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Efficacy of Ciprofloxacin against *Leptospira interrogans* Serogroup Icterohaemorrhagiae

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Ciprofloxacin activity against *Leptospira interrogans* serogroup icterohaemorrhagiae was studied in vitro and in an animal model. The MBC of ciprofloxacin was 0.6 μg/ml. Three of three Syrian hamsters died 8 to 9 days after intraperitoneal challenge with 10^6 leptospires. In contrast, five of six animals given ciprofloxacin 3 or 5 days after challenge survived.

Leptospirosis is a zoonosis of worldwide distribution affecting humans as well as wild and domestic mammals. Human leptospirosis may be asymptomatic or characterized by a mild anicteric disease. However, 5 to 10% of patients develop severe icteric illness with a case fatality rate of up to 10% (2, 3). The recommended antimicrobial therapy is primarily a penicillin or a tetracycline. The effectiveness of such treatment is still the subject of some controversy (6), and onset of treatment beyond day 4 of illness is of doubtful benefit (5). Other antimicrobial agents have been found to be effective in vitro or against experimental leptospirosis (1, 4) but have not gained wide clinical application. A new carboxyquinolone, ciprofloxacin, is well absorbed by the oral route and has a broad spectrum of antimicrobial activity. There have been no data regarding its potential activity against leptospires. In this paper, we report the in vitro activity and preliminary in vivo data regarding ciprofloxacin efficacy against *Leptospira interrogans* serogroup icterohaemorrhagiae.

The strain studied was *L. interrogans* serogroup icterohaemorrhagiae serovar budapest, isolated from a fatal human case of leptospirosis. The virulence of the strain was maintained by three serial passages in Syrian hamsters. The growth medium for leptospires was Leptospira Medium EMJH broth (Difco Laboratories, Detroit, Mich.).

Comparative in vitro activity of ciprofloxacin versus tetracycline against the above *Leptospira* isolate was studied by a broth macrodilution technique and a modified time-kill curve method as follows. Serial twofold dilutions of ciprofloxacin (5 to 0.07 μg/ml) and tetracycline (16 to 0.5 μg/ml) were prepared in tubes containing 5 ml of EMJH broth. Tubes were inoculated with a final concentration of 5×10^5 leptospires per ml and incubated at 30°C in ambient air for 7 days. Samples were obtained on days 2, 5, and 7 postinoculation, and *Leptospira* forms were quantitated by chamber count under dark-field microscopy. Since many forms of drug-exposed leptospires were nonmotile and elongated under dark-field microscopy, the viability of leptospires from each drug-containing tube was quantitated 7 days postinoculation. Quantitation was performed by subculturing 10-fold dilutions (10^{-1} to 10^{-9}) of each tube into antimicrobial agent-free EMJH broth. Growth of leptospires in subcultures was quantitated by chamber counts on days 2, 5, and 7 and compared with growth in control tubes containing known concentrations of viable leptospires. The MBC of each antimicrobial agent was defined as the lowest concentration causing a ≥1,000-fold reduction of viable leptospires compared with the initial inoculum (≥99.9% killing).

The in vivo activity of ciprofloxacin against the above strain was evaluated in 12 Syrian hamsters (80 to 100 g). The animals were divided into four equal groups. The first group was challenged by the intraperitoneal route with 10^6 leptospires per animal and received no antimicrobial treatment. The second group received ciprofloxacin only (10 mg/kg of body weight intraperitoneally twice a day for 5 days) without *Leptospira* challenge. The third group was challenged as described above and treated with ciprofloxacin starting 3 days postinoculation. The fourth group was also challenged as described above, but ciprofloxacin therapy was initiated 5 days after inoculation. Efficacy of antimicrobial therapy was evaluated by comparing animal survival in the various groups and by determining leptospiral growth in cultures obtained from kidney and liver tissues of surviving animals.

The in vitro activities of ciprofloxacin and tetracycline against *L. interrogans* serogroup icterohaemorrhagiae are illustrated by time-kill curves in Fig. 1. The MBCs of ciprofloxacin and tetracycline were 0.6 and 4 μg/ml, respectively.

