Effect of Roxithromycin on Acute Toxoplasmosis in Mice

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Roxithromycin effectively treated acute peritoneal murine toxoplasmosis. After five doses, starting 24 h after challenge, the 100 and 50% survival doses were 540 and 336 mg/kg per day, respectively. After 14 doses, starting 3 h after challenge, the 50% survival dose was 360 mg/kg per day. Toxoplasma gondii was recovered from the brain in 59 and 28% of surviving mice treated with 5 and 14 doses, respectively.

In immunocompromised patients or during pregnancy, toxoplasmosis can cause dramatic consequences, and no therapy is available to eradicate the parasite. The combination of pyrimethamine and sulfadiazine, the recommended treatment, is active against tachyzoites (4) but remains ineffective against the tissue cyst form of Toxoplasma gondii. The mortality among immunocompromised patients with Toxoplasma encephalitis treated with this combination approaches 70% (11). Moreover, the toxicity of the combination precludes its use during pregnancy (8, 13).

Spiramycin, a macrolide antibiotic, is less toxic than pyrimethamine and sulfadiazine, but the lack of well-controlled trials leaves uncertainties about its clinical effectiveness. Spiramycin is active against murine acute toxoplasmosis (5) and has been used effectively for treating pregnant women (3), but showed poor activity against T. gondii in cell cultures (9).

Recently, roxithromycin, a new macrolide with an antimicrobial activity similar to that of erythromycin (1, 6), was effective in a mouse model of acute toxoplasmosis (2). Using a similar model, we conducted the present study for evaluating the activity of roxithromycin under very stringent conditions.

Roxithromycin was provided by Roussel-UCLAF, Paris, France, in powder form, and the other antimicrobial agents were provided by their respective manufacturers. Before being used, the drugs were suspended in 0.25% carboxymethyl cellulose-0.20% Tween 80 in sterile water; they were administered once a day by gavage in a volume of 0.5 ml by means of a feeding needle. Mice were fasted for 1 h before receiving the drugs.

Female Swiss-Webster mice, weighing 25 ± 1 g (Madôrin AG, Füllinsdorf, Switzerland), were used. Experimental infection was established by intraperitoneal injection of 5 × 10³ tachyzoites of the virulent RH strain of T. gondii (16), i.e., 500 times the 100% lethal dose (15), in 0.5 ml of sterile 0.9% NaCl. Animals were randomly allocated in groups of 10 and kept in conventional cages with free access to food and water. At the end of the study period, the surviving mice were sacrificed and autopsied. Peritoneal exudates were examined microscopically (×400) for the presence of T. gondii. When no parasites were seen, the brain was ground with fine glass beads in a mortar containing 5 ml of sterile 0.9% NaCl; a portion (1 ml) of this suspension was injected into each of two naive, untreated mice to determine whether the treatment resulted in eradication of T. gondii from the brain. The donor was considered cured if the two recipient mice survived 30 days after injection without having Toxoplasma infection at autopsy.

Two therapeutic regimens were used. In one protocol, the study period lasted 25 days. The treatment was started 24 h after challenge and was continued for 5 days. In the other protocol, the study period lasted 30 days, and the treatment was started 3 h after challenge and was continued for 14 days. Each experiment included 10 infected mice for each drug regimen, as well as three control groups: infected untreated mice, uninfected mice receiving only drug excipient (all survived), and uninfected mice receiving the drug with excipient.

The 50% survival dose was calculated by using the Reed and Muench method (14), and the 95% confidence limits were calculated by the Litchfield and Wilcoxon method (12). The 100% survival dose was determined as the minimal dose after which a 100% survival rate was effectively observed. The cure rate was determined according to the number of surviving mice yielding negative results after brain transfer.

All infected, untreated control mice died 8 ± 1 days after challenge. At autopsy, numerous tachyzoites were seen in the peritoneal fluid at microscopic examination. Brain transfers were always positive.

The combination pyrimethamine-sulfadiazine (4.4 and 250 mg/kg per day, respectively) protected 90% of mice after 5 doses (Table 1) and 100% of mice after 14 doses (Table 2). Brain transfers were always negative.

In mice treated with roxithromycin for 5 days (Table 1), the 50% and 100% survival doses were calculated, respectively, to be 336 mg/kg per day (95% confidence limits: 302.43 to 373.29) and 540 mg/kg per day (P < 0.001 against infected untreated controls). All roxithromycin-treated uninfected mice survived. The 50% survival dose of spiramycin was calculated to be 300 mg/kg per day. The 100% survival dose of spiramycin could not be determined because early lethals (around day 3 of therapy) occurred both in infected and uninfected spiramycin-treated mice at doses of 420 mg/kg per day or over. This probably reflected a toxic effect of the drug, already mentioned (5). Transfer of brain suspensions from surviving treated mice into naive untreated mice yielded similar results for roxithromycin and spiramycin (overall positivity of 59% in both cases).

In mice treated for 14 days (Table 2), the 50% survival dose of roxithromycin was calculated at 360 mg/kg per day. The transfer of brain suspensions was positive in only 28% of the surviving mice in this series.

During the natural course of our experimental infection, dissemination of T. gondii through the body follows the

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intraperitoneal challenge. All untreated mice had a brain infection before death, but the exact timing of the cerebral infection is unknown. Therefore, the cure rate may reflect either a preventive or an actual therapeutic effect. Nevertheless, roxithromycin appeared less effective than pyrimethamine-sulfadiazine for preventing or treating cerebral toxoplasmosis produced after peritoneal infection with RH tachyzoites.

In similar experiments (2), the 50% survival dose of roxithromycin was 625 to 667 mg/kg per day after 4 weeks of therapy. This represents a much greater amount of roxithromycin than that used here for obtaining the same result. Chan and Luft (2) delivered the antibiotic by mixing it with powdered mouse chow. In our studies we used administration by gavage in fasting mice, ensuring total delivery of the drug into an empty stomach and probably higher peak blood levels.

The intracellular survival of T. gondii is not yet completely understood, but it is clear that toxoplasmas multiply inside intracellular parasitophorous vacuoles, avoiding the phagosome-lysosome fusion (7). It has been reported that roxithromycin concentrates considerably in phagocytic cells, especially in their lysosomes (M. B. Carlier, A. Zenebergh, and P. M. Tulkens, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother. abstr. no. 929, 1986), and probably enhances ingestion and bacterial killing by neutrophils (10) and monocytes (A. Fietta, P. Mangiarotti, C. Bersani, V. de Rose, and G. G. Grassi, Program Abstr. 14th Int. Congr. Chemother., P-46-81, p. 415, 1985). These characteristics could be of importance in the antitoxoplastic activity of roxithromycin.

In conclusion, roxithromycin may represent a worthwhile alternative for treating toxoplasmosis, especially if the drug is proven to be safe in pregnancy. However, clinical evaluation is necessary to determine the position of this compound among the other chemotherapeutic agents available against T. gondii human infection including Toxoplasma encephalitis.

LITERATURE CITED


