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EVALUATION OF CYTOTOXIC ACTIVITY OF LIVE TOXOPLASMA GONDII TACHYZOITES AND TOXOPLASMA ANTIGEN ON MCF-7 HUMAN BREAST CANCER CELL LINE

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Abstract

The aim of this study was to investigate the cytotoxic potency of live *Toxoplasma gondii* tachyzoites as well as *Toxoplasma* antigen on MCF-7 human breast cancer cell line. Cancer cell lines are considered an essential preliminary step towards *in-vitro* investigation of the potential antineoplastic impact of novel chemotherapeutic agents. Pathogens, including viruses, bacteria, and parasites are noticeably under investigation, considering their potential antineoplastic activity. Some have attained a steady position in the clinical field as hepatitis B virus, human papilloma virus and BCG immunization. *Toxoplasma gondii* is an apicomplexan parasite with promising antineoplastic activity. In this study, live *Toxoplasma* tachyzoites provoked a direct cytotoxic effect on MCF-7 in a dose dependent manner, while *Toxoplasma* antigen didn't induce such impact.

Skipping the direct cytotoxic effect of *Toxoplasma* antigen doesn't totally divert the possible antineoplastic activity of *Toxoplasma* antigen. Potential alternative immune mediated mechanisms could be an alternative. Further *in-vivo* studies in different cancer models are mandatory to investigate the underlying mechanisms of antineoplastic activity of *Toxoplasma gondii*.

Keywords: novel chemotherapeutic agents, *Toxoplasma* tachyzoites, *Toxoplasma* antigen, MCF-7 cell line.

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1. Introduction

As an outcome of the modernized lifestyle and adoption of unhealthy trends in our life, dreadful diseases are emerging including cancer [1]. Cancer is a national concern and a global health burden and challenge, negatively impacting individuals, and health systems. According to the WHO, cancer is the second leading cause of death globally [2]. In women, breast cancer is the most common type, since globally around two million women are diagnosed with breast cancer annually. In men, lung, prostate, colorectal, stomach and liver cancers are the most common types [2].

In conjugation with the growing numbers of cancer cases, it is becoming an overwhelming appalling illness, stressing the need to continuously invent new fighting strategies. This can be employed through exploration of new prospects to pursuit safe, efficient, and nature-based drugs and get out of the drawbacks of the traditional cancer therapeutic agents, including chemotherapy, and radiotherapy.

During the hunt for novel therapeutic varieties, parasites, including apicomplexan parasites as *Plasmodium* and *Toxoplasma gondii* (*T. gondii*), were included as promising antineoplastic

agents [3–5]. Relating to *T. gondii*, asymptomatic *T. gondii* infection induces stimulation of the immune system and low titer of anti-*Toxoplasma* antibody was related to cancer resistance [6]. Furthermore, *Toxoplasma* antibodies were able to bind selectively to mouse melanoma and breast cancer cell lines and not to normal lymphocytes [7].

To test for new cancer therapeutic modalities, *in-vitro* cancer cell lines have vastly been exploited as a preliminary step to investigate the cytotoxic potential of the different therapeutic modalities, including both chemical and biological reagents [8]. Wide variety of cancer cell lines are commonly used and multiple human breast cancer cell lines are available but T47D, MDAMB231 and Michigan Cancer Foundation-7 (MCF-7) represent more than two third of cancer cell line related experiments [9].

MCF-7 is a commonly used non-invasive, luminal molecular subtype, poorly aggressive human breast cancer cell line with low metastatic capability. It is estrogen receptor (ER) positive and progesterone receptor (PgR) positive and is a widely conserved source for breast cancer model worldwide [9, 10]. The aim in this study is to investigate the cytotoxic potency of live *Toxoplasma gondii* tachyzoites as well as *Toxoplasma* antigen on MCF-7 human breast cancer cell line.

2. Materials and Methods

Maintenance of live *Toxoplasma gondii* tachyzoites and *Toxoplasma gondii* antigen preparation was performed at the Department of Medical Parasitology, Faculty of Medicine, Alexandria University, Egypt. Maintenance of MCF-7 human breast cancer cell line and conduction of the *in-vitro* experiment was performed inthe Tissue Culture Unit, Medical Technology Center, Medical Research Institute, Alexandria University, Egypt.

2. 1. Maintenance of *Toxoplasma gondii* RH strain

To maintain live *Toxoplasma gondii* tachyzoites (L*Tg*) of virulent RH HXGPRT(–) strain, serial intraperitoneal passages in Swiss albino mice was performed [11]. Three-four days post infection, tachyzoites were harvested, washed several times in sterile phosphate buffered saline (PBS) and centrifuged to obtain a purified population of tachyzoites.