The results of the therapeutic efficacy study of ciprofloxacin in 12 hamsters with experimental leptospirosis were as follows. Three of three untreated animals succumbed on days 8 (two animals) and 9 (one animal) after leptospiral challenge. Ciprofloxacin administered to three control animals that were not challenged had no adverse effect. Three of three hamsters treated with ciprofloxacin starting 3 days after challenge survived. When treatment was delayed until 5 days after challenge, two of three animals survived. Surviving animals were free of leptospires as judged by multiple cultures obtained 15 days after challenge. In contrast, leptospires were isolated in every culture obtained from kidney and liver tissues of dead hamsters.

The results of our study indicate that ciprofloxacin is effective both in vitro and in vivo against a virulent strain of *L. interrogans* serogroup icterohaemorrhagiae. The in vitro methods used in our study were selected in view of the unique nature of leptospires (e.g., slow division rate, requirement for special growth medium, etc.). Other conventional techniques for quantitation of leptospiral growth are problematic. Specifically, subculture to agar plates yields an erratic, nonreproducible number of colonies. Turbidity of leptospire-containing tubes is also not reliable, since concentrations of up to 10^7 leptospires per ml cause no turbidity in

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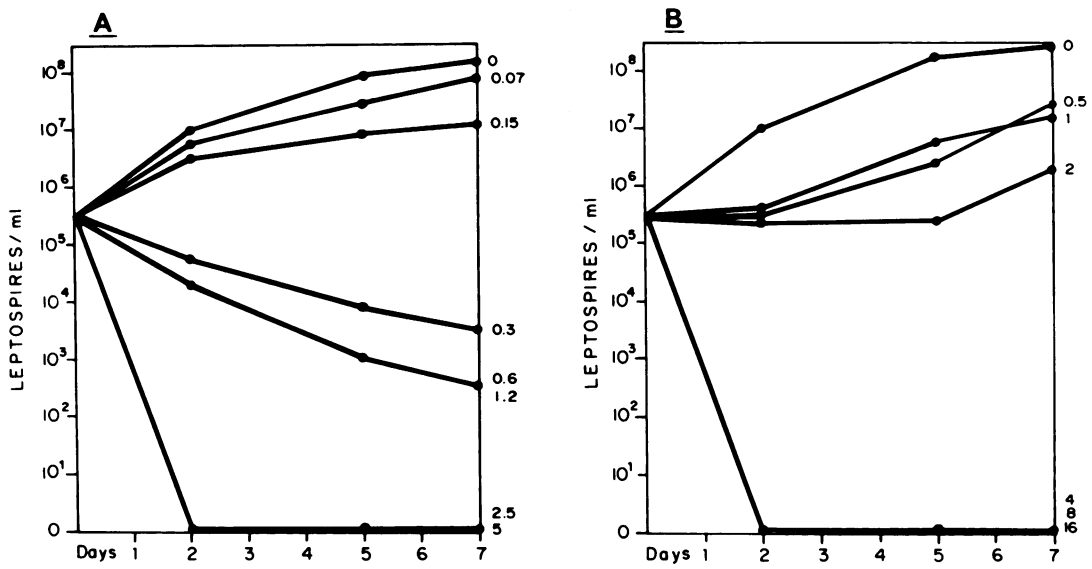

FIG. 1. Time-kill curves of *L. interrogans* serogroup icterohaemorrhagiae exposed to various concentrations (shown in micrograms per milliliter on right axes) of ciprofloxacin (A) and tetracycline (B).

the special *Leptospira* broth. The time-kill curve method utilized in our study enabled us to precisely quantitate the number of viable organisms in each tube; thus, an MBC could be defined.

The data derived from our study are limited due to the fact that only a single organism was tested and only a small number of animals were challenged. However, the positive results should prompt additional animal studies with various strains of leptospires comparing ciprofloxacin efficacy with that of tetracyclines and penicillins.

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