Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading

Great efficacy of sulfachloropyrazine-sodium against acute murine toxoplasmosis

Yan-Bo Zeng, Shun-Hai Zhu, Hui Dong, Hong-Yu Han, Lian-Lian Jiang, Quan Wang, Jun Cheng, Qi-Ping Zhao, Wei-Jiao Ma, Bing Huang*

Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Science, Key Laboratory for Animal Parasitology of Ministry of Agriculture, Shanghai 200241

ARTICLE INFO

Article history:
Received 15 May 2011
Received in revised form 27 June 2011
Accepted 15 July 2011
Available online 28 January 2012

Keywords:
Toxoplasmosis
Sulfonamides
Sulfachloropyrazine-sodium
Efficacy
Therapy
Toxoplasma gondii
Murine
Therapeutic effect

ABSTRACT

Objective: To identify more effective and less toxic drugs to treat animal toxoplasmosis. **Methods:** Efficacy of seven kinds of sulfonamides against *Toxoplasma gondii* (*T. gondii*) in an acute murine model was evaluated. The mice used throughout the study were randomly assigned to many groups (10 mice each), which either remained uninfected or were infected intraperitoneally with tachyzoites of *T. gondii* (strains RH and CN). All groups were then treated with different sulfonamides and the optimal treatment protocol was determined candidates. Sulfadiazine-sodium (SD) was used for comparison. **Results:** The optimal therapy involved gavaging mice twice per day with 250 mg/kg bw of sulfachloropyrazine-sodium (SPZ) for five days. Using this protocol, the average survival time and the time-point of 50% fatalities were prolonged significantly compared with SD treatment. Treatment with SPZ protected 40% of mice from death, and the heart and kidney tissue of these animals was parasite-free, as determined by nested-PCR. SPZ showed excellent therapeutic effects in the treatment of *T. gondii* in an acute murine model and is therefore a promising drug candidate for the treatment and prevention of *T. gondii* in animals. **Conclusions:** It can be concluded that the effective drug sulfachloropyrazine may be the new therapeutic options against animal toxoplasmosis.

1. Introduction

Toxoplasma gondii (*T. gondii*) has been identified as a major opportunistic pathogen in immunocompromised individuals (*e.g.*, patients with AIDS or organ transplant recipients) and neonates. Infection can result in encephalitis, chorioretinitis or congenital transmission if a seronegative pregnant women is infected[1,2]. *T. gondii* is an obligate intracellular protozoan pathogen with worldwide distribution that can infect almost all warmed-blooded animals.

Other members of the same phylum, Apicomplexa, include the human pathogens *Plasmodium* and *Cryptosporidium*, and the animal pathogens *Eimeria* and *Sarcocystis*[3-5]. *T. gondii* has become a model organism for the study of the Apicomplexa, as it is the most experimentally tractable organism in this important group of intracellular parasites that includes *Plasmodium*, *Eimeria*, *Cryptosporidium*, *Neospora*, and *Theileria*. Felids are the key animal species in the life cycle of this parasite because they are the definitive hosts that can excrete the environmentally-resistant stage, the oocyst[6]. According to the serological investigations, about 30% of the human population is infected with this parasite[5]. A recent serosurvey using samples from the population-based National Health and Examination Nutrition Study found a decrease in the age-adjusted *T. gondii* prevalence in USA-born persons, of 12-49 years of age, from 14.1% in 1988-1994 to 9% in 1999-2004[6,7]. In the mainland of China, the rate of human infection with *T. gondii* has been reported to be 7.88% nationwide, as detected by ELISA[8].

At present, there are no non-infective commercial vaccines to prevent clinical toxoplasmosis in humans and animals[9,10]. Research into potential drug treatments have excluded many antibiotics, synthetic drugs and traditional Chinese medicines as being ineffective against the *T. gondii*. Sulfonamides are currently considered as the best therapeutic option for *T. gondii* infection. Sulfonamides

*Corresponding author: Bing Huang, Professor, Key Laboratory for Animal Parasitology of Ministry of Agriculture, Shanghai Veterinary Research Institute, Chinese Academic of Agricultural Science.
Tel: +86 21 3429 3464
Fax: +86 21 5408 1818
E-mail: hb@shvri.ac.cn

Foundation Project: Supported by a grant from The National Special Research Program for Non-Profit Trades (Agriculture, China) (grant No. 200803017).

are a class of broad spectrum synthetic antibiotics that are effective against many kinds of microorganisms and have been used clinically for over 60 years[10]. The sulfonamides that have been used against *T. gondii* include the sulfonamides, sulfamethoxazole, sulfanilanilides, sulfathiazole, sulfisoxazine and sulfadiazine[11-13]. The synergistic combination of sulfadiazine-sodium (SD) and pyrimethamine is currently considered to be the most effective first-line antitoxoplasma therapy, and is also used as a standard control therapy. This type of therapy has been used after the diagnosis of prenatal infection[2]. However, these drugs are not always efficacious and often cannot be tolerated due to severe toxic side effects[14]. Therapy is hampered by hematologic toxicity and allergic reactions in 5%-15% of patients, and some patients require continuous treatment[15,16]. Furthermore, some patients are intolerant of one of these regimens and require alternative therapies[17].

