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Short Communication

Efficacy of antiretroviral compounds against *Toxoplasma gondii* in vitro

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ABSTRACT

The obligate intracellular parasite *Toxoplasma gondii* can infect nearly all warm-blooded animals, including humans. Although infection with this parasite is generally benign, severe illness may occur in infected individuals if their immunity becomes less competent, such as in human immunodeficiency virus (HIV)-infected patients. In this study, the inhibitory activity of 44 commonly used antiretroviral compounds was determined against *T. gondii* in vitro. Of the 44 tested antiretroviral compounds, 14 showed potency against *T. gondii* at IC_{50} concentrations (concentration inhibiting *T. gondii* tachyzoite growth by 50%) ranging from 1.18 ± 2.21 μM (nelfinavir) to 18.89 ± 1.87 μM (trovirdine). Of the 14 potent antiretroviral compounds, 7 are HIV-1 protease inhibitors. This study also investigated whether co-administration of these 14 antiretroviral compounds interferes with the anti-*T. gondii* activity of existing anti-*T. gondii* drugs, namely sulfadiazine and pyrimethamine. The results showed no significant interaction between any of the 14 tested antiretroviral compounds and pyrimethamine or sulfadiazine. These results warrant investigation of whether administration of the lead antiretroviral drugs with highly potent anti-*T. gondii* activity to HIV patients may help to limit the occurrence of toxoplasmic encephalitis.

1. Introduction

Toxoplasma gondii can infect humans and a wide range of warm-blooded animals. Infection with this parasite is mostly benign, however in immunocompromised individuals, such as human immunodeficiency virus (HIV) patients or individuals receiving suppressive chemotherapy, latent bradyzoites residing within tissue cysts may transform to actively replicating tachyzoites, leading to acute toxoplasmic encephalitis and even death [1,2]. Nearly 37 million people are infected with HIV/AIDS worldwide [3]. The burden of HIV/AIDS is compounded by infection with many opportunistic pathogens, including *T. gondii*. A high prevalence of toxoplasmosis exists in HIV-infected individuals, and concurrent infection with *T. gondii* and HIV appears to enhance each other's pathogenicity [4]. The primary cause of toxoplasmosis in HIV-infected people is reactivation of latent infection, which occurs when the $CD4^+$ T-cell count decreases to <100 cells/μL. This increases the risk of developing toxoplasmic encephalitis, which manifests as headache, disorientation, drowsiness, hemiparesis, convulsions, loss of memory and seizures. Less often, retinochoroiditis, pneumonia and other systemic pathologies may also occur [1].

The introduction of highly active antiretroviral therapy (HAART) for the treatment of HIV may have helped in significantly reducing opportunistic infections, with some reports suggesting that certain antiretroviral drugs have reduced the morbidity and mortality of toxoplasmic encephalitis in HIV patients [1]. The ART-mediated protection against opportunistic infections can be attributed to modulation of the immune response by ART. Moreover, some antiretroviral drugs can have a direct inhibitory effect on opportunistic (e.g. *Cryptosporidium* spp.) and non-opportunistic (*Plasmodium* spp., *Trypanosoma cruzi* and *Leishmania* spp.) parasite infections [5,6]. Two antiretroviral drugs, the HIV protease inhibitors (PIs) indinavir and nelfinavir, have shown inhibitory effects on *T. gondii* growth in vitro [7]. However, neither of these HIV PI drugs altered the anti-*T. gondii* activity of pyrimethamine or sulfadiazine, the main drugs used to treat *T. gondii* infection [7].

Several classes of antiretroviral drugs, including PIs, integrase inhibitors, entry inhibitors, nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase

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inhibitors, have been developed for suppression of HIV infection, and it is possible that ART administered to HIV patients influences the course of *T. gondii* infection [8]. In an effort to explore new avenues for the treatment of toxoplasmic encephalitis in AIDS patients, in this study the inhibitory effect of 44 antiretroviral drugs on *T. gondii* growth in vitro and the possible interaction between antiretroviral drugs and pyrimethamine or sulfadiazine was assessed.

