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The Co-treatment of Irradiated Tumor Cell Lysate Vaccine and Zinc Oxide Nanoparticles Motivates Antitumor Immunity

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THE USE of gamma radiation in vaccine production has proven to be one of the useful methods. Nanoparticle therapy, specifically the use of ZnO nanoparticles (ZnONPs), is a promising and innovative approach to treating a variety of diseases. The present study is designed to evaluate the effectiveness of irradiated tumor cell lysate vaccine and ZnONPs in treating solid Ehrlich ascites carcinoma tumors. The current study monitored the tumor size, MTT cell viability, and antioxidant markers (NO and SOD) in serum. Additionally, the levels of interleukins IL-10 and IL-6 were examined, as well as TGF-β, Caspase-3, and p53 expression in tumor tissues. The results demonstrated that the co-treatment of irradiated tumor cell lysate vaccine and ZnONPs had a potent effect in reducing tumor size, TGF-β, and P53 expression, and elevating IL-6 and IL-10 levels, and Caspase-3 activity. In conclusion, the combination of irradiated tumor cell lysate vaccine and ZnONPs shows great promise as a potential cancer treatment.

Keywords: Antioxidant, Cancer, Ionizing radiation, Irradiation, Tumor cell lysate vaccine, Zinc oxide nanoparticles.

Introduction

Over the past few decades, several methods have been employed to treat cancer, a condition characterized by uncontrolled cell differentiation (Arneth, 2019). However, chemotherapy treatments, antimetabolites, biological agents, and natural products are not always effective since drug resistance and distinguishability of the cells can lead to systemic toxicity and debilitating adverse effects in normal body tissues (Guðmundsdóttir, 2023). Tumor cell-based vaccines derive tumor antigens from patient tumor cells (Sadeghi Najafabadi et al., 2022). Ex vivo genetic modification of the cells is followed by inactivation with high-dose IR or lysis. Tumor cell-based vaccines derive their tumor antigens from tumor cells obtained from patients (Hayes, 2021). Following ex vivo genetic modification, the cells are inactive via high-dose infrared (IR) (Remic et al., 2022). Irradiated whole-tumor cell vaccines are a type of radio-immunotherapy that can induce a wide range of tumor-associated antigens (TAAs) (Al Saber et al., 2021). They are considered the best immunotherapy choice because the whole tumor cell presents the best source of immunizing antigens (Zhang et al., 2023). These vaccines have the potential to amplify the tumor-reactive T cells from the naive repertoire, reactivate existing tumor-specific T cells, and increase the extensiveness and variety of the tumor-reactive T cell response (Gray, 2021). This, in turn, minimizes immune escape. By stimulating T cell responses to these antigens, TAA in cancer vaccines can indirectly target tumors by releasing spontaneous tumor immunity (Saxena et al., 2021). The recognition of tumor cells by host immune cells has opened up new possibilities for eliminating cancer cells without the severe side effects of traditional cancer treatments (Chen et al., 2021). However, achieving a strong, tumor-specific immune response requires the use of adjuvants; various types of nanosystems have been utilized to protect and deliver vaccine components, showing a promise in achieving prolonged eradication of cancer cells (Yao et al., 2023). Zinc oxide nanoparticles (ZnONPs) are one of the most vital inorganic metal oxide

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nanoparticles utilized in different biomedical applications such as cancer therapy due to their remarkable properties and biocompatibility (Radwan et al., 2021). Zinc oxide nanoparticles have been found to exhibit cytotoxicity both in vitro and in vivo (Sengul & Asmatulu, 2020). These nanomaterials possess unique chemical properties (Czyżowska & Barbasz, 2022).

Furthermore, they have demonstrated a high degree of selectivity towards cancer cells, with the ability to surpass the therapeutic indices of traditional chemotherapeutic agents (Chandra et al., 2023). ZnO nanoparticles preferentially target rapidly dividing cancerous cells, leading to enhanced cytotoxicity through the generation of reactive oxygen species (ROS), which in turn, cause oxidative stress and eventual cell death, as described in the study by Hu et al. (2020). The present study focused on the effectiveness of the irradiated tumor cell lysate vaccine and ZnONPs in treating Ehrlich solid carcinoma by testing their role in apoptotic activation and host immunogenic activity enhancement.

Material and methods

Chemicals

Zinc oxide nanoparticles (ZnONPs) with a 20-50 nm diameter were purchased from Sigma–Aldrich Company Ltd. (purities 99.5 wt. %., surface areas 50m2/g).

Animals

Fifty female Swiss albino mice (20-25g) purchased from the National Center for Radiation Research and Technology, EAEA, Cairo, Egypt, were used in this study. Animals were maintained on a commercial standard pellet diet and tap water ad libitum. Care was consistent with the guidelines of Ethics by the Public Health Guide for the Care and Use of Laboratory Animals (National Research Center, 1996) by the proper care and use of laboratory animals approved by the animal care committee of the National Center for Radiation Research and Technology, Cairo, Egypt.

Tumor Transplantation.

Ehrlich Ascites Carcinoma (EAC) cell line was obtained from the National Cancer Institute (NCI), Cairo University. It was propagated in vivo as Ascites in female Swiss albino mice after intraperitoneal (i.p) inoculation of 2.5×10^6 cells/mouse once a week for the time of the experiment (Salem et al., 2011). The solid Ehrlich carcinoma induction was performed by injecting $2.5x10^6$ (7 days old) in the left thigh (Fahim et al., 1997).

