

Activity of Trovafloxacin in Combination with Other Drugs for Treatment of Acute Murine Toxoplasmosis

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Current therapy for toxoplasmosis with a synergistic combination of pyrimethamine plus sulfadiazine or pyrimethamine plus clindamycin is not always efficacious and is frequently discontinued due to intolerable toxic effects in immunocompromised individuals, particularly those with AIDS. Trovafloxacin, a new fluoroquinolone with potent activity against *Toxoplasma gondii*, was examined for potential synergistic activity when combined with other drugs used for treatment of human toxoplasmosis. Combinations of trovafloxacin with clarithromycin, pyrimethamine, or sulfadiazine demonstrated significantly enhanced activities compared to those observed with each drug alone. Our results suggest that combinations of trovafloxacin and other anti-toxoplasma drugs should be further explored for treatment of toxoplasmosis in humans.

The synergistic combination of pyrimethamine plus sulfadiazine or clindamycin is currently considered the optimal drug regimen for treatment of toxoplasmosis (2, 9, 14). Unfortunately, in immunocompromised individuals, particularly those with AIDS, use of this combination is limited because of the untoward side effects of all three drugs (3, 13). In addition, this combination has only limited efficacy in patients with ocular toxoplasmosis and in congenitally infected infants and children, and pyrimethamine must be avoided for women who acquire the infection during pregnancy because the drug is potentially teratogenic.

We recently found that trovafloxacin, a novel fluoroquinolone compound, has potent activity against *Toxoplasma gondii* in vitro in cell culture and in vivo in a mouse model of acute toxoplasmosis (8). In our earlier experiments, we observed that trovafloxacin administered orally at 25 mg/kg of body weight/day resulted in prolongation of time to death of the infected mice. At 50 mg/kg/day, the rate of protection against death was 90%, and at doses above 50 mg/kg/day all mice survived. Because we consider it unlikely that single-drug therapy will be effective against all forms of toxoplasmosis, it was considered of interest to examine trovafloxacin at suboptimal doses in combination with several drugs being used for treatment of this disease.

MATERIALS AND METHODS

***T. gondii*.** Tachyzoites of the RH strain were obtained from the peritoneal cavities of infected mice and prepared as previously described (1, 4).

Mice. Outbred, female Swiss Webster mice (Simonsen Laboratories, Gilroy, Calif.), weighing approximately 20 g at the beginning of each experiment, were used. Food and water were available at all times.

Drugs. The drugs used in these studies and their sources were trovafloxacin mesylate (CP-99,219-27), Pfizer Inc., Groton, Conn.; atovaquone and pyrimethamine, Burroughs Wellcome Co., Research Triangle Park, N.C.; clarithromycin,

Abbott Laboratories, Abbott Park, Ill.; rifabutin, Pharmacia Laboratories, Dublin, Ohio; and sulfadiazine, Sigma Chemical Co., St. Louis, Mo.

Drug combinations. Solutions of drugs were made according to the manufacturers' directions. Atovaquone was suspended in 0.25% carboxymethyl cellulose and sonicated. Clarithromycin was dissolved in phosphate-buffered saline (pH 7.2) and sonicated prior to administration. Pyrimethamine was dissolved in 0.25% carboxymethyl cellulose. Trovafloxacin was dissolved in double-distilled water. Since trovafloxacin is light sensitive, solutions were made and stored in the dark. Sulfadiazine was dissolved in double-distilled water and administered to mice in their drinking water. Doses of the drugs were chosen such that they either resulted in survival of up to approximately 30% of treated mice or effected only prolongation of time to death. Trovafloxacin was used at 25 mg/kg/day in combination with one of the following drugs: atovaquone at 5 and 10 mg/kg/day, clarithromycin at 200 mg/kg/day, pyrimethamine at 10 mg/kg/day, rifabutin at 50 mg/kg/day, and sulfadiazine at 150 mg/liter of drinking water. Each of the drugs was also evaluated alone at these same doses.

In vivo studies. Mice were infected intraperitoneally (i.p.) with 2.5×10^3 tachyzoites of the RH strain of *T. gondii* as previously described (8). Treatment by gavage with a single daily oral dose was initiated 24 h later and continued for 10 days. There were 10 mice in each group. Mice were observed for mortality and time to death for 30 days from the day of infection. At the end of the 30-day period, portions of brain, liver, and spleen were pooled and homogenized in sterile phosphate-buffered saline, and 0.2 ml of the suspensions was inoculated i.p. into each of three normal mice. Organs were pooled since we were solely interested in determining whether there was residual infection in the mice. Subinoculated mice were observed for mortality for 30 days. Surviving mice from the subinoculated groups were examined for toxoplasma antibodies by a direct agglutination assay (5).

