Veterinary Parasitology 159 (2009) 249–252

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Trichinella spiralis—A potential anti-tumor agent

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ARTICLE INFO

Keywords:
Trichinella spiralis
Anti-tumor
Anti-proliferation
Apoptosis

ABSTRACT

Murine forestomach carcinoma (cell line MFC), ascitic hepatoma (cell line H22) and sarcoma (cell line S180) solid tumor models were used to test the anti-tumor effect of *Trichinella spiralis in vivo*. Mice previously infected by oral administration of 400 viable *T. spiralis* larvae per mouse for 7 days were grafted with various solid tumor cell lines. Other groups of tumor-bearing mice were given caudal vein injection of crude extracts of adult and newborn larvae at 17.5, 35.0 or 70.0 mg kg^{-1}. These treatments to inhibit tumor growth were dose-dependent ($p < 0.05$). The anti-proliferative activity of crude *T. spiralis* extract was examined *in vitro* at 0.035, 0.070 or 0.140 mg ml^{-1} using MFC, H22, S180, human chronic myeloid leukemia cell line (K562) and hepatoma cell line (H7402), tumor cell proliferation *in vitro* was measured by methyl thiazolium stain and was inhibited in dose-dependent manner ($p < 0.05$). At the same doses, crude *T. spiralis* extracts induced apoptosis of K562 and H7402 as detected by DNA fragmentation. Cell cycle analysis indicated that crude *T. spiralis* extracts, at 0.140 mg ml^{-1}, arrested the cell cycle of K562 and H7402 in G1 or S phase. It is concluded that *T. spiralis* contains anti-tumor active agent.

1. Introduction

Co-infection with different pathogens is a common occurrence that can alter the progression of disease (Furze et al., 2006). Helminths have been experimentally associated with protection against a number of autoimmune disorders and allergic airway inflammation (Saunders et al., 2007). *Trichinella spiralis* has a powerful cytotoxic effect on R1 leukemic cells, syngeneic E1-4 tumor cells, and allogeneic P815 tumor cells (Pocock and Meerovitch, 1982). *Trichinella spiralis* infection activates immunologically competent cells to produce cytokines that can inhibit tumor growth. The mechanism of action may include the role of c-Ski, an oncoprotein, in cooperation with transforming growth factor beta (TGF-beta) signaling pathway genes (TGF-beta, Smad2 and Smad4). A recent study provided evidence that the expression of c-Ski protein is increased as a tumor suppressor, and involved in nurse cell formation through the TGF-beta signaling pathway process in the host cell nucleus during co-infection with *T. spiralis* (Wu et al., 2006). Most studies on the anti-tumor mechanism of *T. spiralis* have focused on the immune system. Other possible anti-tumor effects have not been widely explored. This lack of information has been an impediment to its clinical application.

In the present study, a spectrum of five tumor cancer cell lines was used to examine the range of anti-tumor activities of *T. spiralis in vivo*, and to obtain insights into the effects of crude *T. spiralis* extract on cell proliferation, cell cycle distribution, and apoptosis *in vitro*.

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doi:10.1016/j.vetpar.2008.10.052

2. Material and methods

2.1. Parasite and crude extract

Muscle larvae (ML), adult worms, and newborn larvae of *T. spiralis* (ISS 534) were prepared according to Fu et al. (2005). A mixture of adult worms and newborn larvae was homogenized and centrifuged at 10,000 × *g* for 5 min. The supernatant was concentrated (contain protein 1.40 mg ml^{-1}) and stored at −20 °C.

2.2. Tumor cell lines and model mice

Murine forestomach carcinoma (MFC), murine ascitic hepatoma (H22), murine sarcoma (S180), human chronic myeloid leukemia (K562), human hepatoma (H7402) cell lines and Institute of Cancer Research (ICR) mice were used as tumor models, and were provided by the Bethune Basic Medicine Faculty of Jilin University. The cells and tumor model were prepared according to the method of Luciani et al. (2004). Mice received proper care and maintenance in accordance with institutional guidelines.

