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In Vitro and In Vivo Effects of Doxycycline on *Toxoplasma gondii*

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We investigated the effects of doxycycline on *Toxoplasma gondii* infections in vitro and in vivo. Resident peritoneal macrophages were infected with the virulent RH strain of *T. gondii* and exposed to doxycycline at different concentrations. The antitoxoplasmic activity of doxycycline was first assessed with [^{3}H]uracil, which is incorporated by the parasite but not the host cell. The concentration of doxycycline that inhibited 50% of the radioactive uptake was calculated to be 6.4 μg/ml (95% confidence limits, 5.07 to 8.06 μg/ml); the concentration of doxycycline that inhibited 90% of the radioactive uptake was 14 μg/ml. Tetracycline was ineffective up to 40 μg/ml. Furthermore, microscopic examination of the infected macrophages after treatment with doxycycline confirmed the inhibition of intracellular growth of *T. gondii*. Mice acutely infected by the intraperitoneal route with 5×10^3 tachyzoites of *T. gondii* were protected against death with a dose of 300 mg of doxycycline per kg (body weight) administered by the oral route for 10 days, starting 24 h after challenge. When mice were infected with 10^5 tachyzoites of *T. gondii* and treated 12 days starting 2 h after challenge, the protection and the cure rates were, respectively, 100 and 0% after doxycycline alone (300 mg/kg per day), 0 and 0% after pyrimethamine alone (12.5 mg/kg per day), and 100 and 60% after the combination of these two drugs at the same dosages given above. These results suggest that doxycycline may prove to be useful in the treatment of toxoplasmic infections.

Infection with *Toxoplasma gondii* is a potential hazard for immunocompromised patients, such as those suffering from the acquired immune deficiency syndrome (AIDS) (30). Currently, the treatment of choice for toxoplasmosis is the synergistic combination of pyrimethamine with sulfonamides (20, 29, 43). The treatment of patients with AIDS suffering from toxoplasmic encephalitis with this combination entails a significant mortality rate, usually associated with relapse after withdrawal of therapy for reasons of toxicity, mainly because of the sulfonamide component of the combination (27, 30). Moreover, it has been proposed that patients with AIDS suffering from toxoplasmic encephalitis should be maintained on a chronic suppressive (clinical prophylactic) regimen to avoid relapses (23). Thus, the development of effective and safer compounds for treating this disease is critically needed. As alternative treatments, the experimental in vitro and in vivo activities of some new macrolides have been assessed (1, 5, 7–9), but their efficacies in human toxoplasmosis, however, remain to be confirmed in clinical trials. Clindamycin has been shown to have activity in animal models of toxoplasmosis (2, 32, 41), and its use as an alternative drug in the treatment of patients who do not tolerate sulfonamides has been reported recently (12).

Among the other possible therapies, the antitoxoplasmic activities of tetracyclines have been studied in animal models. The results of those studies with tetracycline (11, 16, 38, 39), chlortetracycline (16, 28, 33), terramycin (25, 33), and dimethylchlortetracycline (21, 33) have led to disparate conclusions on the activity of these drugs, with some studies finding activity and others finding no activity. From the semisynthetic tetracycline analogs, only the activity of minocycline has been studied in murine models of toxoplasmosis (40). In the studies reported here, we evaluated the activity of doxycycline against intracellular *T. gondii* by using in vitro assays and murine models of acute toxoplasmosis.

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MATERIALS AND METHODS

Antimicrobial agents. Doxycycline hyclate (22 mg/ml), doxycycline hydrochloride (874 μg/ml), and tetracycline hydrochloride (980 μg/ml) were obtained from commercial sources. Pyrimethamine and sulfadiazine were provided in powder form by F. Hoffmann-La Roche & Cie. S.A., Basel, Switzerland. For in vitro studies doxycycline hyclate (injectable form; 20 mg/ml) was first diluted in sterile distilled water and then in culture medium; tetracycline hydrochloride was dissolved into a small amount of 0.1 N HCl, diluted in sterile distilled water, and then diluted in culture medium. Pyrimethamine was dissolved in 95% ethanol and diluted in 0.1 M phosphate buffer, whereas sulfadiazine was dissolved with 0.1 M NaOH and diluted in 0.1 M phosphate buffer; both compounds were further diluted in culture medium prior to their use. The final pH of all drug-containing media was 7.2.

