Journal of Antimicrobial Chemotherapy (1987) **20**, *Suppl. B*, 69–74

Activity of roxithromycin against *Toxoplasma gondii* in murine models

Hernan R. Chang and Jean-Claude F. Pechère

Department of Microbiology, University of Geneva Medical School, C.M.U., 9 avenue de Champel, 1211 Geneva 4, Switzerland

Investigations into the activity of roxithromycin against murine toxoplasma infections are reviewed. Roxithromycin is an active drug against murine toxoplasmosis after intraperitoneal challenge with the RH strain of *Toxoplasma gondii*. Roxithromycin protected 100% of mice after five daily doses of 540 mg per kg administered by gavage. The cure rate after treatment of peritoneal infections seemed to be related to the length of the therapy. Roxithromycin also decreased the number of toxoplasma cysts, after intracerebral infection with the C56 strain and showed synergistic activity when combined with gamma interferon. Thus, roxithromycin could be a worthwhile alternative to current therapy against toxoplasma infections. Clinical studies on its activity and safety, especially in pregnancy, are warranted.

Introduction

Infections caused by *Toxoplasma gondii* are very common all over the world, but only a small percentage of those infections produce severe symptoms and significant illness. Infections in the immunocompromised host and in pregnant women are of special significance because they can lead to the development of very severe toxoplasmosis. The immunocompromised host, especially with AIDS, can develop severe toxoplasma encephalitis and up to 12% of AIDS patients who have antibodies to *T. gondii* do indeed develop the disease (Hofflin, Conley & Remington, 1987). The infected fetus often develops serious sensorial and neurological disorders. In France, there is an estimated 6·3% risk of infection during pregnancy, and an estimated 33% incidence of fetal infection in those women who become infected during their pregnancies (Desmonts & Couvreur, 1974).

The most effective treatment for both acquired and congenital toxoplasmosis is the combination of pyrimethamine and sulphadiazine (or triple sulphonamides) (Eyles & Coleman, 1955). However, the mortality rate among immunodeficient patients with toxoplasma encephalitis treated with this combination approaches 70% (Levy, Bredesen & Rosemblum, 1985), and the toxicity of the combination precludes its use during pregnancy (Kutscher, Lane & Segael, 1954; Kaufman & Geisler, 1960). Therefore, there is a need for alternative therapy for this disease.

Spiramycin, a macrolide antibiotic, is less toxic than pyrimethamine and sulphadiazine, but the lack of well controlled trials leaves uncertainties about its clinical effectiveness. Spiramycin is active against murine acute toxoplasmosis (Garin & Eyles, 1958) and has been used effectively for treating pregnant women (Desmonts & Couvreur, 1979).

0305–7453/87/20B069+06 $02.00/0

Roxithromycin is a new macrolide antibiotic with an in-vitro antimicrobial spectrum similar to that of erythromycin (Barlam & Neu, 1984); but roxithromycin is distinct in that it produces higher serum levels and has a longer half-life (Puri *et al.*, 1985). Hence, the antitoxoplasmic activity of roxithromycin has been evaluated by several authors in murine models. The results of these investigations will be reviewed here.

Murine models of toxoplasmosis

Mice are very susceptible to *T. gondii* infection; although results may vary with the parasite and the strain of mouse. Two strains of *T. gondii* have been selected in the studies reviewed here: the highly virulent RH strain described by Sabin (1941) and the relatively virulent C56 strain. Young Swiss Webster mice weighing 15–25 g were used in all studies. The inoculum was obtained by harvesting tachyzoites from the peritoneal fluid of mice heavily infected for this purpose. This inoculum was standardized by appropriate dilution after microscopic examination.

