Anti- *Toxoplasma gondii* effect of Lumefantrine *in vitro* and *in vivo*

Dawei Wang
Shenyang Agricultural University
Mengen Xing
Shenyang Agricultural University
Saeed El-Ashram
Foshan University
Yingying Ding
Shenyang Agricultural University
Xiaoyu Sang
Shenyang Agricultural University
Ying Feng
Shenyang Agricultural University
Ran Chen
Shenyang Agricultural University
Xinyi Wang
Shenyang Agricultural University
Ning Jiang
Shenyang Agricultural University
Qijun Chen
Shenyang Agricultural University
Na Yang (dayangna@163.com)
Laboratory of Zoonosis of Liaoning Province, College of Animal Science & Veterinary Medicine, Shenyang Agricultural University https://orcid.org/0000-0002-8384-7404

Research

Keywords: Toxoplasma gondii, Lumefantrine, anti-Toxoplasma gondii, Invasion, Proliferation

Posted Date: February 25th, 2020

DOI: https://doi.org/10.21203/rs.2.24422/v1

Abstract

Background: *Toxoplasma gondii* is an obligate intracellular protozoan parasite, which can infect almost all warm-blooded animals, including humans, leading to toxoplasmosis. Currently, the effective treatment for human toxoplasmosis is the combination of sulfadiazine and pyrimethamine. However, both drugs have serious side effects and toxicity in the host. Therefore, there is an urgent need for the discovery of new anti-*Toxoplasma* drugs with high potency and less or no side-effects.

Methods: The cytotoxicity of sulfadiazine and lumefantrine to Vero cells was evaluated by the methyl thiazolyl tetrazolium (MTT) assay. And MTT assay was also used to detect the inhibitory effects of lumefantrine on parasites invasion and proliferation. Flow cytometry was conducted to further verify parasites proliferation. qPCR was performed to evaluate the parasite load in the mice after lumefantrine treatment. In order to determine whether lumefantrine treatment enhances Th1 or Th2 cytokine response, IFN-γ, IL-4, and IL-10 levels in the serum of mice were determined.

Results: Our findings suggest that lumefantrine exerts activity against *T. gondii* by inhibiting its replication and invasion of Vero cells *in vitro* without being toxic to the cells. Furthermore, lumefantrine protected mice with acute toxoplasmosis from death to a certain extent and reduced the parasite burden in mouse tissues *in vivo*. In addition, a significant increase in IFN-γ production was observed in high dose lumefantrine-treated mice while IL-10 and IL-4 levels increased in low dose lumefantrine-treated mice.

Conclusions: The results of this study demonstrated that lumefantrine may be a promising agent to treat toxoplamosis, and more experiments on the protective mechanism of lumefantrine should be undertaken in further studies.

Key words: *Toxoplasma gondii*, Lumefantrine, anti-*Toxoplasma gondii*, Invasion, Proliferation

Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite, which can infect almost all warm-blooded, including humans, leading to toxoplasmosis [1, 2, 3, 4, 5]. Approximately 30% of the world's population has serological evidence of Toxoplasma infection [6]. T. gondii is a potential threat to both human and animal health. Toxoplasmosis is normally innocuous in individuals with a good immune system, however, T. gondii is quite severe or even fatal for immunocompromised patients, such as those with AIDS, tumour and organ transplant recipients [7–9]. In women, primary infection during pregnancy can cause severe damage to fetus and newborns, including blindness, abortion, and stillbirth.

Several anti-T. gondii drugs, including sulphonamides and pyrimethamine to control toxoplasmosis [10]. Both sulphonamides and pyrimethamine prevent the synthesis of folate by inhibiting the dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) that are essential for the survival and multiplication of parasites [11, 12]. However, these drugs can not completely inactivate encysted bradyzoites or treat congenital toxoplasmosis, and their use is also limited by their side effects, including

haematological toxicity (pyrimethamine), cutaneous rash, leukopenia and thrombocytopenia (sulphonamides) [13–16]. There is increasing evidence of treatment failures in patients affected by toxoplasmosis suggesting the existence of drug resistance in clinical therapy against sulphonamides and pyrimethamine [17].

Continuous efforts have been made to develop drugs for the treatment of toxoplasmosis. However, drug development is an expensive and lengthy process [18]. In an attempt to accelerate the process of drug discovery, older drugs, which are being tested and developed for new activities are making a comeback. Lumefantrine (LF), which was previously named benflumetol, is an antimalarial drug synthesized in 1970s in China [19]. Lumefantrine, which exhibits potent antimalarial activities, with a half-life of 2–4 days, is capable of eliminating the residual parasites that remain in the blood, thereby preventing recrudescence [20].

In Guyana, the combination of lumefantrine and artemisinin has shown a better treatment effect for plasmodium vivax [21]. Plasmodium is an apicomplexa intracellular protozoa, which has similar infection mechanisms to T. gondii. However, the effect of lumefantrine on T. gondii has never been studied. Currently, there is an urgent need for the discovery of new anti-Toxoplasma drugs with high potency and less or no side-effects. Therefore, the aim of this study was to evaluate the activity of Lumefantrine against T. gondii using cell culture and mice infected with T. gondii (RH strain) as in vitro and in vivo experimental models, respectively.

Methods

Cells and parasites

Cells were cultured in 25 cm^2 culture flasks in DMEM medium (Macgene, China) supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin (Macgene, China), and 10% heat-inactivated foetal bovine serum (FBS) (BI, Israel) at 37 °C in a 5% CO_2 atmosphere. T. gondii tachyzoites (RH strain) were maintained in Vero cells cultured in DMEM medium supplemented with penicillin, streptomycin and 2% FBS at 37 °C and 5% CO_2.