2. 2. *Toxoplasma gondii* antigen preparation

Heat killed *Toxoplasma* antigen (*Tg*A) was prepared as previously described [12]. Tachyzoites were suspended in PBS and incubated at 90 °C for15 minutes [12]. The antigen was stored in –20 °C for later use and protein concentration was quantified using NanoDrop™ 2000 spectrophotometer (Thermo Scientific, USA).

2. 3. MCF-7 Human breast cancer cell line

A frozen vial of MCF-7 (Human breast cancer cell line, HTB-22) was purchased from VACSERA Co., Cell Culture Unit, Dokky, Giza, Egypt, originally supplied from the American Type Culture Collection (ATCC).

2. 3. 1. Maintenance of MCF-7 human breast cancer cell line

Cells were cultured as previously described [13], in the complete Dulbecco's Modified Eagle Medium (DMEM), 10 % fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, streptomycin (100 mg/ml), and amphotericin-B (5 mg/ml) and incubated under a humidified atmosphere of 5 % CO_2 at 37 °C until confluent in 25 cm^2 cell-culture flasks. Five days before the experiment, MCF-7 cells were seeded in a sterile 24 well-plate in complete culture media and were regularly checked using an inverted microscope until confluent. One day before the experiment, cells were trypsinized, washed, resuspended in complete media, and plated at 20,000 cells/well in 96 well-microtiter plate in a volume of 100 µl/well [14].

2. 3. 2. Exposure of MCF-7 to different experimental treatments

After 24-hour (h), cells were exposed for 24 h to various concentrations of L*Tg,* ranging from $7.5 \cdot 10^4$ to $12 \cdot 10^5$ live tachyzoite/ml [15]. Other wells were exposed to different concentrations

of *Tg*A, ranging from 14 to 54 μg/ml [16]. Each concentration was done in triplicate. The negative control included only culture media.

2. 3. 3. MCF-7 cell viability assessment using MTT

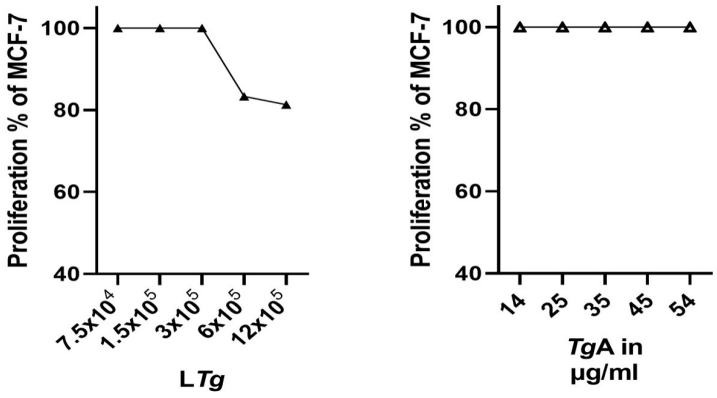
At the end of the 24 h, cell proliferation was assessed by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay as previously described [16]. Briefly, the following protocol was applied [16]. The supernatant was discarded, and cells were incubated for the last two hours with 50 μl of MTT (MTT formazan, 2 mg/ml; Sigma), added to each well according to manufacturer instructions. One hundred μl of dimethyl sulfoxide (DMSO) (Sigma) was added to dissolve the formazan, and the absorbance values were read at 490 nm using an ELISA plate reader [17]. The proliferation was checked in three wells for each treatment group. To calculate proliferation percentage, the absorbance of the treated group was divided by the absorbance of the negative control (media only control wells) and multiplied by 100. The following equation was applied as follow [16]:

$$(A_{etc}/A_{ncw})\times 100,$$

where A_{etc} is the absorbance of each treatment concentration and A_{ncw} is the absorbance of the negative control wells.

3. Results

Results showed that the exposure of MCF-7 cells to the highest concentrations of L*Tg* tachyzoites ($6\cdot10^5$ and $12\cdot10^5$) reduced the proliferative rate of MCF-7 cells to 83.3 % and 81.3 %, respectively compared to the negative control wells. On the other hand, the exposure of the cells to different concentrations of *Tg*A, ranging from 14 to 54 μg/ml, did not induce a detectable difference in MCF-7 cell proliferation compared to the negative control (**Fig. 1**).



a *b*

Fig. 1. Cytotoxicity test measured using MTT: *a* – L*Tg:* Live *Toxoplasma* tachyzoites of RH strain; *b* – *Tg*A: *Toxoplasma* antigen

Cells of MCF-7 human breast cancer cell line were incubated for 24 hours with different concentrations of live *Toxoplasma* tachyzoites (**Fig. 1, *a***) and *Toxoplasma* antigen (**Fig. 1, *b***).

