In Vivo Activity of the Macrolide Antibiotics Azithromycin, Roxithromycin and Spiramycin against *Toxoplasma gondii*

F.G. Araujo[1], R.M. Shepard[2], J.S. Remington[1,3]*

The macrolide antibiotics azithromycin, roxithromycin and spiramycin were examined in parallel for in vivo activity against *Toxoplasma gondii.* Azithromycin was considerably more active in protecting mice against death due to acute toxoplasmosis even when the other two antibiotics were used at twice its dose. The higher activity of azithromycin prompted a further examination of its activity against five different strains of *Toxoplasma gondii,* including two isolated from patients with AIDS. Although variable degrees of protection against death were noted, treatment with 200 mg/kg/day for ten days was sufficient to promote survival of 100 % of mice infected with inocula as high as 1 x 10^5 tachyzoites of *Toxoplasma gondii.* 90 % of mice inoculated with 1 x 10^5 tachyzoites of strain MO, isolated from an AIDS patient, and treated orally with 200 mg/kg/day for ten days survived the infection whereas only 40 % of mice infected with the same inoculum of the SOU strain, also isolated from an AIDS patient, survived. Tissue concentrations of azithromycin were examined in treated infected and non-infected mice. In both groups of mice azithromycin attained high concentrations in liver, spleen and heart, which exceeded concurrent serum levels by 25- to 200-fold. The concentrations in the brain were almost tenfold higher than the concentrations in serum after treatment with 200 mg/kg/day for ten days. Moreover, the concentrations in brains of infected mice were approximately twofold higher than in brains of non-infected mice.

[1] Department of Immunology and Infectious Diseases, Research Institute, Palo Alto Medical Foundation, Palo Alto, California 94301, USA.
[2] Pfizer Inc., Central Research Division, Eastern Point Road, Groton, Connecticut 06340, USA.
[3] Stanford University School of Medicine, Divison of Infectious Diseases, Stanford, California 94305, USA.

Toxoplasma gondii is a major opportunistic pathogen in immunocompromised patients including heart transplant recipients and individuals with the acquired immunodeficiency syndrome (AIDS) (1, 2). Moreover, infection with *Toxoplasma gondii* acquired during pregnancy poses a great risk of infection of the fetus (3). Because of the remarkable synergistic effect of pyrimethamine and sulfadiazine against *Toxoplasma gondii* (4), this drug combination is most commonly used for treatment of toxoplasmosis (5). Both drugs, however, are potentially toxic; pyrimethamine may be teratogenic (3). Approximately 50 % of AIDS patients under treatment with the pyrimethamine-sulfadiazine combination manifest signs of toxicity sufficiently severe that the combination is discontinued (6, 7). Relapse following treatment of acute encephalitis in AIDS patients occurs in most patients when treatment is discontinued. Therefore, the search for newer therapeutic agents for treatment of toxoplasmosis, particularly for toxoplasmic encephalitis in AIDS patients, is critical. Recently, the erythromycin-derived macrolide antibiotics roxithromycin (an ether oxime derivative), azithromycin (an azalide with a methyl-substituted nitrogen inserted into the 9a position), and clarithromycin (a 6-O-methyl derivative) were demonstrated to possess activity against *Toxoplasma gondii* both in vitro an in vivo (8–11). Because different strains of *Toxoplasma gondii* and different mouse models of acute toxoplasmosis were used for the in vivo experiments described in these reports, we considered it of interest to use identical experimental conditions in the same murine model of acute toxoplasmosis to compare the activity of azithromycin and roxithromycin with that of spiramycin, another macrolide antibiotic commonly used to prevent congenital toxoplasmosis (3).

Materials and Methods. Outbreed Swiss-Webster female mice (Simonsen Laboratories, USA), weighing 20 grams at the beginning of each experiment, were used for all experiments to determine the activity of the various macrolide antibiotics against *Toxoplasma gondii.* Inbred CBA/Ca mice (Bantin and Kingman Laboratories, USA) were used for experiments to determine serum and tissue levels of azithromycin. These mice develop extensive parasitism and inflammation in their brains as early as ten days after infection with trophozoites of the ME49 strain of *Toxoplasma gondii* (12). Thus, they represent a good model to study the activity of drugs for treatment of toxoplasmic encephalitis. Water and food pellets were available at all times.