2. Materials and methods

2.1. Parasite and cell culture

Toxoplasma gondii tachyzoites of strain RH were maintained in confluent monolayers of human foreskin fibroblasts (HFFs) as described previously [9]. To isolate tachyzoites, heavily infected monolayers were harvested using a cell scraper, were lysed by passing three times through a syringe (27G needle), and the host cell debris was removed using a 3-μm polycarbonate filter.

2.2. Drugs and chemicals

Antiretroviral compounds (Table 1) were purchased from Med Chem Express (Monmouth Junction, NJ, USA). Pyrimethamine and sulfadiazine were obtained from Sigma Chemical Co. (St Louis, MO, USA). Four antiretroviral drug tablets were obtained from the teaching hospital of Qingdao University (Qingdao, China). All compounds were dissolved in dimethyl sulfoxide (DMSO) at a final concentration of 10 mM of active ingredient. The final concentration of DMSO in the medium did not exceed 0.3% (v/v), which had no cytotoxic effect on HFFs based on preliminary testing. Pyrimethamine and sulfadiazine dissolved in DMSO were used as positive controls.

2.3. Cytotoxicity assay

The cytotoxic effect of all antiretroviral compounds on HFFs was determined using a CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega Corp., Madison, WI, USA) according to the manufacturer's instructions [10]. Briefly, HFFs [10^4 cells/100 μL of Dulbecco's modified Eagle medium (DMEM)/well] were seeded into 96-well tissue culture plates and were incubated at 37 °C for 48 h in a humidified chamber containing 5% CO_2. Then, 4 h after seeding each compound was added into the wells to reach final concentrations of 1, 2.5, 5, 7.5, 10, 15, 20, 25 and 30 μM. DMSO at a final concentration of 0.3% in medium was used as the vehicle control. Following 48 h of incubation, 20 μL of MTS solution containing phenazine ethyl sulfate was added to each well and was further incubated for 3 h at 37 °C. Absorbance was measured at 490 nm using an iMark™ Microplate Absorbance Reader (Bio-Rad, Hercules, CA, USA). Negative control wells contained cells treated with DMSO only. Results were expressed as the percentage reduction of cell viability in the compound-treated cells compared with control cells. The 50% cytotoxic concentration (CC_{50}), i.e. the concentration of a chemical that kills one-half of the treated cells, was calculated by plotting drug concentration versus cell viability curves using GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA). The cytotoxicity experiment was performed in triplicate wells on three separate plates.

2.4. In vitro antiretroviral drug susceptibility

HFFs were seeded into 24-well culture plates with DMEM supplemented with 10% fetal bovine serum (FBS) until a confluent monolayer was formed. The medium was replaced with fresh DMEM supplemented with 2% dialysed FBS and then approximately 5×10^4 freshly egressed tachyzoites were added to each well and were allowed to invade cell monolayers for 4 h. The medium containing extracellular parasites was removed and fresh medium containing various concentrations of antiretroviral compounds (0–30 μM), DMSO (vehicle control) or positive control (pyrimethamine or sulfadiazine) were added. After 5 days, infected cells were collected with a cell scraper, were centrifuged and the pellet was used to extract genomic DNA using a TIANamp Genomic DNA Kit (TianGen Biotech, Beijing, China) according to the manufacturer's instructions. Extracted DNA was stored at –80 °C until determination of parasite load in HFF cultures using real-time quantitative PCR (qPCR) targeting the B1 gene. Oligonucleotides used for amplification of the *T. gondii* B1 gene were B1-F (GGAGGACTGGCAACCTGGTGTCG) and B1-R (TTGTTTCACCCGGACCGTTTAGCAG). The qPCR assay was performed as described previously [11]. The number of parasites was calculated by interpolation from a standard curve of known amounts of DNA equivalent to 10^5, 10^4, 10^3, 10^2 and 10^1 tachyzoites included in each PCR run. Parasite growth rates compared with the negative control were calculated based on the estimated number of parasites. The concentration that inhibited *T. gondii* tachyzoite growth by 50% (IC_{50}) was determined for each compound by non-linear regression of log-transformed compound concentrations using GraphPad Prism v.7. The results are presented as the mean ± standard deviation of data from six independent experiments with at least three replicates per experiment. The difference in parasite growth rate was compared using a two-tailed, unpaired Student's *t*-test. A *P*-value of <0.05 was considered statistically significant.

