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MINIREVIEW

Clinically Available Medicines Demonstrating Anti-*Toxoplasma* Activity

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***Toxoplasma gondii* is an apicomplexan parasite of humans and other mammals, including livestock and companion animals. While chemotherapeutic regimens, including pyrimethamine and sulfadiazine regimens, ameliorate acute or recrudescent disease such as toxoplasmic encephalitis or ocular toxoplasmosis, these drugs are often toxic to the host. Moreover, no approved options are available to treat infected women who are pregnant. Lastly, no drug regimen has shown the ability to eradicate the chronic stage of infection, which is characterized by chemoresistant intracellular cysts that persist for the life of the host. In an effort to promote additional chemotherapeutic options, we now evaluate clinically available drugs that have shown efficacy in disease models but which lack clinical case reports. Ideally, less-toxic treatments for the acute disease can be identified and developed, with an additional goal of cyst clearance from human and animal hosts.**

Known for more than 100 years, the apicomplexan parasite *Toxoplasma gondii* is distributed throughout the world in a great variety of mammalian hosts, including humans (1). Initial exposure to the parasite leads to lifelong chronic infection that is established within cells of the central nervous system (CNS) and that, until recently, has been considered largely asymptomatic in otherwise healthy human populations (2). Recent data from studies in humans and in model organisms now suggest that chronic infection by *T. gondii* may be capable of inducing behavioral changes, such as impaired response times (3) or impaired learning (4), and is associated with psychiatric disorders such as schizophrenia (5). More classically, *Toxoplasma* infection is known as a leading cause of birth defects, brought about when the woman receives a primary infection during pregnancy. These trans-utero infections often cause life-threatening encephalitis and/or other lifelong neurological or ocular illnesses in congenitally infected newborns (6). In addition, people with weakened immune systems, such as AIDS patients or organ transplant recipients who undergo lifelong immunosuppression, are at significant risk of developing life-threatening toxoplasmic encephalitis from primary or recrudescent infection.

It is estimated that approximately 30% to 50% of adults worldwide are infected with *T. gondii*, which is acquired most frequently from eating infected, undercooked meats or via exposure to infected cat fecal matter. Studies report that toxoplasmosis causes the highest disease burden of food-borne pathogens in developed countries and, ultimately, is the second leading cause of death due to food-borne illness (7, 8). Other than fully cooking meats and changing cat litter frequently, there are few interventions that can impede human infection: the only available vaccine is not licensed in North America and is approved solely for sheep (9). Indeed, the vaccine's primary effect is to reduce spontaneous fetal abortion in agricultural mammals, a common outcome of *Toxoplasma* infection in some livestock species. It is estimated that the associated disease has an annual economic impact of $7.7 billion in the United States alone (10). Similarly, feline companion animals can be tested for *Toxoplasma* infection, but treatment to eliminate feline cyst-shedding ability is unavailable.

Despite the well-established maladies resulting from infection by this parasite and the recent associations of chronic infection with altered host behavior, only nonideal treatment options exist (11). For example, in the United States, there are no approved therapies for maternal and fetal infections. Moreover, common medications used synergistically for the treatment of acute toxoplasmosis (i.e., toxoplasmic encephalitis) have well-known side effects: pyrimethamine induces bone marrow toxicity, and many patients are hypersensitive to sulfadiazine (12). The relatively well-tolerated drug atovaquone is increasingly used in acute infections, but only as an adjunctive therapy. Finally, no therapeutic agent or regimen evaluated to date is capable of clearing the chronic infection in humans or in livestock animals.

In an effort to spur the development of treatment regimens with reduced toxicity and/or the capacity to clear chronic infection in human or animal populations, the purpose of this review is to comprehensively profile clinically available drugs that have shown promising activity against *T. gondii in vivo* and *in vitro* but that lack clinical case reports. Such medications may be repurposed for effective use against *Toxoplasma* infections (13). Historically, the chronic infection was not associated with frank disease in otherwise healthy human patients, so therapeutic attempts at clearance were not undertaken. However, there is growing awareness of the value in eradicating chronic infection: in addition to interrupting the infectious route (if regimens were to be applied to

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TABLE 1 A list of drugs commonly used against toxoplasmosis[a]