Few medications are currently available to treat animal toxoplasmosis and those that are available are limited due to poor efficacy. There is therefore an urgent need for new, effective drugs to treat *T. gondii*, particularly in livestock and other warm-blooded animals. In this study, a variety of candidate sulfonamides, some of which are widely used as human and animal antibiotics[18] were used to treat *T. gondii* in an acute murine infection model and the potency and safety of these sulfonamides against infection were evaluated.

2. Materials and methods

2.1. Animals

Outbred, strain Kunming (KM), female mice (Slack Shanghai Laboratory Animal Co., Ltd., China), weighing between 20 and 24 g, were used in each experiment. The animals were acclimatized for two days, then maintained in a suitable rearing environment with free access to water and rodent food throughout the experiment[19].

2.2. Parasites

Tachyzoites of *T. gondii* of the swine strain, Changning (CN), and the human strain RH, were propagated in our laboratory (Shanghai Veterinary Research Institute, CAAS, China). Highly virulent tachyzoites were maintained in the peritoneal cavities by propagating every three or four days in KM mice, three times. The harvested tachyzoites were resuspended in physiological saline and each experimental mouse was inoculated intraperitoneally with 5×10^3 organisms (in 0.2 mL).

2.3. Drugs

Sulfadiazine-sodium (SD, minimum purity 99.53%), sulfadimidine-sodium (SDD, 99.55%), suflamathoxydiazine-sodium (SMD, 99.23%) and trimethoprimlactate (TMP, 99.55%) were obtained from Yanshi Shuoda Pharmaceutical & Co., Ltd. China. Sulfamathoxypyridazine-sodium (SMP, 99.50%), sulfachlorpryridazine-sodium (SPDZ, 99.81%) and sulfachloropyrazine-sodium (SPZ, 99.97%) were produced by Shanghai Baoshan Zhenzong Biochemistry Engineering Factory, Henan, China.

All agents were provided in the powder form of the pharmaceutical raw materials (containing minimum purity), and were dissolved in physiological saline. Then the mixtures were sonicated to produce a smooth dispersion and the desired concentration was prepared daily in physiological saline and administered to mice at a dosage of 250 mg/kg/day, according to the animal's body weight, for 5 or 10 days, 24 h after infection.

2.4. Evaluating the efficacy of sulfonamides

Ninety mice were randomly allocated into nine groups (10 mice each) designated G1-G9. Eight of these groups (G1-G8) were infected with RH strains, the other group (G9) remained uninfected. Each drug was dissolved in physiological saline and prepared daily as a liquid suspension. 24 h after intraperitoneal infection, the G1-G7 mice were administered each drug intragastrically daily for five days using a 16-gauge blunt feeding needle. Mice from the untreated group (G8) and the uninfected group (G9) were administered physiological saline intragastrically daily. Over the course of the experiment (30 days), mice were observed daily and the mortality rate was recorded.

2.5. Different methods of drug administration

Ninety mice were randomized into nine groups (G1-G9). All mice were intraperitoneally infected with RH strains, except for group G5 and G8 mice who were infected with the CN strain as a control, and G9 mice remained uninfected. After 24 h, the mice were administered orally the specified drugs. G1-G5 mice were administered the SPZ at the same dosage via different methods: G1 mice received SPZ by drinking water (100 mL) daily for five days, G2 mice by gavage once per day for ten days, G3 mice by gavage twice a day (morning and night) for five days, G4 mice by gavage once a day for five days, G5 mice by gavage once a day for 10 days. G6 mice received SD by gavage once a day for 10 days as a control. G7 and G8 mice received physiological saline solution as infected untreated controls. G9 mice remained uninfected and received physiological saline solution. All mice were observed and their condition was recorded twice daily. The experiment continued for 65 days.

2.6. Nested-PCR detection

At the end of the 65-day experiment described above, live mice were euthanized and the viscera organs and brain tissue were excised. These tissues were then digested in trypsin overnight at 37 ℃, and genomic DNA was extracted using the Resin-based™ Genomic DNA Extraction Kit (Shanghai SBS Genetech Co., Ltd., China). The presence of parasites was then detected by nested-PCR, using the extracted genomic DNA as a template, to estimate the effectiveness of the treatment.

For specific *T. gondii* detection, nested-PCR was performed by two rounds of PCR with two pairs of primers targeting a 433 bp fragment of the ITS1-5.8S rRNA-ITS2 gene. The primers included: an external forward primer: