Clinically Available Medicines Demonstrating Anti-
Toxoplasma Activity

Andrew J. Neville, a Sydney J. Zach, a Xiaofang Wang, b Joshua J. Larson, a Abigail K. Judge, a Lisa A. Davis, c,d,e Jonathan L. Vennerstrom, b, Paul H. Davis a

Department of Biology, University of Nebraska at Omaha, Omaha, Nebraska, USA a; College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska, USA b; Rheumatology, Denver Health Medical Center, Denver, Colorado, USA c; University of Colorado School of Medicine, Aurora, Colorado, USA d; Department of Internal Medicine, Denver Veterans Affairs Medical Center (VAMC), Denver, Colorado, USA e

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 chromoresistant 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-uterine 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...
TABLE 1 A list of drugs commonly used against toxoplasmosis

<table>
<thead>
<tr>
<th>Drug(s)</th>
<th>References</th>
<th>Mechanism of action</th>
<th>In vitro IC_{50}</th>
<th>Murine lethal challenge(s) and outcome (reference(s))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrimethamine</td>
<td>15–20</td>
<td>Antifolate</td>
<td>0.4 μM</td>
<td>10 mg/kg/day, 20–90% survival, depending on parasite strain (18)</td>
</tr>
<tr>
<td>Dapsone, sulfadiazine, sulfadoxine, sulfamethoxazole</td>
<td>17, 19, 21–27</td>
<td>Antifolate</td>
<td>Dapsone, 1.2 μM; sulfadiazine, 1.6 μM; sulfamethoxazole, 395 μM</td>
<td>Dapsone, 100 mg/kg/day, 100% survival (24); sulfadiazine, 375 mg/kg/day, 100% survival (17); sulfamethoxazole, 600 mg/kg/day, 100% survival (26)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>17, 27–30</td>
<td>Protein synthesis inhibitor</td>
<td>0.005 μM</td>
<td>300 mg/kg/day, increased survival by 11 days (17)</td>
</tr>
<tr>
<td>Trimethoprim (typically combined with sulfamethoxazole)</td>
<td>21, 25, 26, 31</td>
<td>Antifolate</td>
<td>17.2 μM</td>
<td>70 mg/kg/day, 20% survival (26)</td>
</tr>
<tr>
<td>Atovaquone</td>
<td>27, 32, 33</td>
<td>Mitochondrial electron transfer chain inhibitor</td>
<td>0.82 μM</td>
<td>5 mg/kg/day, 20% survival (19)</td>
</tr>
<tr>
<td>Doxycycline, minocycline</td>
<td>34, 35</td>
<td>Apicoplast division inhibitor</td>
<td>Doxycycline, 14.4 μM</td>
<td>Doxycycline, 300 mg/kg/day, 100% survival (34)</td>
</tr>
<tr>
<td>Acetylspiramycin, azithromycin, clarithromycin, dirithromycin, erythromycin, roxithromycin, spiramycin, telithromycin, tylosin</td>
<td>18, 29, 36–41</td>
<td>Protein synthesis inhibitor</td>
<td>Azithromycin, 11.5 μM; clarithromycin, 401 μM; erythromycin, 19.6 μM; roxithromycin, 32.3 μM; spiramycin, 17.8 μM; telithromycin, 0.20 μM</td>
<td>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)</td>
</tr>
</tbody>
</table>

*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 parasitidal 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 “toxoplasma 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...
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 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 "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 statistically significant; ND, not determined. The term "the lowest observed IC50 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 M; zuclopenthixol, 8.3 M, chlorpromazine, trifluoperazine, 3.8 ND.

<table>
<thead>
<tr>
<th>Drug or drug class</th>
<th>Common clinical use</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Didanosine, 0.21 M</td>
<td>Antiretroviral</td>
<td>71–74</td>
</tr>
<tr>
<td>Itraconazole, 10 mg/kg/day</td>
<td>Antifungal</td>
<td>68–70</td>
</tr>
<tr>
<td>Ciclosporine, 16,77,78</td>
<td>Immunosuppression</td>
<td>0.83 M</td>
</tr>
<tr>
<td>Azathioprine, 10 mg/kg/day</td>
<td>Anticancer</td>
<td>15</td>
</tr>
<tr>
<td>Piritrexim, 0.02 M</td>
<td>Anticancer</td>
<td>ND</td>
</tr>
<tr>
<td>Gefitinib</td>
<td>Anticancer</td>
<td>76</td>
</tr>
<tr>
<td>Crizotinib</td>
<td>Anticancer</td>
<td>76</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>Anticancer</td>
<td>M</td>
</tr>
<tr>
<td>Didanosine, 0.21 M</td>
<td>Antiretroviral</td>
<td>71–74</td>
</tr>
<tr>
<td>Itraconazole, 0.05 M</td>
<td>Antifungal</td>
<td>M; atazanavir, 1.97 M</td>
</tr>
<tr>
<td>Gatifloxacin, 0.56 M</td>
<td>Antibacterial</td>
<td>65–67 Gatifloxacin, 400 mg/kg/day, 20%</td>
</tr>
<tr>
<td>Rifabutin, 300 mg/kg/day</td>
<td>Antibacterial</td>
<td>32, 40, 63, 64 ND</td>
</tr>
<tr>
<td>Synercid, 200 mg/kg/day</td>
<td>Antibacterial</td>
<td>62 Synercid, 1.57 M</td>
</tr>
<tr>
<td>Halofuginone, 0.00094 M</td>
<td>Antiprotozoal</td>
<td>53–60 Halofuginone, 0.00094 M; arprinocid, 7.2 M</td>
</tr>
<tr>
<td>Artemisinin, 0.64 M</td>
<td>Antiprotozoal</td>
<td>49–52 Artemisinin, 0.64 M; artesunate, 0.213 M</td>
</tr>
<tr>
<td>Triclosan</td>
<td>Antibacterial</td>
<td>44–48 0.02 M</td>
</tr>
<tr>
<td>Miltefosine, 20 mg/kg/day</td>
<td>Antimicrobial</td>
<td>ND</td>
</tr>
</tbody>
</table>

