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In Vivo Effects of Rufloxacin and Ciprofloxacin on T-Cell Subsets and Tumor Necrosis Factor Production in Mice Infected with *Bacteroides fragilis*

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We investigated the in vivo effects of rufloxacin and ciprofloxacin on T-cell subsets and tumor necrosis factor production in mice infected with *Bacteroides fragilis*. These quinolones did not alter the helper/suppressor ratio but did modulate the kinetics of tumor necrosis factor production in infected animals. This result correlated with the observed therapeutic efficacies of the quinolones.

Several recent reports indicate that quinolone antibiotics at clinically achievable levels modulate the in vitro growth of T cells and the production of cytokines by activated lymphoid cells (1, 2, 5, 7–9). The purpose of this investigation was to determine whether quinolones in vivo alter the T-cell response and cytokine production in an infection model. Intra-abdominal abscesses produced by *Bacteroides fragilis* are a good model to study the in vivo effects of quinolones because, first, abscess formation and elimination of abscesses require T-cell function (10, 11) and, second, the cytokines play an important role in elimination of infection (3, 11).

Five- to six-week-old female C3H/HeN mice were infected with an abscess-forming mixture of *B. fragilis* with sterile cecal contents and $BaSO_4$ as previously described (3). Six hours after the inoculation, the mice were treated with rufloxacin (50 mg/kg of body weight) once a day or with ciprofloxacin (40 mg/kg) twice daily for 10 days. Each group contained 11 animals. The antibiotics were given intraperitoneally. Rufloxacin was given once a day because of its long half-life (6). As a control, a group of mice were treated with saline. In addition, a group of uninfected animals used as controls received either ciprofloxacin or rufloxacin so as to determine the effect of the quinolones on T-cell subsets without the associated effects of infection with *B. fragilis*. On day 11 posttreatment, mice were sacrificed by cervical dislocation under anesthesia, the spleens were removed, and a single cell suspension was prepared as previously described (4). Cells were stained with fluorescein isothiocyanate-labeled Thy 1.2 (total T-cell), L3T4 (helper), or LYT2 (suppressor) antibody (Becton Dickinson, San Jose, Calif.). The binding of antibodies to T lymphocytes was analyzed with a FACScan flow cytometer (Becton Dickinson). Mice treated with or without drugs were bled at 2, 24, 48, and 72 h posttreatment, sera were pooled, and tumor necrosis factor (TNF) levels in the serum were determined by enzyme-linked immunosorbent assay as suggested by the manufacturer (Genzyme, Cambridge, Mass.). Mice were killed 1 day after completion of therapy; their abdomens were opened and examined for the presence or absence of abscesses. If abscesses were present, then pus was cultured to confirm the presence of *B. fragilis*. *B. fragilis* was recovered from the abdominal abscess pus of all control untreated animals. The absence of *B. fragilis* was considered to indicate that the animals had been cured. The results were analyzed for statistical significance by chi-square analysis, and P values of <0.05 were considered significant.

Infection leads to increased splenic weight due to increased cell proliferation. To determine whether the quinolones had an effect on cell proliferation in vivo, we examined the splenic weights of the mice. The splenic weights of ciprofloxacin- and rufloxacin-treated (77 ± 14 and 87 ± 22 mg, respectively) animals were similar to those of untreated controls (85 ± 11 mg). This result suggests that, in vivo, neither rufloxacin nor ciprofloxacin interfered with the normal splenic response to *B. fragilis* infection.

To study whether quinolone antibiotics induce subtle changes in the T-cell response, we investigated the effect of treatment with the quinolones on T-cell subsets. The helper/suppressor T-cell ratio in infected animals (ratio, 2.0) was higher than in uninfected controls (ratio, 1.6). The percentages of helper (L3T4) and suppressor (LYT2) T cells in mice treated with rufloxacin (ratio, 2.0) and ciprofloxacin (ratio, 2.12) were essentially similar to those in untreated animals (ratio, 2). This result suggests that these two quinolones did not induce substantial changes in the T-cell response to infection.

In control mice, TNF levels in serum peaked 2 h posttreatment and decreased rapidly thereafter (Table 1). In mice treated with rufloxacin or ciprofloxacin, the magnitude of the TNF response 2 h posttreatment was lower than in untreated animals, but the TNF levels in serum persisted for up to 72 h. This result suggests that the quinolones modulate TNF production in vivo.

The treatment of mice with rufloxacin (50 mg/kg once a day) or ciprofloxacin (40 mg/kg twice daily) resulted in the elimination of *B. fragilis* from 66.6 and 63.5% of the animals, respectively (Table 2). The serum antibiotic levels were measured by bioassay (12) after one dose of 50 mg of rufloxacin per kg or after 40 mg of ciprofloxacin per kg twice daily in the infected as well as the uninfected mice. They were similar in both groups: 0.7 to 0.8 mg/liter for rufloxacin and 1.6 to 1.8 mg/liter for ciprofloxacin 1.5 h after the study

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TABLE 1. Effects of rufloxacin and ciprofloxacin on TNF production in mice infected with *B. fragilis*

Treatment	TNF levels in serum (pg/ml) at time posttreatment[a]			
	2 h	24 h	48 h	72 h
Saline	350	180	96	54
Rufloxacin (50 mg/kg once a day)	240	155	165	127
Ciprofloxacin (40 mg/kg twice daily)	135	110	145	150

[a] Mice were bled at the indicated time intervals, and TNF levels were determined by enzyme-linked immunosorbent assay.

drug was injected. The MICs of rufloxacin and ciprofloxacin were 4 mg/liter for *B. fragilis* used in this study.

The data presented in this study demonstrate that, in vivo, quinolones such as rufloxacin and ciprofloxacin appear not to adversely affect T-cell growth but modulate cytokine production. Furthermore, we show that despite their inactivities against *B. fragilis* in vitro, these two quinolones eliminated *B. fragilis* infection effectively in nearly two-thirds of the animals. We propose that the in vivo efficacies of these quinolones with *B. fragilis* may be related to their ability to modulate cytokine production. Several reports indicate that low levels of TNF protect animals from experimental infections caused by extracellular pathogenic strains of bacteria. In this study, we showed that quinolones, i.e., rufloxacin and ciprofloxacin, alter the kinetics of TNF production in infected animals, thus allowing low levels of TNF to be present for a prolonged period. Quinolones in general are regarded to be ineffective against *B. fragilis* because the MIC for 90% of the isolates of this organism tested ranges from 4 to 16 mg/liter, a concentration that is not achieved with therapeutic doses of quinolones. Despite their lack of activity in vitro, we have shown that some quinolones indeed are effective against *B. fragilis* in vivo (12, 13). The in vivo efficacies of quinolones in *B. fragilis* infections as observed in these animal models may be related to the ability of quinolones to modulate cytokine production. Synergy between antibiotics and cytokines has been reported previously (3). In that study, when clindamycin was given by itself, it failed to eliminate *B. fragilis* infections in mice. When those animals were treated with cytokines alone derived from splenic cells of *B. fragilis*-infected mice, the cytokines also failed to cure the infection. However, when clindamycin was given together with the cytokines, the infection was eliminated in the majority of the animals. An alternative explanation for the observed therapeutic efficacies of the quinolones in the present study could be due to the sub-MIC effect. It is known that bacteria preexposed to sub-MICs of antibiotics are prone to be eliminated more efficiently by host phagocytes. Cytokines also enhance phagocyte function to facilitate bacterial clearance. These two possibilities are not mutually exclusive.

In summary, rufloxacin in vivo appears not to alter T-cell responses but modulates cytokine production favorably in eliminating *B. fragilis* infections.

TABLE 2. Therapeutic effect of rufloxacin versus ciprofloxacin in experimental *B. fragilis* intra-abdominal abscesses

Treatment	No. of animals cured/ no. of animals infected	% Cured
Saline	0/9	0
Rufloxacin (50 mg/kg once a day)	8/12	66.6
Ciprofloxacin (40 mg/kg twice daily)	7/11	63.6

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