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REVIEW

Activities of anti-*Toxoplasma* drugs and compounds against tissue cysts in the last three decades (1987 to 2017), a systematic review

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Abstract

Currently, there is no approved therapy that can eradicate *Toxoplasma gondii* tissue cysts, which are responsible for chronic infection. This systematic review was performed to assess drugs or compounds that can be used as anti-*T. gondii* tissue cysts in vitro and in vivo. English electronic databases (i.e., PubMed, Science Direct, Scopus, Google Scholar, and Web of Science) were systematically searched for articles published up to 2017. A total of 55 papers published from 1987 to 2017 were eligible for inclusion in this systematic review. Among the drugs, atovaquone and azithromycin were found effective after long-term inoculation into mice; however, clinical cases of resistance to these drugs have been reported. Also, FR235222, QUI-11, tanshinone IIA, and hydroxyzine were shown to be effective against *Toxoplasma* cysts, but their effectiveness in vivo remains unknown. Additionally, compound 32, endochin-like quinolones, miltefosine, and guanabenz can be used as effective antiparasitic with the unique ability to reduce brain tissue cysts in chronically infected mice. Importantly, these antimicrobial agents are significant criteria for drug candidates. Future studies should focus on the biology and drug susceptibility of the cyst form of *T. gondii* in chronic toxoplasmosis patients to find more effective strategies that have sterilizing activity for eliminating *T. gondii* tissue cysts from the host, preventing disease relapse and potentially shortening the required duration of drug administration.

Keywords *Toxoplasma gondii* · Toxoplasmosis · Drugs · Compounds · Tissue cysts

Introduction

Toxoplasma gondii, an obligate intracellular parasite belonging to the phylum Apicomplexa, is one of the most successful parasitic organisms in the world, which can infect many warm-blooded vertebrates including humans (Dubey and Jones 2008). It is estimated that up to one third of the human population is infected with *T. gondii*, which is acquired mainly by ingesting tissue cysts from undercooked meat or ingestion of food or water contaminated with oocysts shed from cats (as the definitive hosts). However, other routes of transmission include vertical transmission from mother to child, organ transplantation, blood transfusion, and inhalation of oocyst-contaminated dust. This ubiquitous parasitic protozoan is the etiologic agent of toxoplasmosis, which causes the greatest disease burden of foodborne pathogens in the developed countries. *T. gondii*, if not treated, is the second leading cause of death due to foodborne diseases in these countries (Havelaar et al. 2012; Scallan et al. 2011).

Tachyzoites, bradyzoites (tissue cyst form), and sporozoites are three infectious forms of the *T. gondii* parasite (Dubey et al. 1998). Tissue cysts, intracellular structures formed by bradyzoites, divide by endodyogeny (Ferguson et al. 1994). The size of a tissue cyst is variable, but on average, a mature cyst can range from less than 10 to 70 μm in diameter and consist of several to hundreds or even thousands of

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bradyzoites (Dubey 1977). The development of tissue cysts is more common in the brain, eyes, and muscles (e.g., skeletal and cardiac tissues); however, they can also develop in visceral organs (e.g., lungs, liver, and kidneys). Tissue cysts are especially prevalent in the central nervous system (CNS). They have been detected in neurons, astrocytes, and microglia (Dubey 1988). Stage conversion from tachyzoites to bradyzoites allows the life-long persistence of the *T. gondii* parasite in the host (Weiss and Kim 2000).

When chronically infected patients become immunocompromised with conditions such as AIDS or due to the medication process after organ transplantation, bradyzoites get released from tissue cysts, multiply, and spread to other organs, predominantly the brain and muscles, resulting in severe morbidity and mortality (Luft and Remington 1992). Unfortunately, cyst walls are resistant to both the immune system and drugs (Gormley et al. 1998). While several drugs are available that can control acute toxoplasmosis (Montazeri et al. 2017), there is no approved therapy that eliminates the tissue cysts responsible for chronic infections (Montazeri et al. 2016). Accordingly, the current systematic review was aimed at retrieving published studies related to in vitro and in vivo evaluation of antimicrobial agents for the treatment of chronic toxoplasmosis in humans or animals against tissue cysts in order to prepare comprehensive data for designing more accurate investigations in the future.

Methods

This review followed the Preferred Reporting Items for Systematic Reviews (PRISMA) guidelines (Moher et al. 2009). A protocol of this systematic review is available in PROSPERO International prospective register of systematic reviews (2013), CRD42017072655 (https://www.crd.york.ac.uk/prospero/).

Search strategy

English databases, including PubMed, Science Direct, Scopus, Google Scholar, and Web of Science, were systematically searched for articles related to in vitro or in vivo evaluation of anti-*Toxoplasma* activities of drugs and compounds published up to December 2017. The used keywords consisted of "*Toxoplasma gondii*," "*T. gondii*," "toxoplasmosis," "drugs," "compounds," "tissue cysts," "bradyzoites," and "chronic infection."

Study selection and data extraction

According to the inclusion criteria, papers written in English were selected and carefully reviewed for eligibility. Gray literature and abstracts of articles that were published in congresses were not explored. In addition, to avoid missing any articles, entire references of the papers were meticulously hand-searched. Among English articles that were found using the mentioned strategies, full-text papers that used laboratory methods both in vitro and in vivo for the detection of chronic infections were included. The included papers were precisely investigated, and the main information was extracted.

Results

Analysis of the included literature

A total of 55 papers (13 in vitro studies, 49 in vivo, and 7 both in vitro and in vivo) published in three decades from 1987 to 2017 were included in the systematic review. Figure 1 briefly shows this article's search process.

In vitro and in vivo results

The efficacy of 50 drugs and several new compounds against *T. gondii* in vitro and in vivo was evaluated (Tables 1 and 2). Also, drugs or compounds with more than 20 pathways or mechanisms of action are shown in Table 3. Notably, several targets were identified against *T. gondii* including mitochondrial electron transport chain, calcium-dependent protein kinase 1, type II fatty acid synthesis, DNA synthesis, and protein synthesis. Our collected data indicated that many of the drugs or compounds evaluated against *T. gondii* cysts act on the mitochondria and apicoplast. Therefore, these organelles represent a potential drug target for new chemotherapy.

Most of the in vitro and in vivo investigations on the activity of drugs against the *T. gondii* cyst were based on their infectivity by subinoculation to mice (5 studies) and counting the number of brain cysts (40 studies). Most of the surveys used the ME49 strain of *T. gondii* (4 in vitro studies and 28 in vivo) while in some studies, Prugniaud, EGS, and VEG strains were also used. The animal model used in the reviewed papers was mostly Swiss Webster mice (22 studies).

Discussion

The aim of this systematic review was to investigate the in vitro and in vivo effects of anti-*Toxoplasma* drugs and synthetic compounds against tissue cysts. The previous studies in 1992, 1994, and 1998 suggested that atovaquone had activity on cyst tissue in vivo (Araujo et al. 1992a; Ferguson et al. 1994; Gormley et al. 1998); however, clinical cases of resistance to this drug have been reported (Baatz et al. 2006; Megged et al. 2008). Unfortunately, *Toxoplasma* has a strong ability to spontaneously develop drug resistance by mutation of the atovaquone binding site on cytochrome b, and thus, this