Drug(s)	References	Mechanism of action	*In vitro* IC_{50}	Murine lethal challenge(s) and outcome (reference[s])
Pyrimethamine	15–20	Antifolate	0.4 μM	10 mg/kg/day, 20–90% survival, depending on parasite strain (18)
Dapsone, sulfadiazine, sulfadoxine, sulfamethoxazole	17, 19, 21–27	Antifolate	Dapsone, 1.2 μM; sulfadiazine, 1.6 μM; sulfamethoxazole, 395 μM	Dapsone, 100 mg/kg/day, 100% survival (24); sulfadiazine, 375 mg/kg/day, 100% survival (17); sulfamethoxazole, 600 mg/kg/day, 100% survival (26)
Clindamycin	17, 27–30	Protein synthesis inhibitor	0.005 μM	300 mg/kg/day, increased survival by 11 days (17)
Trimethoprim (typically combined with sulfamethoxazole)	21, 25, 26, 31	Antifolate	17.2 μM	70 mg/kg/day, 20% survival (26)
Atovaquone	27, 32, 33	Mitochondrial electron transfer chain inhibitor	0.82 μM	5 mg/kg/day, 20% survival (19)
Doxycycline, minocycline	34, 35	Apicoplast division inhibitor	Doxycycline, 14.4 μM	Doxycycline, 300 mg/kg/day, 100% survival (34)
Acetylspiramycin, azithromycin, clarithromycin, dirithromycin, erythromycin, roxithromycin, spiramycin, telithromycin, tylosin	18, 29, 36–41	Protein synthesis inhibitor	Azithromycin, 11.5 μM; clarithromycin, 401 μM; erythromycin, 19.6 μM; roxithromycin, 32.3 μM; spiramycin, 17.8 μM; telithromycin, 0.20 μM	Azithromycin, 200 mg/kg/day, 40–100% survival, depending on parasite strain (38); clarithromycin, 200 mg/kg/day, 80% survival (40); clarithromycin, 300 mg/kg/day, 100% survival (18); roxithromycin, 10 mg/day, 80–100% survival (36, 37); spiramycin, 400 mg/kg/day, no survival (38)

[a] The lowest observed IC_{50} values are reported in cases in which multiple values were found in the literature. Moreover, wide variations exist in the method of murine lethal challenge between studies: differences include infectious dose, parasite strain, drug delivery route, time postinfection before treatment initiation, treatment length, and time of observed survival.

companion and/or livestock animals), it would reduce the risk of reactivation in immunocompromised patients, and the possibility exists that clearing infection from the brain might reverse any parasite-induced behavioral phenotypes. Despite its known side effects, no treatment has been as effective against the acute stage as the synergistic combination of pyrimethamine and sulfadiazine, which target the folate synthesis pathway of the parasite. As an addition to or a replacement of the clinical use of pyrimethamine and sulfadiazine against acute infection, the following are commonly employed: clindamycin, doxycycline, dapsone, and an erythromycin derivative (e.g., clarithromycin or azithromycin) (14). These are summarized briefly in Table 1.

The characteristics of an ideal anti-*Toxoplasma* drug or combination would be severalfold. First, treatment should be effective against both stages of parasite growth in all mammals: the fast-growing tachyzoite stage associated with acute disease and rapid cell invasion and division (and easily cultivated *in vitro*) and the chronic bradyzoite stage associated with parasite formation of an intracellular chemoresistant cyst wall within infected brain and muscle cells. Often, measurements in model organisms to evaluate tachyzoite susceptibility to the drug treatment do so by quantifying parasite counts ("parasite burden") of internal organs (heart, liver, and/or spleen) typically 3 to 10 days following the initial infection (notably, where the degree of parasite clearance is positively correlated with the length of treatment) or by providing a lethal challenge and counting mice spared due to drug treatment. In contrast, bradyzoite cyst reduction resulting from drug treatment is typically evaluated by quantifying cysts that form in the brain by at least 30 days postinfection. Although not uncommon, trials evaluating cyst levels when the drug is administered early in the infective process (<30 days after infection) do not accurately measure the effect on the cyst stage but instead measure the reduction of the count of parasites entering the brain—a process that is known to take several days, depending on the parasite and host species/strain. Second, the ideal treatment would be parasiticidal against these two stages, but parasitostatic capability against the tachyzoite stage, which has greater difficulty resisting the host adaptive immune response, may be sufficient. Mice are used almost universally in these evaluations, which is quite appropriate and natural for this parasite: carnivorism of infected mice is likely the most common infection route for cats, the definitive host of *Toxoplasma gondii*. For compounds to effectively eliminate parasitic brain cysts, they would likely need to penetrate the blood-brain barrier. Lastly, an ideal treatment would show high efficacy and low toxicity across a range of hosts, including humans but also livestock and companion animals.

