bone type, that should be considered in determining dosage for the antibiotic treatment of osteomyelitis.

Summary. The penetration of lincomycin into serum, femur, and mandible was compared with penicillin G and tetracycline following intramuscular injection. The concentrations were assayed by the serial tube dilution method using *S. aureus*. Lincomycin produced higher concentrations than penicillin in serum and bone. Although tetracycline gave higher serum levels than the other two antibiotics, a sufficient quantity could not be extracted from the bone by the method used to permit detection by the test organism. However, its presence was confirmed by the bacteriostatic effect of the bone powder upon inoculated broth cultures.

[2] MIC (lincomycin) = 1.26 μg/ml; MIC (penicillin) = 0.024 μg/ml.

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