For in vivo studies doxycycline hydrochloride was dissolved in a small amount of 0.1 N HCl and then diluted in sterile distilled water. Pyrimethamine and sulfadiazine were suspended in 0.25% carboxymethyl cellulose–0.20% Tween 80 in sterile water. In studies in which pyrimethamine was used in combination with doxycycline, pyrimethamine was first dissolved in 95% ethanol and diluted with sterile distilled water before being mixed with the doxycycline solution.

Animals. Female Swiss-Webster mice (weight, 25 ± 1 g; Madörin AG, Füllinsdorf, Switzerland) were used.

***T. gondii*.** The highly virulent RH strain of *T. gondii* was used for all studies. Tachyzoites were obtained from the peritoneal cavities of mice that were heavily infected 2 days before, as described previously (8).

In vitro studies. (i) Macrophages and media. Resident peritoneal macrophages were obtained from the peritoneal cavities of normal female Swiss-Webster mice as described previously (6). The purified cell suspensions were suspended at a concentration of 2×10^6 cells per ml in medium 199 (M199; Fakola AG, Basel, Switzerland) containing 10% heat-inactivated (60 min, 56°C) fetal bovine serum (FBS; Fakola). Cell viability was always >95%, as tested by using the trypan blue exclusion test.

An amount of 200 μl of the suspension containing 4×10^5 cells was placed into each well of 96-well plates (0.32 cm^2; Costar, Cambridge, Mass.), and an amount of 1 ml (2×10^6 cells) was placed into each chamber of four-chamber slides (1.78 cm^2; Lab-Tek; Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill.) and then incubated at 37°C (in a 5% CO_2–95% air atmosphere) for 3 h to allow macrophages to adhere. The nonadherent cells were removed by two gentle washings with prewarmed Hanks balanced salt solution. The monolayers were then reincubated with M199–10% FBS and used for the experiments. The phagocytic ability of macrophages was tested by their phagocytosis of heat-killed *Candida albicans* (30 min, 100°C). The number of adherent macrophages in different wells from both 96-well plates and four-chamber slides was found to be similar, according to protein concentration measurements in three different experiments.

(ii) Infectious challenge and quantitation of antitoxoplasmic activity. (a) [^{3}H]uracil incorporation. Adherent macrophage monolayers on 96-well dishes were challenged for 1 h with 4×10^5 tachyzoites of the RH strain in M199–3% FBS. After uningested parasites were removed by washing the dishes with prewarmed Hanks balanced salt solution, the infected monolayers received the drugs at various concentrations in M199–10% FBS. Control monolayers received drug-free medium and medium containing solvents. The intracellular growth of *T. gondii* was determined by measuring the incorporation of [^{3}H]uracil into acid-precipitable material by a previously described procedure (6). The [^{3}H]uracil is not incorporated by the host cells because of a deficiency of the respective enzymes (35). Briefly, the monolayers were pulsed with 2.5 μl of [5,6-^{3}H]uracil (specific activity, 46–50 Ci/mmol; Amersham International, Buckinghamshire, England) and reincubated for 24 h. A similar incubation time of 24 h was used in the light microscopy studies described below. The supernatant of each well was discarded, and the monolayers were dissolved with 50 μl of 1% sodium dodecyl sulfate containing 100 μg of unlabeled uracil (Sigma Chemie, Deisenhofen, Federal Republic of Germany) per ml, stored at 4°C for 30 min, and then precipitated with 50 μl of ice-cold 10% trichloroacetic acid (final concentration in the mixture, 5%). The precipitates were then collected in glass filters by using a cell harvester (Nunc, Roskilde, Denmark), washed with 5% trichloroacetic acid, rinsed with 95% ethanol, dried, and counted in a liquid scintillation spectrophotometer.

(b) Light microscopy. Adherent macrophage monolayers (four-chamber slides) were infected with 2×10^6 *T. gondii* (in M199–3% FBS) for 1 h, followed by addition of the drugs with M199–10% FBS over 24 h. Monolayers were fixed immediately after the 1-h challenge (time zero) and 24 h after challenge with 0.4% 9-aminoacridine (Sigma) in 50% (vol/vol) ethanol-water, Giemsa-stained (30 min), and then mounted. The percentage of infected cells, the mean number of *T. gondii* per infected vacuole, and the number of *T. gondii* per 100 infected cells (in multiple infected cells only, a single vacuole containing the highest number of parasites was counted) were determined by microscopic examination (×1,000) of 200 to 300 cells.