CLINICALLY AVAILABLE MEDICATIONS WITH ANTI-*TOXOPLASMA* ACTIVITY

Table 2 represents a comprehensive review of the literature accessed from PubMed using a search with the keywords "toxoplasm* AND (drug* OR treatment*)." Reviewed publication dates were limited to 1 January 1980 to 4 July 2015. A total of 5,222 items were filtered for primary literature evaluating the *in vitro* or *in vivo* efficacy of clinically available compounds, excluding publications evaluating any drug with a 50% inhibitory concentration (IC_{50}) that was >10 μM or that was determined solely by the less reliable enzyme-linked immunosorbent assay (ELISA) method (81) and those already listed in Table 1. Treatments for ocular infection were not included, as these have been recently reviewed

TABLE 2 A comprehensive list of clinically available human or veterinary drugs evaluated against the parasite *Toxoplasma gondii*, ordered by common clinical uses[a]

Drug or drug class	Common clinical use	Reference(s)	*In vitro* IC_{50}	*In vivo* lethal challenge(s)	*In vivo* parasite burden for indicated tissue or animal and treatment (reference[s])
Miltefosine	Antimicrobial	42	ND	20 mg/kg/day, 5% increased survival*	78% significant reduction of brain cyst count; treatment lasted 15 days, starting 60 days postinfection
Niclosamide	Antimicrobial	43	~0.25 μM	ND	ND
Triclosan	Antimicrobial	44–48	0.02 μM	200–300 mg/day, no difference in survival compared to control (45, 46)	Significant reduction in parasite burden (45, 47, 48); treatment lasted 4 days (45)
Artemisinins	Antiprotozoal	49–52	Artemisinin, 0.64 μM; artesunate, 0.213 μM; artemether, 0.31 μM; dihydroartemisinin, 0.35 μM	Artemisinin, 10 mg/kg/day, 20% survival (49)	ND
Veterinary anticoccidials	Antiprotozoal	53–60	Halofuginone, 0.00094 μM; monensin, 0.0015 μM; diclazuril, 0.006 μM; decoquinate, 0.011 μM; arprinocid N-oxide, 0.05 μM; salinomycin, 0.053 μM; robenidine, 0.09 μM; toltrazuril, 0.94 μM; clopidol, 5.21 μM; arprinocid, 7.2 μM	Arprinocid, 100 μg/day, 100% survival (53); ponazuril, 10 mg/kg/day, 100% survival (58); diclazuril, 1 mg/kg/day, 100% survival (56, 57)	Toltrazuril (sheep), significant reduction in muscle and heart cyst burden (59); treatment lasted 14 days
Fusidic acid	Antibacterial	61	7.74 μM	60 mg/kg/day, no difference in survival	No reduction in parasite burden; treatment lasted 5 days
Pristinamycins	Antibacterial	62	Synercid, 1.57 μM	Synercid, 200 mg/kg/day, 100% survival	ND
Rifamycins	Antibacterial	32, 40, 63, 64	ND	Rifabutin, 300 mg/kg/day, 100% survival (63); rifapentine, 50–200 mg/kg/day, 100% survival (32)	ND
Quinolones	Antibacterial	65–67	Gatifloxacin, 0.56 μM; trovafloxacin, 1.85–2.35 μM; enrofloxacin, <69.5 μM	Gatifloxacin, 400 mg/kg/day, 20% survival (66); enrofloxacin, 3 mg/kg/day, 17% survival (67); trovafloxacin, 100 or 200 mg/kg/day, 100% survival (65)	Enrofloxacin, 68% parasite cyst reduction (67); treatment lasted 25 days
Azoles	Antifungal	68–70	Itraconazole, 0.05 μM; fluconazole, 3.1 μM	Itraconazole, 10 mg/kg/day, 12% increased survival* (69); fluconazole, 20 mg/kg/day, 37% increased survival (69)	Itraconazole, significant reduction of brain cysts (69), treatment lasted 10 days; fluconazole, no significant reduction of brain cysts (69), treatment was carried out for 10 consecutive days, starting 4 days postinfection
Antiretrovirals	Antiretroviral	71–74	Didanosine, 0.21 μM; atazanavir, 1.97 μM; saquinavir, 3.88 μM; fosamprenavir, 5.29 μM	Didanosine, ~1 mg/day, 100% survival (71)	Didanosine, <70% reduction in cerebral cysts (71); treatment lasted 30 days
5-Fluorouracil	Anticancer	75	0.08–0.77 μM	ND	ND
Crizotinib	Anticancer	76	0.4–4 μM	ND	ND
Gefitinib	Anticancer	76	5–10 μM	ND	ND
Methotrexate	Anticancer	15	Piritrexim, 0.02 μM; trimetrexate, 0.02 μM	ND	ND
Cyclosporine	Immunosuppression	16, 77, 78	0.83 μM	150 mg/kg/day, treatment led to earlier time of death (78)	Variable effects (77)
Purine nucleoside analogues	Immunosuppression	77, 79	Adenine arabinoside, 1.5 μM	Azathioprine, 10 mg/kg/day, 100% survival (77)	Azathioprine, no significant reduction in parasite burden (77) after treatment for 7–100 days
Auranofin	Immunosuppression	80	0.28 μM	ND	Significantly reduced parasite burden in chicken embryo; one dose was administered
Antischizophrenic, antipsychotic, or mood-stabilizing agents	Psychiatric disorder treatment	81–83	Thioridazine, 1.2 μM; fluphenazine, 1.7 μM, trifluoperazine, 3.8 μM, chlorpromazine, 7.3 μM; zuclopenthixol, 8 μM	ND	ND

