Veterinary Parasitology 187 (2012) 99–104

Contents lists available at SciVerse ScienceDirect

Veterinary Parasitology

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

Neospora caninum: *In vivo* and *in vitro* treatment with artemisone

Monica L. Mazuz[a], Richard Haynes[b], Varda Shkap[a], Leah Fish[a], Ricardo Wollkomirsky[a], Benjamin Leibovich[a], Thea Molad[a], Igor Savitsky[a], Jacob Golenser[c,d,*]

[a] *Division of Parasitology, Kimron Veterinary Institute, Bet Dagan 50250, Israel*
[b] *Department of Chemistry, Institute of Molecular Technology for Drug Discovery and Synthesis, The Hong Kong University of Science and Technology, Hong Kong*
[c] *Department of Microbiology and Molecular Genetics, The Kuvin Centre for the Study of Infectious and Tropical Diseases, The Hebrew University of Jerusalem, Jerusalem, Israel*
[d] *Department of Pathology and Bosch Institute, The University of Sydney, Sydney, Australia*

ARTICLE INFO

Article history:
Received 4 September 2011
Received in revised form 8 December 2011
Accepted 16 December 2011

Keywords:
Neospora caninum
Treatment
Artemisinin
Artemisone
Gerbil

ABSTRACT

Neosporosis caused by *Neospora caninum* has global economic, clinical, and epidemiological impacts, mainly in the cattle industry. Currently, there is no useful drug for treatment of neosporosis. This publication is the first to describe the significant benefits that artemisone has on *Neospora* infections both *in vitro* and *in vivo*.

Artemisone is a new semi-synthetic 10-alkylamino artemisinin that is superior to other artemisinin derivatives in terms of its significantly higher antimalarial activity, its tolerance *in vivo*, lack of detectable neurotoxic potential, improved *in vivo* pharmacokinetics and metabolic stability. Low micromolar concentrations of artemisone inhibited *in vitro Neospora* development. Prophylactic and post-infection treatment profoundly reduced the number of infected cells and parasites per cell. In the *in vivo* gerbil model, a non-toxic dose prevented typical cerebral symptoms, in most animals. There were no signs of clinical symptoms and brain PCR was negative. Most treated gerbils produced high specific antibody titer and were protected against a challenge. Overall, artemisone could be considered as a future drug for neosporosis.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Neospora caninum is an obligate intracellular apicomplexan protozoan parasite of worldwide distribution that infects a wide range of livestock and domestic animals, including cattle, dogs, sheep, goats, horses and others. *N. caninum* is recognized as one of the major causes of abortion, stillbirth and reproductive failure in cattle (Anderson et al., 2000; Innes et al., 2000; Dubey et al., 2007). Cattle can be infected horizontally by ingestion of sporulated oocysts excreted by the definitive host (dogs and related canids), or vertically (congenital infection) during pregnancy (Dubey et al., 2007; López-Pérez et al., 2010). The vertical transmission is highly efficient; up to 95% of calves from positive dams are born infected (Dubey et al., 2007). In infected cows the infection persists for life and so also are their offspring in consecutive pregnancies (Fioretti et al., 2003).

The economic impact of the loss of livestock due to bovine neosporosis is estimated to be in the order of hundreds of millions of $US (Trees et al., 1999; Reichel, 2000; Dubey et al., 2007). In dairy farms in Israel more than 45% of dams were carrying specific *N. caninum* antibodies and 18% of abortions were due to *N. caninum*, as confirmed by serological and molecular assays on aborted fetuses (Fish et al., 2007; Mazuz et al., 2011).

Although the parasites cause serious economic losses in the dairy and beef cattle industries, little information is available on the efficacy of therapeutic drugs that can be

* Corresponding author at: Department of Microbiology and Molecular Genetics, The Hebrew University of Jerusalem, Jerusalem, Israel. Tel.: +972 26758090; fax: +972 26757425.
E-mail address: golenser@md.huji.ac.il (J. Golenser).

0304-4017/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2011.12.020

used to treat neosporosis. Some coccidiostatic compounds, sulfonamides, anti-folates and antibiotics are active *in vitro* against certain stages of the parasite (Lindsay et al., 1994). Ponazuril (toltrazuril sulfone), a metabolite of toltrazuril, displays significant efficacy against *N. caninum* infection in mice and cattle (Kritzner et al., 2002; Strohbusch et al., 2009), and in taxonomically related *Toxoplasma gondii* and *Sarcocystis neurona* (Mitchell et al., 2003; Furr et al., 2001). However, the drug is relatively toxic and is not recommended for use, especially given the absence of knowledge concerning public health associated with residues in milk and meat products (Dubey and Schares, 2011). Therefore, treatment of cattle with these drugs appears not to be practical. Use in prophylaxis would require long- or life-long treatment, that would result in unacceptable level of residues in products, that in turn would require extended withdrawal periods prior to slaughter (Reichel and Ellis, 2002; Dubey et al., 2007; Dubey and Schares, 2011). An effect against *N. caninum* has been demonstrated *in vitro* by artemisinin (Kim et al., 2002). However, although artemisinin (Dunay et al., 2009) is still clinically used in China for treatment of the apicomplexan parasite *Plasmodium*, it does possess lower efficacy than other artemisinin derivatives *in vivo* and there are potential toxicity issues associated with its use (Schmuck et al., 2002, 2009).

