Use of ketolides in combination with other drugs to treat experimental toxoplasmosis

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Because combination therapy is required to treat human toxoplasmosis, we examined combinations of the ketolides HMR 3004 and HMR 3647 with atovaquone, clindamycin or sulphadiazine in a murine model of toxoplasmosis. An oral dose of 50 mg/kg/day of HMR 3004 protected 30% of mice lethally infected with \textit{Toxoplasma gondii}. The same dose protected 100% of infected mice when administered in combination with non-protective doses of atovaquone, clindamycin or sulphadiazine. Similar results were noted with 25 mg/kg/day of HMR 3647. These results demonstrate that these drug combinations are highly effective for treating toxoplasmosis in mice.

Introduction

We have previously demonstrated that ketolides, a new class of derivatives of 14-membered ring macrolides, are active both in vitro and in vivo against \textit{Toxoplasma gondii}.\textsuperscript{1} Because therapy with a single drug is often ineffective, particularly in acute toxoplasmosis or toxoplasmic encephalitis in severely immunocompromised individuals,\textsuperscript{2} we examined whether the activity of drugs frequently used to treat human toxoplasmosis is enhanced when used in combination with the ketolides HMR 3004 and HMR 3647.

Materials and methods

Mice

Swiss-Webster female mice (Simonsen Laboratories, Gilroy, CA, USA), weighing 20 g at the beginning of each experiment, were used. They were given water and food ad libitum throughout the experiments. Groups of ten mice were used in all experiments.

Toxoplasma gondii

Tachyzoites of the RH strain were obtained from the peritoneal cavities of mice infected for 2 days as previously described.\textsuperscript{3} Each mouse was infected intraperitoneally (ip) with 2.5 x 10\textsuperscript{6} tachyzoites.

Drugs and treatment

The ketolides HMR 3004 and HMR 3647 were obtained from Roussel UCLAF (Romainville, France). Atovaquone was obtained from Burroughs Wellcome Co. (Research Triangle Park, NC, USA) and clindamycin from the Pharmacia–Upjohn Co. (Kalamazoo, MI, USA). Sulphadiazine was obtained from Sigma Chemical Co. (St Louis, MO, USA). Ketolides and atovaquone were prepared by adding them to a solution of 0.25% carboxymethyl cellulose (CMC) followed by sonication for 30 s. The suspensions were kept at 4°C and were sonicated briefly before each dosing of the mice. Clindamycin–HCl was dissolved in double distilled water and administered in CMC. Sulphadiazine was dissolved in drinking water.

Ketolides, atovaquone or clindamycin–HCl alone or in combination were administered orally, by gavage, as a single daily dose. Sulphadiazine was administered in the drinking water and was available at all times during treatment. Controls were treated with 0.25% CMC administered by gavage. Treatment with each drug alone or in combination was started 24 h after infection and continued for 10 days. Mice were observed for 30 days from the day of infection to determine whether reactivation of the infection would occur following discontinuation of therapy. Surviving mice were examined for residual infection by ip inoculation of suspensions of portions of their liver and spleen into healthy mice. The toxicities of the ketolides for mice were determined by treating healthy mice with dosages of each ketolide as high as 300 mg/kg/day for 10
days. These mice were observed daily for signs of toxicity such as piloerection, lethargy and weight loss.

Statistical analysis

Statistical analysis was performed using the Mann-Whitney U-test using statistical software (GraphPad Software Inc., San Diego, CA, USA).

Results and discussion

Uninfected mice treated with doses as high as 300 mg/kg/day of each ketolide administered daily for 10 days did not show any signs of toxicity. Mortality in infected and untreated control mice was 100% by day 9 of infection whereas 70% of mice treated with 50 mg/kg/day of HMR 3004 alone died. Survival was 100% when this same dose was used in combination with doses of atovaquone, sulphadiazine or pyrimethamine that were not protective when administered alone (P = 0.01). Figure 1 shows the results of one of the two experiments performed. The results were essentially the same in the other experiment. Mortality in mice treated with 25 mg/kg/day of ketolide HMR 3647 administered alone was 50% whereas survival was 100% (P = 0.01) in mice treated with this same dose of ketolide administered in combination with doses of atovaquone, sulfadiazine or clindamycin that were not protective when used alone. Three experiments were conducted with different batches of HMR 3647. Batch-to-batch variation was noted but each batch demonstrated excellent activity. Figure 2 shows representative results of one of these experiments. Inoculation of tissues from surviving mice into healthy mice did not result in the development of acute toxoplasmosis in the recipient mice. This suggests that the treatment had eradicated the parasites from the treated mice since an inoculum calculated to contain a single tachyzoite of the RH strain is lethal for these mice.

The above results demonstrate that the activity of HMR 3004 and HMR 3647 and of atovaquone, sulphadiazine or clindamycin against T. gondii is significantly enhanced when individual combinations of these drugs are used. Ketolides are a new generation of macrolide antibiotics that have been shown to be far more stable in acid conditions than clarithromycin, roxithromycin or azithromycin. Both the ketolides employed in the present study are highly active against streptococci and staphylococci resistant to other macrolides. In bacteria, the mechanism of action of ketolides appears to be similar to that of macrolides, i.e. inhibition of protein synthesis. It should be noted, however, that treatment of extracellular or intracellular tachyzoites with 100 μM of azithromycin or clindamycin for 24 h did not result in inhibition of protein synthesis by the parasites. Thus, the mechanism(s) of the activity of ketolides against T. gondii may involve steps other than inhibition of protein synthesis. Other macrolides including clarithromycin and azithromycin have been shown to be active against T. gondii and their activity is significantly enhanced by combining them with other drugs or with cytokines. However, when macrolides are used alone, relatively high concentrations are required to demonstrate in-vivo activity. That HMR 3004 and HMR 3647 are active at relatively low doses and that significant enhancement in activity resulted from their combination with ineffective doses of other anti-toxoplasma drugs is of considerable interest. Our results indicate that studies to examine the activity of ketolides either alone or in combination with other drugs in the treatment of human toxoplasmosis are warranted.

Figure 1. Survival in mice infected intraperitoneally with tachyzoites of the RH strain of T. gondii and treated with ketolide HMR 3004 50 mg/kg (□, panels a–c) or with atovaquone 5 mg/kg (a, ○), sulphadiazine 150 mg/L (b, ◦) or clindamycin 25 mg/kg (c, ●), or with a combination of HMR 3004 with atovaquone (a, ●), sulphadiazine (b, ◦) or clindamycin (c, ●). Controls are represented by solid squares (■) in each panel. Treatment was administered orally, begun 24 h after infection and continued for 10 days. There were ten mice in each group.
Ketolides and combinations against toxoplasmosis

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References


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Figure 2. Survival in mice infected intraperitoneally with tachyzoites of the RH strain of T. gondii and treated with ketolide HMR 3647 (a–d, 25 mg/kg, □) alone, or with (a) atovaquone, (b) sulphadiazine or (c) clindamycin or with a combination of HMR 3647 with (a) atovaquone, (b) sulphadiazine or (c) clindamycin. See legend to Figure 1 for other symbols and for the concentrations of the other drugs. Treatment was administered orally, begun 24 h after infection and continued for 10 days. There were ten mice in each group.