(c) Effect of doxycycline on extracellular *T. gondii* and on phagocytosis of *T. gondii* by macrophages. To test whether doxycycline could also affect extracellular *T. gondii*, fresh parasite suspensions were incubated with 10 and 30 μg of doxycycline per ml for 30 min at 37°C, before they were washed with Hanks balanced salt solution and used to challenge macrophage monolayers. Also, to test whether doxycycline could affect the phagocytosis of the parasites, the same concentrations of doxycycline (10 and 30 μg/ml) were added to the parasite suspensions and allowed to remain there during the contact time (1 h) between parasites and macrophages. For both experiments, monolayers were washed 1 h after the infectious challenge and received medium without antibiotics. Some monolayers were fixed immediately after challenge (time zero), and other monolayers were fixed 24 h after challenge.

(d) Effect of doxycycline on antimicrobial activity of macrophages. To ascertain whether doxycycline could enhance the natural antimicrobial activities of resident macrophages, monolayers were incubated during 24 h at 37°C with 10 and 30 μg of doxycycline per ml, washed three times with prewarmed Hanks balanced salt solution, challenged with *T. gondii* in medium without antibiotic during 1 h, and incubated over a period of 24 h in medium without antibiotic. Monolayers were fixed at time zero and 24 h after infection.

(iii) Controls for toxicity. Controls for toxicity were prepared by using the trypan blue dye exclusion test (4), as described previously (8).

(iv) Incubations. Unless indicated otherwise, incubations were performed at 37°C in a humid atmosphere of 5% CO_2–95% air. The pH of the media was adjusted to 7.2.

In vivo studies. (i) Animal models. Mice were infected intraperitoneally with 5×10^3 or 1×10^5 tachyzoites of the RH strain of *T. gondii*, i.e., 500 and 50,000 times the 100% lethal dose (7), respectively, in 0.5 ml of sterile 0.9% NaCl. Animals were randomly allocated in groups of 10 with free access to food and water. At the end of the study period (30 days), the surviving mice were sacrificed and autopsied. Peritoneal exudates were examined microscopically (×400) for the presence of *T. gondii* tachyzoites. When no parasites were seen, the brain was triturated and used for subinoculation into each of two naive mice. The donor was considered cured if the two recipient mice survived 30 days after injection without having *T. gondii* present at autopsy.

(ii) Therapeutic regimens. Two therapeutic regimens were used. In the first one, mice infected with 5×10^3 tachyzoites were treated with different doses of doxycycline, starting 24 h after challenge (delayed treatment). Treatment was administered by gavage once daily during 10 days, and mice were fasted for 1 h prior to receiving the drugs. In the second regimen, mice were infected with 10^5 *T. gondii* tachyzoites, and the treatment was administered by gavage, starting 2 h after challenge and during 12 days. Treatment consisted of the administration of a dose of doxycycline that afforded 100% protection in the delayed-treatment experiments. In some experiments administration of treatment was divided into two daily dosages (every 12 h). Pyrimethamine alone was administered by gavage, and pyrimethamine was also administered in combination with doxycycline. Control groups of infected mice received only the vehicle, and uninfected mice received drug treatment in all regimens.

(iii) Levels of doxycycline in serum. Groups of three normal fasting (1 h) mice were given by gavage a single dose of 300 mg of doxycycline per kg; and blood samples were collected

TABLE 1. Activities of doxycycline, tetracycline, pyrimethamine, sulfadiazine, and pyrimethamine in combination with sulfadiazine against intracellular *T. gondii* as assessed by [^{3}H]uracil incorporation[a]

Preparation	Drug (concn [μg/ml])	Uracil uptake (cpm ± SEM)[b]	*P* value[c]
Infected macrophages	None (0)	99,910 ± 4,996	
Uninfected macrophages	None (0)	356 ± 192	<0.001
Extracellular *T. gondii*	None (0)	5,721 ± 270	<0.001
Infected macrophages + solvents	None (0)	102,425 ± 4,337	NS[d]
Infected macrophages	Pyrimethamine		
	0.1	99,756 ± 5,003	NS
	1	9,812 ± 347	<0.001
	Sulfadiazine (25)	87,552 ± 2,557	NS
	Pyrimethamine (0.1) + sulfadiazine (25)	10,247 ± 1,010	<0.001
	Tetracycline		
	1	96,761 ± 2,318	NS
	20	92,736 ± 2,009	NS
	40	88,936 ± 3,412	NS
	Doxycycline		
	1	90,305 ± 6,468	NS
	2	90,951 ± 673	NS
	3	89,245 ± 2,093	NS
	4	74,319 ± 4,482	<0.01
	5	77,613 ± 3,522	<0.01
	6	56,347 ± 3,239	<0.001
	8	33,417 ± 1,839	<0.001
	9	32,882 ± 642	<0.001
	10	14,575 ± 2,555	<0.001
	20	12,043 ± 801	<0.001

[a] Values are of one representative experiment of three experiments.
[b] Results are presented as means ± standard errors of the mean for eight replicate wells.
[c] Compared with infected macrophages with no drug by Student's *t* test.
[d] NS, Not significant.