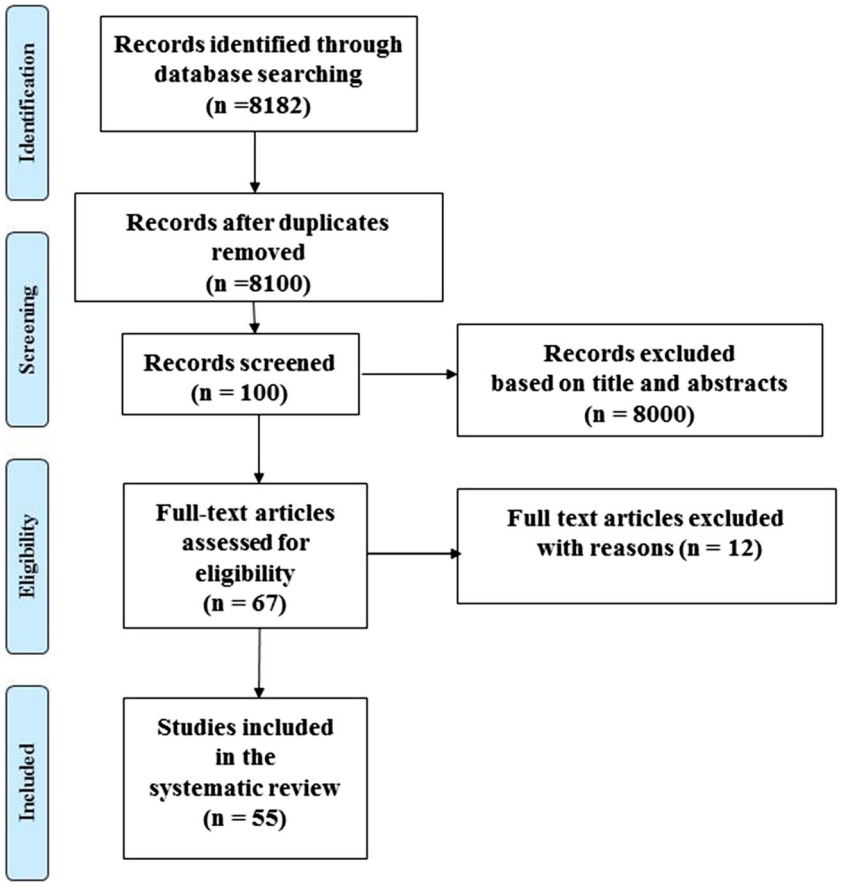

Fig. 1 The PRISMA flowchart describing the study design process

drug has never become a first-line treatment for chronic toxoplasmosis (Chirgwin et al. 2002).

In a new study, Vidadala et al. (2016) optimized compound 32 (*T. gondii* calcium-dependent protein kinase 1 inhibitor [CDPK1]), a promising lead for the development of a new antitoxoplasmosis therapy in the acute and latent stages of infection. Interestingly, this compound does not have human Ether-à-go-go-Related Gene (hERG) inhibitory activity. Moreover, compound 32 is a CNS-penetrant and can significantly reduce brain cysts by 88.7%.

In a recent study by Rutaganira et al. (2017), the effect of small molecule inhibitors of CDPK1 for the treatment of CNS toxoplasmosis was examined. In this study, compound 24 was effective in treating acute and chronic infection, reducing propagation to the CNS, and decreasing reactivation of chronic toxoplasmosis in immunocompromised mice. CDPK1, an essential enzyme in *T. gondii*, controls multiple processes that are critical for the intracellular replicative cycle of the parasite, including secretion of adhesins, motility, invasion, and egression. Based on these studies, CDPK1 inhibitors can be represented as the potential drugs for new chemotherapy methods.

In another study by Doggett et al. (2012), researchers showed the remarkable efficacy of endochin-like quinolone (ELQ-271 and ELQ-316) in decreasing *T. gondii* brain cysts by up to 88% at low doses, suggesting that they have the potential to eradicate latent infection at clinically applicable doses. ELQ-271 and ELQ-316 are inhibitors of the Qi site of the *T. gondii* cytochrome bc1 complex, and their mechanism of action differs from that of current clinically used anti-*Toxoplasma* therapies.

Recently, investigators have focused on miltefosine, an anticancer agent, which was demonstrated to result in significant reduction in brain cyst load in the chronic stage of toxoplasmosis. Also, the survived cysts were noticeably smaller upon microscopic examination, suggesting that this drug can effectively penetrate the blood–brain barrier and that the prolongation of treatment time may result in greater effects (Eissa et al. 2015). Additionally, future studies should focus on the mechanism of action of miltefosine against the *T. gondii* cyst form in chronic toxoplasmosis.

In two studies performed by Afifi et al., on the *Toxoplasma* brain cyst load, 74% reduction in cystic forms in the chronic phase of toxoplasmosis after treatment with rolipram, a phosphodiesterase-4 (PDE4) inhibitor, has been shown. Cyclic nucleotide phosphodiesterases are critical modulators of cellular levels of cAMP, which catalyzes cyclic nucleotide hydrolysis, since rolipram causing high cAMP levels can inhibit *Toxoplasma*'s conversion to the bradyzoite form. Additionally, rolipram could interfere with tachyzoite–bradyzoite interconversion due to suppression of cytokines

Table 1 Summary of in vitro studies that evaluated the anti-*Toxoplasma* activity of drugs/compounds against tissue cysts

No.	Drug	Strain	Cell/medium	Culture	Evaluation	Main results	Effectivity	Ref
1	Compound 566C80	ME49	RPMI	24 and 72 h	Inoculation “”””into mice (IP)	None of the mice were infected with cysts	Effective	(Araujo et al. 1991)
2	Arprinocid-N-oxide, azithromycin and the hydroxynaphthoquinone 566C80	ME49	RPMI	24 and 72 h	Inoculation into mice (IP)	The most active compounds against the cyst form were arprinocid-N-oxide, azithromycin, and hydroxynaphthoquinone 566C80	Effective	(Huskinson-Mark et al. 1991)
3	Hydroxynaphthoquinone 566C80	ME49	RPMI	3 or 6 days	Inoculation into mice (IP)	Loss of viability of the cysts	Effective	(Huskinson-Mark et al. 1991)
4	CAMP PYR MTX	ME49	Peritoneal macrophages	1, 3, 5, and 10 days	[3H]-Uracil uptake assay and Giemsa stain	CAMP and PYR: the number of pseudocysts were decreased but the size was not changed. MTX: no effect was noted	Effective Effective Ineffective	(Choi et al. 1994)
5	Monensin	PLK, 76 K	Vero	6 or 48 h	Immunofluorescence assay, electron microscopy, and inoculation into mice	Significant cytological alterations of the monensin-treated bradyzoites were seen. Mice inoculated with cysts were treated 6 or 48 h; no antibodies and no cysts were recovered from their brains	Effective	(Couzinet et al. 2000)
6	PHNQ6 alone and combined with sulfadiazin	EGS	DMEM	24 or 48 h	Inoculation into mice (IP)	Infectivity of bradyzoites treated with PHNQ6 alone or combined with sulfadiazine was inhibited after in vitro incubation	Effective	(Ferreira et al. 2006)
7	Tetrapeptide FR235222	PRU	HFF	24, 48, and 72 h	Immunofluorescence assay (IFA)	100% altered cysts 24 h after treatment with the lowest concentration (30 nM) of FR235222	Effective	(Maubon et al. 2010)
8	New naphthoquinones (QUI-11, QUI-6, and QUI-5) and liriodenine	EGS	DMEM	24 and 48 h	Inoculation into mice (IP)	In vitro incubation with QUI-6, QUI-11, and liriodenine inhibited the infectivity of the bradyzoites. None of the surviving animals had detectable cysts in the brain	Effective	(Ferreira et al. 2012)
9	Spiramycin coadministered with metronidazole	ME49	Vero E6 cells	1 week	Counting the number of cysts	Spiramycin reduced the numbers of cysts 44 and 42%. Coadministration of drugs reduced the numbers of cysts (68 and 58% reductions)	Effective	(Chew et al. 2012)
10	Atovaquone and 3-bromopyruvate	RH	LLC-MK2	24 or 48 h, or 6 days	Indirect immunofluorescent assays	Atovaquone and 3-bromopyruvate in combination led to fewer parasite-infected cells with no evidence of cystogenesis	Atovaquone, effective	(de Lima et al. 2015)
11	Guanabenz	PRU	HFF	32 h	Stained with Diff-Quick and evaluated by light microscopy	4 and 15% of cysts were abnormal in the guanabenz and salubrinal	Effective	(Benmerzouga et al. 2015)
12	Tanshinone IIA and hydroxyzine	PLK/DLUC1C9	HFF	3 days	Dual-Glo luciferase assay	Both compounds reduced the bradyzoite number	Effective	(Murata et al. 2017)
13	Aureobasidin A and compound 20	PRU	HFF	3 days	ED_{50} determination	Aureobasidin A demonstrated slightly higher efficacy (ED_{50} 2·51, μg/ml) than compound 20 (ED_{50} 3·74, μg/ml)	Effective	(Alqaisi et al. 2017)

IP intraperitoneal, *PYR* pyrimethamine, *MTX* methotrexate, *PHNQ6* 2-hydroxy-3-(1′-propen-3-phenyl)-1,4-naphthoquinone

Table 2 Summary of in vivo studies that evaluated the anti-*Toxoplasma* activity of drugs/compounds against tissue cysts

No.	Drug	Animal	Strain	Type of infection	Inoculum
1	CLI	Swiss Webster female mice	C56	Chronic	10^4 tachyzoites (IP)
2	TMP and SMX	Murine	PRU	Chronic	10 cysts (IP)
3	Minocycline	Swiss Webster mice	ME49	Chronic	20 cysts (IP)
4	Compound 566C80	Female CBA/Ca	ME49	Chronic	20 cysts (IP)
5	Clarithromycin alone or in combination with minocycline	CBA/Ca	ME49	Chronic	10 cysts (orally)
6	Hydroxynaphthoquinone 566C80	Outbred female Swiss Webster and inbred female CBA/Ca	ME49	Chronic	10 cysts (CBA/Ca) and 20 cysts (Swiss Webster) (IP)
7	Rifabutin alone and combined with sulfadiazine, PYR, CLI, or ATO	Inbred CBA/Ca females	ME49	Chronic	20 cysts, orally
8	Epiroprim (Ro 11-8958), alone and in combination with dapsone	Female Swiss Webster	ME49	Chronic	10 cysts
9	Azithromycin	Mice	PRU	Chronic	9 cysts (IP)
10	ATO	Inbred female CBA/Ca mice	ME49	Chronic	10 tissue cysts (IP)
11	Rifapentine	Swiss Webster female mice	C56	Chronic	10 tissue cysts (orally)
12	Copolymers CRL 8131 and CRL 8142 plus ATO	Inbred female CBA/Ca	ME49	Chronic	30 tissue cysts
13	Corticoids, azathioprine, and cyclosporine	Swiss Webster mice	C strain	Chronic	High-level inoculum of cysts, infected per orally
14	DDI	OF1 mice	DUR	Chronic	10 cysts by gavage
15	Ketolide antibiotics HMR 3647 and HMR 3004	Female Swiss Webster mice	C56	Chronic	10 cysts by gavage
16	Phenylalanine derivatives	Female OF1 mice	DUR	Chronic	10 cysts/0.2 ml by gavage
17	ATO, PYR, sulfadiazine sodium, CLI, vancomycin, spiramycin, and Rhodiafarma	Female outbred Syrian golden hamsters	ME49	Chronic	0.2 ml mouse brain (IP)
18	ATO and ATO-loaded nanocapsules	Female Swiss Webster mice	COUL and ME49	Chronic	10 cysts by gavage
19	CLI and recombinant IL-12 (rIL-12)	Mouse	–	Chronic	–
20	Garlicin and minocycline	Mice	Fukaya	Chronic	(IP)
21	ATO combined with CLI	Female Swiss Webster mice	ME49	Chronic	10 or 20 cysts
22	PHNQ4, PHNQ5, and PHNQ6	Outbred female Swiss mice	EGS	Chronic	10 cysts (orally)
23	PHNQ6 alone and combined with sulfadiazine	Outbred female Swiss mice	P strain	Chronic	10 cysts (IP)
24	Valproic acid	Mice	ME49	Chronic	1×10^3 tachyzoites
25	FR235222 and the five FR235222 derivative compounds	Outbred female Swiss mice	PRU	Chronic	25 to 50 cysts
26	ITZ and FLZ	Outbred female Swiss mice	ME49	Chronic	20 cysts (IP)
27	Endochin-like quinolone (ELQ-271 and ELQ-316)	CBA/J mice	ME49	Chronic	18 cysts (IP)
28	Spiramycin coadministered with metronidazole	Male BALB/c mice	ME49	Chronic	1000 tachyzoites (orally)
29	Toltrazuril	Akkaraman lambs	ME49	Chronic	1×10^5 oocysts (IP)
30	4 new derivatives of 3-methyl-benzyl-PP (3-MB-PP, or 1)	Female BALB/c mice	Pru-LUC	Chronic	10^4 PRU-Luc-GFP tachyzoites (IP)
31	Rolipram	Female Swiss albino mice	KSU strain	Chronic	20 cysts (IP)
32	Guanabenz	Female BALB/c mice	PRU	Chronic	10^6 tachyzoites (IP)
33	Corticosteroids (hydrocortisone sodium succinate)	Swiss Webster	ME-49	Chronic	10^3 bradyzoites (IP)
34	Triclosan and TS liposomal nanoparticles	Swiss albino mice	ME49	Chronic	10 cysts (orally)
35	Miltefosine	Swiss albino mice	ME49	Chronic	10 cysts by gavage
36	Aripiprazole	Male BALB/c mice	Tehran	Chronic	50 cysts (IP)
37	Rolipram	Female Swiss albino mice	KSU strain	Chronic	20 cysts (IP)
38	*T. gondii* biotherapies (BIOT-TG 200)	Swiss male mice	ME49	Chronic	20 cysts (orally)
39	Resveratrol and ST	Swiss male mice	VEG	Chronic	50 cysts (orally)
40	TgCDPK1 inhibitor 32	CBA/J mice	ME49	Chronic	30 mg/kg once daily for 14 days (orally)
41	Fluphenazine and thioridazine	Male BALB/c mice	Tehran	Chronic	20 cysts (IP)
42	Etanercept (TNF-α antagonist)	Swiss albino mice	ME49	Chronic	10 cysts (IP)

Table 2 (continued)

43	Diphenyl diselenide	Swiss albino mice	ME49	Chronic	50 cysts (orally)
44	Artesunate	Female Swiss albino mice	ME49	Chronic	20 cysts/0.2 ml/mouse by nasogastric feeding tube
45	DLE	Female NIH mice	ME49	Chronic	25 cysts (orally)
46	Diphenyl diselenide	Swiss albino mice	ME49	Chronic	50 cysts (orally)
47	Diphenyl diselenide	Swiss albino mice	ME49	Chronic	50 cysts (orally)
48	Pyrazolopyrimidine analog, compound 24	Mice lacking the IFN-γ receptor (Ifngr1−/−)	ME49Δhx::FLUC line	Chronic	5 cysts (orally)
49	DXM	Female BALB/c	A local isolate of chicken brain	Chronic	15 cysts (IP)

