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In Vitro and *in Vivo* Activities of Ofloxacin Against *Mycobacterium leprae* Infection Induced in Mice[1]

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Ofloxacin, a quinolone agent newly developed by Daiichi Pharmaceutical Co., Tokyo, Japan, has an appreciable antimycobacterial activity *in vitro,* particularly against pathogenic acid-fast bacilli such as *Mycobacterium tuberculosis, M. bovis, M. kansasii, M. marinum, M. xenopi,* and *M. fortuitum* ([2, 5]). We reported that ofloxacin is highly efficacious against *M. fortuitum* infection in mice ([2]). More recently, Tsukamura ([6, 7]) found that this agent is efficacious in the treatment of clinical pulmonary tuberculosis. Such being the case, we determined the effects of ofloxacin on *M. leprae* infection in mice.

MATERIALS AND METHODS

Mice. Five-week-old, female ddY mice were purchased from the Shizuoka Agricultural Cooperative for Experimental Animals, Shizuoka, Japan.

Bacteria. *M. leprae* Kyoto-1 derived from the hindfoot pads of nude mice infected with the organisms were used.

Assay for *in vivo* activity of ofloxacin. Eighty mice were infected subcutaneously with 1×10^4 *M. leprae* in 0.03 ml of saline into the left hindfoot pad. Thirty days later five mice were killed to estimate the number of *M. leprae* in the left hindfoot pad. Twenty-five animals each were treated by gavage with ofloxacin (Daiichi) dissolved in saline in a dose of 0.1 or 1 mg/mouse/administration for a total of 50 days (day 31 through day 80 after infection). This was done by giving the drugs six times per week (once daily) from day 31 to day 45 and, thereafter, twice daily from day 46 to day 80 after infection. The remaining 25 mice served as untreated controls. At intervals, for up to 230 days after infection, five mice in each group were killed and the number of acid-fast bacilli (AFB) in the left hindfoot pad was counted, as described previously ([3]).

Assay for *in vitro* activity of ofloxacin. *M. leprae* Kyoto-1 harvested from the infected nude mice were suspended in saline, with or without 0.02, 0.2, or 2 mg/ml of ofloxacin at the concentration of 5×10^5 bacilli/ml, and the preparation incubated at 30°C for 1 hr. Then, 0.02 ml (1×10^4 *M. leprae*/mouse) of the resultant bacterial suspension was injected into the left hindfoot pad of ddY mice, and the number of AFB in the infected foot pad tissue of 4–5 mice in each group was counted on days 30, 90, 180, and 240.

RESULTS

The growth of *M. leprae* in the left hindfoot pads of mice given or not given ofloxacin during the course of infection is shown in Figure 1. In the untreated group of mice (open circles), $3.14 \pm 0.63 \times 10^3$, $3.10 \pm 0.78 \times 10^4$, $1.45 \pm 2.34 \times 10^5$, and $1.73 \pm 0.25 \times 10^5$ organisms were recovered from infected tissue on days 80, 130, 180, and 230, respectively. In mice given ofloxacin in a dose of 0.1 mg/injection (closed circles), $3.14 \pm 0.63 \times 10^3$, $5.40 \pm 0.61 \times 10^3$, $3.99 \pm 1.50 \times 10^4$, and $7.10 \pm 0.98 \times 10^4$ organisms were recovered from infected tissue on the same days as above, respectively, indicating a significant inhibition of growth in the period from day 80 to day 130 after infection. In mice given ofloxacin in a dose of 1 mg/injection (closed triangles), the values were $1.58 \pm 0.29 \times 10^3$, $2.80 \pm 0.55 \times 10^3$, $4.92 \pm 0.41 \times 10^3$, and $6.86 \pm 0.26 \times 10^3$, respectively, on the same days as above, thereby indicating an enhancement of the elimination from the infected site during the course of drug administration (days 31 to

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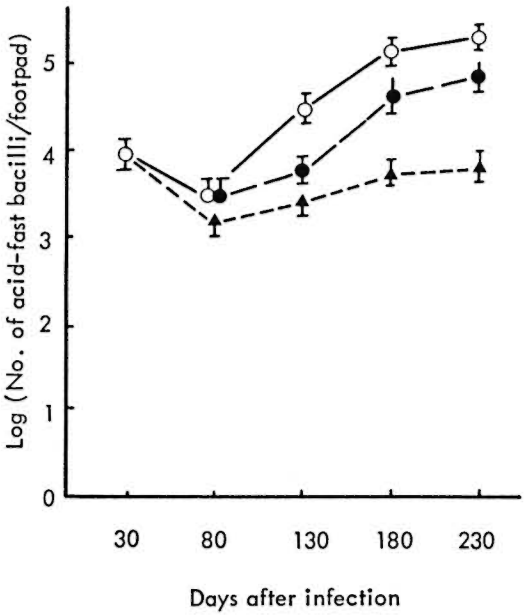

FIG. 1. Therapeutic efficacy of ofloxacin against *M. leprae* infection in mice. Mice were given saline (○) or 0.1 mg (●) or 1 mg (▲)/mouse/injection dosage of ofloxacin from day 31 to day 80 after infection, as described in Materials and Methods. Each symbol indicates the mean ± standard error of the mean (N = 5).