A comprehensive list of clinically available human or veterinary drugs evaluated against the parasite Toxoplasma gondii, ordered by common clinical uses.

TABLE 2 A comprehensive list of clinically available human or veterinary drugs evaluated against the parasite Toxoplasma gondii, ordered by common clinical uses.
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 non-apicomplexan 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 IC50 of 6 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 (IC50 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 antococcial mechanism of action, if known) demonstrated in vitro IC50 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 (IC50 7 μM; unknown mechanism of action) and the mitochondrial inhibitors ponazuril and dichlorozuril (IC50 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 (IC50 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 IC50 value of 0.64 μM, this is more than 100-fold higher than the corresponding IC50 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 (IC50 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 treat-
ment using the combination is thought to be bactericidal but
treatment using each of the individual drugs is thought to be bac-
teriostatic. 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, lead-
ing to DNA fragmentation. Several newer fluoroquinolone deriv-
atives 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-dependent RNA syn-
thesis, 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 dur-
ing 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, es-
specially, 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, rifa-
pentine 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 com-
pared to other rifamycin derivatives is unknown, suggesting that
this group of antibiotics may be affecting one or more novel tar-
gets 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). Keto-
conazole, fluconazole, and itraconazole work by inhibiting ergos-
terol synthesis, a key component of the fungal cell membrane.
Though fluconazole and itraconazole have IC_{50} values of 3 and 0.5
\mu M, 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 individ-
uals with AIDS. Symptomatic infection can occur in immuno-
compromised individuals via primary infection or by reactivation
of a latent, chronic infection. To prevent this, patients are often
prescribed the trimethoprim-sulfadiazine combination prophyl-
actically. Surprisingly, even patients who have been on this pro-
phylactic antiparasitic regimen for more than a decade still retain
viable parasite tissue cysts, which typically reassert, often pro-
foundly, when prophylactic therapy is removed. Frequently, coin-
cident with T. gondii prophylaxis is antiretroviral therapy against
HIV, thus ameliorating a primary concern of Toxoplasma symp-
томatic 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 nu-
cleic 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) re-
duced levels of chronic brain cysts by approximately 65%; nota-
sibly, 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 pro-
tases 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 \mu M. 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 as-
associated with CNS toxicity (96).

Crizotinib is a kinase inhibitor targeting multiple receptor ty-
rosine 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 \mu M; how-
ever, the host HeLa cells detached from the plate, indicating host
toxicity. Lower doses of crizotinib (0.4 \mu M) had no effect on the
parasite. Gefitinib, another kinase inhibitor, is postulated to in-
hbit the intracellular tyrosine kinase domain of epidermal growth
factor receptor (EGFR), resulting in cell cycle arrest and inhibition
of angiogenesis. By inhibiting EGFR, 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 par-
site-specific kinases are targeted is unknown (97), but this is a
distinct possibility. Gefitinib at 20 \mu M inhibits T. gondii com-
pletely, without detachment of the host HeLa cells from the plate
(76). However, gefitinib concentrations of <5 \mu M 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 antimetab-
olite 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 rheuma-
tology as a disease-modifying antirheumatic drug (DMARD).
While parasite DHFR is an essential enzyme in purine and thymi-
dylate metabolism, mammalian cells can use leucovorin (folinic
acid), a reduced folate, to perform MTX “rescue.” Enzymatically, pyritrexim (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. Folic acid rescue did not diminish the efficacy of pyritrexim 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 between CsA and cyclosporine-binding proteins (cyclphin); 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 anti-psychotic 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.

**ACKNOWLEDGMENTS**

This report was supported by the following NIH grants: GM103427 (P.H.D.) and AI116723 (J.L.V.). Additionally, the following support is acknowledged: the Rheumatology Research Foundation Scientist Development Award (L.A.D.) and the Nebraska Research Initiative (P.H.D. and J.L.V.).

**REFERENCES**


10. Buzby JC, Roberts T. 1997. Economic costs and trade impacts of micr...


73. Jones-Brando L, Torrey EF, Yolken R. 2003. Drugs used in the treatment of schizophrenia and bipolar disorders inhibit the replication of...