Strains RH, isolated from a human (13), C56 from a chicken (14), M7741 from a sheep (15), ME49 from a lamb (12), and MO and SOU isolated recently in our laboratory from tissues of two patients with AIDS and acute toxoplasmosis were used. Strain RH is maintained in our laboratory by intraperitoneal passage in mice every two or three days and strains C56, M7741, ME49, MO and SOU are maintained as cysts in brains of chronically infected mice. Trophozoites of the various strains were obtained from the peritoneal cavity of mice previously injected with an antiserum against gamma-interferon and inoculated with *Toxoplasma gondii* cysts as described (16). The parasites were washed twice with 0.1 M phosphate buffered saline (PBS) of pH 7.2, counted in a hemocytometer, and the desired number for intraperitoneal (i.p.) inoculation into each mouse was suspended in 0.2 ml of PBS.

Azithromycin (Pfizer, USA), roxithromycin (Hoechst-Roussel Pharmaceuticals, USA) and spiramycin (Rhone-Poulenc, USA) were dissolved in a small volume of 70 % ethanol, and the appropriate concentrations were prepared in polyethylene glycol 200 (PEG, J.T. Baker Chemical, USA). Treatment was by gavage, and was initiated 24 hours after infection and continued for ten days. Mice dying during treatment were examined for *Toxoplasma gondii* trophozoites in their peritoneal fluid. Survivors were examined for antibodies to the organism using the Sabin-Feldman dye test (17) and for the presence of *Toxoplasma gondii* cysts in their brains (9). Control mice received PEG only. The chi-square test and the group t-test were used for statistical analysis of the data (18).

Serum samples and portions of liver, spleen, heart and brain of CBA/Ca mice which were not infected or infected with parasites of the ME49 strain of *Toxoplasma gondii* were used to determine levels of azithromycin in tissues. The samples were collected and immediately frozen at -80 °C. Analysis was conducted at Pfizer Central Research (Groton, USA) using a high-performance liquid chromatography assay with electrochemical detection (19). The dynamic range of this assay was 0.01–2.0 µg/ml in serum and 0.1–10,000 µg/g in tissues.

Results and Discussion. Mice infected with 2.5 x 10^3 trophozoites of the RH strain and treated for ten days with 200 mg/kg/day of roxithromycin or spiramycin had 100 % and 80 % mortality on day 7 and 8, respectively (Table 1). At this time, none of the mice treated with 200 mg/kg/day of azithromycin also for ten days had died ($p = 0.0001$). All of the mice treated with azithromycin survived longer than 30 days, whereas none of those treated with roxithromycin or spiramycin survived longer than eight days. When dosages of azithromycin, roxithromycin and spiramycin reported to be effective against *Toxoplasma gondii* (9, 20, 21) were used in parallel to treat for ten days mice infected i.p. with 2.5 x 10^3 RH trophozoites, 100 % of the controls were dead at a time when none of the mice treated with 200 mg/kg/day of azithromycin had died ($p < 0.0001$), 10 % treated with 500 mg/kg/day of roxithromycin had died ($p < 0.003$), and 30 % treated with 400 mg/kg/day of spiramycin had died ($p < 0.004$). Whereas all animals treated with azithromycin were alive at day 15 of infection (five days after discontinuation of treatment), 100 % of those treated with spiramycin and 30 % of those treated with roxithromycin were dead. Each of the azithromycin-treated mice was still alive 35 days after infection, at which time the experiment was terminated (Table 2). All surviving mice developed antibodies to *Toxoplasma gondii*. *Toxoplasma gondii* cysts were not demonstrable in the brains of surviving mice either by light microscopy or by subinoculation. In addition, to determine whether surviving mice had residual infection in other organs, spleens and livers of two

Table 1: In vivo activity of equal doses of azithromycin, roxithromycin and spiramycin in experimental murine toxoplasmosis.

