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Toxoplasma gondii but Not *Leishmania major* or *Trichomonas vaginalis* Decreases Cell Proliferation and Increases Cell Death on Fibrosarcoma Cancer Cells in Culture Medium

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ABSTRACT

It has been shown that some parasites interfere with malignant tumor cells growth *in vivo* or *in vitro*. In this work anticancer activity of three live protozoan parasites; *Toxoplasma gondii*, *Trichomonas vaginalis* and *Leishmania major* on fibrosarcoma cells growth has been investigated in cell culture medium. In this experimental study, WEHI-164 fibrosarcoma cells treated with alive *Toxoplasma gondii* tachyzoite, *Trichomonas vaginalis* trophozoite or *Leishmania major* promastigote as case groups or left intact as control groups. Following 24 hours incubation the number of cells, lactate dehydrogenase (LDH) and apoptosis were determined in case and control groups. *Toxoplasma gondii* tachyzoite decreased cell proliferation and increased cell lyses' but it did not induce apoptosis. *Trichomonas vaginalis* or *Leishmania major* didn't show any effects on cell proliferation, cell lyses or apoptosis. Therefore *Toxoplasma gondii* may have anticancer activity and further works is recommended to understand the mechanisms of action.

Keywords: *Toxoplasma gondii*; *Trichomonas vaginalis*; *Leishmania major*; Fibrosarcoma

1. Introduction

It has been shown that some parasitic infections induce antitumor activity against certain types of cancers [1-6]. We previously showed that mice immunized with *Toxoplasma gondii* tachyzoiter or *Toxocara canis* egg antigens and challenged with fibrosarcoma cells showed less solid tumor growth in comparison with mice that were not immunized but challenged with the same cells [7]. Also apoptosis induction or inhibition of proliferation of tumor cells has been shown by some parasites [8]. These findings are in agreement with the hygiene theory [9-11]. Moreover effects of some other substances such as diphosphate [12], Parazosin [13] and dopamin [14] on malignant cells proliferation have been demonstrated. In this work in order to explore anticancer activity of parasites further, in cell culture the effects of *Toxoplasma gondii* and two other protozoan parasites; *Trichomonas vaginalis* and *Leishmania major*; on proliferation, apoptosis and death of mouse fibrosarcoma cells have been investigated.

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2. Materials and Methods

2.1. Parasites

Toxoplasma tachyzoites harvested from experimentally infected mice, *Trichomonas vaginalis* was isoloated from patients referred to gynecology and midwifery clinic in Shahrekord and maintained in TYIS medium. *Leishmania major* was isolated from skin sore of patient's referred to cutaneous leishmaniasis clinic in Isfahan, Iran and maintained in NNN medium.

2.2. Culture of Tumour Cells

WEHI-164, Balb/C mouse fibrosarcoma cells provided by Pasture Institute, Tehran, Iran and cultured in Dulbecco's Modification of Eagles Medium (DMEM) purchased from Sigma company and was supplemented with 20 mm HEPES 0.2 mM L-glutamine, 50 μM 2-mercaptoethanol 0.15% sodium bicarbonate, 50 μg/ml gen-

tamicin and 10% fetal calf serum (FCS) as reported previously [15]. Viability of tumor cells was checked by trypan blue staining.

2.3. Lactate Dehydrogenase Assay (LDH)

The *in vitro* lysis of WEHI-164 tumor cells by parasites was assayed by LDH-release as reported previously [16]. Briefly 100,000 WEHI-164 cells were incubated with each appropriate parasite at 37˚C in 5% CO_2 and 90% relative humidity. At appropriate time points in the assay, the cells were centrifuged and 50 µl of supernatant was harvested and transferred to a flat bottomed 96-well plate. LDH-release determined using a kit according to manufacturer's instructions. LDH-release measurements were calculated by spectrophotometery. The controls were WEHI-164 tumor cells culture without parasite treatment.

2.4. Apoptosis Study

Apoptosis of tumor cells was detected according to previous study [16]. Briefly, 1×10^5 WEHI-164 cells pre incubated with appropriate parasite for 24 hours. The cells were harvested and suspended in 50 µl media and subjected to mild hypotonic treatment with 50 µl of 50% DH_2O in tissue culture media for 10 min before fixing with 100 µl of 25% acetic acid in methanol for 10 min. Prepared cytospots were stained with 0.1 µg/ml Hoechst 33258 (Sigma) for 10 min in dark, washed with water and dried. Specimens were viewed under 50% glycerol in PBS immersion by fluorescence microscopy using a UG-1 excitation filter and UV-absorbing barrier filter.

2.5. Experiments Designs

In each experiment six culture flasks containing 10 ml culture medium were prepared and 100,000 fresh, alive WEHI-164 cells were added to every flask. Flasks numbers 1 - 3 treated with 1000 live *Toxoplasma gondii* tachysoite, *Leishmarnia majo* promastigote or *Trichomonas vaginalis* protoscolices as case groups and flasks 4-6 left intact as control groups. All flasks were incubated overnight at 37˚C and 90% humidity and examined after 24 hr. Tumor cell proliferation was calculated by counting the number of cells in 1 ml of suspended tumor cells of culture mediums. Each experiment was performed in triplicate. Kruskal Wallis and Down statistical tests used to compare the means of data between case and control groups.

3. Results

Effect of Toxoplasma tachyzoites on proliferation, apoptosis or death of fibrosarcoma WEHI-164 cells (case group) in comparison with the same cells without parasite treatment (control group) were investigated *in-vitro*. This parasite inhibited cell proliferation and caused cell lyses but it did not induce apoptosis. Results of cell count and LDH estimation presented in **Table 1** and **Figures 1** and **2** respectively.

In other experiments the effects of *Trichomonas vaginalis* or *Leishmania major* on proliferation, apoptosis or death of fibrosarcoma WEHI-164 cells (case group) in comparison with the same cells without parasite treatment (control group) were investigated *in-vitro*. These

Table 1. The number of dead or alive cells and LDH estimation (unit/litter) in flasks with WEHI-164 fibrosarcoma cells treated with *Toxoplasma gondii* tachyzoite (case groups) or left intact (control groups).

Cells or LDH	Case groups mean	Control groups mean	P
Number of alive cells	82,500	420,000	0.046
Number of dead cells	210,000	82,500	0.046
LDH estimation (U/L)	130.3	89.83	0.049

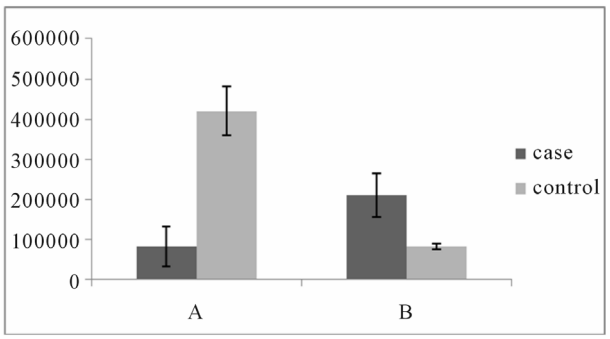

Figure 1. Mean and standard Error of alive (A) or dead (B) cells in flasks with WEHI-164 fibrosarcoma cells treated with *Toxoplasma gondii* tachyzoite (case group) or left intact (control group).

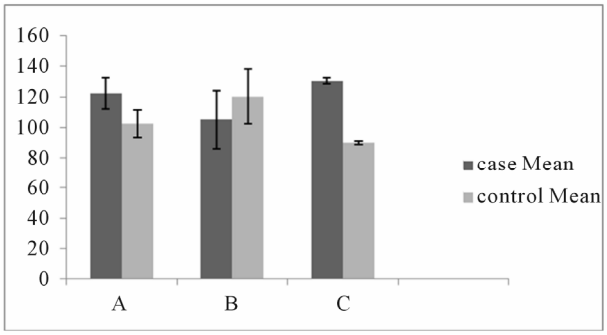

Figure 2. LDH estimation in flasks with WEHI-164 fibrosarcoma cells treated with *Trichomonas vaginalis* (A), *Leishmania major* (B) or *Toxoplasma gondii* (C) as case groups or left intact as control groups.

Table 2. The number of dead or alive cells and LDH estimation (unit/litter) in flasks with WEHI-164 fibrosarcoma cells treated with *Trichmonas vaginalis* or *Leishmania major* (case groups) or left intact (control groups).

	Leishmania major		*Trichomonas vaginalis*	
	Mean case flasks	Mean control flasks	Mean case flasks	Mean control flasks
Number of alive cells	313333.3	243333.3	872,000	800,000
Number of dead cells	47333.3	41666.6	86666.6	33333.3
LDH estimation (U/l)	105.166	120.33	122.5	102.66

two parasites didn't show any affect on cell proliferation, cell death or apoptosis. Results of cell count and LDH estimation presented in **Table 2** and **Figure 2** respectively.

4. Discussion

Results of this investigation revealed that *Toxoplasma gondii* tachyzoite but not *Trichomonas vaginalis* trophozoite or *Leishmania major* promastigote decreased fibrosarcoma WEHI-164 cells proliferation and increased cell lyses in cell culture medium. There are scientific evidences indicating that some parasitic and microbial infections interfere with tumor growth and have anticancer activities [1-8] , and it has been shown that some extracts of *Trypanosoma cruzi* have toxic effects on cancer cells in cell cultures [1,2]. Kim *et al.* (2007) showed that *Toxoplasma gondii* inhibited Lewis lung carcinoma growth through induction of Th1 immune responses and inhibition of angiogenesis [3]. This result is in agreement with our findings. Although both *Toxoplasma gondii* and *Leishmania major* are intracellular parasites they treat fibrosarcoma cells differently. Further works is recommended to understand different behaviors of these to protozoa on fibrosarcoma cells.

In this work none of parasite induced apoptosis, this finding is in agreement with previous works indicating that *Toxoplasma gondii* inhibits apoptosis induction in host cells [17,18]. Conversely induction of apoptosis by this parasite is reported by Zhang *et al.* [8]. How *Toxoplasma gondii* tachyzoite inhibits tumor cells proliferation needs to be discovered by further investigations.

Sayed *et al.* (2002) Showed that high relative risk of cervical cancer was associated with *Trichomonas vaginalis* infection [19]. However, our results revealed that this parasite had no effect on fibrosarcoma cells growth.

It seems that no publication is available about anticancer activity of *Leishmania major* parasite. However it has been shown that anticancer drugs have anti-leishmania activity [20]. Result s of our work also showed that *Leishmania major* parasite didn't influence fibrosarcoma growth.

5. Conclusion

Results of this investigation revealed that *Toxoplasma gondii* but not *Leishmania major or Trichomonas vaginalis* decreases cell proliferation and increases cell death on fibrosarcoma cells. However many more experiments would be performed to get strict findings.

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