[a] The lowest observed IC_{50} values are reported when multiple values were found in the literature. With one exception (didanosine), none of these drugs have been described in published toxoplasmosis clinical case reports. *, not statistically significant; ND, not determined. The term "*in vivo* lethal challenge" used here refers to an experiment where model organisms are exposed to a 100% lethal infectious dose of parasite, often via intraperitoneal injection. The strain of the parasite and the recipient host affect the absolute number of parasites required to produce a lethal infection, which often manifests 7 to 15 days after infection. "*In vivo* parasite burden" refers to the tissue or fluid count of parasites isolated from a host following a nonlethal infection (typically accomplished by administration of a low dose of parasites and/or delivery of parasites via the oral route and/or the use of a low-virulence strain). Notably, parasite burden measurements are performed in a manner which typically quantifies both tachyzoite and bradyzoite parasite stages (most often by PCR). However, studies which aim to evaluate bradyzoite "cyst" counts (usually determined >30 day postinfection, since by this time the tachyzoites have been cleared by the immune system) are noted in the parasite burden column as such.

elsewhere (84). Articles not available in English were also excluded. The following includes a brief discussion on the demonstrated activity of these drugs against *Toxoplasma*.

ANTIMICROBIAL AGENTS

A number of relatively distinct compounds used clinically against an array of predominantly eukaryotic pathogens have shown some efficacy against *T. gondii*. Miltefosine is an analog of the ubiquitous compound phosphatidyl choline found in eukaryotic cell membranes and was initially developed to treat tumors. Subsequently, it was discovered to display potent efficacy against nonapicomplexan *Leishmania* protozoans, and it is now used clinically for treatment of *Leishmania* infections. Results of additional *in vitro* studies, including a recent study investigating *Toxoplasma* sensitivity, suggest that it may have much broader antimicrobial properties (42). The study showed that miltefosine had little efficacy in controlling acute infection after 5 days of treatment; however, a 15-day treatment against the established chronic stage led to a 78% reduction of the level of cysts in the brain. Moreover, the remaining cysts were noticeably smaller upon microscopic examination, suggesting that the drug effectively penetrates the blood-brain barrier and that extension of treatment time may produce greater effects. In an effort to provide functional options for treatment of infections by the unrelated parasite *Naegleria fowleri*, expanded investigational access to miltefosine for use against this uncommon but deadly infection has been granted by the United States Centers for Disease Control and Prevention. While the mechanism of action is not established in these antimicrobial roles, an appealing feature of miltefosine is an extended half-life of approximately 7 days in humans.