No.	Treatment	Assessment of efficacy	Main results	Effectivity	Ref
1	150 mg of CLI phosphate per kg twice daily for 14 days (IP)	Number of cysts was counted	The average number of cysts in the brains of the CLI-treated mice were 9	Effective	(Hofflin and Remington 1987)
2	The combination from day 5 for 15 days or from day 28 for 288 days (60/300 mg/kg per day)	Survival rates and histopathology	The combination gave protection and even apparent toxoplasmal eradication at the highest dosing (60/300 mg/kg per day)	Effective	(Dumas et al. 1999)
3	50 mg/kg per day of minocycline for 3 weeks	Number of cysts was counted	A significant reduction in the number of brain cysts was seen	Effective	(Chang et al. 1991)
4	100 mg/kg per day for 8 weeks by gavage	Number of cysts was counted	Fewer cysts in their brains were seen. Mortality as well as clinical signs of brain infection was absent from treated mice	Effective	(Araujo et al. 1991)
5	300 mg/kg/day, orally	Counting the number of brains cysts and histopathology	The combination that resulted was significantly greater than the activity of drugs alone	Effective	(Araujo et al. 1992b)
6	200 mg/kg per day by gavage	Counting the number of brain cysts and histopathology	The reduction in the number of cysts in the brain was rarely seen in the histopathology sections of treated mice	Effective	(Araujo et al. 1992a)
7	200 mg/kg per day by gavage	Counting the number of cysts and histological examination	The inflammatory responses were significantly reduced	Effective	(Araujo et al. 1994)
8	50 mg/kg/day 3 weeks by gavage	Counting the number of cysts and evaluation of inflammation	Dapsone and epiroprim alone reduced the number of brain cysts and the inflammation in the brains	Effective	(Chang et al. 1994)
9	100 mg/kg/day, 100 days	Counting the number of cysts and survival rates	Enhanced survival and a reduction in brain inflammation	Effective	(Dumas et al. 1994)
10	200 mg/kg/day, 4 weeks by gavage	Counting the number of cysts	This significant reduction in tissue cyst numbers and the average size of the tissue cysts	Effective	(Ferguson et al. 1994)
11	50 or 100 mg/kg/day for 10 days, beginning 3 days after infection	Survival rates	Resulted in 100% survival	Effective	(Araujo et al. 1996)
12	2 mg of ATO and 100 mg of CRL 8131 per kg/day by gavage	Counting the number of cysts and histological studies	Reduction in the number of cysts and in the inflammation in the brains of mice	Effective	(Araujo and Slifer 1995)
13		Brain parasite load estimation	Did not significantly affect parasite loads in the brain	Ineffective	(Sumyuen et al. 1996)
14	2 mg of DDI/ml, 30-day treatment	Microscopical and histological studies	The number of cyst in the brains of mice treated with DDI was lower than that in the controls	Effective	(Sarciron et al. 1997)
15	5 to 200 mg/kg/day for 10 days, beginning 3 days after infection	Survival rates	Lower doses of each ketolide protected the mice	Effective	(Araujo et al. 1997)
16	150 mg/kg/day under 0.2 ml volume per animal for 5 days	Cysts counted and histological studies	Phe-Phe-OMe: 67, Boc-L-Phe: 77, L-Phe-OMe: 62, Boc-L-Phe-L-Phe-OMe: 62, and PYR 77% decrease the number of cysts	Effective	(Sarciron et al. 1998)
17	PYR 50, CLI 1200 mg, sulfadiazine 4, spiramycin, and ATO 3 g for 4 weeks in the drinking water	Counting the number of cerebral and retina cysts	The ATO-treated group had a significantly lower mean number of cysts than the control group	Effective	(Gormley et al. 1998)
18	15 mg/kg/day during 6 weeks, 5 days a week	Counting the number of brain cysts and parasitic burdens	A decrease of brain parasitic burden, which was significantly more pronounced in ME49-infected mice	Effective	(Sordet et al. 1998)

Table 2 (continued)

19	CLI (5 mg/kg orally), rIL-12 (0.25 microg IP), or the combination of both, once weekly for 3 months	Counting the number of brain cysts and survival rate	Simultaneous administration of CLI and rIL-12 resulted in prevention of reactivation in 73.3%	Effective	(Tawfeek et al. 2001)
20	–	Counting the number of brain cysts	Either garlicin or minocycline had no effect on elimination of tissue cysts	Ineffective	(Shu and Jiang 2002)
21	ATO 100, CLI 400, and ATO + CLI 100 + 400 mg/kg/day for 14 consecutive days	Microscopically counted	The cyst burdens in ATO and CLI alone and in combination were decreased to 75.9, 76.4, and 43%	Effective	(Djurković-Djaković et al. 2002)
22	10, 50, and 100 mg/kg/day, for 10 days, beginning 2 days after infection orally	Survival rate	A prolongation of the time to death or up to 30 days after treatment was seen	Effective	(Ferreira et al. 2002)
23	Sulfadiazine (40 mg/L), PHNQ6 (50 mg/kg/day), and sulfadiazine plus PHNQ6, for 4 weeks	Counting the number of brain cysts and liver histological studies	The number of brain cysts was lower in mice treated with PHNQ6 alone or combined with sulfadiazine. Liver histology being normal after treatment	Effective	(Ferreira et al. 2006)
24	800 mg/kg for 4 weeks in the drinking water	Counted the number of tissue cysts	No significant differences were present in tissue cyst numbers in valproic-acid-treated mice	Ineffective	(Goodwin et al. 2008)
25	FR235222 (200 nM), and PYR (1 μM)	Dolichos lectin, acridine orange, and ethidium bromide staining	No cysts were detected in mice inoculated with FR235222-treated cysts	Effective	(Maubon et al. 2010)
26	10 and 20 mg/kg/day alone and in combination for 10 consecutive days, (orally)	Counting the number of brain cysts and survival rate	ITZ significantly reduced brain cyst numbers. FLZ significantly enhanced protection in mice	Effective	(Martins-Duarte et al. 2010)
27	Atovaquone, ELQ-271 5, and ELQ-316 25 mg/kg (IP)	Counting the number of brain cysts	ELQ-271 and ELQ-316 reduced the brain cysts by 87–84 and 76–88%, respectively	Effective	(Doggett et al. 2012)
28	400 and 500 mg/kg alone and in combination for 7 days	Counting the number of brain cysts	Spiramycin reduced the brain cysts (58 and 36%). The combined administration showed a large and significant reduction of brain cysts	Effective	(Chew et al. 2012)
29	20 and 40 mg/kg twice, once every week (orally)	Counting the number of cysts	The toltrazuril treatment efficacy on the cyst presence was determined as 44.4%	Effective	(Kul et al. 2013)
30	1–5 mg/kg containing 5% DMSO for 10 days (IP)	Counting the number of cysts	Compound 24 showed a large and significant reduction of brain cysts	Effective	(Lourido et al. 2013)
31	10 mg/kg daily for 3 weeks	Life expectancy, Alt, histopathology of liver and brain	Rolipram exerts a significant lowering effect on ALT levels	Partially effective	(Afifi et al. 2014)
32	5 mg/kg/day (IP)	Count the number of tissue cysts and qPCR	Guanabenz representing a 69% reduction in the number of cysts	Effective	(Benmerzouga et al. 2015)
33	2.5 mg/kg/day (orally)	Brain parasite load estimation and histopathological examination	Toxoplasmic encephalitis in corticosteroid-treated animals was seen	Ineffective	(Elfadaly et al. 2015)
34	200 and 120 mg/kg for 30 days (orally)	Measurement of the parasite burden, ultrastructural and biochemical study	Reduction in mice mortality, brain parasite burden, and infectivity of cysts obtained from the brains of treated mice	Effective	(El-Zawawy et al. 2015)
35	Milteforan 20 and sulfadiazine 200 mg/kg for 15 days (orally)	Counting the number of cysts and measured and survival rate	Reduction of brain cysts 77.7%	Effective	(Eissa et al. 2015)
36	10 and 20 mg/kg (IP)	Counting the number of cysts	The cysts were observed in brains of all mice	Ineffective	(Saraei et al. 2015)
37	10 mg/kg daily for 3 weeks	Tissue injury scoring, brain cyst count, specific IgG titers, TNF-α, IFN-γ, and IL-12 assays	Significant reduction of TNF-α (84.6%), IFN-γ (76.7%), and IL-12 (71%)	Partially effective	(Afifi and Al-Rabia 2015)
38	The medicines were diluted in water (1 ml/100 ml of water) for 24 h	Counting the number of brain cysts	The number of brain cysts was reduced in the BIOT-TG200-pretreated group	Effective	(Braga-Silva et al. 2016)
39	Resveratrol 100 and ST 0.5 mg/kg (orally)	Counting the number of brain cysts	The drug combination showed a significant reduction in the number of brain cysts	Effective	(Bottari et al. 2016)
40	14 days via oral gavage with either 30 mg/kg of 32	Counting the number of brain cysts	Reducing of brain cysts by 88.7%	Effective	(Vidadala et al. 2016)
41		Counting the number of cysts		Ineffective	(Saraei et al. 2016)

Table 2 (continued)

