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Ciprofloxacin Concentrations in Bone and Muscle after Oral Dosing

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Ciprofloxacin, a quinoline derivative with marked gram-negative and staphylococcal activity, may be a valuable orally administered agent for use against soft-tissue and bone infections. The concentrations of this antibiotic in serum, bone, and muscle samples were determined in patients undergoing orthopedic surgery. A total of 18 patients undergoing hip or knee replacement surgery or osteotomy were randomized to receive single oral doses of ciprofloxacin (500 mg, 750 mg, or 1 g); 10 patients with osteomyelitis were given single doses of 500 or 750 mg. Mean levels in bone of more than 1 μg/g were achieved with the 750-mg ciprofloxacin doses in patients with osteomyelitis (1.4 ± 1 μg/g) or with the 1-g doses in patients without infections (1.6 ± 0.6 μg/g). The levels in muscle were significantly higher with each increasing dose level. Orally administered ciprofloxacin (750 mg given every 12 h) should provide adequate concentrations in bones and soft tissues to treat most osteomyelitis and soft-tissue infections.

Ciprofloxacin, the most potent in vitro oxyquinolone derivative, has marked activity against gram-negative bacteria, including *Pseudomonas*, *Serratia*, and *Enterobacter* spp., and staphylococci (1, 5, 6, 11, 15). The MICs of ciprofloxacin for 90% of the members of the *Enterobacteriaceae*, *Pseudomonas* spp., and *Neisseria* spp. are between 0.005 and 0.8 μg/ml (5, 6). This antibiotic also has significant gram-positive activity; it inhibits all strains of staphylococci at concentrations of ≤1.0 μg/ml (5, 6).

Ciprofloxacin has been shown to be bactericidal at concentrations close to the MIC and to be active against multiply resistant strains of *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and methicillin-resistant *Staphylococcus aureus*. Unlike nalidixic acid, emergence of resistance in vitro is low and occurs at frequency of between 10^{-7} and 10^{-9} (5). In view of its excellent activity and wide spectrum, orally administered ciprofloxacin may be suitable for treatment of some systemic infections besides urinary tract infections. One area where an orally administered broad-spectrum drug would be of great benefit to patients and result in tremendous cost savings to the health care system is in the treatment of gram-negative or mixed bacterial osteomyelitis, for which prolonged hospitalization and parenteral therapy are usually required.

The purpose of this study was to examine the adequacy of penetration and actual concentrations of ciprofloxacin in bones and muscles after single oral doses.

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MATERIALS AND METHODS

A total of 28 patients requiring orthopedic surgery were studied after obtaining informed, written consent. Eighteen patients undergoing hip or knee replacement surgery or osteotomy were randomized to receive single oral doses of 500 mg, 750 mg, or 1 g of ciprofloxacin 1.5 to 4.75 h before surgery. Ten patients with chronic osteomyelitis requiring debridement were given single doses of 500 or 750 mg of ciprofloxacin 2 to 4.5 h before surgery. An attempt was made to standardize the dosing 2 h before the scheduled operating time. Control bone and muscle samples were obtained from patients who were not enrolled in the study.

Bone and muscle preparation. Strips of cortical bone were removed during surgery and rinsed in sterile saline to remove excess blood. The bone was dissected free of soft tissue, split lengthwise, and scraped free of marrow. Then it was rinsed again in sterile saline and dried. Any remaining marrow was removed, and, after freezing at −70°C, the bone was crushed to a fine powder by using a recoil action mortar and pestle (Thermovac Industries Corp., Copiaque, N.Y.). Strips of muscle were also removed (dissected free of blood vessels) and rinsed in sterile saline; these samples were kept frozen at −70°C until they were ready for analysis, when they were ground to a fine mixture.

The prepared bone and muscle samples were weighed and then extracted with a solution containing 0.1 mol of phosphate buffer (pH 6) per liter at the ratio of 2 ml of phosphate buffer to 1 g of solid. The extraction was done at room temperature by using 1 h of magnetic stirring, followed by 23 h of mixing on a rotary mixer. After centrifugation the supernatant was analyzed for hemoglobin, myoglobin, and ciprofloxacin. The residual bone or muscle was washed three times with 2 ml of phosphate buffer and centrifuged after each wash, and the supernatant was removed by suction. Additional extractions were then carried out by using the same procedure.