Niclosamide is a salicylanilide which is approved for treatment of parasitic worm infection, where it appears to decouple oxidative phosphorylation. Noting that niclosamide inhibits *T. gondii* growth *in vitro* at approximately 250 nM, Fomovska et al. designed a number of niclosamide derivatives and evaluated them against *T. gondii* (43). These showed efficacy against *in vitro* parasite growth; the most potent of these had an IC_{50} of 8 nM but was found to be parasitostatic, not parasiticidal. Efficacy against the cyst stage of *Toxoplasma* has not been studied.

Triclosan is a broad-spectrum agent used topically to inhibit fatty acid synthesis in susceptible organisms. Several reports indicate that triclosan (IC_{50}, 0.02 μM) decreases *in vitro* parasite growth of not only *Toxoplasma* (see Table 2) but also *Plasmodium* (44) and *Babesia* (85). Investigators reported that the drug targets enoyl reductase, an enzyme not found in mammals. However, parasite survival studies are either lacking or disappointing; triclosan did not extend mouse survival during a lethal challenge. This hydrophobic drug may hold greater promise if it can be more effectively delivered to the target enzyme (which resides in the parasite's quadruple-membraned organelle, the apicoplast): proof-of-concept approaches investigated triclosan conjugated to octa-arginine or encased in liposomal nanoparticles, the latter conferring greater reductions of parasite counts in peritoneal fluid than triclosan alone (45, 46).

ANTIPROTOZOAL AGENTS

A number of antiprotozoal agents (typically folate synthesis inhibitors) approved for human use have already been leveraged for clinical use against the acute stage. It is notable, though, that an extensive pool of drug-like compounds screened against *Plasmodium falciparum* followed by secondary screening against additional parasites (*T. gondii*, *Leishmania major*, and *Trypanosoma brucei*) showed that *T. gondii* was the least responsive of that group to this compound subset, suggesting that it may be more difficult to target chemotherapeutically than other tested human parasites (86).

Several structurally unrelated veterinary agents used on livestock (poultry, cattle, sheep, etc.) and companion animals have shown *in vitro* efficacy against *T. gondii*, although those agents are not commonly used for treatment of acute toxoplasmosis in these animals (87). Rather, they are used to treat or prevent a number of coccidian infections caused by organisms from the genera *Eimeria*, *Neospora*, *Hammondia*, *Sarcocystis*, and others. The following agents (along with their anticoccidial mechanism of action, if known) demonstrated *in vitro* IC_{50} values below 1 μM but were not evaluated further for *in vivo* effectiveness: the mitochondrial inhibitors decoquinate and robenidine; the ionophores monensin and salinomycin; and the protein synthesis inhibitor halofuginone (see Table 2). Arprinocid (IC_{50}, 7 μM; unknown mechanism of action) and the mitochondrial inhibitors ponazuril and diclazuril (IC_{50}, 6 nM), after administration at doses of 10 mg/kg of body weight/day or lower, each showed 100% survival of mice groups challenged with *T. gondii* acute lethal infection. Separately, *in vivo* testing of the mitochondrial inhibitor toltrazuril (IC_{50}, 0.94 μM) in sheep showed reductions in levels of microscopically counted tissue and brain cysts.

Isolated from the plant *Artemisia annua*, artemisinin and its semisynthetic derivatives have become part of a mainstay combination therapy for malaria infections. Although the mechanism of action of these compounds is not completely clear, their activity is hemoglobin digestion dependent (88). A recent study identified the malaria parasite's phosphatidylinositol-3-kinase as another possible target (89). While *Toxoplasma* is sensitive to artemisinin, with an IC_{50} value of 0.64 μM, this is more than 100-fold higher than the corresponding IC_{50} against *Plasmodium falciparum* (68). Additionally, treatment with artemisinin during mouse lethal challenges increased survival by only 20%, whereas treatment with artemisone, a synthetic derivative with reduced side effects that is undergoing clinical trials, permitted 50% mouse survival in the same study (49). Studies evaluating the effect of these compounds on the bradyzoite stage are lacking.