Cytotoxicity assay

The cytotoxicity of sulfadiazine and lumefantrine (Sigma, USA) to Vero cells was evaluated by the methyl thiazolyl tetrazolium (MTT) assay[22, 23]. Vero cells (2×10^5) were seeded in 96-well plates and cultured in 10% FBS DMEM for 12 h to obtain a monolayer. Monolayer cells were washed and directly subjected to lumefantrine (dilution from 94.5 nmol/L to 2.9531 nmol/L) or sulfadiazine(dilution from 500 mg/L to 15.625 mg/L, from 100 mg/L to 3.125 mg/L, from 30 mg/L to 0.9375 mg/L, respectively), which were diluted with 10% FBS DMEM. The cells were subsequently cultured for 24 h and 48 h. As a control, cells were treated with 200 μL 10% FBS DMEM (Negative control) and 20 μL DMSO (1 μL/mL) (Sigma,USA) together with 180 μL 10% FBS DMEM (Solvent control/DMSO group). Supernatants were removed after culturing for 24 h or 48 h, and the plates were washed twice by PBS and pulsed by adding 10 μL of MTT

(Solarbio, China) together with 90 μL 10% FBS DMEM for 4 h in the same culture conditions. The supernatants were removed gently with pipettes and 110 μL formazan was added to each well. The plates were vibrated on a low-speed oscillator, and optical density (OD) was measured at 490 nm by a microplate reader after 30 min (Tecan, Switzerland).

Invasion assay in vitro

T. gondii tachyzoites (1×10^6) were separately pre-treated with sulfadiazine (10 μg/mL) (positive control), or lumefantrine (94.5 nmol/L, 17.7188 nmol/L or 2.9531 nmol/L) for 2 h. After treatment, tachyzoites were washed three times with PBS and added to Vero cells in a 96-well plate (200 μL/well) (parasites per host cell ratio = 5:1). After 2 h post-infection, extracellular parasites were washed away and then incubated with DMEM (2% FBS) containing sulfadiazine (10 μg/mL) or lumefantrine (94.5 nmol/L, 17.7188 nmol/L or 2.9531 nmol/L) for another 18 h. MTT assay was used to detect the inhibitory effect of lumefantrine on parasites invasion.

Proliferation assay in vitro

The anti-proliferation effect of lumefantrine on T. gondii was also detected using the MTT assay. Vero cell monolayers in 96-well plates were infected with 1×10^6 fresh RH tachyzoites per well and incubated for 2 h. Then, the cell monolayers were washed twice with PBS to remove extracellular tachyzoites and incubated with DMEM (2% FBS) containing different concentrations of lumefantrine (94.5 nmol/L, 17.7188 nmol/L, or 2.9531 nmol/L) for 24 h and 48 h. The sulfadiazine (10 μg/mL) was added as a positive control. Infected cells without drugs were used as a negative control. The MTT assay was carried out to evaluate parasite proliferation as previously described.

Flow cytometry

To further verify parasites proliferation, flow cytometry was conducted. In brief, Toxoplasma-infected cells were digested by trypsin without EDTA after culturing for 24 h, washed twice with PBS, stained with annexin V-FITC (Biolegend, USA) and Propidium Iodide (Biolegend, USA), and incubated at room temperature for 10–15 min without light. Parasite proliferation was measured using a flow cytometer (BD, USA) [24].

Effect of lumefantrine on mice infected by T. gondii

Kunming mice were purchased from Liaoning Changsheng Biotecnology Company, China. Ninety female mice (4–6 weeks, weighing 18–20 g) were divided into 6 treatment groups (15 mice per group). All the mice except the blank control group were infected with fresh T. gondii (100 tachyzoites/mice). After 24 h post-infection, the mice were injected intragastrically with sulfadiazine (10 μg/mL) or lumefantrine(94.5 nmol/L, 17.7188 nmol/L or 2.9531 nmol/L)every two days. Mice in both the blank and

parasite control groups were injected intragastrically with the equal amounts of PBS. Mice were observed daily to record the death time and rate. All mice were humanely killed by cervical dislocation to collect blood at 11 days post-infection. Liver, heart, spleen, and lung tissues were collected and stored in liquid nitrogen for RNA extraction.

Real-time quantitative PCR

Tissue RNAs in different groups were extracted using Trizol ((Invitrogen, USA), and the extracted RNAs were treated with DNase I (TaKaRa, China) to completely remove the genomic DNA. The mRNA was reverse transcribed from Oligo (dT) and used as templates for quantitative RT-PCR.

Specific primers (F: TCCGGCTTGGCTGCTTT, R: TTCAATTCTCTCCGCCATCAC) were designed according to the gene sequence of Toxoplasma repeat region (AF146527.1). Quantitative RT-PCR was performed on an ABI PRISM 7500 Real-Time PCR System (Applied Biosystems) using the SYBR Premix Ex Taq kit (TaKaRa, China). Experiment was repeated three times, and transcription levels were represented by the mean values of the three parallel experiments.

Detection of IL-4, IL-10, IFN-γ

The changes of IL-4, IL-10 and IFN-γin mice treated with lumefantrine or sulfadiazine were evaluated using the cytokine ELISA kits (Beyotime, China) according to the manufacturer's instructions. Sera of different treatment groups were used to detect the changes of cytokine levels through three independent experiments. Absorbance at 450 nm was measured by a microplate reader (Tecan, Switzerland).

Statistical analysis

Data were analyzed using SPSS (ver18.0) computer software (SPSS for Windows, SPSS Inc., 2009). All values are expressed as the mean ± S.D.. Statistical analysis was performed using ANOVA. Differences were considered statistically significant when P-values were ≤ 0.05.

Results

Cytotoxicity activity

The MTT assay revealed that different concentrations of both lumefantrine and sulfadiazine had no cytotoxicity compared with the negative control cells (Fig. 1a and 1b). Thus, different concentrations of lumefantrine (high 94.5 nmol/L, medium 17.7188 nmol/L, and low 2.9531 nmol/L) and sulfadiazine (10 μg/mL) were used to carry out further experiments against T. gondii in vitro.

Anti-invasion activity

Vero cell counts using the MTT assay showed that pre-treatment with 94.5 nmol/L, 17.7188 nmol/L and 2.9531 nmol/L lumefantrine reduced tachyzoites invasion by 7.12%, 7.08%, and 6.72% for 18 h,

respectively (Fig. 2A). Sulfadiazine caused a 4.15% reduction after 18 h treatment (Fig. 2A). The invasion ability of pre-treated tachyzoites with different concentrations of lumefantrine and sulfadiazine were significantly reduced compared with the untreated group at 18 h (Toxoplasma group) ($P \leq 0.01$).

Anti-Proliferation activity

Further evaluation of the ability of lumefantrine and sulfadiazine to inhibit the intracellular tachyzoite replication within Vero cells was examined using the MTT assay at 24 h and 48 h post-treatment (Fig. 2B). Post-treatment with 94.5 nmol/L, 17.7188 nmol/L or 2.9531 nmol/L lumefantrine reduced the proliferation of tachyzoites by 27.31%, 22.79%, and 20.85% at 24 h and 47.18%, 42.34% and 42.48% at 48 h, respectively (Fig. 2B). A 21.12% reduction at 24 h and 41.2% reduction at 48 h for tachyzoite post-treatment with sulfadiazine were observed (Fig. 2B). This was an indication that lumefantrine could significantly inhibit tachyzoite proliferation compared with the Toxoplasma group ($P \leq 0.01$).

Flow cytometry

The anti-proliferation activity of lumefantrine was further examined using flow cytometry. Samples were stained with annexin V-FITC and Propidium Iodide after treatment with lumefantrine and sulfadiazine for 24 h. Different quadrants represent different states of the cells (Q1:Necrotic and damaged cells. Q2: Late apoptotic cells. Q3: Living cells. Q4: Early apoptotic cells). The more living cells in Q3 quadrant, reflects the good effect of lumefantrine on anti-parasite proliferation (Fig. 3a). These results indicated that different concentrations of lumefantrine could inhibit the proliferation of T. gondii by flow cytometry in Fig. 3a and 3b.

Survival rate of acutely infected mice treated with lumefantrine

Mice were observed daily and survival time was recorded at 11 days post-treatment. Compared with the Toxoplasma group, mice started to die at 6 days post-treatment. However, mice treated with 94.5 nmol/L, 17.7188 nmol/L, and 2.9531 nmol/L lumefantrine started to die at day 7, 8, and 9 post-treatemt, respectively. The positive group (sulfadiazine group) started to die at day 7 post-treatment. After 11 days, 80%, 66.7% and 53.3% of mice treated with 94.5 nmol/L, 17.7188 nmol/L, 2.9531 nmol/L lumefantrine, respectively had survived, while only 46.7% living mice treated with 10 μg/mL sulfadiazine had survived (Fig. 4).

Parasite load in mice tissues

To further evaluate the parasite load in the mice after lumefantrine treatment, liver, heart, spleen, and lung samples from infected mice were determined by qPCR, and the results are shown in Fig. 5. Treatment with different concentrations of lumefantrine significantly (**$p \leq 0.01$ and *$p \leq 0.05$) reduced the parasite load in the liver, heart, spleen and lung tissues compared with the Toxoplasma group (PBS group). The parasite load in different tissues except the liver was also reduced in the positive control group (sulfadiazine group).

Regulation of cytokine levels by lumefantrine in mice infected by T. gondii

In order to determine whether lumefantrine treatment enhances Th1 or Th2 cytokine response, IFN-γ, IL-4, and IL-10 levels in the serum of mice were determined in Fig. 6. Significantly higher levels of IFN-γ were observed in mice treated with a high concentration lumefantrine compared to the control groups ($p \leq 0.01$). In addition, IL-4 and IL-10 were significantly produced in mice treated with a low concentration lumefantrine compared to the control groups ($p \leq 0.01$).

Discussion

Lumefantrine has been shown to have a prominent inhibition effect on P. vivax (sexual and asexual stages) in China [21, 25]. Lumefantrine can reduce gametocyte rates in blood and inhibit the development of gametocyte in mosquitoes [21, 25]. T. gondii is an apicomplexa intracellular protozoa, which has a similar infection mechanism to Plasmodium. However, studies that have shown successful treatment for toxoplasmosis patients are limited, indicating the urgent need to identify and develop new therapies [26]. In addition, data about the inhibition of T. gondii using lumefantrine is not available. Therefore, in this study, we evaluated the effect of lumefantrine treatment on T. gondii infection in vivo and in vitro .

Cytotoxicity assays showed that lumefantrine was not cytotoxic to Vero cells. Anti-invasion assay showed that the invasion inhibition rate of lumefantrine was about 7% at 18 h post-treatment ($p \geq 0.05$), and anti-proliferation assay showed that a 21.12% reduction at 24 h and a 41.2% reduction at 48 h post-treatment with lumefantrine were recorded ($p \leq 0.01$). These results indicated that lumefantrine could significantly inhibit the proliferation of T. gondii, which was also verified by Flow cytometry.

Evaluation of anti-T. gondii effects of lumefantrine in mice acutely infected by the RH strain of T. gondii revealed 80%, 66.7%, and 53.3% of mice treated with 94.5 nmol/L, 17.7188 nmol/L, 2.9531 nmol/L lumefantrine, respectively had survived at 11 days post-treatment, and only 46.7% living mice treated with 10 μg/mL sulfadiazine had survived. Furthermore, the parasite burdens in the liver, heart, spleen, and lung after lumefantrine treatment were significantly decreased compared with those in the parasite control group, indicating that lumefantrine exerts an inhibitory effect on T. gondii, partially provides protection against death due to T. gondii infection, and reduces the parasite burden in the tissues of mice. High levels of Th1 (IFN-γ) and Th2 (IL-4, IL-10) cytokines were detected in lumefantrine-treated mice. IFN-γ was

the key cytokine in resistance against T. gondii infection [27]. IFN-γ can inhibit the replication of T. gondii in infected cells through various mechanisms, including induction of the inhibitory protein guanamine 2,3-dioxygenase (IDO), inducible nitric oxide synthase (iNOS), the effector proteins immunity-related GTPases (IRGs), and guanylate binding proteins (GBPs) [28]. In the present study, a significant increase in IFN-γ production in mice treated with a high dose lumefantrineimproved mouse survival. These results indicate that lumefantrine can trigger an increased IFN-γ production and contribute to the prevention of acute T. gondii infection. Meanwhile, an increase in IL-10 and IL-4 levels was also observed in mice, which received a low dose of lumefantrine. IL-10 has a central role in limiting inflammation and inhibiting CD4 + T cell-mediated severe immunopathology [29], and IL-4 functions to enhance IFN-γ production in the late stage of infection [30].

Conclusions

In conclusion, our findings suggest that lumefantrine exerts activity against T. gondii by inhibiting its replication and invasion in vitro in the absence of host toxicity in this study. Furthermore, lumefantrine protected mice with acute toxoplasmosis from death to a certain extent and decreased parasite burden in mice tissues in vivo. Therefore, our results clearly demonstrate that lumefantrine may be a promising agent to treat toxoplamosis in the future, However, more experiments on the protective and therapeutic mechanisms of lumefantrine should be undertaken to fully understand the effects of lumefantrine on Toxoplasma gondii.

Abbreviations

AIDS: acquired immune deficiency syndrome; DHFR: dihydrofolate reductase; DHPS: dihydropteroate synthase; LF: Lumefantrine; DMEM: Dulbecco's modified Eagle's medium; FBS: foetal bovine serum; MTT: methyl thiazolyl tetrazolium; DMSO: dimethylsulfoxide; OD: optical density; EDTA: Ethylene Diamine Tetraacetic Acid; annexin V-FITC: annexin V fluorescein isothiocyanate; RT-PCR: reverse transcription polymerase chain reaction; qPCR: Quantitative real-time PCR; SYBR: Synergy Brands; FCM: Flow cytometry; IL-4: interleukin-4; IL-10: interleukin-10; IFN-γ: gamma interferon; ANOVA: analysis of variance; IDO: inhibitory protein guanamine 2,3-dioxygenase; iNOS: inducible nitric oxide synthase; IRGs: immunity-related GTPases; GBPs: guanylate binding proteins.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

The study was designed by NY. Experiments were performed by DW. The manuscript was revised by El-Ashram S. Data were analyzed by MX, YD, XS, YF, RC, XW, NJ, QC. Manuscript was written by NY and DW.

All authors have read and approved the final manuscript.

Funding

This research was supported by grants of the National Key Research and Development Program of China (2017YFD0500400), the National Natural Science Foundation of China (Grant Numbers 31672546) and LiaoNing Revitalization Talents Program (XLYC1907091).

Availability of data and materials

Data supporting the conclusions of this article are included within the article.

Ethics approval and consent to participate

The experiments were performed in strict according to the recommendations of the Guide for the Care and Use of Laboratory Animals of the Ministry of Science and Technology of China. All experimental animal procedures and protocols were approved by the Institutional Animal Care and Use Committee of Shenyang Agricultural University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Key Laboratory of Livestock Infectious Diseases in Northeast China, Ministry of Education, Key Laboratory of Zoonosis, College of Animal Science and Veterinary Medicine, Shenyang Agricultural University, Dongling Road 120, 110866, Shengyang, China. [2]College of Food Science, Shenyang Agricultural University, Dongling Road 120, 110866, Shengyang, China. [3]College of Life Science and Engineering, Foshan University, 18 Jiangwan Street, Foshan, 528231, Guangdong Province, China. [4]Department of Parasitology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt.

References

1. Dubey JP. Toxoplasmosis of animals and humans. 2nd ed. Boca Raton: CRC Press; 2010.
2. El-Ashram S, Yin Q, Barta JR, Khan J, Liu X, Suo X. Immunoproteomic technology offers an extraordinary diagnostic approach for *Toxoplasma gondii* J Microbiol Methods. 2015;119:18-30.

3. El-Ashram S, Sun X, Yin Q, Liu X, Suo X. Exploring early and late Toxoplasma gondii strain RH infection by two-dimensional immunoblots of chicken immunoglobulin G and M profiles. PLoS One. 2015;10:e0121647.
4. Yin Q, El-Ashram S, Liu H, Sun X, Zhao X, Liu X, Suo X. Interferon-Gamma Release Assay: An Effective Tool to Detect Early *Toxoplasma gondii* Infection in Mice. PLoS One. 2015;10:e0137808.
5. Yin Q, El-Ashram S, Liu XY, Suo X. Early detection of *Toxoplasma gondii*-infected cats by interferon-gamma release assay. Exp Parasitol. 2015;157:145-9.
6. Zhou P, Chen Z, Li HL, Zheng H, He S, Lin RQ, Zhu XQ. Toxoplasma gondii infection in humans in China. Parasit Vectors. 2011;4:105.
7. Tian YM, Dai FY, Huang SY, Deng ZH, Duan G, Zhou DH, Yang JF, Weng YB, Zhu XQ, Zou FC. First report of *Toxoplasma gondii* seroprevalence in peafowls in Yunnan Province, Southwestern China. Parasit Vectors. 2012;5:205.
8. Qin SY, Cong W, Liu Y, Li N, Wang ZD, Zhang FK, Huang SY, Zhu XQ, Liu Q. Molecular detection and genotypic characterization of *Toxoplasma gondii* infection in bats in four provinces of China. Parasit Vectors. 2014;7:558.
9. Wang DW, Liu Y, Jiang TT, Zhang GX, Yuan GM, He JB, Su CL, Yang N. Seroprevalence and genotypes of *Toxoplasma gondii* isolated from pigs intended for human consumption in Liaoning province, northeastern China. Parasites Vectors. 2016;9:248.
10. Montoya JG, Liesenfeld O. Toxoplasmosis. Lancet. 2004;363:1965-76.
11. Anderson AC. Targeting DHFR in parasitic protozoa. Drug Discov. 2005;10:121-8.
12. Derouin F. Anti-toxoplasmosis drugs. Curr Opin Investig Drugs. 2001; 2:1368-74.
13. Agha F, Sadaruddin A, Ghafoor A. Human toxoplasmosis. J Pakistan Med Assoc. 1992;42:224-6.
14. Torre A, Stanford M, Curi A, Jaffe GJ, Gomez-Marin JE. Therapy for ocular toxoplasmosis. Ocul Immunol Inflamm. 2011;19:314-20.
15. Kim YA, Sharon A, Chu CK, Rais RH, Al Safarjalani ON, Naguib FN, el Kouni MH. Synthesis, biological evaluation and molecular modeling studies of N6- benzyladenosine analogues as potential anti-*toxoplasma* Biochem Pharmacol. 2007;73:1558-72.
16. Subauste CS, Remington JS. Immunity to *toxoplasma gondii*. Curr Opin Immunol. 1993;5:532-
17. Doliwa C, Xia D, Escotte-Binet S, Newsham EL, Sanya JS, Aubert D, Randle N, Wastling JM, Villena I. Identifification of diffffferentially expressed proteins in sulfadiazine resistant and sensitive strains of *Toxoplasma gondii* using diffffference-gel electrophoresis (DIGE). Int J Parasitol Drugs Drug Resist. 2013;3:35-44.
18. Hoelder S, Clarke PA, Workman P. Discovery of small molecule cancer drugs: successes, challenges and opportunities. Mol Oncol. 2012;6:155-176.
19. Olliaro PL, Trigg PI. Status of antimalarial drugs under development. Bull World Health Organ. 1995;73:565-71.

20. 20. Richard MH, Pauline BK, Mohammed L, Concepta M, Michael A, Warunee H, Nicholas PJ, Nicholas JW, Angela A, Joel T. Artemether-lumefantrine co-administration with antiretrovirals: population pharmacokinetics and dosing implications. Brit J Clin Pharmaco. 2014;79:636-49.
21. Eibach D. Therapeutic efficacy of artemether-Lumefantrine for *Plasmodium vivax* infections in a prospective study in Guyana. Malar J. 2012;11:347.
22. Chen SX, Wu L, Jiang XG, Feng YY, Cao JP. Anti-*Toxoplasma gondii* activity of GAS in vitro. J Ethnopharmacol. 2008;118:503-7.
23. Kavitha N, Noordin R, Chan KL, Sasidharan S. Cytotoxicity activity of root extract/fractions of *Eurycoma longifolia* Jack root against vero and Hs27cells. J Med Plant Res. 2010;4:2383-7.
24. Hou N, Piao XY, Liu S, Wu C, Chen QJ. Tim-3 induces Th2-biased immunity and alternative macrophage activation during *Schistosoma japonicum* infection. Infect Immun. 2015;83:3074-82.
25. Artemether-lumefantrine treatment of uncomplicated *Plasmodium falciparum* malaria: a systematic review and meta-analysis of day 7 lumefantrine concentrations and therapeutic response using individual patient data. BMC Medicine. 2015;13:227.
26. 26. Adeyemi OS, Sugi T, Han Y, Kato K. Screening of chemical compound libraries identified new anti-*Toxoplasma gondii* Parasitol Res. 2018;117:355-63.
27. Dautu G, Munyaka B, Carmen G, Zhang G, Omata Y, Xuenan X. *Toxoplasma gondii*: DNA vaccination with genes encoding antigens MIC2, M2AP, AMA1 and BAG1 and evaluation of their immunogenic potential. EXP Parasitol. 2007;116:273-82.
28. Zheng B, Ding JZ, Lou D, Tong QB, Zhuo XH, Ding HJ, Kong QM, Lu SH. The Virulence-Related MYR1 Protein of *Toxoplasma gondii* as a Novel DNA Vaccine Against Toxoplasmosis in Mice. Front Microbiol. 2019;10:734.
29. Dupont CD, Christian DA, Hunter CA. Immune response and immunopathology during toxoplasmosis. Semin Immunopathol. 2012;34:793-813.
30. Zheng B, Lou D, Ding JZ, Zhuo XH, Ding HJ, Kong QM, Lu SH. GRA24-Based DNA Vaccine Prolongs Survival in Mice Challenged With a Virulent *Toxoplasma gondii* Strain. Front Microbiol. 2019;10:418.

Figures

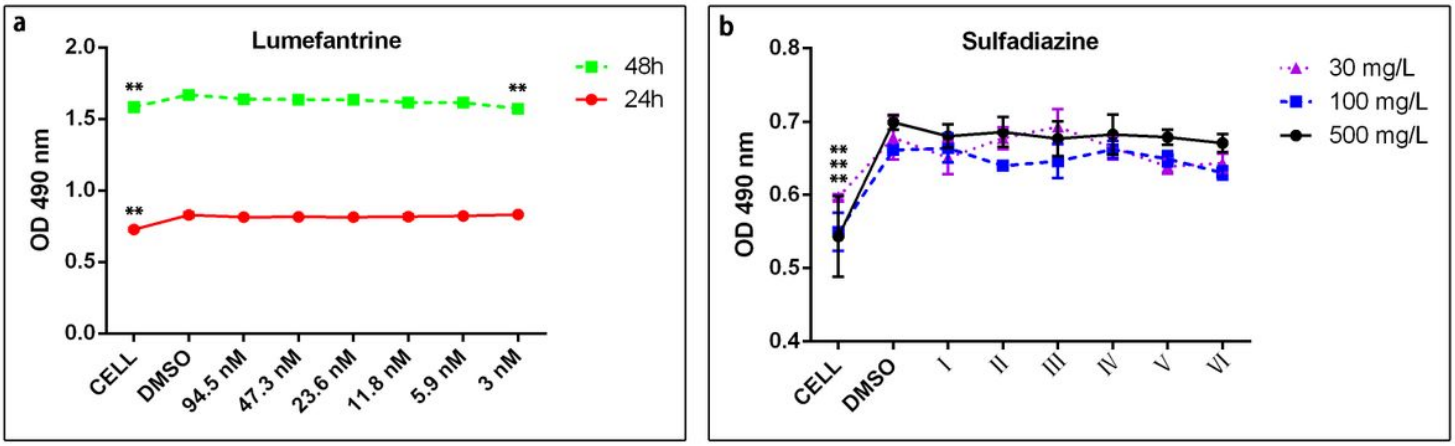

Figure 1

Cytotoxicity of lumefantrine and sulfadiazine on the viability of Vero cells. Vero cells were treated with different concentrations of lumefantrine and for 24 h and 48 h, respectively. Vero cells treated with DMEM were used as a negative control and treated with DMSO as a solvent control. All data are presented as mean ± S.D., and the experiments were performed in triplicate (compared with DMSO group, **p≤0.01 *p≤0.05).

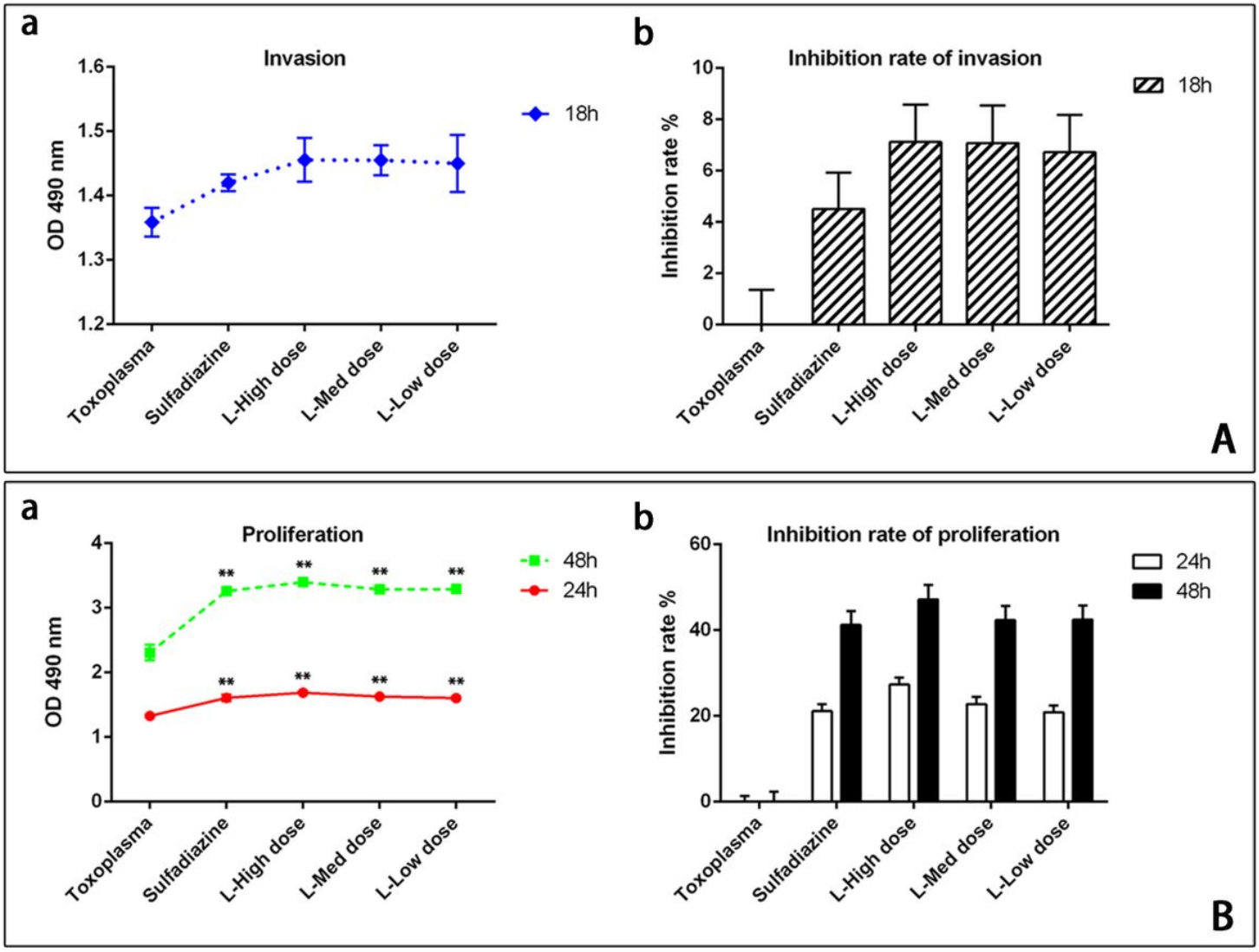

Figure 2

Effects of lumefantrine and sulfadiazine on T. gondii invasion and proliferation. A. Tachyzoites were pretreated with lumefantrine and sulfadiazine for 2 h, tachyzoites were subsequently seeded into Vero cells and counted for invasion assays. Tachyzoites treated only with DMEM were defined as the negative control. As a positive control, tachyzoites were treated with sulfadiazine (10 μg/mL). B. After 2 h pre-treatment of tachyzoites, Vero cells were treated with lumefantrine or sulfadiazine for another 24 h and 48 h, respectively. Tachyzoites treated only with DMEM were defined as the negative control and those treated with sulfadiazine (10 μg/mL) as the positive control. The inhibition rates of T. gondii invasion and proliferation were calculated by formula:{(Group treatments OD - Group Toxoplasma OD)/ Group Toxoplasma OD}. Data represent the mean ± S.D. of three independent experiments performed in triplicate. Significantly different from the negative control (Compared with Toxoplasma group, **$p \leq 0.01$ *$p \leq 0.05$).