Hemoglobin and myoglobin analysis. Patient blood hemoglobin levels were determined with a model S Plus IV counter (Coulter Electronics, Inc., Hialeah, Fla.). The hemoglobin levels in muscle and bone extracts were determined spectrophotometrically at 580 nm. The relative amounts of myoglobin and hemoglobin in muscle samples were measured by using electrophoretic separation on cellulose acetate with Tris-barbital buffer (pH 8.6) at 265 V for 30 min (8). Bands were visualized by using a benzidine-nitroprusside-peroxide stain. Since the extinction coefficients of hemoglobin and myoglobin in the region of 580 nm are very similar (4, 16), the amount of hemoglobin determined by absorption at 580 nm was reduced by the percentage of myoglobin found by electrophoresis. The amounts of drug in the bone and muscle samples were corrected for

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TABLE 1. Demographic characteristics of the patients studied

No. of patients	Dosage (mg)	Mean age (yr)	Ratio of males to females	Mean wt (kg)
Patients without infections				
7	500	43.9 ± 19.1	6:1	75.9 ± 18.1
7	750	58.1 ± 13.3	6:1	86.7 ± 21.5
4	1,000	63.0 ± 12.7	3:1	76.8 ± 25.8
Patients with osteomyelitis				
6	500	50.3 ± 14.6	6:0	72.3 ± 9.3
4	750	57.0 ± 8.7	4:0	90.0 ± 18.8

blood contamination by using the following formula (12): serum drug level × (tissue hemoglobin level/blood hemoglobin level) = correction factor.

The correction factor represents the drug concentration contributed by blood contamination and thus was subtracted from the actual concentrations measured from bone and muscle samples to arrive at the true (corrected) concentrations.

Chemicals. Analytical-grade phosphoric acid (85%), tetrabutylammonium hydroxide (40% wt/wt), and high-pressure liquid chromatography-grade acetonitrile were used in the mobile phase. Sodium hydrogen phosphate and sodium dihydrogen phosphate were used in the extraction solutions. Hydrochloric acid was used in the sample dilution mixture. Ciprofloxacin standard powder (potency, 840 μg/mg) was supplied by Miles Laboratories Canada, Ltd.

Liquid chromatography. The chromatography apparatus which we used consisted of a model M45 pump, a column heater, a model WISP 710B autosampler, and a data module recorder-integrator (Waters Associates, Inc., Milford, Mass.). The column (30 cm by 4 mm) was packed with Techsil 10 C18 (P M Instruments Inc., Toronto, Ontario, Canada) and was preceded by a guard column packed with Bondapak C_{18}/Corasil (Waters Associates). A model RF-530 fluorescence spectrophotometer (Shimadzu Corp., Kyoto, Japan) was used as the detector in the system.

The chromatographic conditions used were based on those described by Wingender et al. (17). Elution was carried out by using a mobile phase containing 6% acetonitrile at a temperature of 50°C. The retention time was 4.3 min with a run time of 7 min. All samples were diluted 1:1 with a solution containing 0.16 mol of HCl per liter before injection. Each sample was analyzed in triplicate. Samples and spiked standards were analyzed simultaneously. Concentrations were calculated from calibration curves that were constructed by plotting peak areas against graded concentrations of ciprofloxacin in aqueous, serum, bone, and muscle standards.

Tests for hemoglobin, leukocyte count, with differential, platelet count, alkaline phosphatase, bilirubin, and serum aspartate aminotransferase were done on all patients before and after they received ciprofloxacin.

RESULTS

The high-pressure liquid chromatography analysis was very specific and sensitive. There were no interfering peaks in pooled serum, control bone, or muscle samples. When an injection volume of 10 μl was used, the limit of detection was 0.02 μg/ml.