Acute toxoplasmosis after intraperitoneal inoculation

Intraperitoneal inoculation of the RH strain was used in a first model (Chan & Luft, 1986; Chang & Pechère, 1987). Less than ten tachyzoites are able to kill all the mice, and the inoculum is standardized to be lethal in seven to nine days (5000 tachyzoites in our model). The experimental disease is characterized by the production of ascites containing numerous tachyzoites. At autopsy, tachyzoites are also found in most organs, including the spleen, liver and brain. For assessing the activity of a drug in this model the protective dose is calculated. Moreover, when no parasites are seen in the surviving animals, the organs (especially the brain of these animals), are ground in a mortar and portions of these suspensions are injected into untreated mice not previously exposed to toxoplasma. The donors can be considered as cured when the recipient mice survive 30 days after injection without having toxoplasma at autopsy.

Toxoplasma encephalitis

Toxoplasmic encephalitis can be established by intraperitoneal injection of 10,000 tachyzoites of the C56 strain into mice immunocompromised with cortisone acetate or cyclophosphamide (Luft *et al.*, 1986). However, the same group of researchers developed a particularly suitable model (Hofflin, Conley & Remington, 1987). Here, an encephalitis is produced after intracerebral inoculation of 10,000 C56 tachyzoites. With the use of a stereotactic unit, injections are delivered into the right frontal lobe of the brain. The mice develop necrosis at the site of inoculation surrounded by areas with cyst formation. The therapeutic activity is assessed by survival rate, time until death and number of toxoplasma cysts in the brain.

Effect of roxithromycin

Roxithromycin in the peritoneal model

Two studies (Chan & Luft, 1986; Chang & Pechère, 1987), dealt with the activity of roxithromycin in this acute type of toxoplasma infection (Table I). In the study of

Chan & Luft (1986) roxithromycin was mixed in powdered mouse-feed after the observation that the mice consumed 4 g of feed per day. In treatments starting 24 h after challenge and given for 28 days the 50% survival dose was 625 to 660 mg/kg per day. The 100% survival dose was not obtained.

Chang & Pechère (1987) administered roxithromycin once a day by gavage. After five doses, starting 24 h after challenge, the 50% and 100% survival doses were 336 and 540 mg/kg per day, respectively. The cure rate, determined according to the number of surviving mice yielding negative results after brain transfer, seemed to be associated with the duration of the therapy: 41% and 72%, after five and 14 days of therapy, respectively (Chang & Pechère, 1987), and 90% after 28 days of therapy (Chan & Luft, 1986).

Roxithromycin against toxoplasma encephalitis

In immunocompromised mice infected by the peritoneal route, roxithromycin at a dose of 10 mg per day, mechanically mixed with chow, significantly protected mice compared with untreated controls (Table I). Even when therapy was delayed for as long as four days results were similar (Luft *et al.*, 1986).

In the model of toxoplasma encephalitis after intracerebral inoculation, roxithromycin at a daily dosage of 35 or 50 mg per mouse decreased the rate of mortality (Hofflin & Remington, 1987). Moreover, the median number of cysts in the treated mice that survived was 142 compared with 484 in the untreated that survived. The same study showed very interestingly that gamma interferon acted synergistically with roxithromycin. When combined, the two agents strikingly increased the survival of the mice.

Discussion

Roxithromycin was active in treating acute infection with the virulent RH strain of *T. gondii* in mice. For the first time it has been possible to report total protection with a macrolide in such an infection (Chang & Pechère, 1987). Of particular interest was the fact that some surviving mice seemed completely cured according to the subinoculation studies. However, in that study, roxithromycin appeared less effective than the combination of pyrimethamine and sulphadiazine for treating cerebral toxoplasmosis produced after peritoneal infection of mice with RH tachyzoites.

In the two studies dealing with acute peritoneal toxoplasmosis, the 50% survival doses were different. The mode of administration of roxithromycin probably accounted for this difference. In the study by Chan & Luft (1986) the total dose required to protect 50% of the animals was approximately 18 g/kg; the antibiotic was mixed with feed. In the study by Chang & Pechère (1987) the total dose was only 1·6 g/kg; roxithromycin was administered by gavage in fasting mice. This procedure assured total delivery of the drug into an empty stomach during the entire treatment period and was likely to produce higher peak blood levels. In healthy volunteers, roxithromycin bioavailability was reduced two-fold after a meal, compared with the fasting state (Tremblay *et al.*, 1986). Also, higher blood peaks could possibly produce higher intracellular concentrations.