Statistical analysis was performed with survival tools for StatView version 4.02 (Abacus Concepts, Berkeley, Calif.). *P* values were obtained by the log-rank test of the Kaplan-Meier product limited survival analysis.

Pharmacokinetic studies. High-performance liquid chromatography assays were performed to determine trovafloxacin concentrations in sera and various tissues of mice left uninfected or infected with tachyzoites. The ranges of trovafloxacin concentrations for the calibration assay were 1.0 to 20.0 $\mu\text{g/ml}$ for sera and 0.2 to 50.0 $\mu\text{g/g}$ for tissues. Quality control samples, three concentrations in duplicate, were within 10% of their normal concentrations, and all were acceptable. Trovafloxacin was administered in single oral doses of 10, 25, or 50 mg/kg/day for 3 days. Treatment of infected mice was begun 24 h postinfection. Blood samples were collected at 0.25, 0.5, and 1 h after drug administration on days 1 and 3 of treatment. Blood samples were allowed to coagulate at 4°C for 45 min, and thereafter sera were separated by centrifugation. Brain, liver, and spleen tissues were collected at the same time. Tissues were weighed and snap frozen in liquid nitrogen immediately after collection. Three mice were used in each group. These samples were stored at -80°C until further analysis. Peak concentrations (C_{max}) of trovafloxacin were determined directly from the concentration-time profile. The area under the concentration-time curve from 0 to 1 h after drug administration (AUC_{0-1}) was calculated by the trapezoidal method.

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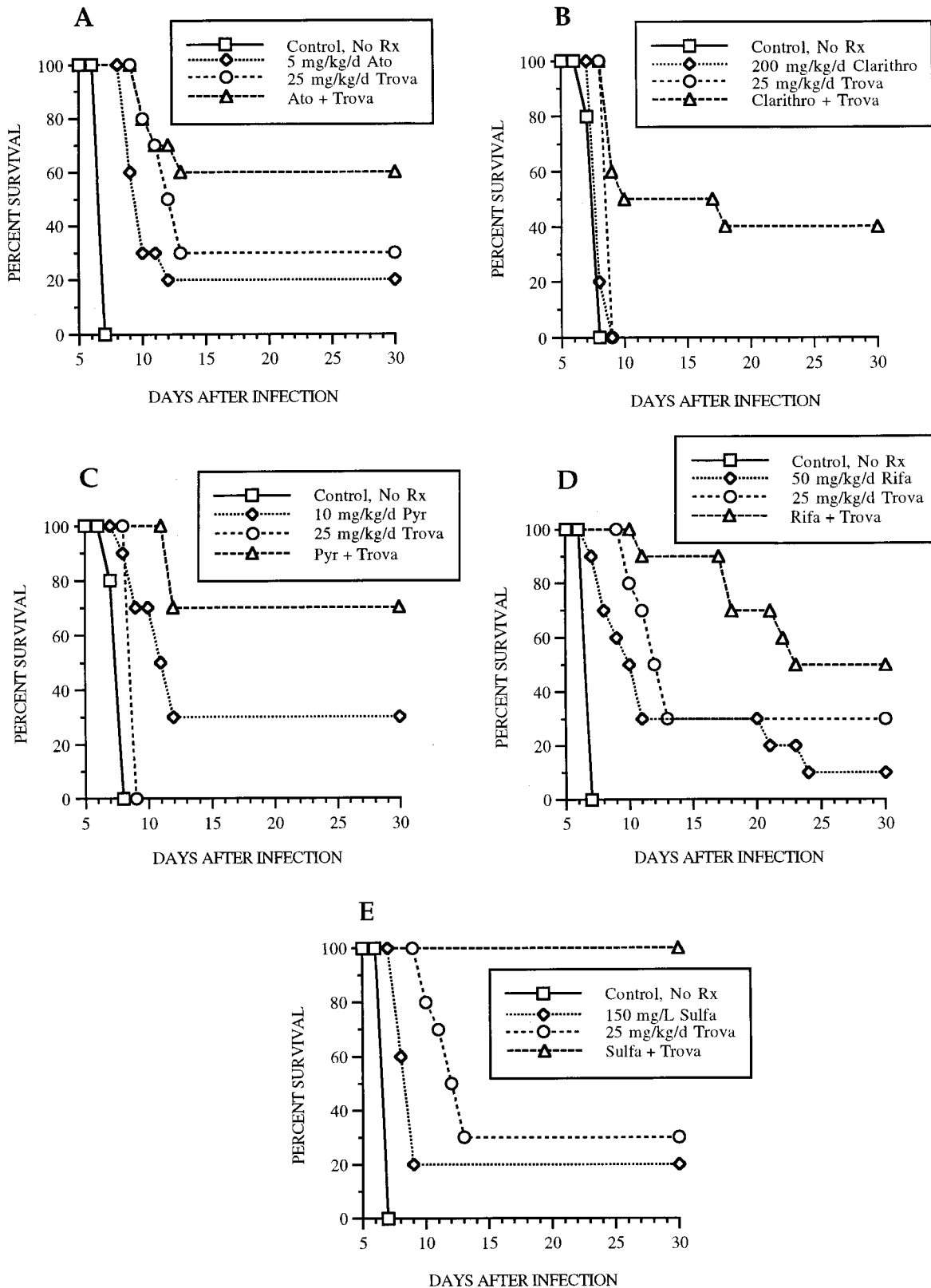