Treatment[a]	Percent mortality on stated day after infection[b]					
	4	5	6	7	8	30
Controls	0	0	30	100		
Azithromycin	0	0	0	0	0	0
Roxithromycin	10	10	20	100		
Spiramycin	10	20	40	80	100	

[a] Each mouse received 200 mg/kg/day of one of the drugs administered orally.
[b] There were 10 mice in each group.

Table 2: In vivo activity of different doses of azithromycin, roxithromycin and spiramycin in experimental murine toxoplasmosis.

Treatment	Dose (mg/kg/day)[a]	Percent mortality on stated day after infection[b]						
		8	9	10	11	12	15	35
Controls		50	100					
Azithromycin	200	0	0	0	0	0	0	0
Roxithromycin	500	0	10	10	20	30	30	30
Spiramycin	400	0	30	50	80	90	100	

[a] Treatment by gavage started 24 hours after infection and continued for 10 days.
[b] There were 10 mice in each group.

Table 3: Percent mortality in mice infected with various inocula of different strains of *Toxoplasma gondii* and treated with 200 mg/kg/day of azithromycin for ten days.

Strain	Inoculum	Treated[a]	Percent mortality on stated day after infection				
			7	9	15	20	30
M7741[b]	1 x 105	yes	0	0	0	0	0
		no	0	30	70	80	100
MO	1×10^5	yes	10	10	10	10	10
		no	20	40	100		
	1×10^4	yes	0	0	20	20	20
		no	10	50	100		
	1×10^3	yes	0	0	0	0	0
		no	0	0	0	20	80
SOU	1×10^5	yes	0	20	40	60	60
		no	10	60	100		
	1×10^4	yes	0	20	30	50	80
		no	0	50	100		
	1×10^3	yes	0	0	0	20	40
		no	20	60	60	80	100
C56	1×10^5	yes	0	10	20	40	40
		no	50	70	90	100	
	1×10^4	yes	0	0	20	30	50
		no	10	40	60	100	
	1×10^3	yes	0	0	0	0	0
		no	0	0	0	20	80
RH	1×10^5	yes	0	10	30	60	60
		no	40	100			
	1×10^4	yes	0	0	0	0	0
		no	80	100			
	1×10^3	yes	0	0	0	0	0
		no	0	100			

[a] Treatment by gavage started 24 hours after infection. There were 10 mice in each group.
[b] All mice inoculated with lower inocula survived the infection.

mice that survived after treatment with any of the macrolides were subinoculated into five normal mice 50 days after completion of the treatment. Residual infection was not demonstrable in any of the mice.

Because the results of the above experiments revealed higher activity for azithromycin than for the other two macrolides, further experiments were conducted to determine its activity against different inocula of five strains of *Toxoplasma gondii*. The results revealed variable degrees of protection against death caused by infection with the different strains (Table 3). Thus, 100 % of mice infected with 1 x 10^5 trophozoites of strain M7741 and treated with 200 mg/kg/day for ten days survived the infection, whereas only 40 %, 20 % and 60 % of mice infected with 1 x 10^5, 1 x 10^4 and 1 x 10^3 trophozoites, respectively, of the SOU strain survived. The effect of azithromycin on mortality of mice infected with the other strains was intermediate between those observed with strains M7741 and SOU. Surprisingly, 40 % of mice infected with 1 x 10^5 and 100 % of mice infected with 1 x 10^4 trophozoites of the highly virulent RH strain survived the infection. All mice that survived infection with any of the strains had antibodies to *Toxoplasma gondii*. Toxoplasma cysts were not demonstrated in brains of treated mice but were seen in brains of surviving control mice.

Serum and tissue levels of azithromycin were examined in two groups of mice, one uninfected and the other infected i.p. with ten cysts of the ME49 strain. Mice in both groups were treated orally with 200 mg of azithromycin/day for ten days. Treatment was started ten days after infection in infected mice to allow time for development of an inflammatory response in their brains. In both groups of mice, three animals were killed before initiation of treatment and three after one, five and ten days following initiation of treatment. An additional three mice were killed five days after treatment was discontinued. Serum and fragments of liver, spleen, heart and brain of each mouse were collected and examined individually for concentrations of azithromycin. The results are shown in Table 4. Azithromycin attained high tissue concentrations in liver, spleen, and heart in both groups of mice, exceeding concurrent serum levels by 25- to 200-fold. Concentrations in brains were 9-fold greater than those in serum after ten doses of the drug. Azithromycin concentrations in liver, spleen, heart and serum of infected mice were 3- to 5-fold higher than those in control uninfected mice ($p < 0.04$) following the first and fifth doses. Brain concentrations were approximately twofold higher in infected mice although this difference did not achieve statistical significance.

The above results demonstrated that azithromycin is considerably more active than roxithromycin and spiramycin in treatment of acute murine toxoplasmosis. Each one of these macrolides has been shown to have in vitro and in vivo activity against *Toxoplasma gondii* (10, 20, 21). Our results demonstrated that the activity of azithromycin in protecting mice against death due to acute toxoplasmosis was considerably higher than that of roxithromycin and spiramycin even when these two latter macrolides were used at twice the dose of azithromycin. Although it has been demonstrated that spiramycin is not very active against acute murine toxoplasmosis (22, 23), this antibiotic has been widely used in Europe to prevent congenital toxoplasmosis (23). We did not ascertain whether the differences in activity of the different macrolides were due to differences in their pharmacokinetics or to actual differences in their anti-toxoplasma activity. Their activity, in addition to the activity of other macrolides, in humans would necessarily require evaluation of optimal route of administration and pharmacokinetics.

Because of the remarkable diversity among different strains of *Toxoplasma gondii* (12, 24), examination of the activity of any drug against a single strain may not allow prediction of its role in treatment of toxoplasmosis. For this reason we used a number of different strains of *Toxoplasma gondii*. The excellent in vivo activity of azithromycin extended to five different strains of the parasite, including two isolated from the patients with AIDS.

The mechanism of action of macrolides antibiotics against *Toxoplasma gondii* has not been defined yet. It has been suggested that they may act by a mechanism similar to the antibacterial action of erythromycin which involves interference with protein synthesis by binding to the 50S ribosomal unit and thereby preventing elongation of the peptide chain (10). Spiramycin has also been shown to act in this manner (25). Other mechanisms of action unrelated to ribosome-binding have also been suggested. Thus, a stimulating effect of the macrolide on the microbicidal activity of phagocytes has been suggested to explain the activity of macrolides against non-bacterial intracellular parasites (10). In this context, Gladue et al. (26) demonstrated that concentrations of azithromycin within human and mouse polymorpho-

Table 4: Concentrations of azithromycin in serum and tissues of control and *Toxoplasma gondii*-infected mice administered 200 mg/kg/day of the drug for 10 days. Results are mean (standard deviation) for three mice.

Number of doses	Days after last dose	Group	Concentrations (μg/ml)				
			Liver	Spleen	Heart	Brain	Serum
1	1	control	36 (22)	14 (5.8)	3.4 (1.4)	0.48 (0.14)	0.16 (0.064)
		infected	107 (34)[a]	47 (6.4)b	11 (0.58)c	0.85 (0.57)	0.45 (0.035)[b]
5	1	control	26 (18)	24 (11)	3.3 (2.7)	1.3 (0.20)	0.14 (0.094)
		infected	114 (46)[a]	79 (23)[d]	16 (4.0)[e]	3.0 (1.2)	0.36 (0.072)[e]
10	1	control	43 (35)	22 (7.5)	5.0 (3.9)	1.0 (0.44)	0.15 (0.077)
		infected	25 (4.6)	42 (13)	4.2 (0.85)	1.7 (0.51)	0.021 (0.005)
10	5	control	0.24 (0.064)	1.2 (0.48)	0.17 (0.065)	0.70 (0.20)	0.0 (0.0)
		infected	3.3 (2.2)	4.6 (0.12)[f]	0.24 (0.036)	1.5 (0.66)	0.021 (0.005)

[a]p = < 0.04; [b]p = < 0.003; [c]p = < 0.0008; [d]p = < 0.02; [e]p = 0.01; [f]p = < 0.001.

nuclear leukocytes, murine peritoneal macrophages, and mouse and rat alveolar macrophages in vitro reached levels of up to 226 times the external levels. Thus, uptake and release of azithromycin by phagocytes may serve as a means of delivering this macrolide to sites of active infection. Our results revealed that during daily oral administration of azithromycin to either control uninfected or infected mice, this antibiotic became extensively distributed into tissues, attaining concentrations much greater than in serum. Although penetration of most antibiotics into the brain is poor, our results revealed that azithromycin penetration into the brain was substantial. In addition, azithromycin concentrations during the first five days of dosing were two- to five-fold higher in the inflamed tissues of infected mice than in tissues of control mice.

Although azithromycin had excellent activity against all strains of *Toxoplasma gondii* used in this study, it was of interest that there was a wide strain variation in the response to the antibiotic. This observation may have important implications for therapy of toxoplasmosis, particularly in individuals with AIDS, since one of the strains showing least sensitivity was isolated from an AIDS patient. Further work to define more clearly the significance and mechanism of the variation of the response of *Toxoplasma gondii* strains to therapeutic agents is needed.

Acknowledgements

We thank Teri Lin and Richard A. Ferraina for their excellent technical assistance.
This work was supported in part by grants AI30230 and AI04717 from the National Institutes of Health.

References

1. **Araujo FG, Remington JS:** Toxoplasmosis in immunocompromised patients. European Journal of Clinical Microbiology and Infectious Diseases 1987, 6: 1–2.
2. **Mills J:** *Pneumocystis carinii* and *Toxoplasma gondii* infections in patients with AIDS. Review of Infectious Diseases 1986, 8: 1001–1011.
3. **Remington JS, Desmonts G:** Toxoplasmosis. In: Remington JS, Klein JO (ed): Infectious Diseases of the fetus and newborn infant. W.B. Saunders, Philadelphia, 1990, p. 89–195.
4. **Eyles DE, Coleman M:** Synergistic effect of sulfadiazine and daraprin against experimental toxoplasmosis in the mouse. Antibiotics and Chemotherapy 1953, 3: 483–490.
5. **Brooks RG, Remington JS, Luft BJ:** Drugs used in the treatment of toxoplasmosis. In: Peterson PK, Verhoef J (ed): The Antimicrobial Agents Annual. Volume 2. Elsevier, Amsterdam, 1987, p. 297–306.
6. **Israelski DM, Remington JS:** Toxoplasmic encephalitis in patients with AIDS. Infectious Diseases Clinics of North America 1988, 2: 429–445.
7. **Haverkos HW:** Toxoplasmic encephalitis study group. Assessment of therapy for toxoplasmic encephalitis. American Journal of Medicine 1987, 82: 907–914.
8. **Luft BJ:** In vivo and in vitro activity of roxithromycin against *Toxoplasma gondii* in mice. European Journal of Clinical Microbiology and Infectious Diseases 1987, 6: 479–481.
9. **Araujo FG, Guptill DR, Remington JS:** Azithromycin: a macrolide antibiotic with potent activity against *Toxoplasma gondii*. Antimicrobial Agents and Chemotherapy 1988, 32: 755–757.
10. **Chang HR, Pechère JC:** In vitro effects of four macrolides (roxithromycin, spiramycin, azithromycin [CP-62, 993], and A-56268) on *Toxoplasma gondii*. Antimicrobial Agents and Chemotherapy 1988, 32: 524–529.
11. **Hofflin JM, Remington JS:** In vivo synergism of Roxithromycin (RU 965) and interferon against *Toxoplasma gondii*. Antimicrobial Agents and Chemotherapy 1987, 31: 346–348.

12. **Suzuki Y, Conley FK, Remington JS:** Differences in virulence and development of encephalitis during chronic infection vary with the strain of *Toxoplasma gondii.* Journal of Infectious Diseases 1989, 159: 790–794.
13. **Sabin AB:** Toxoplasmic encephalitis in children Journal of the American Medical Association 1941, 116: 801–807.
14. **Jacobs L, Melton ML:** Toxoplasmosis in chickens. Journal of Parasitology 1966, 52: 1158–1162.
15. **Jacobs L, Remington JS, Melton ML:** A survey of meat samples from swine, cattle and sheep for the presence of encysted toxoplasma. Journal of Parasitology 1960, 46: 23–28.
16. **Suzuki Y, Remington JS:** A method for obtaining large numbers of trophozoites of avirulent strains of *Toxoplasma gondii* using an antibody to interferon gamma. Journal of Parasitology 1989, 75: 174–176.
17. **Sabin AB, Feldman HA:** Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoan parasite *(Toxoplasma).* Science 1948, 108: 660–663.
18. **Snedecor G, Cochran W:** Stastical methods. Iowa State University Press, Ames, IA, 1980.
19. **Shepard RM, Duthu GS, Ferraina RA, Mullins MA:** High-performance liquid chromatographic assay with electro chemical detection for azithromycin in serum and tissues. Journal of Chromatography 1991, 565: 321–337.
20. **Chan J, Luft BJ:** Activity of roxithromycin (RU28965), a macrolide, against *Toxoplasma gondii* infection in mice. Antimicrobial Agents and Chemotherapy 1986, 30: 323–324.
21. **Garin JP, Eyles DE:** Le traitement de la toxoplasmose experimentale de la souris par la spiramycine. Presse Medicale 1958, 66: 957–958.
22. **Mas Bakal P, Intveld N:** Postponed spiramycin treatment of acute toxoplasmosis in white mice. Tropical and Geographical Medicine 1965, 17: 254–260.
23. **Garin JP, Pellerat J, Maillard Mme:** Bases theoriques de la prevention par la spiramycine de la toxoplasmose congenitale chez la femme enceinte. Presse Medicale 1968, 76: 2266.
24. **Ware PL, Kasper L:** Strain specific antigens of *Toxoplasma gondii.* Infection and Immunity 1987, 55: 778–783.
25. **Menninger JR, Otto DP:** Erythromycin carbomycin, and spiramycin inhibit protein synthesis by stimulating the dissociation of peptidyl-tRNA from ribosomes. Antimicrobial Agents and Chemotherapy 1982, 21: 811–818.
26. **Gladue RP, Bright GM, Isaacson RE, Newborg MF:** In vitro and in vivo uptake of azithromycin (CP-62, 993) by phagocytic cells: possible mechanism of delivery and release at sites of infection. Antimicrobial Agents and Chemotherapy 1989, 33: 277–282.

Effect of Meropenem on the Intestinal Microflora

T. Bergan[1,2], C.E. Nord[3]*, S.B. Thorsteinsson[4]

Ten healthy volunteers were given 500 mg of meropenem by intravenous infusion over 30 min three times daily for seven days. Stool specimens were collected before, during and after meropenem administration. The numbers of enterobacteria and streptococci decreased during the administration period, while the numbers of enterococci increased. There was a decrease in the numbers of clostridia, bacteroides and gram-negative cocci, while the numbers of gram-positive cocci and rods were not changed by the administration of meropenem. The intestinal flora returned to normal in all volunteers within two weeks after the termination of meropenem administration.

Meropenem (SM 7388; Sumitomo Pharmaceuticals, Japan) is a new carbapenem antibiotic with a broad spectrum of in vitro activity against gram-positive and gram-negative aerobic and anaerobic bacteria (1). Meropenem is more stable than imipenem in the presence of renal dehydropeptidase I and the coadministration of a dehydropeptidase I enzyme inhibitor, such as cilastatin, is not considered necessary.

The administration of antimicrobial agents may influence the human intestinal microflora in different ways (2). One is overgrowth of microorganisms already present, such as yeasts, which may produce systemic infections in immunocompromised patients, and of *Clostridium difficile,* which may lead to diarrhoea or colitis. A second consequence is the development of antimicrobial resistance among the bacteria in the normal microflora. A third effect is the reduction

[1] Department of Microbiology, Institute of Pharmacy, University of Oslo, Oslo, Norway.
[2] Department of Microbiology, Aker Hospital, Oslo, Norway.
[3] Department of Microbiology, Karolinska Institute, Huddinge University Hospital, S-141 86 Huddinge, Sweden.
[4] Department of Medicine, Landspitalinn, Reykjavik, Iceland.