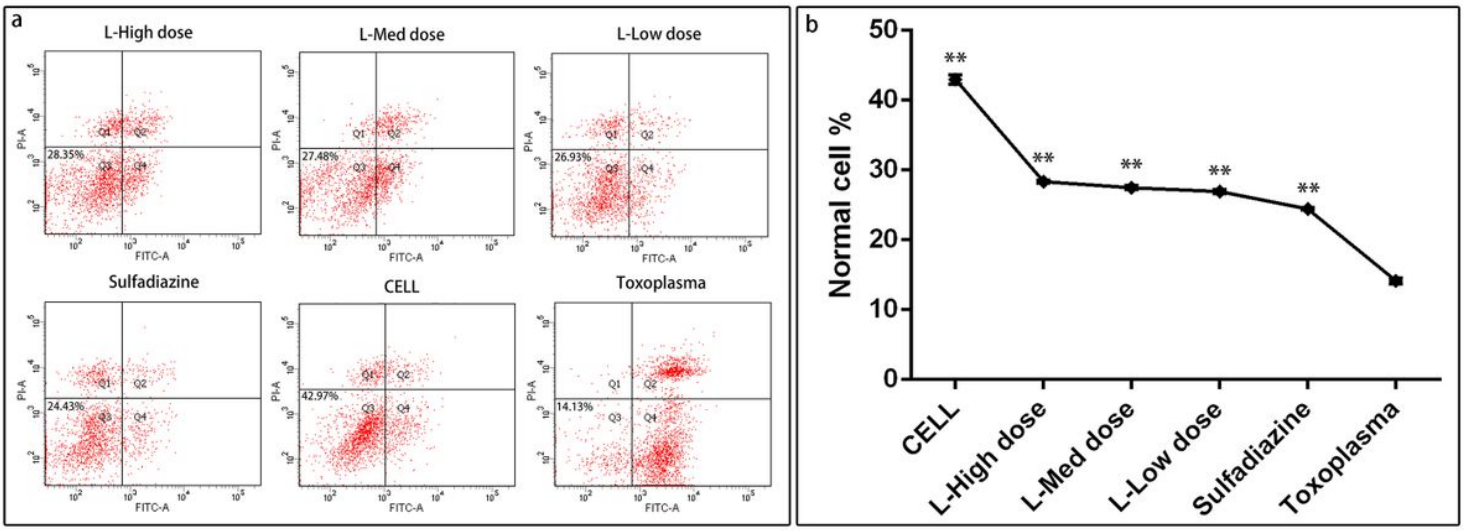

Figure 3

Lumefantrine inhibition of proliferation of T. gondii at 24 h post-treatment by Flow cytometry. Tachyzoites were treated with lumefantrine for 24 h. Cells treated only with DMEM were defined as the blank control, and tachyzoites treated only with DMEM were defined as the negative control. As a positive control, tachyzoites were treated with sulfadiazine. Samples were stained with annexin V-FITC and Propidium Iodide, and the percentage of cells in each group was determined by FCM. Data represent the mean ± S.D. of three independent experiments performed in triplicate Significantly different from the negative control (Compared with Toxoplasma group, **p≤0.01 *p≤0.05).

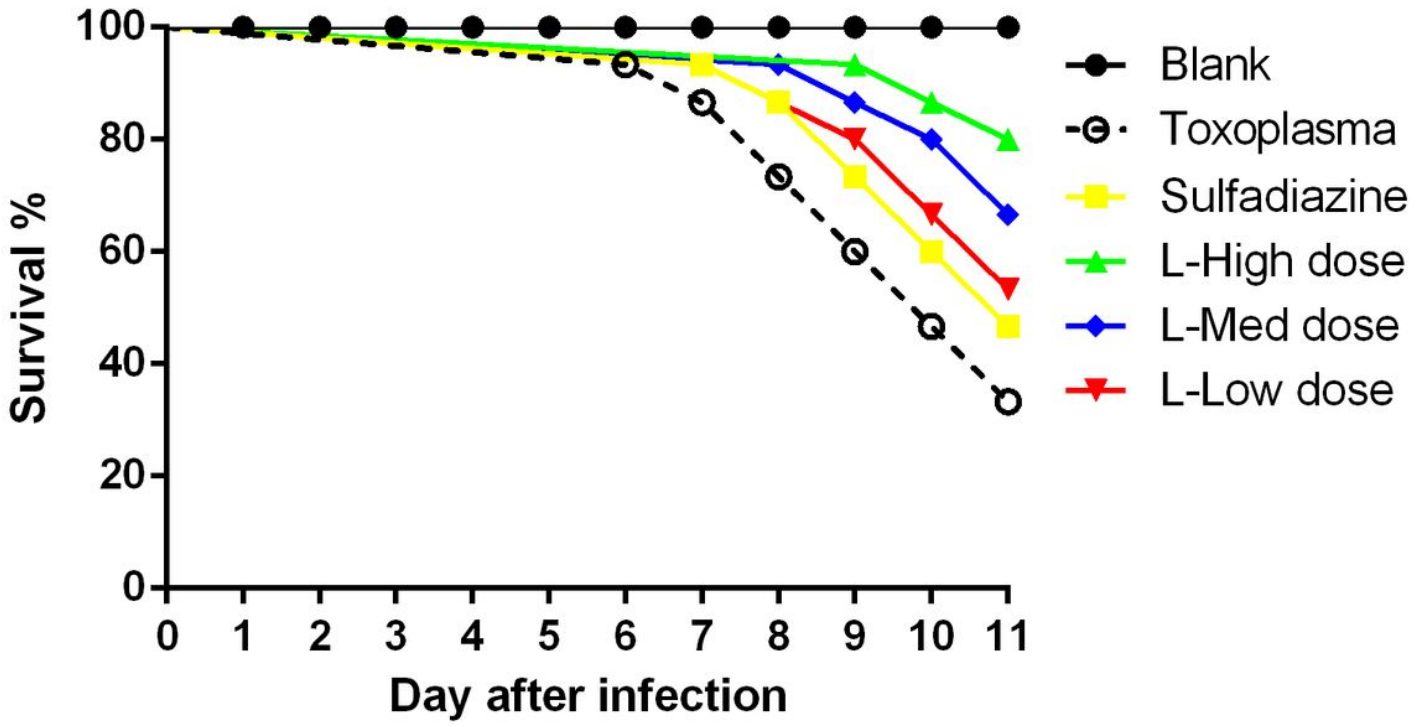

Figure 4

Effect of lumefantrine on the survival rate of acutely infected mice. All the mice were infected with 100 T. gondii tachyzoites and then treated with lumefantrine (94.5 nmol/L, 17.7188 nmol/L, and 2.9531 nmol/L), a positive control (10 μg/mL sulfadiazine) and PBS (Toxoplasma group) every two days for 10 days. Mice were then observed for 11 days, and the survival times of the infected mice were recorded for 11 days.