FIG. 1. Effects of treatments with trovafloxacin (Trova) alone and in combination with atovaquone (Ato), (A), clarithromycin (Clarithro) (B), pyrimethamine (Pyr) (C), rifabutin (Rifa) (D), and sulfadiazine (Sulfa) (E) on survival of mice infected i.p. with tachyzoites of the RH strain of *T. gondii*. Ten mice per group were used. Rx, treatment; d, day.

RESULTS

Trovafloxacin plus atovaquone. Following infection, mice were treated with 5-mg/kg/day atovaquone alone or in combination with 25-mg/kg/day trovafloxacin (Fig. 1A). One hundred percent of the untreated control mice died by day 7 of infection. Treatment with atovaquone alone at 5 mg/kg/day or trovafloxacin alone at 25 mg/kg/day resulted in survival rates of 20% ($P < 0.001$) and 30% ($P < 0.001$), respectively. Treatment with the combination resulted in 60% survival. This effect was significantly different from the survival rate obtained with atovaquone alone ($P = 0.028$) but not from that obtained with trovafloxacin alone ($P = 0.255$).

Trovafloxacin plus clarithromycin. One hundred percent of the untreated control mice died by day 8 of infection (Fig. 1B). All of the mice treated with clarithromycin alone at 200 mg/kg/day or trovafloxacin alone at 25 mg/kg/day died by day 9. Treatment with the combination resulted in 40% survival. This effect was significantly different from the effect of treatment with either drug alone ($P < 0.005$).

Trovafloxacin plus pyrimethamine. One hundred percent of the untreated control mice died by day 8 of infection (Fig. 1C). Treatment with pyrimethamine alone at 10 mg/kg/day resulted in 30% survival ($P = 0.002$). Treatment with trovafloxacin alone at 25 mg/kg/day resulted in prolongation of time to death of the infected mice. Treatment with the combination resulted in 70% survival. This effect was significantly different from the 30% survival rate of mice treated with pyrimethamine alone ($P = 0.029$) and the prolongation of time to death with trovafloxacin alone ($P < 0.0001$).

Trovafloxacin plus rifabutin. One hundred percent of the untreated control mice died by day 7 of infection (Fig. 1D). Treatment with rifabutin alone at 50 mg/kg/day and treatment with trovafloxacin alone at 25 mg/kg/day resulted in survival of 10% ($P < 0.001$) and 30% ($P < 0.001$) of the treated mice, respectively. Treatment with the combination resulted in 50% survival. This effect was significantly different from that afforded by treatment with rifabutin alone ($P = 0.013$) but not from that afforded by trovafloxacin alone ($P = 0.138$).

Trovafloxacin plus sulfadiazine. One hundred percent of the untreated control mice died by day 7 of infection (Fig. 1E). Treatment with sulfadiazine alone at 150 mg/liter and treatment with trovafloxacin alone at 25 mg/kg/day resulted in 20% ($P < 0.001$) and 30% ($P < 0.001$) survival, respectively. Treatment with the combination resulted in 100% survival of the mice ($P < 0.001$).

Residual infection as measured by death of subinoculated mice or their seroconversion revealed that none of the drug combinations at the dosages used eradicated the parasite from the tissues.

