

Effect of *Toxoplasma gondii* on colon cancer growth in mouse model

Farideh Sadat Esbah Zavareh¹, Mahboubeh Hadiipour², Reza Kalantari², Somayeh Mousavi², Negah Tavakolifard³, Hossein Yousofi Darani²

Abstract

Despite all advances in cancer treatment methods, failure of treatment is a major concern. This failure can be caused by *tumor environment* made by tumor cells and prevents immune system to reach neoplastic cells. So, cancer immunotherapy and target therapy are in the focus of scientists. Due to the inverse relationship shown between parasites and cancer, parasites are a candidate for use in cancer immunotherapy. *Toxoplasma gondii* is an intracellular parasite invades many cells of vertebrate species but make symptoms only in fetus and immuno-deficient person. Studies have shown *T. gondii* can stimulate immune system against neoplastic cells and break fort of *tumor environment*. In this experimental work, Colon cancer bearing mice randomly divided into three groups. Groups 1 and 2 were injected with either lysate or irradiated tachyzoite of *T. gondii* respectively. The third group were left intact as control group. Our resulted data showed that in irradiated tachyzoite or lysate treated groups there was a significant reduction in tumor growth in comparison with control group. However, the difference in survival time was not statistically significant. In conclusion, treatment with *T. gondii* antigens resulted in suppression of tumor growth.

Keywords: *Toxoplasma gondii*, Cancer, Immunotherapy, Tumor, Mouse model

*Corresponding author email: yousofidarani@gmail.com

¹Medical student, Isfahan medical school, Isfahan University of Medical Sciences, Isfahan, Iran

²Department of Parasitology and Mycology, Isfahan medical school, Isfahan University of Medical Sciences, Isfahan, Iran

³Community and family medicine group, Medical school, Isfahan University of Medical Sciences, Isfahan, Iran

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Introduction

Toxoplasma gondii (*T. gondii*) is an obligate parasite which can invades many of nucleated cells of vertebrate species. *T. gondii* infection in healthy people usually doesn't cause symptoms, but it can lead to serious complication in fetus and immuno-deficient individuals [1]. *T.gondii* has three infectious stage including; 1- *tachyzoites* that multiply in any cell of the intermediate host -like

human- and in non-intestinal cells of the definitive host. 2- *Bradyzoites*, in this form *T. gondii* remains in tissue and multiplies slowly in tissue cyst by endodyogeny replication. 3- oocytes containing *sporozoites* which excreted through cat feces [2].

In the few last years, some researchers have shown adverse relationship between parasite and cancers [3]. On the other hand, cancers mortality and morbidity are becoming a major concern for our world [4]. Cancers develop when immune system fails to control tumors. Neoplastic cells create tumor environment, which immune system can't attack tumor and is forced to tolerate them [5]. As a result, many therapeutic strategies such as surgery, radiotherapy, chemotherapy have failed and have not achieved their goals. So, nowadays cancer immunotherapy is used to eliminate tumor cells [5].

There are many evidences demonstrating that *Toxoplasma gondii* can stimulate immune system and has positive effects in cancer treatment [6-8]. Recent studies have shown that *T. gondii* when loss its replication capacity can't cause symptoms despite it invades cells. In these situation immune system raised against the parasite may have anti-tumor effects [6-8]. So, in this work effect of *Toxoplasma gondii* tachyzoite on growth of mouse colon cancer has been investigated.

Materials and Method

In this experimental research study population were inbred Balb-C mice.

Antigen preparation: *T. gondii* tachyzoites were purchased from the Pasture Institute, Tehran, Iran. Ten mice were infected with *T. gondii* RH species. After one week, tachyzoites were aspirated from mice's peritoneum and washed with normal saline then sonicated in PBS and kept at -20°C as tachyzoite lysate antigen.

Irradiated parasites: Some tachyzoites aspirated from mice's peritoneum were irradiated using UV light to make attenuated tachyzoites.

Cell culture: CT26 cell line is derived from colon carcinoma in Balb-C mice. This cell line was purchased from Pasture Institute, Tehran, Iran and transferred to our laboratory. Cells were cultured in RPMI medium supplemented with FBS 10% and ampicillin and were kept in 37°C temperature and 5% CO₂ humidity.

Animal experiments: This study was conducted on 3 groups of inbred Balb-C female 5-8 weeks-old mice (6 mice in each group). Each mouse was numbered using a random number table. After adapting to animal lab, each mouse was injected subcutaneously with 10⁵ colon carcinoma cell line CT26 in its chest area. After 3-4 days when tumor was palpable in small size; mice were randomly assigned to the following three groups:

- 1) Tachyzoite lysate antigen was injected into the tumor margin.
- 2) Irradiation attenuated tachyzoite were injected into the tumor margin.
- 3) The third group kept intact without any intervention .