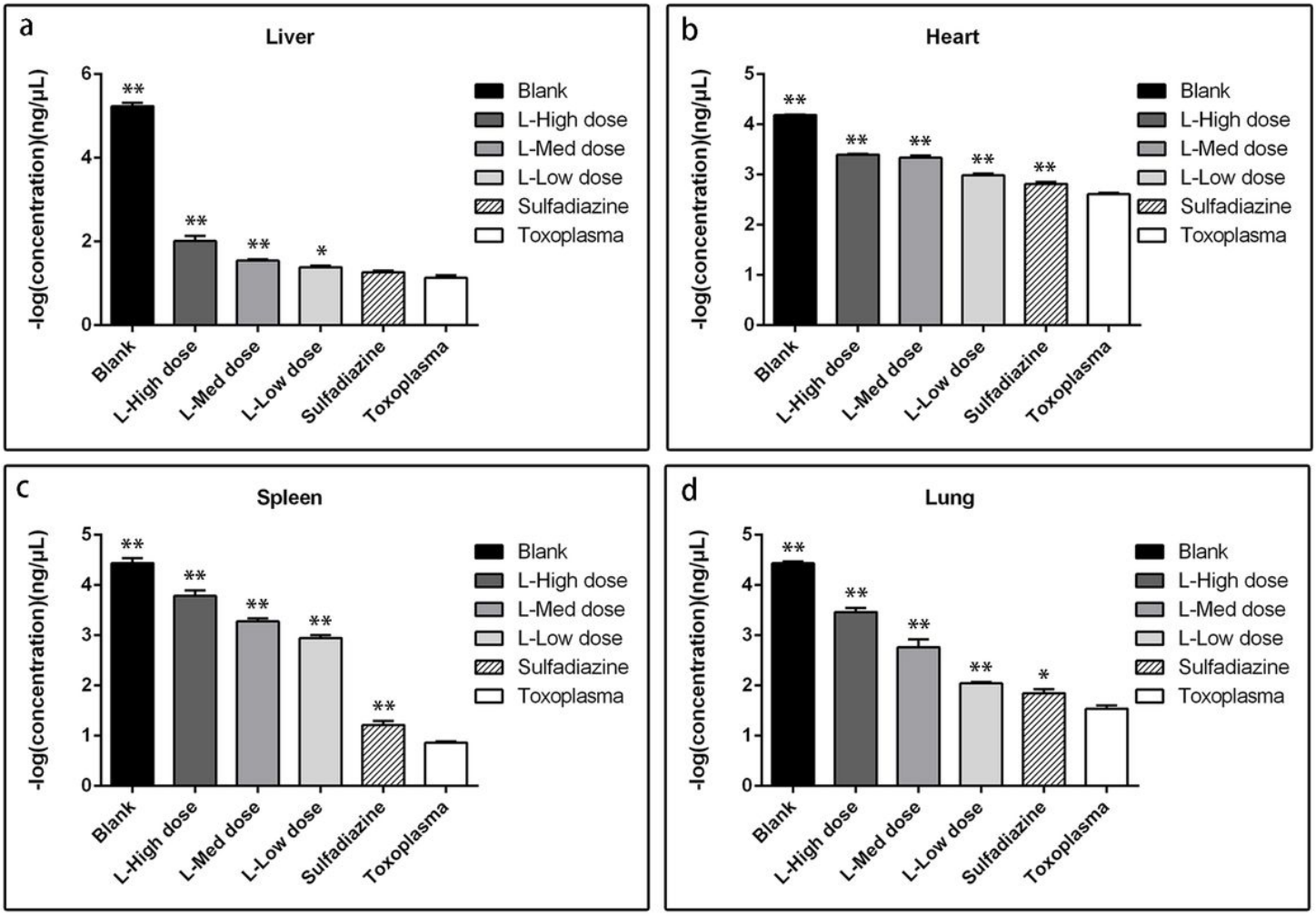

Figure 5

Parasite burden in tissues from the acutely infected mice. Mice were challenged intraperitoneally with 100 T. gondii tachyzoites, treated with lumefantrine (94.5 nmol/L, 17.7188 nmol/L, and 2.9531 nmol/L), a positive drug (10 μg/mL sulfadiazine) and PBS (Toxoplasma group) every two days for 10 days. The parasite loads in the liver, heart, spleen, and lung tissues of the infected mice were isolated and homogenized. Total RNA was isolated, and the T. gondii repeat region was detected by qPCR. The quantified parasite loads in the tissues of mice are presented as the -log10 values of the numbers of tachyzoites per 20 mg of tissues. Data represent the mean ± S.D. of three independent experiments performed in triplicate. Significantly different from the negative control (Compared with Toxoplasma group, **$p \leq 0.01$ *$p \leq 0.05$)

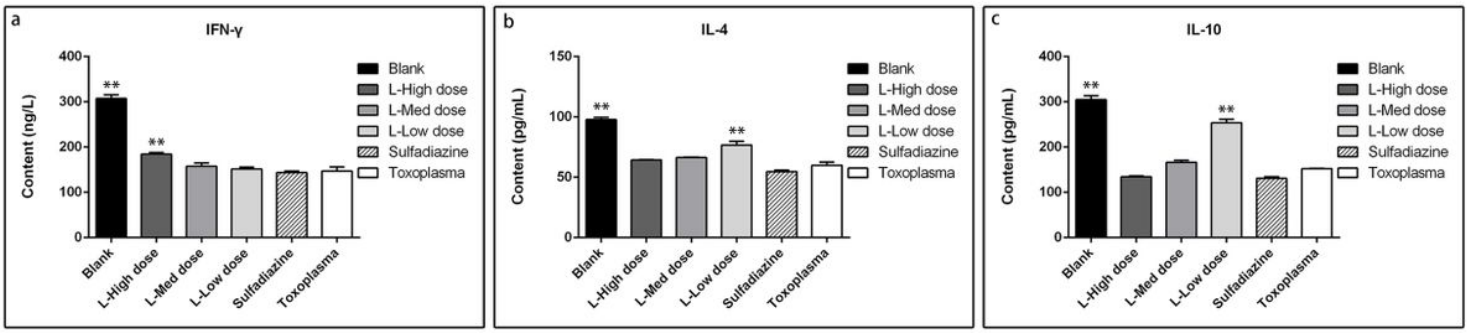

Figure 6

Lumefantrine regulates the change of cytokines. Mice were challenged intraperitoneally with 100 T. gondii tachyzoites and treated with lumefantrine (94.5 nmol/L, 17.7188 nmol/L, and 2.9531 nmol/L), a positive drug (10 μg/mL sulfadiazine) and PBS (Toxoplasma group) every two days for 10 days. Serum of infected mice were collected, and the cytokines levels were detected using a microplate reader. Cytokines levels were expressed as ng/L or pg/mL. Three independent experiments were performed, and data are presented as the means ± S.D. Significantly different from the negative control (Compared with Toxoplasma group, **$p \leq 0.01$ *$p \leq 0.05$).