This study also examined whether interaction occurs when antiretroviral drugs are used in combination with existing drugs with known anti-*T. gondii* efficacy (e.g. pyrimethamine and sulfadiazine). As shown in Supplementary Table S1, the drug interaction analysis was performed by testing various concentrations of 14 antiretroviral compounds in combination with serial concentrations of sulfadiazine (0, 10, 20 and 30 μM) or pyrimethamine (0, 0.1, 0.25 and 0.5 μM). The antiretroviral compounds included 11 antiretroviral compounds that were not cytotoxic to HFFs even at 30 μM (tested at concentrations 0, 5, 10 and 15 μM) and 3 antiretroviral compounds that were cytotoxic at low concentrations (tested at concentrations 0, 1, 2.5 and 5 μM). The effect of drug combinations both on cytotoxicity and the anti-*T. gondii* activity of pyrimethamine or sulfadiazine was determined using the same assays described above. A two-way analysis of variance (ANOVA) was performed to identify the level of drug–drug interactions between the 14 antiretroviral compounds and pyrimethamine or sulfadiazine.

Finally, the antiparasitic activity of four antiretroviral prescription drug tablets widely used in practice was tested. These included dolutegravir sodium (DTG), dolutegravir sodium–abacavir sulfate–lamivudine (DTG/ABC/3TC), tenofovir disoproxil fumarate (TDF) and emtricitabine–tenofovir disoproxil fumarate (FTC/TDF). For the drugs that contained more than one ingredient (i.e. DTG/ABC/3TC and FTC/TDF), the tested concentrations were calculated based on DTG and TDF, respectively.

3. Results

3.1. Cytotoxicity of antiretroviral compounds

It was important to determine the cytotoxic potential of the antiretroviral compounds on the same cell line as used in the antiparasitic assays. The results showed that at 30 μM, 35 antiretroviral compounds were not cytotoxic to host cells (Table 1). Three compounds, including lopinavir ($CC_{50} = 24.39 \pm 2.02$ μM), nelfinavir ($CC_{50} = 22.44 \pm 1.97$ μM) and saquinavir ($CC_{50} = 16.23$

Table 1
In vitro anti-*Toxoplasma gondii* activity of 44 antiretroviral drugs tested in the present study

Class	Compound	IC_{50} (μM)	CC_{50} against HFFs (μM)	Human plasma concentration [C_{min}–C_{max} (μM)]
Nucleoside reverse transcriptase inhibitors (NRTIs)	Abacavir	– [c]	– [d]	– [e]
	Didanosine	– [c]	– [d]	– [e]
	Emtricitabine	– [c]	– [d]	– [e]
	Lamivudine	– [c]	– [d]	– [e]
	Stavudine	– [c]	– [d]	– [e]
	Zalcitabine	– [c]	– [d]	– [e]
	Zidovudine	– [c]	– [d]	– [e]
	Tenofovir	– [c]	– [d]	– [e]
	Tenofovir disoproxil fumarate (TDF) [a]	**5.10 ± 1.01**	– [d]	0.1–0.6
Non-nucleoside reverse transcriptase inhibitors (NNRTIs)	Dapivirine	– [c]	<15	– [e]
	Delavirdine mesylate	– [c]	– [d]	– [e]
	Doravirine	– [c]	– [d]	– [e]
	Efavirenz	– [c]	<15	– [e]
	Etravirine	– [c]	<15	– [e]
	Nevirapine	– [c]	– [d]	– [e]
	Rilpivirine	– [c]	<15	– [e]
Protease inhibitors (PIs)	Amprenavir	– [c]	– [d]	– [e]
	Atazanavir [a]	**14.62 ± 1.01**	– [d]	0.3–6.7
	Darunavir	– [c]	– [d]	– [e]
	Fosamprenavir calcium salt	– [c]	– [d]	– [e]
	Indinavir sulfate	– [c]	– [d]	– [e]
	Lopinavir [a]	**6.70 ± 1.10**	24.39 ± 2.02	8.8–15.3
	Nelfinavir [a]	**1.18 ± 2.21**	22.44 ± 1.97	1.2–6.0
	Ritonavir [a]	**8.04 ± 1.02**	– [d]	2.9– 15.6
	Saquinavir [a]	**3.76 ± 1.02**	16.23 ± 1.11	0.1– 3.3
	Telaprevir [a]	**13.65 ± 1.04**	– [d]	2.7– 6.2
	Tipranavir [a]	**9.69 ± 0.91**	– [d]	16.6– 99.5
	Pepstatin trifluoroacetate	– [c]	– [d]	– [e]
Integrase inhibitors (IIs)	BMS-707035 [b]	– [c]	– [d]	– [e]
	Cabotegravir	– [c]	– [d]	– [e]
	Dolutegravir	– [c]	– [d]	– [e]
	Elvitegravir [a]	**5.21 ± 1.06**	– [d]	0.04– 3.6
	HIV-1 integrase inhibitor 1 [b]	– [c]	– [d]	– [e]
	HIV-1 integrase inhibitor 2 [b]	– [c]	– [d]	– [e]
	Salicylanilide [a]	**16.76 ±1.12**	– [d]	UN [f]
Entry inhibitors (EIs)	**NBD-557** [a,b]	**13.90 ± 0.92**	– [d]	UN [f]
	BMS-378806 [b]	– [c]	– [d]	– [e]
	Enfuvirtide	– [c]	– [d]	– [e]
	Maraviroc	– [c]	– [d]	– [e]
Other inhibitors	**Bevirimat** [a]	**4.66 ± 1.20**	– [d]	53.6–86.7
	Clofarabine	– [c]	<15	– [e]
	Ebselen	– [c]	<15	– [e]
	Triciribine [a]	**12.69 ± 0.71**	– [d]	UN [f]
	Trovirdine [a]	**18.89 ± 1.87**	– [d]	UN [f]
Positive controls	Pyrimethamine	0.36 ± 0.04	– [d]	– [e]
	Sulfadiazine	22.21 ± 3.41	– [d]	– [e]