In this study we examined the effect of artemisone (Dunay et al., 2009) on *N. caninum* infection in gerbils, a susceptible appropriate model for acute neosporosis (Ramamoorthy et al., 2005). Artemisone is a recent artemisinin derivative, which was found highly effective against *Plasmodium falciparum* (Haynes et al., 2006; Waknine-Grinberg et al., 2010), and *Toxoplasma gondii* (Dunay et al., 2009) that is more closely related to *N. caninum*. Artemisone is highly effective against *P. falciparum* in monkeys and has been used in Phase IIa clinical trial for non-severe malaria in humans (Nagelschmitz et al., 2008).

2. Materials and methods

2.1. Cell culture and parasites

Vero cells were cultured in Leibovitz-15/McCoy medium (at 1:1 ratio), supplemented with 10% inactivated newborn calf serum (Bet Haemek, Israel), penicillin (200 IU/ml), streptomycin (100 μg/ml) and mycostatin (75 IU/ml) (BioLab, Israel). The cultures were kept at 37 °C under 5% CO_2 grown in 25 cm^2 tissue culture flasks (Shkap et al., 1991). The medium was replaced after 24–48 h by a maintenance media consisting of the same commercial media with 2% inactivated newborn calf serum. Subcultures of the Vero cells (1:4) were performed every 5 days using trypsin (0.25%)/EDTA (0.02%) solution for cell dissociation. *N. caninum* tachyzoites (NcIS491) from an Israeli isolate (Fish et al., 2007) were propagated in Vero cell monolayer culture as described (Shkap et al., 1991). Free parasites were collected from media of heavily infected Vero cells into which the parasites were released. The medium was passed through 25-gauge needle followed by centrifugation at $70 \times g$ for 5 min to remove cell debris. The supernatant containing the parasites was centrifuged at $900 \times g$ for 20 min, the pelleted parasites were resuspended in PBS and counted in a Neubauer haematocytometer.

2.2. In vitro assays

Vero cells were seeded into Leighton tubes with cover slips at concentration of 6×10^5 cells/tube, in duplicates. Four hours after the cells were seeded they were infected with 6.0×10^5 freshly prepared parasites semi-purified from the host cells. Artemisone was dissolved in DMSO (final concentration of DMSO in media was <0.2%) and added to the cultures at concentrations shown below. The medium containing the drug, or DMSO in control cultures was changed daily. Cultures grown on glass cover slips were fixed in methanol and stained with 10% Giemsa. The efficacy of the drug on the parasite growth rate at various concentrations was evaluated under light microscopy by counting of infected cells and the number of parasites per cell. A total of at least 1000 cells for each concentration were recorded. The percent inhibition of the parasite growth was calculated as follows: [(% of control infected cells − % of treated infected cells)/(% of control infected cells)] × 100.

The effect of artemisone was evaluated as follows: (i) artemisone at 0.1, 0.5, 5.0, 10.0 or 15.0 μg/ml was added to the medium 5 h prior to infection and daily upon changing the growth medium; (ii) artemisone at concentration of 25.0 or 50.0 μg/ml was added to the medium 24 h after infection.

2.3. Serology

Serum was collected by bleeding of gerbils from the retro orbital sinus under ketamine-xylazine anesthesia. The blood collected into 1.5 ml tubes was allowed to clot at room temperature and centrifuged at $5000 \times g$ for 10 min. The serum was stored at 20 °C until use. The presence of antibodies to *N. caninum* was examined by indirect fluorescent antibody test (IFAT) as previously described (Shkap et al., 2002).

2.4. In vivo treatment

All animal experimentations were approved by the Animal Welfare Committee of the Kimron Veterinary Institute. Eighteen gerbils (*Meriones tristrami*), seronegative for *N. caninum* by IFA were randomly divided to artemisone-treated and control (non-treated) groups and infected intraperitoneally (i.p.) with 10^6 parasites. The treated group was injected i.p. twice a day for 4 consecutive days with 20 mg/kg artemisone diluted in DMSO (in a total volume of 40 μl). The animals were monitored daily for clinical signs described for neosporosis in gerbils. In the surviving animals antibodies were examined monthly by IFA. PCR analyses of brains were performed upon post-mortem. Surviving animals were challenged with 10^7 parasites, 45 days post-infection (40 days after the end of the treatment), together with two *N. caninum* seronegative gerbils of the same gerbil group.

Download English Version:

https://daneshyari.com/en/article/2470127

Download Persian Version:

https://daneshyari.com/article/2470127

Daneshyari.com