ANTIBACTERIAL AGENTS

T. gondii is a single-cell eukaryote, which obviously limits the likely efficacy of many prokaryotic-specific drugs. However, a number of antibacterial agents have shown *in vitro* efficacy against *T. gondii*. Almost all antibacterials used in the clinic to treat acute or recrudescent *Toxoplasma* patients are macrolides; clarithromycin and azithromycin are among the macrolides often used (see Table 1). Clindamycin, a lincosamide antibiotic, has also been used for this purpose. While the mechanism of action against *Toxoplasma* is not established for these agents, they are known to inhibit the ribosome in target organisms. Fusidic acid, a bacteriostatic compound used outside the United States to treat skin, bone, and joint infections by inhibiting microbial protein synthesis, shows relatively weak activity *in vitro* (IC_{50}, 7 μM) and no efficacy against *Toxoplasma in vivo*. Administration of the potent combination drug quinupristin-dalfopristin (Synercid) used to treat antibiotic-resistant *Enterococcus* infections via protein synthesis inhibition resulted in 100% survival in the acute infection model. Notably, when administered alone, each drug was much

less effective, thus mimicking observations in bacteria where treatment using the combination is thought to be bactericidal but treatment using each of the individual drugs is thought to be bacteriostatic. However, no cyst reduction studies were conducted using quinupristin-dalfopristin or its components.

Another group of potent antibiotics are the fluoroquinolones, which function by inhibiting prokaryotic topoisomerase II, leading to DNA fragmentation. Several newer fluoroquinolone derivatives have shown efficacy against experimental fungal infections (90); however, their routine clinical use in toxoplasmosis cases is uncommon. A number of fluoroquinolone derivatives showed *in vitro* and *in vivo* efficacy: trovafloxacin permitted 100% survival of infected mice in an acute infection model.

The drugs in the rifamycin group of antibiotics work against prokaryotic organisms by inhibiting DNA-depending RNA synthesis, and rifamycin derivatives are particularly effective against *Mycobacterium* infections. Moreover, rifamycins typically operate as bactericidal agents and show some ability to penetrate the blood-brain barrier (91). Although the classic drug rifampin showed no efficacy against *Toxoplasma in vitro* (92), a number of derivatives demonstrated growth inhibition. At relatively high (300 mg/kg) doses in mice, rifabutin protected 100% of mice during a lethal challenge with the hypervirulent RH strain; notably, lower (50 to 100 mg/kg) doses used in combination with known anti-*Toxoplasma* drugs such as pyrimethamine, sulfadiazine, and, especially, clindamycin showed potential synergistic effects (63). Another rifamycin derivative, rifapentine, is known for its long half-life in mice and humans and, evaluated against *T. gondii* acute lethal challenge in a mouse model, was 90 and 100% effective at doses of 100 and 200 mg/kg, respectively. Due to its half-life, rifapentine would be an exciting drug to evaluate against the cyst stage; however, such studies evaluating any of the drugs in the rifamycin group are lacking. Why rifampin is ineffective compared to other rifamycin derivatives is unknown, suggesting that this group of antibiotics may be affecting one or more novel targets in the parasite.

ANTIFUNGAL AGENTS

Antifungal agents effectively target a broad range of eukaryotic fungal pathogens of humans; chief among these agents are the azoles, which were first used clinically in the 1980s (93). Ketoconazole, fluconazole, and itraconazole work by inhibiting ergosterol synthesis, a key component of the fungal cell membrane. Though fluconazole and itraconazole have IC_{50} values of 3 and 0.5 μM, respectively, the mechanism responsible for their effect against *Toxoplasma* is unknown. While both antifungals, at doses of up to 20 mg/kg/day, increased survival only marginally (<40%) in an acute mouse infection model, they significantly reduced cyst levels, albeit the effect was seen when administration began 5 days following the initial infection.