Thus far we have no data about the penetration of roxithromycin into the brain, but the reduced numbers of toxoplasma cysts in the encephalitis model suggest that the

Table I. Studies on the activity of roxythromycin against *T. gondii* in mice

Mouse strain/weight	*T. gondii* strain	Inoculum/ route	Therapy/route of administration	Time of therapy	Survival rate %	Cure rate among survivors	Reference
?	C56		oral, with mouse food				Luft *et al.*
		10^4 ip	cortisone acetate, non-ROX-treated	NM	0	ND	(1986)
			cortisone acetate + ROX 10 mg/day	NM	100	ND	
			cyclophosphamide, non-ROX-treated	NM	0	ND	
			cyclophosphamide + ROX 10 mg/day	NM	80	ND	
		10^4 ic	untreated controls	0	0	0	
			ROX 10 mg/day	NM	85	ND	
Swiss Webster	C56		oral, with mouse food				Hofflin &
female 18–20 g		10^4 ic	untreated controls	0	11	0	Remington, (1987)
			ROX 50 mg/day[c]	22 days	44	ND	
			untreated controls	0	10	0	
			ROX 35 mg/day[b]	22 days	38	ND	
		10^4 ic	untreated controls	0	0	0	
			ROX 35 mg/day[a]	16 days	0	ND	
			gamma interferon 5×10^4 U[a]	16 days	0	ND	
			gamma interferon 5×10^4 U + ROX 35 mg/day[a]	16 days	40	ND	
		10^4 ic	untreated controls	0	0	0	
			ROX 25 mg/day[a]	16 days	0	ND	
			gamma interferon 5×10^4 U[a]	16 days	0	ND	
			gamma interferon 5×10^4 U + ROX 25 mg/day[a]	16 days	100	ND	

Swiss Webster	RH		oral, with mouse food				Chan &
female 15–16 g		2×10^2	untreated controls	0	0	0	Luft, (1986)
		ip	ROX 10 mg/day[b]	28 days	100	70	
		2×10^3	untreated controls	0	0	0	
		ip	ROX 10 mg/day[b]	28 days	90	100	
			untreated controls	0	0	0	
			ROX 10 mg/day[d]	28 days	80	70	
			untreated controls	0	0	0	
			ROX 10 mg/day[e]	28 days	50	90	
			P-S (0·044 mg/day-5 mg/day)[e]	28 days	90	100	
		2×10^4	untreated controls	0	0	0	
		ip	ROX 10 mg/day[b]	28 days	100	80	
			untreated controls	0	0	0	
			ROX 10 mg/day[d]	28 days	80	100	
Swiss Webster	RH		oral, by gavage				Chang &
female 25 ± 1 g		5×10^3	untreated controls	0	0	0	Pechère (1987)
		ip	ROX SD_{50}: 336 mg/kg/day[e]	5 days	50	59	
			ROX SD_{100}: 540 mg/kg/day[e]	5 days	100	50	
			SPI SD_{50}: 300 mg/kg/day[e]	5 days	50	59	
			P-S (4·4 mg/kg/day–250 mg/kg/day)[e]	5 days	90	100	
		5×10^3	untreated controls	0	0	0	
		ip	ROX SD_{50} 360 mg/kg/day[d]	14 days	50	28	
			P-S (4·4 mg/kg/day–250 mg/kg/day)[d]	14 days	100	100	

ROX, Roxythromycin; SPI, spiramycin; P-S, pyrimethamine-sulphadiazine; ip, intraperitoneal; ic, intracerebral; SD_{50}, survival dose 50%; SD_{100}, survival dose 100%.

[a] Treatment started 2 h before challenge; [b] 24 h before challenge (prophylactic treatment); [c] 48 h before challenge; [d] 2–3 h after challenge (dose-response study); [e] 24 h after challenge (delayed treatment).

antibiotic exerts an effect in the brain parenchyma. These findings raise the hope that roxithromycin may have an activity against toxoplasma encephalitis in AIDS patients. This hope, however, must be confirmed by clinical studies.

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