IC_{50}, concentration inhibiting *T. gondii* tachyzoite growth by 50%; CC_{50}, 50% cytotoxic concentration; HFF, human foreskin fibroblast; C_{min}, minimum (or trough) serum concentration; C_{max}, maximum (or peak) serum concentration.
[a] The 14 potent anti-*T. gondii* lead antiretroviral compounds and their corresponding IC_{50} are shown in bold.
[b] Structures of these five compounds are shown in Supplementary Fig. S1.
[c] Compounds that did not have antiparasitic effect at the maximum concentration (30 μM) examined.
[d] Compounds that showed no cytotoxicity even at 30 μM.
[e] Not determined because compounds did not have anti-*T. gondii* activity.
[f] UN, unknown based on literature search.

± 1.11 μM), were cytotoxic. In addition, six compounds, including dapivirine, efavirenz, etravirine, rilpivirine, clofarabine and ebselen, were cytotoxic at CC_{50} < 15 μM.

3.2. Antiretroviral compounds exhibited antiparasitic activity in vitro

To test whether antiretroviral compounds had any inhibitory activity against *T. gondii* growth, the effect of a series of concentrations of each antiretroviral compound up to the highest concentration that showed no cytotoxic effect (i.e. 30 μM) was examined. Of the 44 antiretroviral drugs, 14 showed in vitro activity against tachyzoites of *T. gondii* at a dose that was not cytotoxic to the host cells (Fig. 1; Table 1). The top five most potent compounds included nelfinavir (IC_{50} = 1.18 ± 2.21 μM), saquinavir (IC_{50} = 3.76 ± 1.02 μM), bevirimat (IC_{50} = 4.66 ± 1.20 μM), TDF (IC_{50} = 5.10 ± 1.01 μM) and elvitegravir (IC_{50} = 5.21 ± 1.06 μM). Of the 14 potent antiretroviral compounds, 7 belong to HIV-1 PIs. Interestingly, the IC_{50} values of two PIs (lopinavir and ritonavir) fit within the range of plasma concentrations of both compounds in humans (Table 1). Also, the IC_{50} values of another two PIs (nelfinavir and saquinavir) are approximate to the minimum and maximum range, respectively, of the plasma concentrations of both compounds in humans.

