Synergistic Activity of Azithromycin and Gamma Interferon in Murine Toxoplasmosis

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A dose of 75 mg of azithromycin per kg of body weight per day combined with a dose of 2 μg of gamma interferon per day and administered for 10 days protected at least 40% of mice infected with a lethal inoculum of Toxoplasma gondii. Azithromycin administered alone protected less than 10% of the mice; gamma interferon had no protective effect.

Toxoplasma gondii is one of the major opportunistic agents causing infections in immunocompromised individuals, particularly in those with AIDS (3, 6, 9). Treatment of the infection with the combination pyrimethamine-sulfonamide may result in strong adverse reactions requiring discontinuation of the treatment before cure has been achieved (5, 6). Because AIDS patients with toxoplasmosis, particularly toxoplasmic encephalitis, must be maintained on prolonged therapy to prevent recrudescence of the infection, it is important to develop safe and effective therapeutic regimens as an alternative to the pyrimethamine-sulfonamide combination (3, 6). We have previously demonstrated that the macrolide antibiotic azithromycin has potent activity against T. gondii in mice (2). Further observations have indicated that its activity is significantly superior to that of other macrolide antibiotics which have been used to treat human toxoplasmosis (1, 4). In addition, work from our laboratory has demonstrated that gamma interferon (IFN-γ) has protective activity against murine toxoplasmosis (7, 8) and has a critical role as a mediator of host immunity to toxoplasmosis (9). Thus, it was considered of interest to investigate the activity of the combination of IFN-γ-azithromycin in the treatment of murine toxoplasmosis.

Azithromycin (lot 14, 462-120-1F) and recombinant murine IFN-γ (rMuIFN-γ) (lot 2271-68; 2.3 × 10^5 U/mg) were from Pfizer Inc., Groton, Conn., and Genentech Co., South San Francisco, Calif., respectively. Female Swiss Webster mice (Simonsen Laboratories, Gilroy, Calif.), weighing 20 g each at the beginning of the experiment, were injected intraperitoneally with 2 μg of rMuIFN-γ in 0.2 ml of endotoxin-free phosphate-buffered saline (PBS). Controls were injected with PBS only. Twenty-four hours later, each mouse was infected intraperitoneally with 2.5 × 10^3 trophozoites of the RH strain of T. gondii. This inoculum is equal to at least 250 100% lethal doses for mice. Thereafter, the mice were divided into four groups. Group A (control) was not given any treatment, group B (azithromycin alone) received 75 mg of azithromycin per kg of body weight per day administered orally for 10 days, group C (rMuIFN-γ alone) received a daily intraperitoneal injection of 2 μg of rMuIFN-γ for 10 days, and group D (combination group) received azithromycin and rMuIFN-γ as described above for groups B and C. Treatment with azithromycin was started 24 h after infection. These doses of rMuIFN-γ and azithromycin were used because preliminary experiments showed them to be optimal for this type of experiment. In repeated experiments none of these doses, when used alone, protected more than 10% of mice against death caused by the inoculum of T. gondii employed in the study.

Three experiments were performed at different times with a total of 90 mice. The results of one of two experiments with identical results are shown in Fig. 1. Protection induced by the combination rMuIFN-γ-azithromycin was significantly higher than protection induced by either azithromycin alone or rMuIFN-γ alone. Thus, at the time when 100% of mice treated with rMuIFN-γ alone were dead, 90% of the mice treated with the combination rMuIFN-γ-azithromycin were alive (P < 0.01, Student’s t test). Similarly, at the time when 90% of the mice treated with azithromycin alone were dead, only 20% of those treated with the combination IFN-γ-azithromycin had died (P < 0.05). Thirty days after infection, when the experiment was terminated, only 1 of 10 mice treated with azithromycin alone was alive, whereas 4 of 10 mice treated with the combination were alive. None of the mice treated with IFN-γ alone survived longer than 9 days.

![FIG. 1. Synergistic effect of azithromycin and IFN-γ against T. gondii infection in mice. There were five mice each in the control and azithromycin groups and 10 mice each in the rMuIFN and azithromycin + rMuIFN groups.](http://aac.asm.org/)

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These results demonstrate that in murine toxoplasmosis, the combination rMuIFN-γ-azithromycin provides significant protection against death compared with treatment with either of these agents alone. In other reports (2, 4), it was demonstrated that, by using the model employed in this study, significant protection was achieved with an azithromycin dose of 200 mg/kg per day for 10 days. A dose of 5 × 10^4 U of IFN-γ induced significant protection in mice infected with *T. gondii* C56 (8). In the present study, a similar dose did not protect mice infected with the more virulent RH strain. A dose of IFN-γ twice as high as that used in the present study also was not protective (1). Although there are a number of limitations which prevent extrapolating results obtained with murine models to the situation in humans, the results described above are relevant in the search for alternative therapies for treatment of toxoplasmosis in immunocompromised hosts.

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REFERENCES

1. Araujo, F. G. Unpublished data.