ANTRETROVIRAL AGENTS

Beyond the classic association of *Toxoplasma* with fetal infections via primary infection of the mother, *Toxoplasma* infections have become a leading cause of mortality among HIV-positive individuals with AIDS. Symptomatic infection can occur in immunocompromised individuals via primary infection or by reactivation of a latent, chronic infection. To prevent this, patients are often prescribed the trimethoprim-sulfadiazine combination prophylactically. Surprisingly, even patients who have been on this prophylactic antiparasitic regimen for more than a decade still retain viable parasite tissue cysts, which typically reassert, often profoundly, when prophylactic therapy is removed. Frequently, coincident with *T. gondii* prophylaxis is antiretroviral therapy against HIV, thus ameliorating a primary concern of *Toxoplasma* symptomatic disease. However, observant researchers hypothesized that some antiretroviral therapies were more directly affecting the *Toxoplasma gondii* parasite. Indeed, studies show that multiple-antiretroviral therapies (using both protease inhibitors and nucleic acid analogues) appear to inhibit parasite growth *in vitro* through mechanisms that are not established. Single 100 mg/kg oral doses of didanosine (a reverse transcriptase inhibitor) reduced levels of chronic brain cysts by approximately 65%; notably, a single clinical review showed a reduction in reactivation of disease in HIV patients treated with didanosine, suggesting that it may reduce cyst levels in the human brain (94). No *in vivo* studies have been conducted using antiretroviral protease inhibitors, which may target parasite proteases or may modulate host proteases required for parasite egress (95).

ANTICANCER AGENTS

Fluorouracil (5-FU) is a pyrimidine antimetabolite analog used to treat ranges of malignancies. 5-FU undergoes conversion to 5-fluorodeoxyuridylate and interacts covalently with thymidylate synthetase and N5,N10-methylene tetrahydrofolate, thus forming a block for DNA synthesis (75). 5-FU is effective against *T. gondii in vivo* at doses as low as 0.08 μM. Preliminary work by Harris et al. indicated that 5-FU may be effective against *T. gondii* in doses 10-fold lower than those used for malignancies. It is assumed that 5-FU has the capacity to transit the blood-brain barrier, as it associated with CNS toxicity (96).

Crizotinib is a kinase inhibitor targeting multiple receptor tyrosine kinases, including anaplastic lymphoma kinase (ALK), which interferes with tumor cell proliferation and survival. It is approved for use in cases of ALK-positive, metastatic, non-small-cell lung cancer. Crizotinib inhibited *T. gondii* at 4.0 μM; however, the host HeLa cells detached from the plate, indicating host toxicity. Lower doses of crizotinib (0.4 μM) had no effect on the parasite. Gefitinib, another kinase inhibitor, is postulated to inhibit the intracellular tyrosine kinase domain of epidermal growth factor receptor (EGRF), resulting in cell cycle arrest and inhibition of angiogenesis. By inhibiting EGRF, downstream kinases such as AKT, extracellular signal-regulated kinase (ERK), Jun N-terminal protein kinase (JNK), and mitogen-activated protein kinase (MAPK) p38 are also inhibited. Whether one or more of the parasite-specific kinases are targeted is unknown (97), but this is a distinct possibility. Gefitinib at 20 μM inhibits *T. gondii* completely, without detachment of the host HeLa cells from the plate (76). However, gefitinib concentrations of <5 μM had little effect on the parasite. Neither kinase inhibitor was further evaluated in an *in vivo* model.

IMMUNOSUPPRESSANTS AND IMMUNOMODULATORS

Methotrexate (MTX) is an immunosuppressant folate antimetabolite analog and shows polyglutamation in the host cell, where it inhibits dihydrofolate reductase (DHFR) (98). MTX is used in various malignancy treatment protocols and is used in rheumatology as a disease-modifying antirheumatic drug (DMARD). While parasite DHFR is an essential enzyme in purine and thymidylate metabolism, mammalian cells can use leucovorin (folinic

acid), a reduced folate, to perform MTX "rescue." Enzymatically, piritrexim (a lipid-soluble analog of MTX) is at least 10-fold more potent against the parasite than MTX and at concentrations of 0.1 to 1.0 μM was shown to inhibit replication of *T. gondii* in a mouse peritoneal macrophage. Folinic acid rescue did not diminish the efficacy of piritrexim in inhibition of *T. gondii* replication (15, 99).

Cyclosporine (CsA) is an immunosuppressive and DMARD that inhibits T lymphocytes. It is principally used in organ transplant rejection prophylaxis but may also be used to treat rheumatoid arthritis (RA) or recalcitrant plaque psoriasis. Three biochemical processes have been associated with CsA: (i) complexes form between CsA and cyclosporine-binding proteins (cyclophilins) which interfere with calcineurin and inhibit signal transduction; (ii) CsA inhibits the chaperone function of specific proteins; and (iii) CsA has been shown to inhibit P-glycoprotein, a membrane pump that confers multidrug resistance to cancer cells and parasitic protozoa (100). CsA variably affected parasite loads, depending on the time frame being investigated (77). Not surprisingly, mice undergoing an acute lethal challenge experiment perished rapidly when given CsA (78).