4. Discussion

In-vitro studies have long been considered as an essential conventional preliminary step to test the therapeutic potency of different chemical and biological agents. Cancer cell lines have attracted many scientists to deeply investigate both cancer pathogenesis and therapeutic chances, while avoiding *in-vivo* use of an animal model, which may result in misleading outcomes due to differences between human and animal pathophysiology and responses [8].

Beside the use of standard chemotherapeutic agents in cancer treatment, pathogen-based therapeutic trials are getting more consideration. The immunomodulatory potency of some patho-

gens and the reported antineoplastic activity of some of them; both *in-vitro* and *in-vivo* have been the driving force for more investigational studies. This concept has been even promoted to the clinical field where BCG immunization is used as a therapeutic cancer vaccine for bladder cancer [18], while hepatitis B and human papilloma virus immunization are used as preventive vaccines against liver and cervix carcinoma respectively [19].

Pathogen-derived inactivated killed antigens are a safe promising immunization tool against their corresponding infectious diseases. The list extends to include viruses, bacteria as well as parasites. The protective efficacy has been verified in experimental animals as heat killed *Escherichia coli* [20], *Leishmania* [21], and some have already been applied clinically and commonly used as whole-cell Pertussis and inactivated Polio vaccine, Hepatitis A, Rabies, Cholera and Typhoid vaccines [22].

Toxoplasma gondii and some of its antigens successfully showed antineoplastic activity against some cancer models both *in-vitro* and *in-vivo* [4, 5, 15]. The attenuated auxotroph strain of virulent *T. gondii* (cps) that selectively invade cells without replication showed antitumor efficacy in melanoma [23] and pancreatic ductal adenocarcinoma murine models [24]. This activity was established through a powerful immunostimulatory effect [23–25]. The gamma irradiated *T. gondii* ME49 strain suppressed Ehrlich ascites carcinoma cells proliferation in a murine model [26]. Nevertheless, attenuated strains still posses the hazards of inoculation of live parasites in cancer immunosuppressed patients. Thus, killed vaccine may be a safer and clinically applicable alternative. Thus, it was noteworthy to investigate the antineoplastic activity of live *Toxoplasma* tachyzoites as well *Toxoplasma* antigen (*Tg*A) *in-vitro* on MCF-7 human breast cancer cell lines.

LTg were able to induce a direct cytotoxic effect on MCF-7 human breast cancer cell line at a dose-dependent manner. This is in agreement with previously reported data [15, 27]. On the other hand, no direct cytotoxic effect was encountered following *in-vitro* exposure of MCF-7 cells to different concentrations of *Tg*A.

Despite referring to a previous study that emphasized the role of *Toxoplasma* lysate antigens in inhibiting the proliferation of glioma cells [16], the obtained finding agrees with another study. They investigated the antitumor potential of different antigenic *Toxoplasma* variants against the experimental ovarian tumor model and reported that only live invasive non-replicating tachyzoites induced potent antitumor activity [28]. This could be attributed to the value of the invasive organelles and host cell invasion step to induce an antitumor effect. Whereas *Tg*A, used in this study, is a killed, non-living, non-invasive, and non-replicating safe alternative.

Nevertheless, this does not imply overall ignoring the potential antineoplastic activity of *Tg*A. Verification of such hypothesis necessitates considering multiple alternative side-ways instead of direct cytotoxic effect. A proposed immunomodulatory effect as well as an antiangiogenic effect may be alterative mechanisms [4, 5, 26]. Further studies are ongoing to assess such theory using an *in-vivo* animal cancer model to explore the influence of *Toxoplasma* antigen on the tumor microenvironment, its potential immunomodulatory and antiangiogenic roles.

During this study we investigated the cytotoxic effect of *Toxoplasma gondii* and its antigen on MCF-7 human breast cancer cell line using MTT. Due to technical limitations, we were not able to use other assessment parameters, including Matrigel invasion. Future studies are highly recommended to investigate the impact of *Toxoplasma gondii* antigen on different cancer cell lines in addition to its *in-vivo* influence on the tumor microenvironment using different experimental cancer models.

5. Conclusions

Direct cytotoxic effect was elicited upon exposure of MCF-7 human breast cancer cell lines to live *Toxoplasma* tachyzoites. Whereas *Toxoplasma* antigen did not provoke a direct cytotoxic effect on MCF-7 human breast cancer cell lines, suggesting alternative immune mediated antineoplastic mechanisms if any, that needs to be evaluated.

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