3.3. Interaction between antiretroviral compounds and pyrimethamine or sulfadiazine

The extent of interactions between antiretroviral compounds and pyrimethamine or sulfadiazine was examined as well as

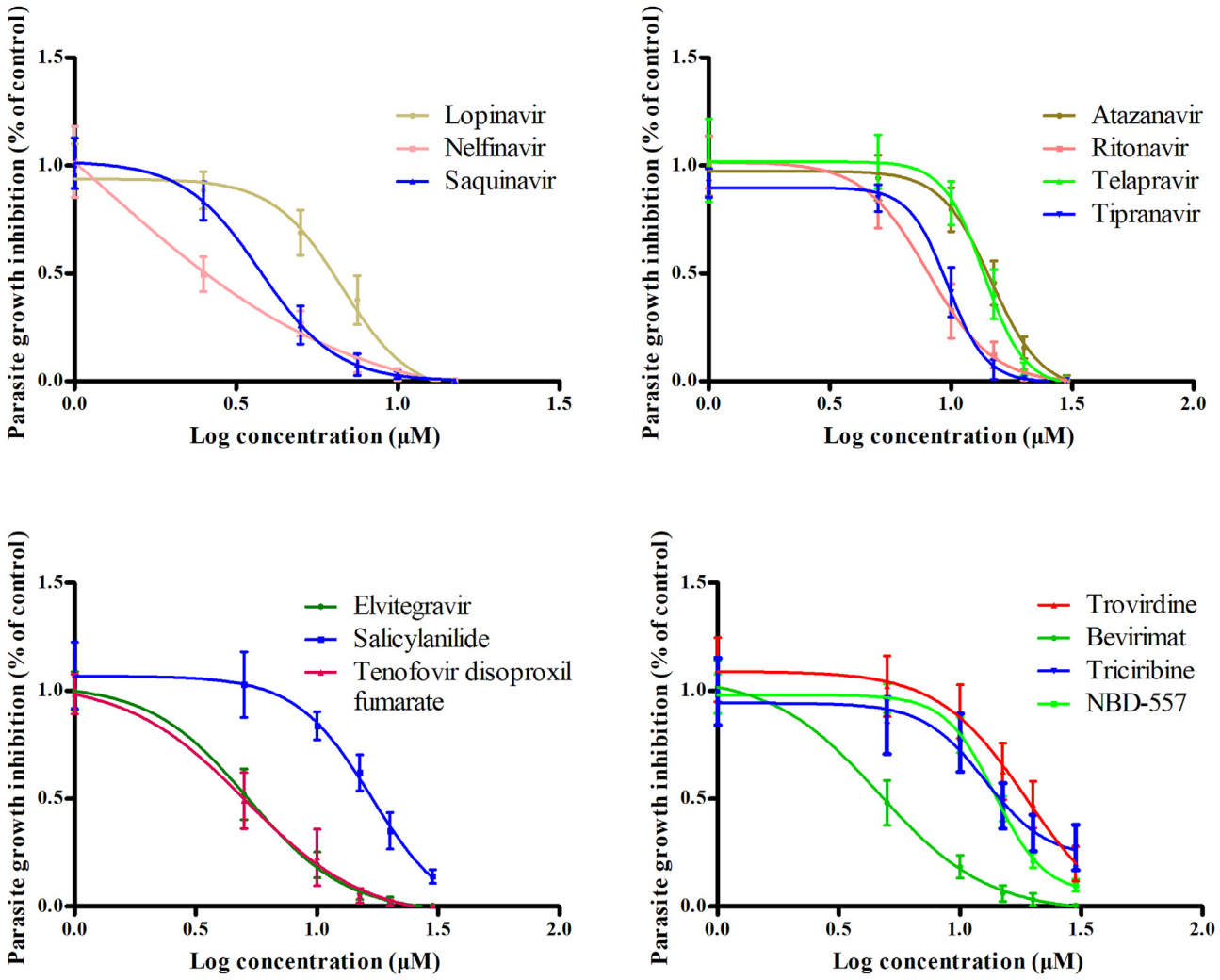

Fig. 1. Dose–response growth inhibition curves of *Toxoplasma gondii* treated with 14 antiretroviral compounds that showed in vitro activity against *T. gondii* tachyzoites at a dose that was not cytotoxic to host cells. Results are presented as the mean, with error bars representing the standard deviation of data from at least six independent experiments.

the influence of this interaction on the potential toxicity to host cells and the antiparasitic activity of pyrimethamine and sulfadiazine. No cytotoxicity to HFFs and no significant interactions were detected in all drug combinations tested (Supplementary Table S1).

3.4. Evaluation of the antiparasitic activity of four commercial antiretroviral drugs

The results showed that DTG and DTG/ABC/3TC had no activity against *T. gondii*, whereas TDF and FTC/TDF displayed a remarkable and similar dose-dependent inhibitory activity against *T. gondii* (Fig. 2).

4. Discussion

Although the use of effective ART has decreased the incidence of toxoplasmic encephalitis, this condition remains a potential threat in HIV patients, particularly in those who do not receive therapy [1]. Thus, understanding the therapeutic potential of antiretroviral compounds against *T. gondii* infection has significant clinical relevance, particularly in areas where both HIV and *T. gondii* infections overlap. In this study, the in vitro anti-*T. gondii* activity of 44 antiretroviral compounds, mainly belonging to five antiretroviral drug classes, was evaluated. The results showed that 14 antiretroviral compounds had marked anti-*T. gondii* activity.

The antiretroviral compounds belonging to the PI class exhibited the highest potency against in vitro *T. gondii* infection (Table 1). Among the PIs, nelfinavir ($IC_{50} = 1.18 \pm 2.21$ μM), saquinavir ($IC_{50} = 3.76 \pm 1.02$ μM), lopinavir ($IC_{50} = 6.70 \pm 1.10$ μM), ritonavir ($IC_{50} = 8.04 \pm 1.02$ μM) and tipranavir ($IC_{50} = 9.69 \pm 0.91$ μM) were the most potent at inhibiting the in vitro growth of *T. gondii* tachyzoites. This finding is in agreement with the previously reported inhibitory activity of ritonavir and nelfinavir against *T. gondii* [7]. Regarding other antiretroviral classes, the HIV-1 maturation inhibitor bevirimat ($IC_{50} = 4.66 \pm 1.20$ μM), the NRTI TDF ($IC_{50} = 5.10 \pm 1.01$ μM) and the integrase inhibitor elvitegravir ($IC_{50} = 5.21 \pm 1.06$ μM) were the most potent compounds identified. Interestingly, two prescription medicines of the NRTI class (FTC/TDF and TDF) were also found to have a significant dose-dependent inhibitory effect on *T. gondii* growth in vitro.

Although ART can help in the control or improvement of certain opportunistic infections [1,5,6,12], co-administration of ART with treatment for certain opportunistic infections can potentially result in drug–drug interactions or increased toxicities, which may compromise the effective management of both the opportunistic infection and HIV. Interestingly, all antiretroviral compounds tested in the present study in combination with sulfadiazine

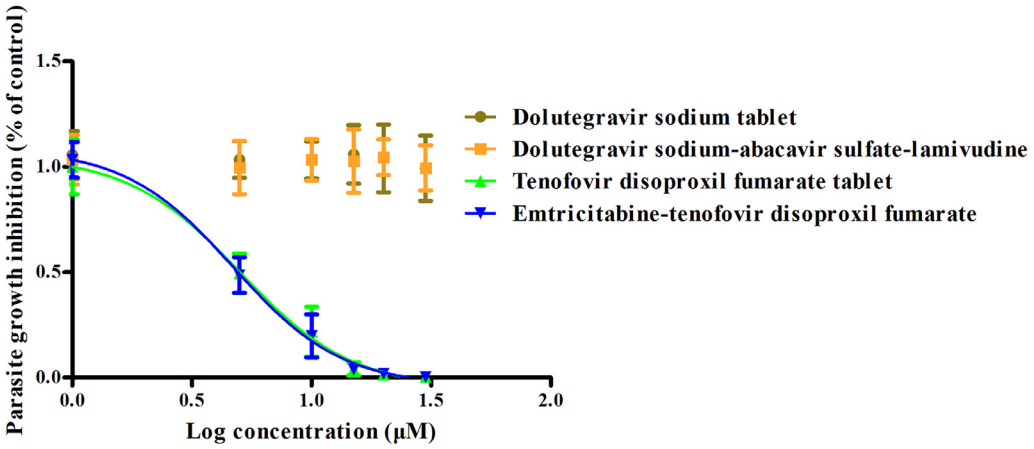

Fig. 2. Inhibitory effect of dolutegravir sodium (DTG), dolutegravir sodium–abacavir sulfate–lamivudine (DTG/ABC/3TC), tenofovir disoproxil fumarate (TDF) and emtricitabine–tenofovir disoproxil fumarate (FTC/TDF) against *Toxoplasma gondii* in vitro. Error bars represent the standard deviation of data from at least six independent experiments.

or pyrimethamine showed no effect on the anti-*T. gondii* activity of sulfadiazine or pyrimethamine, suggesting that co-administration of the antiretroviral compounds with sulfadiazine or pyrimethamine may not influence their activity when used to treat *T. gondii* infection in HIV patients receiving ART. However, this result is preliminary and more robust investigation of drug interactions, including in silico prediction analysis, is required to confirm the observed in vitro interaction data.

It remains unclear how antiretroviral compounds suppress the development of *T. gondii* parasites. PIs target aspartyl protease [13], and protozoan parasites express a number of aspartyl proteases that play various roles in parasite growth, including invasion and egress from host cells, hydrolysis of parasite proteins for virulence and host cell remodelling, and regulation of the immune response. For *T. gondii*, seven putative aspartic proteases have been identified and were found to play roles in parasite survival in vitro and virulence in vivo [14]. PIs have been shown to inhibit other protozoan parasites such as *Trypanosoma, Plasmodium, Cryptosporidium* and *Leishmania* [5,6]. Previous studies showed that aspartic proteinase (Ddi1) of *Leishmania* may be a target of PIs, and in vitro enzyme assays showed that aspartic peptidase activity in *Leishmania* can be inhibited by nelfinavir [15,16]. Also, the enzyme activity of aspartic proteinases in *Plasmodium falciparum* was inhibited by PIs such as lopinavir, ritonavir and saquinavir [17–19]. Interestingly, the drug designed to inhibit the *Plasmodium* aspartic proteinase also targets *T. gondii* aspartic proteinase and interferes with the tachyzoite lytic cycle [20]. Whether PIs affect only aspartyl proteases or also target other non-aspartyl proteases in *T. gondii* remains to be elucidated. In summary, 14 antiretroviral compounds demonstrated a good level of anti-*T. gondii* activity, suggesting that further testing and validation of the efficacy of the most potent leads of these antiretroviral compounds is warranted.

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Competing interests: None declared.

Ethical approval: Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.08.023.

References

[1] Robert-Gangneux F, Dardé ML. Epidemiology of and diagnostic strategies for toxoplasmosis. Clin Microbiol Rev 2012;25:264–96. doi:10.1128/CMR.05013-11.
[2] Conrad A, Le Maréchal M, Dupont D, Ducastelle-Leprêtre S, Balsat M, Labussière-Wallet H, et al. A matched case–control study of toxoplasmosis after allogeneic haematopoietic stem cell transplantation: still a devastating complication. Clin Microbiol Infect 2016;22:636–41. doi:10.1016/j.cmi.2016.04.025.
[3] Bekker LG, Alleyne G, Baral S, Cepeda J, Daskalakis D, Dowdy D, et al. Advancing global health and strengthening the HIV response in the era of the Sustainable Development Goals: the International AIDS Society–Lancet Commission. Lancet 2018;392:312–58. doi:10.1016/S0140-6736(18)31070-5.
[4] Wang ZD, Wang SC, Liu HH, Ma HY, Li ZY, Wei F, et al. Prevalence and burden of *Toxoplasma gondii* infection in HIV-infected people: a systematic review and meta-analysis. Lancet HIV 2017;4:e177–88. doi:10.1016/S2352-3018(17)30005-X.
[5] Pozio E, Morales MA. The impact of HIV-protease inhibitors on opportunistic parasites. Trends Parasitol 2005;21:58–63. doi:10.1016/j.pt.2004.11.003.
[6] van Griensven J, Diro E, Lopez-Velez R, Boelaert M, Lynen L, Zijlstra E, et al. HIV-1 protease inhibitors for treatment of visceral leishmaniasis in HIV-co-infected individuals. Lancet Infect Dis 2013;13:251–9. doi:10.1016/S1473-3099(12)70348-1.
[7] Derouin F, Santillana-Hayat M. Anti-*Toxoplasma* activities of antiretroviral drugs and interactions with pyrimethamine and sulfadiazine in vitro. Antimicrob Agents Chemother 2000;44:2575–7. doi:10.1128/AAC.44.9.2575-2577.2000.
[8] De Clercq E, Li G. Approved antiviral drugs over the past 50 years. Clin Microbiol Rev 2016;29:695–747. doi:10.1128/CMR.00102-15.
[9] Wang JL, Li TT, Elsheikha HM, Chen K, Cong W, Yang WB, et al. Live attenuated Pru:Δ*cdpk2* strain of *Toxoplasma gondii* protects against acute, chronic, and congenital toxoplasmosis. J Infect Dis 2018;218:768–77. doi:10.1093/infdis/jiy211.
[10] Nichol T, Smith TJ, Townsend R, Stockley I, Akid R. Analysis of linezolid and tigecycline as candidates for local prophylaxis via antibiotic-loaded bone cement. J Antimicrob Chemother 2017;72:410–16. doi:10.1093/jac/dkw410.
[11] Jeffers V, Gao H, Checkley LA, Liu Y, Ferdig MT, Sullivan WJ Jr. Garcinol inhibits GCN5-mediated lysine acetyltransferase activity and prevents replication of the parasite *Toxoplasma gondii*. Antimicrob Agents Chemother 2016;60:2164–70. doi:10.1128/AAC.03059-15.
[12] Hobbs CV, Voza T, De La Vega P, Vanvliet J, Conteh S, Penzak SR, et al. HIV nonnucleoside reverse transcriptase inhibitors and trimethoprim–sulfamethoxazole inhibit *Plasmodium* liver stages. J Infect Dis 2012;206:1706–14. doi:10.1093/infdis/jis602.
[13] Boddey JA, Hodder AN, Günther S, Gilson PR, Patsiouras H, Kapp EA, et al. An aspartyl protease directs malaria effector proteins to the host cell. Nature 2010;463:627–31. doi:10.1038/nature08728.
[14] Shea M, Jäkle U, Liu Q, Berry C, Joiner KA, Soldati-Favre D. A family of aspartic proteases and a novel, dynamic and cell-cycle-dependent protease localization in the secretory pathway of *Toxoplasma gondii*. Traffic 2007;8:1018–34. doi:10.1111/j.1600-0854.2007.00589.x.

[15] Trudel N, Garg R, Messier N, Sundar S, Ouellette M, Tremblay MJ. Intracellular survival of *Leishmania* species that cause visceral leishmaniasis is significantly reduced by HIV-1 protease inhibitors. J Infect Dis 2008;198:1292–9. doi:10.1086/592280.

[16] White RE, Powell DJ, Berry C. HIV proteinase inhibitors target the Ddi1-like protein of *Leishmania* parasites. FASEB J 2011;25:1729–36. doi:10.1096/fj.10-178947.

[17] Andrews KT, Fairlie DP, Madala PK, Ray J, Wyatt DM, Hilton PM, et al. Potencies of human immunodeficiency virus protease inhibitors in vitro against *Plasmodium falciparum* and in vivo against murine malaria. Antimicrob Agents Chemother 2006;50:639–48. doi:10.1128/AAC.50.2.639-648.2006.

[18] Hobbs CV, Tanaka TQ, Muratova O, Van Vliet J, Borkowsky W, Williamson KC, et al. HIV treatments have malaria gametocyte killing and transmission blocking activity. J Infect Dis 2013;208:139–48. doi:10.1093/infdis/jit132.

[19] Goulielmaki E, Kaforou S, Venugopal K, Loukeris TG, Siden-Kiamos I, Koussis K. Distinct effects of HIV protease inhibitors and ERAD inhibitors on zygote to ookinete transition of the malaria parasite. Mol Biochem Parasitol 2018;220:10–14. doi:10.1016/j.molbiopara.2017.12.003.

[20] Dogga SK, Mukherjee B, Jacot D, Kockmann T, Molino L, Hammoudi PM, et al. A druggable secretory protein maturase of *Toxoplasma* essential for invasion and egress. Elife 2017;6 pii: e27480. doi:10.7554/eLife.27480.