Binding of ciprofloxacin to crushed bone and muscle samples was evident when we compared the solution phase concentrations of standards made up with phosphate buffer and control bone and muscle samples mixed at room temperature for 24 h. The total drug levels in muscle and bone samples were calculated in two ways. In method A, the results from a single extraction were calculated by using the standards made with control bone or muscle samples. In method B, the results from multiple extractions in which we used standards made in buffer were added together. In the case of bone samples, the summing of results for multiple (two or three) extractions gave values that were, on the average, 85% of the values calculated by method A. However, in the case of muscle samples, method B gave values that were only 58% of the values calculated by method A. Hence, the estimated concentrations from muscle samples were substantially different, depending on whether the calculations were made from a calibration curve prepared from buffered standards compared with standards containing muscle. The data in this paper are based on method A for muscle and bone samples.

Of the 18 patients without infections who required hip or bone replacement surgery, 7 received 500 mg of ciprofloxacin, 7 received 750 mg of ciprofloxacin, and 4 received 1 g of ciprofloxacin in single doses. Most of these patients had underlying severe osteoarthritis of the affected joints. Ten patients with osteomyelitis were studied; six received 500 mg of ciprofloxacin, and four reveived 750 mg. The infecting organisms causing osteomyelitis included staphylococcal species, *Streptococcus* spp., *Pseudomonas aeruginosa*, and *Proteus mirabilis*. The demographic charateristics of the patients are shown in Table 1.

The mean serum, bone, and muscle concentrations determined by using standards made with serum, bone, and muscle (method A) are shown in Table 2. It appears that the concentrations in bone and muscle samples increased with increasing dose and the presence of infection. The differences between bone concentrations in patients with and without osteomyelitis were not statistically significant; however, the mean serum levels were also higher in patients with osteomyelitis, and this could not be explained by differences in the mean weights of the patients in the groups studied. In general, the muscle levels of ciprofloxacin were 1.4 to 2.75 times greater than the bone levels. Mean concentrations of

TABLE 2. Concentrations of ciprofloxacin in serum, bone, and muscle samples

No. of patients	Dosage (mg)	Bone	Serum concn (μg/ml)	Bone concn (μg/g)	Muscle concn (μg/g)
7	500	Normal	1.4 ± 0.6 (0.4-2.0)[a]	0.4 ± 0.3 (0.2-0.9)	1.1 ± 0.7 (0.5-1.9)
7	750	Normal	2.6 ± 1.1 (0.9-3.8)	0.7 ± 0.4 (0.2-1.4)	1.3 ± 0.8 (0.6-2.4)
4	1,000	Normal	2.9 ± 1.5 (0.9-4.4)	1.6 ± 0.6 (1.0-2.4)	2.6 ± 1.9 (0.8-5.2)
6	500	Infected	2.0 ± 0.9 (0.9-3.2)	0.7 ± 0.4 (0.2-1.4)	1.0 ± 0.2 (0.8-1.4)
4	750	Infected	2.9 ± 2.2 (1.0-6.0)	1.4 ± 1.0 (0.6-2.7)	2.4 ± 1.0 (1.7-5.7)

[a] Mean ± standard deviation. The numbers in parentheses are ranges.

ciprofloxacin in bone samples of more than 1 μg/g were observed with 1-g doses in normal bones or with 750-mg doses in patients with osteomyelitis, whereas concentrations of 1 μg/g or above in muscle samples were achieved with 500-mg doses.

No serious clinical or laboratory adverse effects were observed with ciprofloxacin in this study. One patient vomited the medication and was excluded from the study. Another two patients experienced nausea.

DISCUSSION

In this study we found that significant levels of ciprofloxacin were achieved in bone samples after oral dosing of patients with and without osteomyelitis. The concentration in bone samples increased with higher doses and was 30 to 100% greater in infected bone samples than in noninfected bone samples. This difference was not statistically significant, primarily because of the small number of patients in each group. Our results also emphasize the importance of using the same tissues for preparation of control standards when calculating the calibration curves. There was significant binding of ciprofloxacin to bone samples and more binding to muscle samples, but this could be compensated for by using control muscle and bone samples to prepare the standards.