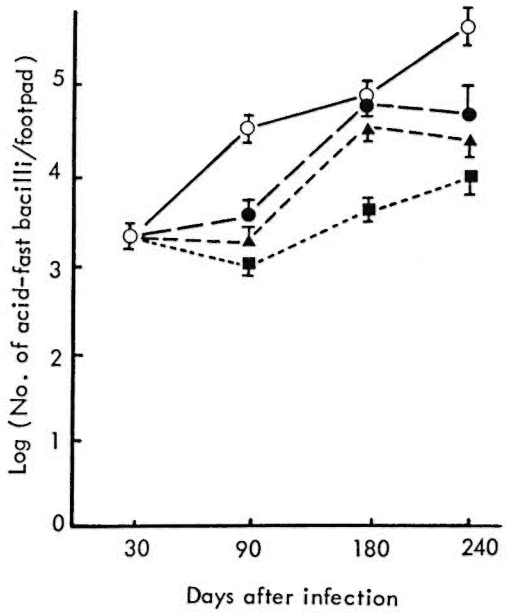

FIG. 2. *In vitro* anti-*M. leprae* activity of ofloxacin. *M. leprae* cells were treated with 0.02 (●), 0.2 (▲), or 2 (■) mg/ml of ofloxacin at 30°C for 1 hr, as described in Materials and Methods, and then infected into the left hindfoot pads of normal mice. The growth of *M. leprae* with or without (○) ofloxacin treatment in the infected tissue was measured at time indicated. Each symbol indicates the mean ± standard error of the mean (N = 4 or 5).

80). Thereafter, there was a marked suppression of growth of *M. leprae* in the infected tissue up to the end of the experiment. It may be noteworthy that ofloxacin caused a marked decrease in the growth rate of *M. leprae,* even after cessation of drug administration.

As shown in Figure 2, *in vitro* treatment of *M. leprae* with ofloxacin caused a marked reduction in the growth rate of the organisms at the site of infection in the host mice in a dose-dependent manner. In particular, *M. leprae* treated with ofloxacin at the concentration of 2 mg/ml resulted in nearly two orders of reduction of growth.

DISCUSSION

Ofloxacin (DL-8280), a potent quinolone derivative, has marked activity against gram-negative and gram-positive bacteria, including obligate anaerobes ([4]). The agent also has an appreciable antimycobacterial activity *in vitro* ([2, 5]) and *in vivo* ([2, 6, 7]). The MIC_{90} of ofloxacin against pathogenic mycobacteria was below 1.6 μg/ml (unpublished data). Such being the case, it was considered that ofloxacin may be active against *M. leprae.* Although diaminodiphenylsulfone (dapsone, DDS) has been the main drug used in the treatment of leprosy, recent studies have revealed a high resistance rate by *M. leprae* to this agent ([1, 8]), and new potent antileprosy drugs have been studied. Because of the rapid elimination of ofloxacin noted in our preliminary studies, *M. leprae*-infected mice were treated with the agent, usually twice daily, after the induced infection. Ofloxacin caused a marked decrease in the growth rate of *M. leprae,* even after cessation of drug administration (Fig. 1). Therefore, this agent seems to possess bactericidal activity against *M. leprae. In vitro* treatment of *M. leprae* with ofloxacin at a high concentration (2 mg/ml) caused a marked reduction in the rate of growth of the organisms at the site of infection (Fig. 2). Thus, ofloxacin seems to possess bactericidal activity *in vitro* against *M. leprae.*

The results of these studies indicate that ofloxacin may be an effective antileprosy

drug. In relation to this conclusion, we noted a recent report by Itoh, *et al.* (summarized reports of conference on research on leprosy, Sasakawa Memorial Health Foundation, Japan, 1986) that ofloxacin orally administered with feeding failed to control leprosy in CBA and BALB/c (nude) mice, although another investigator group (personal communication; Dr. J. Grosset) observed an appreciable efficacy in this agent to control leprosy in mice. Therefore, it may be possible that the efficacy of ofloxacin against *M. leprae* infection in mice is very variable, depending upon the experimental conditions such as mouse strain, administration dosage and protocol, etc. On these problems, current studies are under way.

SUMMARY

Ofloxacin, a new quinolone, exhibits bactericidal activity against *Mycobacterium leprae* in mice, both *in vivo* and *in vitro.*

RESUMEN

La ofloxacina, una nueva quinolona, exhibe actividad bactericida contra el *Mycobacterium leprae* inoculado en el cojinete plantar del ratón y su efecto ocurre tanto *in vivo* como *in vitro.*

RÉSUMÉ

L'ofloxacine, une nouvelle quinolone, témoigne d'une activité bactéricide contre *Mycobacterium leprae* chez la souris, tant *in vivo* qu'*in vitro.*

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