1, 2, and 3 h later. The amount of doxycycline in the serum of animals was assessed with a microbiological assay by using *Staphylococcus aureus* ATCC 6538P.

Statistics. For in vitro studies, differences between test and control groups were analyzed by using Student's *t* test. The 50% inhibitory concentrations (IC_{50}s), the 90% confidence limits, and the 90% inhibitory concentrations (IC_{90}s) were calculated by probit analysis by the method of Litchfield and Wilcoxon (31). For in vivo studies statistical analysis was performed by using the Fisher exact test. A *P* value of <0.05 was considered significant.

RESULTS

In vitro studies. (i) [^{3}H]uracil incorporation. The results of the [^{3}H]uracil incorporation experiments are shown in Table 1. Doxycycline inhibited [^{3}H]uracil incorporation by infected macrophages, in a dose-dependent manner, allowing the calculation of its IC_{50} and its IC_{90}. The calculated IC_{50} of doxycycline was 6.4 μg/ml (95% confidence interval, 5.07 to 8.06 μg/ml), and its IC_{90} was 14 μg/ml. Pyrimethamine (1 μg/ml) and pyrimethamine (0.1 μg/ml) in combination with sulfadiazine (25 μg/ml) also significantly inhibited the incorporation of [^{3}H]uracil by the parasites. Tetracycline was not active up to 40 μg/ml.

(ii) Light microscopy. The results of the light microscopy experiments are shown in Table 2. At time zero (1 h after infection with *T. gondii*) no difference was observed in the percentage of infected cells among monolayers. At the end of the 24-h period of incubation, doxycycline showed again a dose-dependent effect on the multiplication of the parasites, and at a concentration of 10.8 μg/ml it was as effective as pyrimethamine (1 μg/ml) and pyrimethamine (0.1 μg/ml) in combination with sulfadiazine (25 μg/ml) in inhibiting intracellular *T. gondii* with regard to the parameters considered.

(iii) Effect of doxycycline on extracellular *T. gondii* and on phagocytosis of *T. gondii*. Doxycycline did not affect either

TABLE 2. Activities of doxycycline, pyrimethamine, and pyrimethamine in combination with sulfadiazine against intracellular *T. gondii* as assessed by light microscopy[a]

Drug (concn [μg/ml])	% of infected cells at:		Mean no. of *T. gondii*/infected vacuole	No. of *T. gondii*/100 infected cells
	0 h	24 h		
None (0)	46	47	13	632
Doxycycline				
1.8	43	45	11	514
5.4	47	26	8.5	235
10.8	43	12[b]	3[b]	43[b]
14.4	46	11[b]	2[b]	26[b]
Pyrimethamine (1)	43	13[b]	3[b]	41[b]
Pyrimethamine (0.1) + sulfadiazine (25)	44	14[b]	3[b]	44[b]

[a] Results are means of four replicate monolayers.
[b] $P < 0.05$, compared with untreated controls.

TABLE 3. Activities of doxycycline and pyrimethamine in combination with sulfadiazine on murine acute toxoplasmosis[a]

Drug (dose [mg/kg per day])	Mean time (days) to death for 50% of mice	No. of survivors/no. of infected mice	No. of cured/no. of survivors (%)
None	8 ± 1	0/20	
Doxycycline			
100	11	0/10	
200	18	3/10	1/3 (33)
300		10/10	1/10 (10)
300[b]		10/10	4/10 (40)
Pyrimethamine (4.4) + sulfadiazine (250)		10/10	10/10 (100)

[a] Mice were treated by gavage for 10 days, starting 24 h after challenge, and were given an intraperitoneal inoculum of 5×10^3 *T. gondii* tachyzoites.
[b] The daily dose was divided into two equal doses (given every 12 h).

TABLE 4. Activities of doxycycline alone and in combination with pyrimethamine and pyrimethamine alone on murine acute toxoplasmosis[a]

Drug (dose [mg/kg per day])	Mean time (days) to death for 50% of mice	No. of survivors/no. of infected mice	No. of cured/no. of survivors (%)
None	5 ± 1	0/20	
Doxycycline			
300		10/10	0/10 (0)
300[b]		10/10	0/10 (0)
Doxycycline (300) + pyrimethamine (12.5)		10/10	6/10 (60)
Pyrimethamine (12.5)	12	0/10	

[a] Mice were treated by gavage for 12 days, starting 2 h after challenge, and were given an intraperitoneal inoculum of 10^5 *T. gondii* tachyzoites.
[b] The daily dose was divided into two equal doses (every 12 h).

the viability of extracellular *T. gondii*, as assessed by testing its ability to reinfect macrophage monolayers, or the phagocytosis of the parasites by the macrophages (data not shown).

(iv) Effect of doxycycline on antimicrobial activity of macrophages. Macrophages did not restrict the multiplication of intracellular *T. gondii* after preincubation with doxycycline (data not shown).

(v) Toxicity test. Doxycycline did not affect the viability of the host cells after they were incubated, during 24 h, up to a concentration of 43.2 μg/ml, as determined by the trypan blue dye exclusion test and morphological criteria (number of cells per high-power field and shape) by using Giemsa-stained preparations.

In vivo studies. All infected (5×10^3 *T. gondii*), untreated control mice died of acute toxoplasmosis 8 ± 1 days after challenge (Table 3). All doxycycline-treated, uninfected mice survived. Complete protection was obtained when mice were given 300 mg of doxycycline per kg per day for 10 days administered either as single daily doses or in 150-mg/kg doses given every 12 h ($P < 0.001$ against infected untreated controls). According to brain transfers, the cure rate among surviving mice was 10% with the once-a-day regimen and 40% with the twice-a-day regimen. The combination of pyrimethamine (4.4 mg/kg per day) plus sulfadiazine (250 mg/kg per day) protected 100% of the mice, with a cure rate of 100%.

In the group of mice infected with 10^5 tachyzoites of *T. gondii*, 100% of untreated controls died from acute toxoplasmosis 5 ± 1 days after challenge (Table 4). All doxycycline-treated, uninfected mice survived. At the end of the study period, 100% of the mice treated with doxycycline at 300 mg/kg per day for 12 days given either as single daily doses or in 150-mg/kg doses every 12 h were protected. Some mice in the group treated with doxycycline (single daily dosage) presented some degree of ascites near the end of the study period (by day 25 after challenge), and two mice presented signs of neurological disorders (partial paralysis of the back legs). However, all the mice were still alive by day 30 after challenge. At autopsy, the macroscopic appearance of the spleens, livers, and peritoneal cavities of the mice presenting ascites were compatible with toxoplasmic infection; and tachyzoites were seen in the peritoneal fluid. By contrast, mice treated with twice-daily dosages of doxycycline (300 mg/kg per day) remained asymptomatic. However, all brain transfers were positive, indicating that none of these mice were cured by either the once-a-day or the twice-a-day regimen. When pyrimethamine (12.5 mg/kg per day) was administered in combination with doxycycline (300 mg/kg per day), 100% protection was observed and all mice were asymptomatic. The cure rate was 60% at the end of the study. Pyrimethamine alone (12.5 mg/kg per day) was unable to protect the mice against death at the end of the study period.

Levels of doxycycline in serum. Doxycycline reached a peak concentration of 8.7 μg/ml in serum 1 h after a single peroral dose of 300 mg/kg. The concentrations of doxycycline in serum 2 and 3 h after a similar single peroral dose were 6 and 4.8 μg/ml, respectively.

DISCUSSION

Doxycycline was active against intracellular *T. gondii* and was able to protect mice against acute toxoplasmosis that was produced with a high inoculum of a highly virulent strain of *T. gondii*. The amount of doxycycline needed to produce 50% inhibition of nucleotide synthesis by the intracellular parasites (6.4 μg/ml) is in the range of achievable concentrations in the serum of humans given conventional doses of doxycycline. Extrapolation of murine experiments to the human situation is difficult (26). However, we observed that doxycycline levels in the serum of mice that were fully protected from death by doxycycline were quite comparable (actually slightly higher) than those achievable in humans. Mean peak levels of doxycycline in human plasma have been found at of 5.07 and 5.21 μg/ml after single intravenous and oral doses of 200 mg, respectively (19), and at 5.8 and 7.5 μg/ml after repeated oral doses of 200 mg of doxycycline twice a day (13, 44).

The fact that doxycycline was not able to cure the protected mice could be due to limited penetration of the antibiotic into the brain tissue. Doxycycline possesses high lipophilicity and penetrates efficiently into tissues (3), and its achievable mean concentrations in the brain, after intravenous perfusion to dogs, is about 1.2 μg/g (3). However, the actual penetration of the drug into the murine or human brain is unknown. The levels of doxycycline in the cerebrospinal fluid in humans, after repeated doses of 200 mg twice a day in the presence of inflamed meninges, are 1.3 μg/ml (13) and 1.1 μg/ml (44), but concentrations in the cerebrospinal fluid do not necessarily reflect concentrations in the brain tissues.

In addition, the half-life for doxycycline in human serum is from 18.5 to 23 h (17), whereas in mice it is about 4 h (15), which reflects a rapid tissue distribution and less protein binding (15). Incomplete cure rates might have reflected incomplete antibiotic coverage, especially in regimens with a single daily dosage. Indeed, administrations every 12 h, with the low parasite inocula (delayed treatment), performed better than administrations every 24 h did. The degree of protection, however, conferred by doxycycline to mice infected with a high inoculum of *T. gondii* seemed to be remarkably good because other inhibitors of protein synthesis such as roxithromycin and azithromycin are not able to protect mice against death when this high inoculum is used (H. R. Chang, unpublished data). The addition of a relatively low concentration of pyrimethamine in combination with doxycycline enhanced the cure rate of treated mice when compared with treatment with doxycycline alone (60% for the combination versus 0% for doxycycline alone), whereas pyrimethamine alone was ineffective in protecting the mice. Tabbara et al. (40) reported that 100 mg of minocycline per kg per day during 14 days (administered each 12 h) was able to protect 71% of mice infected with 7×10^5 *T. gondii* RH strain parasites. Our studies showed that doxycycline is also active against *T. gondii* in mice challenged with a high inoculum. However, the cure rate observed in the studies of Tabbara et al. (40) was 95.4%. Different intrinsic properties, pharmacokinetics, and the regimens adopted for doxycycline and minocycline may account for these differences.

The mechanism of action of doxycycline against *T. gondii* is as yet unclear. Because doxycycline was not active against extracellular *T. gondii*, it must concentrate within the host cells in order to act on the parasites. Doxycycline acts on the bacterial ribosome, where it inhibits protein synthesis primarily by blocking aminoacyl-tRNA binding (10). If, in *T. gondii*, the target molecule is also the ribosome, doxycycline must cross the host cell cytoplasmic membranes, the vacuolar membrane, and the parasite membrane. Ribosomes in *T. gondii*, however, may be different from bacterial ribosomes. Fractionation of RNA extracted from *T. gondii* showed a pattern different from that of RNA from bacteria (36). Another possibility would be that doxycycline acts on the host cells. In this regard, it has been previously suggested that tetracyclines, at concentrations in the range of those reached in vivo in patients or experimental animals, may stop protein synthesis in mitochondria of eucaryotic cells without impairing the respective cytosolic protein synthesis (22, 42) and that cytosolic protein synthesis is stopped in concentrations greater than 50 μg/ml (22, 42). It has not been established whether mitochondrial protein synthesis by the host cell is required for the intracellular survival of *T. gondii*. Some investigators have also indicated that doxycycline may impair the phagocytosis of certain bacteria and fungi (18), while others have indicated that doxycycline may alter the surface of human leukocytes (24). In our experiments, however, the presence of doxycycline did not impair the entry of *T. gondii* into the macrophages.

Doxycycline has been used in the prophylaxis of *Plasmodium falciparum* malaria and has been found to be effective and well tolerated (34). Doxycycline does not accumulate in the blood of patients with renal failure (37), does not produce a high rate of vestibular disturbances like those reported for minocycline (14), and is considered to be one of the safest tetracyclines for the treatment of extrarenal infections in patients with renal failure (37). We are not aware of any recent clinical study performed with compounds of this family of drugs in the treatment of toxoplasmic infections. However, in the case of nonpregnant patients with AIDS who do not tolerate sulfonamides, the possible use of doxycycline in combination with pyrimethamine should be considered.

Our observations suggest that doxycycline may prove to be a useful agent for the treatment of toxoplasmic infections in patients with AIDS. Therefore, a clinical trial to test the safety and value of doxycycline in the prophylaxis and/or treatment of patients with AIDS suffering from toxoplasmic encephalitis seems to be warranted.

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