T. gondii lacks the ability to synthesize purines *de novo* and thus utilizes adenosine kinase (AK)-mediated phosphorylation of adenosine salvaged from the host to acquire purines (79). Biochemical assays showed that adenine arabinoside (ara-A) effectively inhibits parasite-derived AK, with an IC_{50} value of 1.5 μM. When azathioprine, another purine derivative, was used to treat mice in an acute lethal infection model, 100% survival was observed (77). Evaluated against the cyst stage, however, azathioprine (the only anticancer or immunosuppressant agent to be evaluated against this stage) showed no significant difference compared to control results.

Auranofin is a gold-containing DMARD previously used for treatment of rheumatoid arthritis (RA). Due to its toxicity, auranofin has largely been supplanted by other DMARDS and biologic medications for the treatment of RA, but it is still commercially available. The mechanism of action of auranofin against parasites is thought to be dissociation of the gold, which then targets thioredoxin reductase. Auranofin has an IC_{50} value of 0.28 μM against *T. gondii* and is effective at 1 mg/kg *in vivo* in a chicken embryo model injected with tachyzoites.

PSYCHIATRIC AGENTS

CNS-acting medications, particularly antischizophrenic or antipsychotic agents, are often prescribed to *Toxoplasma*-positive individuals due to the relatively high coincidence of these mental disorders with parasite infection (101, 102). Similarly to the hypothesis that HIV-positive individuals directly reduce parasite disease potential by taking antiretrovirals, some studies have investigated whether psychoactive drugs affect *T. gondii* growth. As a group, these drugs function against unrelated targets, but many appear to inhibit cultured *Toxoplasma* parasites. The most potent, fluphenazine (IC_{50}, 1.7 μM), is a dopamine receptor antagonist with multiple side effects; it has also shown activity against the *Leishmania* parasite (103). However, there are no animal experiments analyzing the ability of these agents to reduce acute disease spread or morbidity or to reduce activity against the cyst stage.

CONCLUSION

Among the compounds listed in Table 2, none presently possess all of the attributes of a highly promising future drug against *Toxoplasma gondii*. Largely, this is due to missing information regarding their efficacy against the bradyzoite stage. From the characteristics compiled, the compelling efficacy of didanosine warrants further investigation; however, this drug, a nucleoside analog of adenosine, is associated with negative and common side effects, including peripheral neuropathy. Methotrexate and related derivatives are also provocative due to their low IC_{50} values *in vitro*, warranting further *in vivo* work. However, these drugs, too, are associated with dose-dependent negative side effects. Similarly, the veterinary anticoccidial halofuginone demonstrated exceptional potency *in vitro*, warranting its further evaluation as a treatment for *Toxoplasma* infection in animals.

In summary, the need for more-effective and less-toxic anti-*Toxoplasma* drug regimens is becoming increasingly urgent with the globally growing ranks of immunocompromised patients and the continued difficulties with ensuring safe livestock food supplies. More-potent regimens may also contribute to reductions in psychiatric disorders, if *Toxoplasma* is indeed a causal factor. Current research on developing a human or animal *Toxoplasma* vaccine has been an ongoing but incredibly challenging effort (104). Recent advances in immunotherapeutics designed to boost the host response to the parasite or to parasite antigens may aid effective multispecies vaccine development, ideally resulting in disease prevention or reduction and interruption of transmission (105). Immunomodulators in combination with anti-*Toxoplasma* compounds are another area of future promise (106). While there are dozens of small molecules that have shown promise against *T. gondii in vitro* or *in vivo*, these are beyond the scope of this review, which addresses clinically available but uncommonly used options. Additionally, as reviewed elsewhere (107), a number of novel natural products have shown activity against the parasite. The potential exists for other apicomplexan organisms (*Plasmodium*, *Babesia*, *Eimeria*, and *Cryptosporidium*) known to cause human or livestock diseases to be similarly sensitive to the agents described in this review; thus, future studies evaluating the aforementioned drugs against a broader range of parasites may be warranted. Synergistic combinations of the anti-*Toxoplasma* drugs reviewed here may yet produce "the right mix" to completely clear *Toxoplasma* chronic infections from humans and human-raised animal species.

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