2.3. Evaluation of anti-tumor activity in vivo

The mice were sub-grouped (10 mice per group) at random, and infected orally with 400 viable *T. spiralis* ML/ per mouse for 7 days, and then grafted with the MFC, H22 or S180 solid tumor lines. Other groups of tumor-bearing mice were administered *T. spiralis* adult and newborn larvae crude extract in dosages of 17.5, 35.0 or 70.0 mg kg^{-1} daily by caudal vein. Parallel control groups were treated with calf serum (CS). All tumor-bearing mice were observed for 7 days after treatment. Mice were sacrificed according to procedures approved by the Welfare of Animals in Experimental Neoplasia of Jilin University. Tumors were excised and the average tumor weight was calculated. Tumor growth inhibition ratio (%) was calculated in according with Bezerra et al. (2006).

2.4. Cell proliferation assays

The proliferation of five cell lines was measured by methyl thiazolyl tetrazolium (MTT, Sigma, USA). Cells were plated in flat-bottomed, 96 well microtiter plates (1×10^6 cells per 64 mm diameter well). After 24 h, cells were treated with doses of crude *T. spiralis* extract containing total protein concentration 0.035, 0.070 or 0.140 mg ml^{-1}. Control groups were treated with equal volumes of CS. After 24 h of treatment, cells were treated as described by Xiao et al. (2003). Optical density value (OD) was measured using a spectrophotometric microtiter plate reader (ELX808^{TM}, Bio-Tek, USA) at 494 nm wavelength. The inhibition rate (IR) was calculated according to Ruggeri et al. (2003).

2.5. DNA fragmentation

K562 and H7402 cells treated with crude *T. spiralis* extract or CS (as described above) were collected and low molecular weight DNA was extracted using the cell apoptosis DNA ladder extraction kit (Beijing Dingguo Biotechnology Co., Ltd.), electrophoresed in 1% agarose gel containing 0.1% ethidium bromide (Ujibe et al., 2005) and photographed using a UVP imaging system (UVP, USA).

2.6. Cell cycle distribution analysis

K562 and H7402 cell lines were plated in culture dishes at concentrations determined to yield 60–70% confluence within 24 h. Cells were treated with crude *T. spiralis* extract (0.140 mg ml^{-1}). After 8 h, both adherent and floating cells were harvested. The cells were labeled with propidium iodide (PI, Sigma, USA) using methods described by Yu et al. (2005). DNA content was analyzed using a fluorescence activated cell sorter (FACS, Becton Dickinson, USA) equipped with Cell Quest software. All experiments were performed in duplicate.

2.7. Statistical analysis

Data was expressed as mean ± SD. Comparisons between the control group and crude *T. spiralis* extract-treated group were performed by SPSS 10.0 software (SPSS Inc., Chicago, IL), using the Paired-Samples *T* test. Differences between groups were compared using One-Way ANOVA, $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Evaluation of anti-tumor activity in vivo

Oral administration of *T. spiralis* larvae or intravenous injection of crude *T. spiralis* extract significantly inhibited the growth of MFC, H22 and S180 tumors in ICR mice ($p < 0.05$). When larvae were administered as a single dose, MFC, H22 and S180 tumors were inhibited by 59 ± 24%, 75 ± 14%, and 78 ± 18%, respectively ($p < 0.05$). The extracts were administered daily for 7 days and produced a significant dose-dependent inhibition of growth, compared with growth in untreated mice treated with CS ($p < 0.05$; Fig. 1). Both treatments were initiated 7 days after tumor injection.

3.2. Effect of crude T. spiralis extract on cell proliferation

To examine the anti-tumor activity of crude *T. spiralis* extract on the growth of MFC, H22, S180, K562 and H7402 cell lines, cell proliferation was measured in cultures containing increasing concentrations of crude *T. spiralis* extract (Fig. 2). There was significant dose-dependent growth inhibition of cells treated with crude *T. spiralis* extract for 24 h.

3.3. Detection of DNA fragmentation in K562 and H7402 cells treated with crude T. spiralis extract

As shown in Fig. 3, treatment with crude *T. spiralis* extract (containing total protein as described above) resulted in degradation of chromosomal DNA into small inter-nucleosomal fragments, evidenced by the formation of a DNA ladder on agarose gels, a hallmark of cells undergoing apoptosis. No DNA ladder was detected in the