Pharmacokinetic studies of trovafloxacin in mice. Pharmacokinetic studies revealed that significant levels of trovafloxacin in serum are achieved within 30 min following oral administration at a dose of 10, 25, or 50 mg/kg (Fig. 2A and B). Levels of trovafloxacin in uninfected and infected mice following a single oral dose of 50 mg/kg (Fig. 2A) did not differ significantly. No significant increases in levels of the drug in serum were observed following once-daily dosing with trovafloxacin for 3 days at 50 mg/kg compared to levels achieved following the first dose (Fig. 2A). C_{max} and AUC_{0-1} of trovafloxacin in sera of infected mice increased linearly with increasing doses of trovafloxacin (correlation coefficient = 0.951 and 0.952, respectively) (Fig. 2C). Following a single oral dose of 50 mg of trovafloxacin per kg, C_{max} and AUC_{0-1} of the drug in the sera of infected mice were $10.6 \pm 1.9 \mu\text{g/ml}$ and $8.2 \pm 1.3 \mu\text{g} \cdot \text{h/ml}$, respectively (Fig. 3). In contrast, C_{max} and AUC_{0-1}

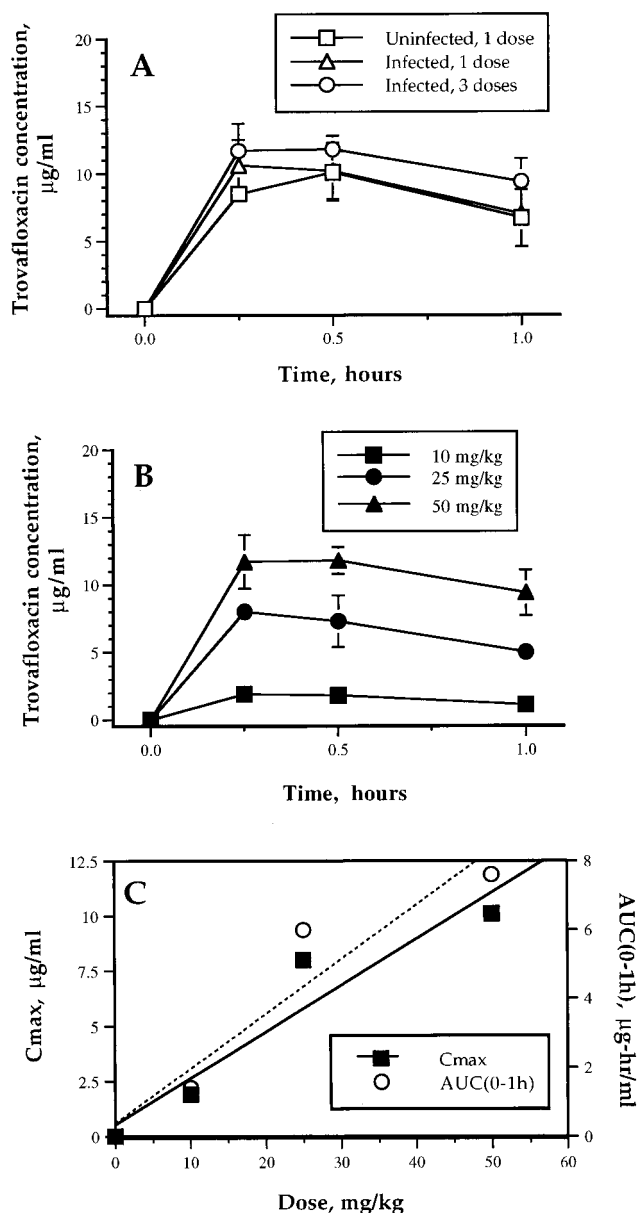


FIG. 2. Concentrations of trovafloxacin in serum following a single oral dose of 50 mg/kg to uninfected mice or mice infected with tachyzoites and following doses of 50 mg/kg/day for 3 days to mice infected with tachyzoites (A) and following increasing single oral doses to mice infected with tachyzoites (B) and the linear relationships between increasing single oral doses of trovafloxacin and pharmacokinetic parameters, C_{max} ($r = 0.951$, $P = 0.049$) and AUC_{0-1} ($r = 0.952$, $P = 0.048$), for mice infected with tachyzoites (C). Three mice per group were used. Mice were treated 24 h postinfection.

were 10-fold lower in the brain, 3-fold higher in the liver, and 2-fold higher in the spleen samples than those achieved in the sera. A similar relationship was also observed following treatment with trovafloxacin for 3 days.

DISCUSSION

The results described above reveal that significant enhancement in protective activity against rapidly multiplying tachyzoites of the RH strain of *T. gondii* occurs in a murine model of acute toxoplasmosis when trovafloxacin, at a suboptimal

dose, is used in combination with other antitoxoplasma drugs at doses which produce a marginal or no protective effect when each drug is used alone. The various drug combinations did not eradicate the parasite from the tissues.

We have previously reported that trovafloxacin is highly active when used alone against the virulent RH strain of *T. gondii* in vitro in cell culture and in vivo in a murine model of acute toxoplasmosis (8). In a separate series of experiments, we have observed similar potent protective activity of trovafloxacin in a murine model of acute toxoplasmosis following oral infection with cysts of the C56 strain of *T. gondii* (unpublished data). The mode of action of most fluoroquinolones against bacteria is based on their ability to convert DNA gyrase, a prokaryotic type II topoisomerase, into a cellular poison by enhancing enzyme-mediated DNA breakage (7, 10, 11). Fluoroquinolones have recently been shown to inhibit activities of the essential enzyme topoisomerase IV in bacteria (6). The mechanism of action of trovafloxacin against *T. gondii* is not known.

In multiple experiments, trovafloxacin administered alone at 25 mg/kg/day resulted in prolongation of time to death of the infected mice or in survival of up to 30% of the mice, when 100% of the control mice had died. Variability in the activity of trovafloxacin (0 to 30%) at 25 mg/kg/day is most likely due to variations in the inoculum size even after the utmost care has been taken in counting. Treatment with higher doses (≥ 50 mg/kg/day) regularly resulted in survival of 90 to 100% of the mice. Survival of 30% of the mice treated with trovafloxacin at 25 mg/kg/day allowed for a sufficient margin to permit observation of enhancement in activity when the drug was used in combination with other drugs at doses which are ineffective or only marginally effective when each drug is used alone.

Significant enhancement of survival of infected mice resulted when trovafloxacin was used in combination with atovaquone, clarithromycin, pyrimethamine, rifabutin, or sulfadiazine compared to treatment with each drug alone. Combinations of trovafloxacin with clarithromycin, pyrimethamine, and sulfadiazine demonstrated significantly higher survival rates than those obtained with each drug alone. However, combinations of trovafloxacin with atovaquone or rifabutin yielded survival rates of mice that were significantly greater than those obtained with atovaquone and rifabutin alone but not significantly different from that obtained with trovafloxacin alone. Use of trovafloxacin in combination with a drug(s) which has a significantly high incidence of side effects when used alone might allow lowering of the dose of the latter drug(s) and at the same time yield comparable or even better therapeutic results.

Pharmacokinetic studies of trovafloxacin in mice revealed that infection with *T. gondii* does not alter the levels of the drug in serum or tissue following 1 or 3 days of oral treatment. We also observed that peak levels and AUCs of trovafloxacin increase linearly with increases in dose. This is in agreement with similar observations for humans (12). Absorption of the drug was rapid, and peak levels were achieved within 15 to 30 min following oral administration to mice. Concentrations of trovafloxacin in serum in mice following an oral dose of 10 or 25 mg/kg were within the range seen in humans following single oral doses of 100 to 600 mg (12). High concentrations achieved in serum, liver, and spleen may help trovafloxacin reduce parasite load in the repository sites and may explain its potent activity against *T. gondii* in acute toxoplasmosis in mice.

Our results suggest that combinations of trovafloxacin with

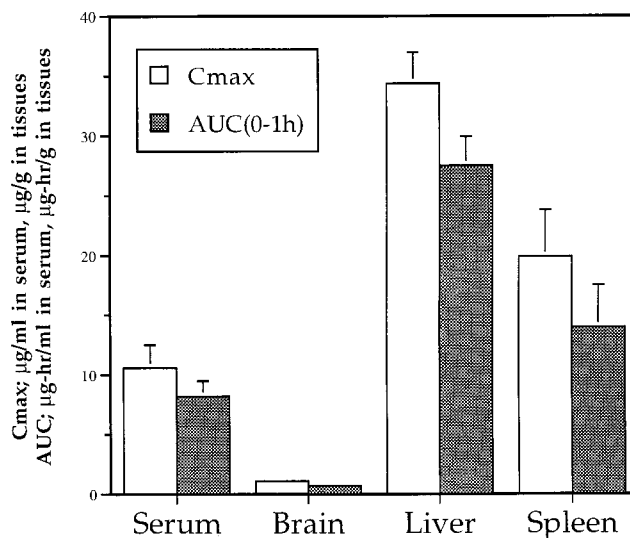


FIG. 3. C_{max} and AUC_{0-1} achieved in sera, brains, livers, and spleens of the mice infected with tachyzoites and treated with a single oral dose of trovafloxacin at 50 mg/kg after 24 h of infection. Data are means + standard deviations for three mice per group.

the drugs described above should be further explored for treatment of toxoplasmosis in humans.

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