It is possible that we could have underestimated the drug levels if the preanesthetic medications which we used impaired gastric motility and affected absorption. We think that this is of minor importance since the preanesthetic medications were usually given 1 h before surgery and the study drug was given 2 to 4 h preoperatively. Moreover, the serum levels achieved in our patients are similar to the levels in healthy volunteers reported previously. For instance, the peak serum concentrations reported 1 h following administration of 500 mg, 750 mg, and 1 g of ciprofloxacin were 1.9 to 2.1, 2.6, and 3.6 μg/ml, respectively (9, 10), whereas the mean serum concentrations achieved in our patients for similar doses were 1.4 to 2.0, 2.6, and 2.9 μg/ml, respectively. The lower levels achieved in our patients after administration of 1 g of ciprofloxacin could be explained by the later sampling time.

Ciprofloxacin, with its broad spectrum and heightened activity, should provide adequate therapy for most cases of gram-negative osteomyelitis (including *Pseudomonas aeruginosa* osteomyelitis) or mixed infections with *S. aureus* at oral doses of 750 to 1,000 mg twice daily. The majority of gram-negative bacilli or staphylococci are inhibited by 0.5 μg of ciprofloxacin per ml or less; with bone concentrations of 1.4 to 1.6 μg/g, the therapeutic ratio would be three to four times the MICs of most bacteria. For a minority of bacteria with higher MICs (more than 0.5 μg/ml), higher doses may be needed to provide a better therapeutic index. Since our study was done after single oral doses, it is possible that higher bone levels may be present when a steady-state is achieved after multiple dosing.

Recently, Norden and Shinners (13) reported that ciprofloxacin was superior to tobramycin for sterilizing the bones of infected animals with chronic osteomyelitis. After 28 days of therapy, only 6% of the rabbits tested had positive cultures for *Pseudomonas* after ciprofloxacin administration, whereas 94% of the rabbits treated with tobramycin were culture positive. In the animal model, after subcutaneous injection of 40 mg of ciprofloxacin per kg, the peak bone concentration was achieved at 1 h after administration and averaged 1.0 ± 0.5 μg/g, with a peak serum level of 2.1 μg/ml. This level of ciprofloxacin, which was sufficient to cure 94% of the rabbits with chronic *Pseudomonas* osteomyelitis, is 30% less than the levels that could be achieved in our patients with osteomyelitis after single 750-mg oral doses.

Gram-negative or mixed bacterial osteomyelitis and soft-tissue infections are not very common but may be on the increase, especially after surgery, after trauma, and in patients with diabetes mellitus and drug addicts. These infections are difficult to treat and require prolonged parenteral therapy with toxic or expensive medications. Although newer cephalosporins (especially ceftriaxone, which has a longer half-life) make once-daily injection possible and are very safe, the inconvenience of injections and the possibility of septic superinfections related to intravenous administration still exist.

Orally administered antibiotics, such as cloxacillin, dicloxacillin, fusidic acid, and clindamycin, have been used in patients with acute and chronic staphylococcal osteomyelitis, usually after short-term parenteral therapy, with good or reasonably good results (2, 3, 7). However, data on oral therapy for gram-negative or mixed bacterial osteomyelitis are sparse.

One of the concerns with the quinoline derivative is the development of bacterial resistance, especially when prolonged therapy is required (such as in osteomyelitis). However, the results of in vitro studies suggest that the frequency of resistance with ciprofloxacin is very low and is no greater than the frequency of resistance with the aminoglycosides and less than the frequency of resistance with norfloxacin (5, 14). Moreover, in the rabbit model of *Pseudomonas* osteomyelitis described previously (13), resistance was not observed after 1 month of ciprofloxacin therapy. However, resistance did develop in 2 of 10 treatment failures in animals that received ciprofloxacin for only 2 weeks.

In summary, ciprofloxacin given orally in 750-mg doses should provide bone and soft-tissue levels that are sufficient to treat most gram-negative and staphylococcal infections. Clinical trials should be performed with orally administered ciprofloxacin in patients with gram-negative or mixed bacterial osteomyelitis and soft-tissue infections.

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