	Thioridazine 10, 20, and fluphenazine 0.06 and 0.6 mg/kg		The number of brain cysts was less at higher dose compared to lower doses for both drugs		
42	A dose of 1 mg/kg/week for 4 weeks alone or combined with sulfadiazine and PYR (subcutaneously)	Counting the number and size of brain cysts, and TNF-α assays	A significant increase in the mean number and sizes of tissue cysts was seen. Also, TNF-α significantly decreased	Ineffective	(El-Sayed et al. 2016)
43	5 μmol/kg	Creatine kinase, MB, troponin, myoglobin, lactate dehydrogenase, and adenylate kinase assays	Treatment had a protective effect on the heart	Effective	(Machado et al. 2016)
44	10 mg/kg/day for 8 days (IP)	Counting the number of brain cysts and survival rate	Lowering brain cyst count (41%) was seen in the artesunate-treated group	Effective	(Mahmoud et al. 2017)
45	35 ng extract per 24–25 g mouse (IP)	Histopathology and counting the number of brain cysts	Less tissue damage, lowering brain cyst count (31%), and the survival rate of 93% was seen in the DLE-treated group	Effective	(Fuentes-Castro et al. 2017)
46	5 μmol/kg	NTPDase, 5′nucleotidase, ADA activities, and ATP/ADO ratio in the liver	Diphenyl diselenide treatment reduced the hepatic inflammation	Effective	(Doleski et al. 2017b)
47	5 μmol/kg	NTPDase, 5′nucleotidase, ADA activities, and ROS levels in the spleen	Diphenyl diselenide reduced histological inflammatory markers, ROS levels, and ADA activity in the spleen	Effective	(Doleski et al. 2017a)
48	25 or 40 mg/kg by oral gavage for a total of 8 days	Weight loss, bioluminescence imaging, and survival for 30 days. Presence of cysts in the brain and by bioassay	Mice treated with 40 mg/kg showed no sign of activation. Treated mice survived significantly longer than controls	Effective	(Rutaganira et al. 2017)
49	DXM: 2.66 and 5.32 mg/kg (daily) at 42 days after infection and monitored for 42 days	Histopathology, counting the number of brain cysts, and IgM and IgG assays	The cysts increased in the brains of all mice, and IgM levels declined but IgG levels continued rising	Ineffective	(Mokua Mose et al. 2017)

CLI clindamycin, *IP* intraperitoneal, *TMP* trimethoprim, *SMX* sulfamethoxazole, *ATO* atovaquone, *DDI* 2′,3′ dideoxyinosine, *ITZ* itraconazole, *FLZ* fluconazole, *ELQ* endochin-like quinolone, *ALT* alanine aminotransferase, *TS* triclosan, *ST* sulfamethoxazole–trimethoprim, *TgCDPK1 T. gondii* calcium-dependent protein kinase 1, *DLE* dialyzable leukocyte extracts, *ADA* adenosine deaminase, *ATP* adenosine triphosphate, *ADO* adenosine, *ROS* reactive oxygen species, *DXM* dexamethasone

Table 3 Pathway/mechanism of action of drugs/compounds used against tissue cysts *T. gondii*

Pathway/mechanism of action	Location	Drugs/compounds	Ref
Protein synthesis inhibitor	Apicoplast	Clindamycin* Spiramycin*	(Chew et al. 2012; Hofflin and Remington 1987)
Type II fatty acid synthesis		Triclosan and triclosan liposomal*	(El-Zawawy et al. 2015)
Electron transport pathway	Mitochondria	Atovaquone* Hydroxynaphthoquinone 566C80* PHNQ6 New naphthoquinones* (QUI-11, QUI-6, and QUI-5) Endochin-like quinolones* (ELQ-271 and ELQ-316) 3-Bromopyruvate	(Doggett et al. 2012; Ferguson et al. 1994; Ferreira et al. 2002, 2006, 2012; Gormley et al. 1998)
Dihydrofolate reductase inhibitor	Cytosol	Epiroprim (Ro 11-8958) Methotrexate Sulfadiazin	(Chang et al. 1994) (Choi et al. 1994) (Ferreira et al. 2006)
DNA synthesis Histone deacetylase enzyme	Core	Metronidazole* Tetrapeptide FR235222 Novel FR235222 derivative compounds*	(Chew et al. 2012) (Maubon et al. 2010)
Inhibitors of calcium-dependent protein kinase 1 (CDPK1)	Apical end	New derivatives of 3-methyl-benzyl-PP (3-MB-PP, or 1) Pyrazolopyrimidine analog, compound 24	(Lourido et al. 2013) (Rutaganira et al. 2017)
Phospholipid metabolism	Cell membrane	Miltefosine	(Eissa et al. 2015)
Activation of protein kinases, transcription of specific genes, and changes in the cytoskeleton structure	Cytoskeleton	CAMP	(Choi et al. 1994)
Different metabolic pathways	–	Monensin	(Couzinet et al. 2000)
Cyclic AMP signaling pathways	–	Rolipram*	(Afifi and Al-Rabia 2015; Afifi et al. 2014)
Calcium signaling pathways	–	Aripiprazole	(Saraei et al. 2015)
Translational control through phosphorylation of parasite eukaryotic initiation factor 2 α a (eIF2α)	–	Guanabenz*	(Benmerzouga et al. 2015)

*Drugs/compounds with known pathway/mechanisms of action against *T. gondii*

TNF-α, IFN-γ, and IL-12. However, rolipram was also partially able to prevent progression to chronic toxoplasmosis. Clinical studies have reported adverse effects of this drug, mostly severe nausea and vomiting (Afifi and Al-Rabia 2015; Afifi et al. 2014; Eissa et al. 2015). It is suggested that investigators should focus on finding safe anti-*Toxoplasma* drugs in the future.

Guanabenz, a Food and Drug Administration (FDA)-approved drug, has excellent solubility and penetration into the CNS (Bougdour et al. 2009; Meacham et al. 1980). In a study by Benmerzougas et al. (2015) in chronically infected mice, guanabenz crossed the blood–brain barrier and reduced the number of brain cysts. Also, guanabenz inhibited the phosphorylation of *T. gondii* eukaryotic initiation factor 2α (eIF2α), a novel antiparasitic drug target, and its ability to kill the *Toxoplasma* parasite did not involve the host's eIF2α. *T. gondii* IF2α phosphorylation occurs in response to stresses, which induces conversion of tachyzoites to bradyzoites during the lytic cycle in tachyzoites (Konrad et al. 2013; Meacham et al. 1980).

The ability of compound 32, endochin-like quinolones, miltefosine, and guanabenz to penetrate the blood-brain barrier is the important criteria for therapeutic intervention as tissye cysts have a propensity to form in the brain (Benmerzouga et al. 2015; Doggett et al. 2012; Eissa et al. 2015; Vidadala et al. 2016).

The cyclopeptide FR235222 appears to be a bradyzoite to tachyzoite conversion inhibitor, and preventing the parasite differentiation process could be an effective way to prevent the parasite from spreading. FR235222 is able to access the bradyzoites within the cyst. The ability of FR235222 to permeate the membrane wall is a major advantage for crossing the blood–brain barrier and the CNS tissues where *Toxoplasma* cysts are located. It is shown that histone acetylation levels are controlled by histone acetylase (HAT) and histone deacetylase (HDAC) enzymes, and the specific

inhibition of *T. gondii* histone deacetylase (TgHDAC3) by FR235222 disrupts the steady-state level of histone 4 (H4) acetylation across the genome, inducing derepression of stage-specific genes. Thus, acetylation of histones plays a substantial role in the control of gene expression during parasite interconversion (Bougdour et al. 2009; Maubon et al. 2010).

In a study by Ferreira et al. (2012), anti-*Toxoplasma* properties of new naphthoquinones (QUI-11, QUI-6, and QUI-5) were evaluated. In vitro incubation with QUI-11 resulted in the inhibition of infectivity of the bradyzoites; none of the surviving animals had detectable cysts in their brains. This suggests that this drug may be useful in treating chronic toxoplasmosis.

Recently, Murata et al. (2017) identified that tanshinone IIA and hydroxyzine represent novel lead compounds in preventing the reactivation of latent infection. These novel anti-*Toxoplasma* compounds can inhibit the growth of intermediately differentiated bradyzoites.

However, FR235222, QUI-11, tanshinone IIA, and hydroxyzine showed anti-*Toxoplasma* cyst effects in vitro (Ferreira et al. 2012; Maubon et al. 2010; Murata et al. 2017); their effectiveness in vivo against chronically infected mice remains to be directly demonstrated. Additionally, future studies should focus on the mechanism of action of QUI-11, tanshinone IIA, and hydroxyzine against the *T. gondii* cyst stage in chronic toxoplasmosis.