Three days after the intervention, tumor size was measured in mice in the three groups and this process was repeated every three days for two months. The tumor volume was calculated for each mouse using following equation:

$$\text{Tumor volume(mm}^3\text{)} = \frac{4}{3} \pi \left(\frac{\text{vertical diameter} + \text{horizontal diameter}}{2} \right)^3$$

During the study, mice mortality was recorded. Ultimately, the survival rates of mice in control and cases groups were compared. Survival of mice was followed for 60 days.

Statics: Kruskal-Wallis way ANOVA test(K-sample) was used to compare data related to tumor volume between triple groups.

P value <0.05 was considered as statistically significant. Result have been presented as mean and standard deviation. Kaplan-Meier test was used to determine the survival rate in each group and the survival function diagram was used to show survival in 3 groups and Log Rank test was used to compare survival in 3 groups. Statistical analysis was performed using IBM SPSS statics 22.0 software.

Results

In this experiment, three groups of mice were injected with colon carcinoma cells. After 4 days when tumor was palpable, there were injected with Tachyzoite lysate antigen and attenuated irradiated tachyzoite and a control group left without any intervention. Tumor volume of every mice was measured every 3 days. Mass tumor volume in weeks 5, 6 and 7 were 26.2 ± 35.34 , 26.2 ± 35.34 and 70.9 ± 79.00 respectively. Result of this measurement have been summarized in table-1

Table 1.

Comparison of tumor volume in lysate and irradiated tachyzoite injected mice and those of control group in weeks 8-13.

Weeks/ groups		Mean \pm SD	P value*
Week 8	lysate	15.9 \pm 24.63	.006
	irradiated	18.2 \pm 34.90	
	control	235.64 \pm 162.16	
Week 9	lysate	15.9 \pm 24.63	.010
	irradiated	22.4 \pm 36.97	
	control	353.1 \pm 313.63	
Week 10	lysate	30.5 \pm 42.47	.005
	irradiated	33.3 \pm 52.34	
	control	1647.2 \pm 695.14	
Week 11	lysate	46.1 \pm 66.33	.010
	irradiated	26.8 \pm 46.38	
	control	2887.7 \pm 1827.28	
Week 12	lysate	46.1 \pm 66.33	.090
	irradiated	58.5 \pm 83.53	
	control	2514.3 \pm 3301.80	
Week 13	lysate	53.3 \pm 80.02	0.296
	irradiated	57.5 \pm 78.75	
	control	179.5 \pm 0	

*Kruskal-Wallis test

There was a significant statically difference between mean of tumor volume among three groups in weeks 8-11 (P value < 0/05) but this have not been seen in weeks 12-13 (P value > 0/05).

In the post-Hoc test, it was found that the mass volume at 8 to 12 weeks in the control group was statistically significantly higher than the other two groups, but this difference was not statistically significant in the lysate group compared to the irradiate tachyzoite injected group.

Another variable that was measured was survival time. Mortality rate in the control group was significantly higher than the other two groups. (83.3% compared to 16.7%) (P-value=0.28). The rate of mortality of the mice have been summarized in table 2.

Table 2.

Comparison of mortality between mice treated with lysate, irradiated tachyzoites and control mice. (n=6)

Outcome	Groups			Total	P-value
	lysate	irradiated	control		
Alive N (%)	5(83.3)	5(83.3)	1(16.7)	11(61.1)	0.024
Death N (%)	1(16.7)	1(16.7)	5(83.3)	7(38.9)	

Means and median and 95% confidence interval for survival time is shown in table 3. A log rank test was run to determine if there were differences in the survival distribution for the different types of intervention; Lysate, irradiated and control group. The survival distributions for the three interventions were not statistically significantly different, $\chi^2 = 2.52$, $p\text{-value} = 0.28$.

Survival time distribution in three groups has been shown in figure1.

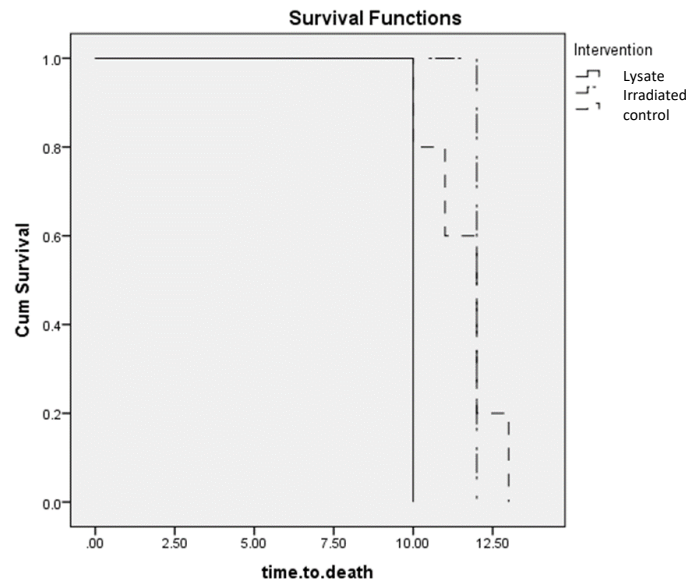
Table .3

means and median for survival time in mice treated with lysate, irradiated tachyzoites and control mice.

Intervention	Mean				Median			
	Estimate	SE	95% CI		Estimate	SE	95% CI	
			Lower	Upper			Lower	Upper
lysate	10.00	0	10.0	10.0	10.0	.	.	.
irradiated	12.00	0	12.0	12.0	12.0	.	.	.
control	11.60	.51	10.60	12.59	12.00	.44	11.12	12.87
Overall	11.42	.42	10.58	12.26	12.00	.30	11.39	12.60

Figure1.

survival time distribution in lysate, irradiated and control group. (n=6)



Discussion

This study showed that treatment of Tumor bearing mice with *Toxoplasma gondii* lysate or irradiated tachyzoite resulted in decreasing tumor volume. In agreement with results of this investigation, there are scientific evidences demonstrating that *T.gondii* have desired effect on tumor suppression [5-9]. In experiments with *Toxoplasma gondii* it has been shown that injection of live tachyzoites of this parasite resulted in decreasing growth of lung [15] and melanoma [16] cancers in mice. Anti-cancer effects of extracts of killed *Toxoplasma gondii* tachyzoites have also been investigated in different works. Results of these researches showed that immunization with tachyzoite extracts of this parasite resulted in reducing growth of Fibrosarcoma [17] or melanoma [18] cancers in mice. Anti-cancer effects of extracts of killed *Toxoplasma gondii* tachyzoites have also been investigated in different works. Results of these researches showed that immunization with tachyzoite extracts of this parasite resulted in reducing growth of Fibrosarcoma [17] melanoma [18] lymphoma [19] and chemically induced [20] tumors in mice.

Moreover, there are a lot of evidences about anti-cancer effects of parasites in human population, in animal model and in laboratory experiments [3, 8, 10, 11].

The exact mechanism of anti-cancer effects of the parasite is not clear. However, immune system raised by the parasite may nonspecifically interfere with tumor growth.

In this context it has been shown that *T.gondii* can stimulate CD8+ T cells, dendritic cells and macrophages response. To determine potency of *T.gondii* to stimulate immune response a

virulent non-replicating vaccine strain(CPS) of the parasite was used. In this investigation, few days after CPS therapy, population of dendritic cells (DCs) increased significantly in tumor environment, mesenteric lymph node and spleen. Systemic administration of IL-12 can also slow down tumor growth but it causes severe toxicity. Treatment with CPS resulted in increasing systemic and local level of IL-12 specially in tumor environment. This increased level can be produced by CPS invaded cells like DCs, macrophage and myeloid cells in tumor environment. Also, levels of CD4+ and CD8+ T-cells increased which can be responsible for increased level of INF- γ in tumor environment [7]. Anti-tumor effect of treatment with CSP *Toxoplasma gondii* has also been reported in another investigation [12, 13].

Toxic effects of *Toxoplasma gondii* molecules on cancer cells is another mechanism that this parasite may induce their anti-cancer activities. SIRT3 is an important protein in mitochondria for ATP synthesis. Protein kinase Ca (PKCa)-phosphorylated *T.gondii* GRA8 enters the mitochondria and interacts with SIRT3. This process leads to mitochondrial dysfunction. Subsequently tumor metabolism will disrupt and can be responsible for antitumor effect [9, 14]. In our work, there wasn't a significant statistically difference in survival time between control and case groups. However, in an investigation performed by Sanders et al. they showed that treatment with *T.gondii* increases survival time of mice [7]. This difference may be related to the type of antigen used or to sample size of experiments. So, more experiments with larger sample size is recommended.

Conclusions

Results of this investigation showed that treatment of colon cancer bearing mice with *Toxoplasma gondii* resulted in suppression of tumor growth. More work should be performed before using *Toxoplasma gondii* for treatment of colon cancer in human.

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Competing interests

The authors declare that they have no competing interests.

Ethical Committee

This work was approved by Isfahan University of Medical Sciences Ethic Committee with approval number of "IR. mui. med. Rec.1398.695. No human sample was used in this work.

Authors' contributions

All authors participated in the conception and design of the study, collected and analyzed the data, read and reviewed the final manuscript.

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