In a new study by El-Zawawy et al. (2015), it was shown that triclosan (TS) significantly reduced mice mortality, parasite load, as well as viability and infectivity of tachyzoites and the cysts that were harvested from infected mice and their brains in the treatment group. Accordingly, TS was proven as an effective, promising, and safe prophylactic drug against chronic murine toxoplasmosis. Liposomal formulation of TS enhanced its efficacy and allowed its use at a lower dose (El-Zawawy et al. 2015; Surolia and Surolia 2001). In *T. gondii*, FAS-II enzymes are present in the apicoplast and are essential for its survival. The key enzyme in this process is the ENR enzyme, which cannot be found in mammals. This enzyme catalyzes the last reductive step of the type II FAS pathway. Significantly, TS inhibits type II FAS, suggesting that apicoplast represents a potential target for new chemotherapy drugs as it is essential for the parasite and it is absent in host cells (El-Zawawy et al. 2015; Surolia and Surolia 2001).

Interestingly, investigators in a study showed the effectiveness of toltrazuril treatment in lambs. The results of this study showed that muscle tissues of lambs receiving toltrazuril were free of tissue cysts (44.4%). The outcomes are promising as one of the paths of getting infected with this parasite is through consumption of undercooked or raw meat containing tissue cysts, and this could be used as a strategy to reduce the cyst exposure of humans (Kul et al. 2013). Given that *Toxoplasma* human infections depend on the prevalence of the parasite in animals and eating habits, production of *T. gondii*-free sheep, lambs, and goats for human consumption is important for public health.

Many studies described anti-*Toxoplasma* effects of different drugs in combination with novel compounds. The compound 2-hydroxy-3-(1′-propen-3-phenyl)-1, 4-naphthoquinone (PHNQ6), combined with sulfadiazine, showed reduction of the brain cysts in vivo (Ferreira et al. 2006).

In another study by Chew et al. (2012), administration of spiramycin and metronidazole, due to the presence of the efflux transporters multidrug-resistant protein 2 and P-glycoprotein spiramycin, did not result in an effective concentration in the brain. Importantly, metronidazole increased brain penetration of spiramycin causing a significant reduction of *T. gondii* brain cysts. According to the information, combination therapy leads to faster recovery, using lower doses of drugs, less relapse, and fewer side effects of the disease. Furthermore, such combinations are highly promising for the development of a drug that can eliminate the cyst form of the parasite and, thus, efficiently impair relapse of the disease in immunocompromised patients (Chew et al. 2012; Ferreira et al. 2006).

The particular resistance of cysts to drugs could be explained by two characteristics: the presence of the cyst walls and the low metabolism of bradyzoites compared to tachyzoites. Despite the importance of the tissue cyst in the life cycle of the parasite, only a few components of the *T. gondii* cyst wall and their functions have been identified. However, bradyzoite pseudokinase 1 (BPK1) is a component of the cyst wall. The expression of BPK1, specifically in the bradyzoite stage, suggests that it may have an important function in the bradyzoite biology and structure or the function of the tissue cyst in the life cycle of *T. gondii* (Buchholz et al. 2013).

Treatment of the *T. gondii*-infected cell cultures with atovaquone in combination with 3-bromopyruvate (3-BrPA), an inhibitor of cellular energy metabolism, led to fewer parasite-infected cells with no evidence of cystogenesis. However, the infection was not completely eliminated, and the apicoplast is possibly another energy source for *T. gondii*. This organelle is important in the parasite metabolism as it is the site of biosynthesis of fatty acid type II, isoprenoids, and some enzymes of carbohydrate metabolism. Based on these results, 3-BrPA can be used as a good tool for the study of cystogenesis in vitro and for gaining more knowledge regarding *T. gondii* parasite metabolism (de Lima et al. 2015).

Conclusions

In conclusion, as bradyzoites located inside the *T. gondii* cysts are resistant to all drugs, development of well-tolerated and

safe specific immunoprophylaxis is a highly valuable goal for global disease control. Importantly, with the increasing number of high-risk individuals, and absence of a proper vaccine, persistent efforts are necessary for the development of novel treatments in patients with *T. gondii* cysts. Future studies should focus on the mechanisms of action of drugs or compounds that have sterilizing activity against the *T. gondii* cyst form in chronic toxoplasmosis in patients with cysts who are at risk for reactivating acute toxoplasmosis.

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Compliance with ethical standards

Conflict interest The authors declare that there is no conflict of interest.

References

Afifi MA, Al-Rabia MW (2015) The immunomodulatory effects of rolipram abolish drug-resistant latent phase of *Toxoplasma gondii* infection in a murine model. JMAU 3(2):86–91

Afifi MA, Jiman-Fatani AA, Al-Rabia MW, Al-Hussainy NH (2014) Application of a phosphodiesterase-4 (PDE4) inhibitor to abort chronic toxoplasmosis and to mitigate consequential pathological changes. JMAU 2(2):94–99

Alqaisi A, Mbekeani A, Llorens MB, Elhammer A, Denny P (2017) The antifungal Aureobasidin A and an analogue are active against the protozoan parasite *Toxoplasma gondii* but do not inhibit sphingolipid biosynthesis. Parasitology 1–8

Araujo F, Huskinson-Mark J, Gutteridge W, Remington J (1992a) *In vitro* and *in vivo* activities of the hydroxynaphthoquinone 566C80 against the cyst form of *Toxoplasma gondii*. Antimicrob Agents Chemother 36(2):326–330

Araujo F, Huskinson J, Remington J (1991) Remarkable *in vitro* and *in vivo* activities of the hydroxynaphthoquinone 566C80 against tachyzoites and tissue cysts of *Toxoplasma gondii*. Antimicrob Agents Chemother 35(2):293–299

Araujo F, Prokocimer P, Lin T, Remington J (1992b) Activity of clarithromycin alone or in combination with other drugs for treatment of murine toxoplasmosis. Antimicrob Agents Chemother 36(11):2454–2457

Araujo F, Slifer T, Remington J (1994) Rifabutin is active in murine models of toxoplasmosis. Antimicrob Agents Chemother 38(3): 570–575

Araujo FG, Khan AA, Remington JS (1996) Rifapentine is active *in vitro* and *in vivo* against *Toxoplasma gondii*. Antimicrob Agents Chemother 40(6):1335–1337

Araujo FG, Khan AA, Slifer TL, Bryskier A, Remington JS (1997) The ketolide antibiotics HMR 3647 and HMR 3004 are active against *Toxoplasma gondii in vitro* and in murine models of infection. Antimicrob Agents Chemother 41(10):2137–2140

Araujo FG, Slifer T (1995) Nonionic block copolymers potentiate activities of drugs for treatment of infections with *Toxoplasma gondii*. Antimicrob Agents Chemother 39(12):2696–2701

Baatz H, Mirshahi A, Puchta J, Gümbel H, Hattenbach L-O (2006) Reactivation of toxoplasma retinochoroiditis under atovaquone therapy in an immunocompetent patient. Ocul Immunol Inflamm 14(3): 185–187

Benmerzouga I, Checkley LA, Ferdig MT, Arrizabalaga G, Wek RC, Sullivan WJ (2015) Guanabenz repurposed as an antiparasitic with activity against acute and latent toxoplasmosis. Antimicrob Agents Chemother 59(11):6939–6945

Bottari NB, Baldissera MD, Tonin AA, Rech VC, Alves CB, D'Avila F, Thomé GR, Guarda NS, Moresco RN, Camillo G, Vogel FF, Luchese C, Schetinger MRC, Morsch VM, Tochetto C, Fighera R, Nishihira VSK, da Silva AS (2016) Synergistic effects of resveratrol (free and inclusion complex) and sulfamethoxazole-trimetropim treatment on pathology, oxidant/antioxidant status and behavior of mice infected with *Toxoplasma gondii*. Microb Pathog 95:166–174

Bougdour A, Maubon D, Baldacci P, Ortet P, Bastien O, Bouillon A, Barale JC, Pelloux H, Ménard R, Hakimi MA (2009) Drug inhibition of HDAC3 and epigenetic control of differentiation in Apicomplexa parasites. J Exp Med 206(4):953–966

Braga-Silva CF, Suhett CSR, Drozino RN, Moreira NM, Sant'Ana DMG, de Araújo SM (2016) Biotherapic of *Toxoplasma gondii* reduces parasite load, improves experimental infection, protects myenteric neurons and modulates the immune response in mice with toxoplasmosis. Eur J Integr Med 8(5):865–874

Buchholz KR, Bowyer PW, Boothroyd JC (2013) Bradyzoite pseudokinase 1 is crucial for efficient oral infectivity of the *Toxoplasma gondii* tissue cyst. Eukaryot Cell 12(3):399–410

Chang HR, Arsenijevic D, Comte R, Polak A, Then RL, Pechere J-C (1994) Activity of epiroprim (Ro 11-8958), a dihydrofolate reductase inhibitor, alone and in combination with dapsone against *Toxoplasma gondii*. Antimicrob Agents Chemother 38(8):1803–1807

Chang HR, Comte R, Piguet P-F, Pechère J-C (1991) Activity of minocycline against *Toxoplasma gondii* infection in mice. J Antimicrob Chemother 27(5):639–645

Chew WK, Segarra I, Ambu S, Mak JW (2012) Significant reduction of brain cysts caused by *Toxoplasma gondii* after treatment with spiramycin coadministered with metronidazole in a mouse model of chronic toxoplasmosis. Antimicrob Agents Chemother 56(4): 1762–1768

Chirgwin K, Hafner R, Leport C, Remington J, Andersen J, Bosler EM, Roque C, Rajicic N, McAuliffe V, Morlat P, Jayaweera DT, Vilde JL, Luft BJ, the AIDS Clinical Trials Group 237/Agence Nationale de Recherche sur le SIDA, Essai 039 Study Team (2002) Randomized phase II trial of atovaquone with pyrimethamine or sulfadiazine for treatment of toxoplasmic encephalitis in patients with acquired immunodeficiency syndrome: ACTG 237/ANRS 039 study. Clin Infect Dis 34(9):1243–1250

Choi W-Y, Park S-K, Nam H-W, Kim D-J (1994) Culture of tissue-cyst forming strain of *Toxoplasma gondii* and the effect of cyclic AMP and pyrimidine salvage inhibitors. Korean J Parasitol 32(1):19–26

Couzinet S, Dubremetz J, Buzoni-Gatel D, Jeminet G, Prensier G (2000) In vitro activity of the polyether ionophorous antibiotic monensin against the cyst form of *Toxoplasma gondii*. Parasitology 121(4): 359–365

De Lima LPO, Seabra SH, Carneiro H, Barbosa HS (2015) Effect of 3-bromopyruvate and atovaquone on infection during *in vitro* interaction of *Toxoplasma gondii* and LLC-MK2 cells. Antimicrob Agents Chemother 59(9):5239–5249

Djurković-Djaković O, Milenković V, Nikolić A, Bobić B, Grujić J (2002) Efficacy of atovaquone combined with clindamycin against

murine infection with a cystogenic (Me49) strain of *Toxoplasma gondii*. J Antimicrob Chemother 50(6):981–987

Doggett JS, Nilsen A, Forquer I, Wegmann KW, Jones-Brando L, Yolken RH, Bordon C, Charman SA, Katneni K, Schultz T, Burrows JN, Hinrichs DJ, Meunier B, Carruthers VB, Riscoe MK (2012) Endochin-like quinolones are highly efficacious against acute and latent experimental toxoplasmosis. Proc Natl Acad Sci 109(39): 15936–15941

Doleski PH, Leal DBR, Machado VS, Bottari NB, Manzoni AG, Casali EA, Moritz CEJ, Rocha ACA, Camillo G, Vogel FF, Stefani LM, Mendes RE, da Silva AS (2017a) Diphenyl diselenide modulates nucleotidases, reducing inflammatory responses in the liver of *Toxoplasma gondii*-infected mice. Purinergic Signal 13(4):489–496

Doleski PH, ten Caten MV, Passos DF, Castilhos LG, Leal DBR, Machado VS, Bottari NB, Vogel FF, Mendes RE, da Silva AS (2017b) Toxoplasmosis treatment with diphenyl diselenide in infected mice modulates the activity of purinergic enzymes and reduces inflammation in spleen. Exp Parasitol 181:7–13

Dubey J (1977) *Toxoplasma, Hammondia, Besnoitia, Sarcocystis,* and other tissue cyst-forming coccidia of man and animals. Parasitic Protozoa 3:101–237

Dubey J (1988) Long-term persistence of *Toxoplasma gondii* in tissues of pigs inoculated with T gondii oocysts and effect of freezing on viability of tissue cysts in pork. Am J Vet Res 49(6):910–913

Dubey J, Jones J (2008) *Toxoplasma gondii* infection in humans and animals in the United States. Int J Parasitol 38(11):1257–1278

Dubey J, Lindsay D, Speer C (1998) Structures of *Toxoplasma gondii* tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. Clin Microbiol Rev 11(2):267–299

Dumas J-L, Chang R, Mermillod B, Piguet PF, Comte R, Pechère J-C (1994) Evaluation of the efficacy of prolonged administration of azithromycin in a murine model of chronic toxoplasmosis. J Antimicrob Chemother 34(1):111–118

Dumas J-L, Pizzolato G, Pechère J-C (1999) Evaluation of trimethoprim and sulphamethoxazole as monotherapy or in combination in the management of toxoplasmosis in murine models. Int J Antimicrob Agents 13(1):35–39

Eissa MM, Barakat AM, Amer EI, Younis LK (2015) Could miltefosine be used as a therapy for toxoplasmosis? Exp Parasitol 157:12–22

El-Sayed NM, Ismail KA, Badawy AF, Elhasanein KF (2016) *In vivo* effect of anti-TNF agent (etanercept) in reactivation of latent toxoplasmosis. J Parasit Dis 40(4):1459–1465

El-Zawawy LA, El-Said D, Mossallam SF, Ramadan HS, Younis SS (2015) Preventive prospective of triclosan and triclosan-liposomal nanoparticles against experimental infection with a cystogenic ME49 strain of *Toxoplasma gondii*. Acta Trop 141:103–111

Elfadaly HA, Hassanain MA, Shaapan RM, Hassanain NA, Barakat AM (2015) Corticosteroids opportunist higher *Toxoplasma gondii* brain cysts in latent infected mice. Int J Zool Res 11(4):169–176

Ferguson D, Huskinson-Mark J, Araujo F, Remington J (1994) An ultrastructural study of the effect of treatment with atovaquone in brains of mice chronically infected with the ME49 strain of *Toxoplasma gondii*. Int J Clin Exp Pathol 75(2):111

Ferreira R, Oliveira A, Gualberto S, Vitor R (2002) Activity of natural and synthetic naphthoquinones against *Toxoplasma gondii*, *in vitro* and in murine models of infection. Parasite 9(3):261–269

Ferreira RA, de Oliveira AB, Gualberto SA, Miguel del Corral JM, Fujiwara RT, Gazzinelli Guimarães PH, de Almeida Vitor RW (2012) New naphthoquinones and an alkaloid with *in vitro* activity against *Toxoplasma gondii* RH and EGS strains. Exp Parasitol 132(4):450–457

Ferreira RA, Oliveira AB, Ribeiro MF, Tafuri WL, Vitor RW (2006) *Toxoplasma gondii*: *in vitro* and *in vivo* activities of the hydroxynaphthoquinone 2-hydroxy-3-(1′-propen-3-phenyl)-1, 4-naphthoquinone alone or combined with sulfadiazine. Exp Parasitol 113(2):125–129

Fuentes-Castro BE, Reyes-García JG, Valenzuela-Vargas MT, Martínez-Gómez F (2017) Histopathology of murine toxoplasmosis under treatment with dialyzable leukocyte extract. Mem Inst Oswaldo Cruz 112(11):741–747

Goodwin DG, Strobl J, Mitchell SM, Zajac AM, Lindsay DS (2008) Evaluation of the mood-stabilizing agent valproic acid as a preventative for toxoplasmosis in mice and activity against tissue cysts in mice. J Parasitol 94(2):555–557

Gormley PD, Pavesio CE, Minnasian D, Lightman S (1998) Effects of drug therapy on *Toxoplasma* cysts in an animal model of acute and chronic disease. Invest Ophthalmol Vis Sci 39(7):1171–1175

Havelaar AH, Haagsma JA, Mangen MJJ, Kemmeren JM, Verhoef LPB, Vijgen SMC, Wilson M, Friesema IHM, Kortbeek LM, van Duynhoven YTHP, van Pelt W (2012) Disease burden of foodborne pathogens in the Netherlands, 2009. Int J Food Microbiol 156(3): 231–238

Hofflin JM, Remington JS (1987) Clindamycin in a murine model of toxoplasmic encephalitis. Antimicrob Agents Chemother 31(4): 492–496

Huskinson-Mark J, Araujo FG, Remington JS (1991) Evaluation of the effect of drugs on the cyst form of *Toxoplasma gondii*. J Infect Dis 164(1):170–177

Konrad C, Queener SF, Wek RC, Sullivan WJ (2013) Inhibitors of eIF2α dephosphorylation slow replication and stabilize latency in *Toxoplasma gondii*. Antimicrob Agents Chemother 57(4):1815–1822

Kul O, Yildiz K, Ocal N, Freyre A, Deniz A, Karahan S, Atmaca HT, Gokpinar S, Dincel GC, Uzunalioğlu T, Terzi OS (2013) *In-vivo* efficacy of toltrazuril on experimentally induced *Toxoplasma gondii* tissue cysts in lambs: a novel strategy for prevention of human exposure to meat-borne toxoplasmosis. Res Vet Sci 94(2):269–276

Lourido S, Zhang C, Lopez MS, Tang K, Barks J, Wang Q, Wildman SA, Shokat KM, Sibley LD (2013) Optimizing small molecule inhibitors of calcium-dependent protein kinase 1 to prevent infection by *Toxoplasma gondii*. J Med Chem 56(7):3068–3077

Luft BJ, Remington JS (1992) Toxoplasmic encephalitis in AIDS. Clin Infect Dis 15(2):211–222

Machado VS, Bottari NB, Baldissera MD, Isabel de Azevedo M, Rech VC, Ianiski FR, Vaucher RA, Mendes RE, Camillo G, Vogel FF, de la Rue ML, Carmo GM, Tonin AA, da Silva AS (2016) *Toxoplasma gondii*: effects of diphenyl diselenide in experimental toxoplasmosis on biomarkers of cardiac function. Exp Parasitol 167:25–31

Mahmoud DM, Mahmoud MS, Ezz-El-Din HM, Abo-Zahra FA, Meselhey RA (2017) Artesunate effect on RH virulent and ME49 non-virulent strains of *Toxoplasma gondii: in vitro* and *in vivo* experimental studies. Sci Parasitol 17:83–92

Martins-Duarte ÉS, Lemgruber L, de Souza W, Vommaro RC (2010) *Toxoplasma gondii:* fluconazole and itraconazole activity against toxoplasmosis in a murine model. Exp Parasitol 124(4):466–469

Maubon D, Bougdour A, Wong YS, Brenier-Pinchart MP, Curt A, Hakimi MA, Pelloux H (2010) Activity of the histone deacetylase inhibitor FR235222 on *Toxoplasma gondii:* inhibition of stage conversion of the parasite cyst form and study of new derivative compounds. Antimicrob Agents Chemother 54(11):4843–4850

Meacham RH et al (1980) Relationship of guanabenz concentrations in brain and plasma to antihypertensive effect in the spontaneously hypertensive rat. J Pharmacol Exp Ther 214(3):594–598

Megged O, Shalit I, Yaniv I, Stein J, Fisher S, Levy I (2008) Breakthrough cerebral toxoplasmosis in a patient receiving atovaquone prophylaxis after a hematopoietic stem cell transplantation. Pediatr Transplant 12(8):902–905

Moher D, Liberati A, Tetzlaff J, Altman DG (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Ann Intern Med 151(4):264–269

Mokua Mose J et al (2017) Development of neurological mouse model for toxoplasmosis using *Toxoplasma gondii* isolated from chicken in Kenya Patholog Res Int 2017

Montazeri M, Ebrahimzadeh MA, Ahmadpour E, Sharif M, Sarvi S, Daryani A (2016) Evaluation of propranolol effect on experimental acute and chronic toxoplasmosis using quantitative PCR. Antimicrob Agents Chemother 60(12):7128–7133

Montazeri M, Sharif M, Sarvi S, Mehrzadi S, Ahmadpour E, Daryani A (2017) A systematic review of *in vitro* and *in vivo* activities of anti-*Toxoplasma* drugs and compounds (2006–2016). Front Microbiol 8: 25

Murata Y, Sugi T, Weiss LM, Kato K (2017) Identification of compounds that suppress *Toxoplasma gondii* tachyzoites and bradyzoites. PLoS One 12(6):e0178203

Rutaganira FU, Barks J, Dhason MS, Wang Q, Lopez MS, Long S, Radke JB, Jones NG, Maddirala AR, Janetka JW, el Bakkouri M, Hui R, Shokat KM, Sibley LD (2017) Inhibition of calcium dependent protein kinase 1 (CDPK1) by pyrazolopyrimidine analogs decreases establishment and reoccurrence of central nervous system disease by *Toxoplasma gondii*. J Med Chem 60(24):9976–9989

Saraei M, Ghaderi Y, Mosavi T, Shahnazi M, Nassiri-Asl M, Jahanihashemi H (2016) The effect of fluphenazine and thioridazine on *Toxoplasma gondii in vivo*. Iran J Parasitol 11(2):226

Saraei M, Samadzadeh N, Khoeini J, Shahnazi M, Nassiri-Asl M, Jahanihashemi H (2015) *In vivo* anti-*Toxoplasma* activity of aripiprazole. Iran J Basic Med Sci 18(9):938

Sarciron M-E, Lawton P, Saccharin C, Petavy A-F, Peyron F (1997) Effects of 2′, 3′-dideoxyinosine on *Toxoplasma gondii* cysts in mice. Antimicrob Agents Chemother 41(7):1531–1536

Sarciron M, Walchshofer N, Paris J, Petavy A, Peyron F (1998) Phenylalanine derivatives active against *Toxoplasma gondii* brain cysts in mice. Parasite 5(4):359–364

Scallan E et al (2011) Foodborne illness acquired in the United States—major pathogens. Emerg Infect Dis 17(1)

Shu H, Jiang L (2002) Effect of garlicin and minocycline on the cyst formation of *Toxoplasma gondii* in mice. Chinese Journal of Zoonoses 18(1):100–101

Sordet F, Aumjaud Y, Fessi H, Derouin F (1998) Assessment of the activity of atovaquone-loaded nanocapsules in the treatment of acute and chronic murine toxoplasmosis. Parasite 5(3):223–229

Sumyuen MH, Garin YJF, Derouin F (1996) Effect of immunosuppressive drug regimens on acute and chronic murine toxoplasmosis. J Parasitol Res 82(8):681–686

Surolia N, Surolia A (2001) Triclosan offers protection against blood stages of malaria by inhibiting enoyl-ACP reductase of *Plasmodium falciparum*. Nat Med 7(2):167–173

Tawfeek G, Oteifa N, Mustafa M (2001) Prophylactic efficacy of recombinant IL-12, clindamycin alone or in combination against experimental reactivated toxoplasmosis. J Egypt Soc Parasitol 31(3):853–866

Vidadala RSR, Rivas KL, Ojo KK, Hulverson MA, Zambriski JA, Bruzual I, Schultz TL, Huang W, Zhang Z, Scheele S, DeRocher AE, Choi R, Barrett LK, Siddaramaiah LK, Hol WGJ, Fan E, Merritt EA, Parsons M, Freiberg G, Marsh K, Kempf DJ, Carruthers VB, Isoherranen N, Doggett JS, van Voorhis WC, Maly DJ (2016) Development of an orally available and central nervous system (CNS) penetrant *Toxoplasma gondii* calcium-dependent protein kinase 1 (Tg CDPK1) inhibitor with minimal human ether-a-go-go-related gene (hERG) activity for the treatment of toxoplasmosis. J Med Chem 59(13):6531–6546

Weiss LM, Kim K (2000) The development and biology of bradyzoites of *Toxoplasma gondii*. Front Biosci 5:D391–d405