Preparation of tumor cell lysate vaccine

According to Scchnurr et al. (2001), EAC cells were aspirated from the peritoneal cavity of EAC-bearing mice. The cell viability was measured using a hemocytometer and adjusted to 2.5x106/mm³. EAC cells were irradiated with 8KGy γ-radiation (Salama and Hassan, 2014). The irradiated cells were incubated with 0.01% EDTA solution for 10 min, washed twice in PBS, and suspended at a density of 5x106/ml in a serum-free medium. The cell suspensions were frozen at -80°C and disrupted by four freeze-thaw cycles (Scchnurr et al., 2001). Mice were injected weekly with 0.2 µl of cell lysate (supernatant) in the right thigh for three sequential weeks.

Experimental design

Fifty mice were divided into five groups, as follows:

1-Control group (C): mice received 0.2 mL saline. 2- EAC group (E): Mice were injected intramuscularly (i.m (with 0.2ml of 2.5×105 ml/mouse viable EAC cells in the left thigh. 3-Ehrlich carcinoma/Lysate vaccine group (EV): Mice were injected i.m with 0.2 ml of tumor cell lysate vaccine in the right thigh one time / week for three weeks (Salama and Hassan, 2014); after two weeks, mice were injected with 0.2ml of 2.5×105ml/mouse viable Ehrlich carcinoma cells in the left thigh 4- Ehrlich carcinoma/ ZnONPs (EZ): mice developed tumors as a group (2) and after seven days; mice were injected intraperitoneally by ZnONPs (10 mg/kg, once daily for three weeks) (Dawood et al., 2019). 5-Ehrlich carcinoma/Tumor cell lysate vaccine group / ZnONPs treated group (EVZ): Mice developed tumors as in group (2) and were immunized as in group (3) and then treated with ZnONPs as in group (4). Two weeks later, after the EAC challenge, mice were sacrificed. Serum and tissues were collected for biochemical assays.

Tumor size mentoring

From the 6th day after Ehrlich carcinoma inoculation, tumor size was monitored using a Vernier caliper according to the following equation: Tumor volume = 0.52 (length × width^2) Where length is the greatest longitudinal diameter and width is the greatest transverse diameter (Jensen al., 2008).

In Vitro MTT assay

MTT cell proliferation and viability assay were applied according to Freimoster et al. (1999).

Biochemical Assays

The present study measured oxidative stress markers (NO Nitric Oxide Assay Kit (Colorimetric) (ab65328) and SOD Superoxide Dismutase Activity Assay Kit (Colorimetric) (ab65354)) in the serum and estimated the immune mediators (IL-6 Rat IL-6 ELISA Kit (ab100772) and IL-10 Rat IL-10 ELISA Kit (ab214566)) in the tumor tissue. Caspase 3 levels were also detected in the tumor tissue using the ELISA kit from LSBio (Rat CASP3 / Caspase 3 (Sandwich ELISA) ELISA Kit - LS-F4138) as per the manufacturer's instructions.

Real-time quantitative polymerase chain reaction

Tissue samples were processed with the RNeasy Mini Kit (Qiagen, Cat. No. 74104) to isolate total RNA under the protocol provided by the manufacturer. The first-strand complementary DNA (cDNA) was synthesized following the manufacturer's instructions using the QuantiTect Reverse Transcription Kit (Qiagen, Cat. No. 205311) and 1 mg RNA as the template. Sequence Detection Software (PE Biosystems, CA) was utilized. 2 mL of cDNA, 900 nM of each primer, and 2 mL of 2X SYBR Green PCR Master Mix (Qiagen, Cat. No. 204143) comprised the reaction mixture, which had a total volume of 25 mL. The PCR thermal cycling conditions contained 40 cycles of 95_C for 20 s, 60_C for 30 s, and 72_C for 20 s, with an initial step at 95_C for 5 min. Utilizing the DDCt method, the relative expression of real-time reverse transcriptase PCR products was ascertained. This method calculates a relative expression to the housekeeping gene using the equation: fold induction ¼2_(DDCt). Where DD Ct ¼ Ct [gene of interest (unknown sample) -Ct housekeeping gene (unknown sample)] - [Ct gene of interest (calibrator sample) -Ct housekeeping gene (calibrator sample)] (Flamand et al. 2008).

Statistical analysis

Tests of significance were performed using the statistical package graph prism. All data are expressed as a mean of n= 6 values; SE and difference between means were considered significant at $P < 0.05$.

Results

Tumor size monitoring

The solid tumor volume was measured during the experiment. The solid tumor size was 131.37 mm3 on the 8th day after tumor injection. Tumor volume exceeded 349.40 mm3 on the 11th day; it continues to increase in size and reach 739.67 mm3 on the 13th day of tumor injection. However, mice treated with ZnONPs (EZ) showed an inhibition rate of 79.23%. Moreover, the tumor volume was diminished in mice immunized with the vaccine (EV) regarding the E group. The inhibition rate was 84.09% and the tumor volumes were 41.65, 56.56, and 75.35 mm3 on the 8th, 11th, and 13th day respectively. The group immunized with the vaccine and treated with ZnONPs (EVZ) showed a maximum percentage of inhibition by 89.85%, compared to the E group, also the tumor volume recorded at 31.82, 28.28, 48.19 mm3 on 8th, 11th, and 13th days respectively Fig (1).

TABLE 1. Primer Sequences Used for RT-PCR. FW: Forward; Rv: Reverse.

Gene	Primer sequence	accession number
TGF-β	Fw TGATACGCCTGAGTGGCTGTCT Rv CACAAGAGCAGTGAGCGCTGAA	NM_011577
P53	Fw GTATTTCACCCTCAAGATCC Rv TGGGCATCCTTTAACTCTA	NM_001127233.1

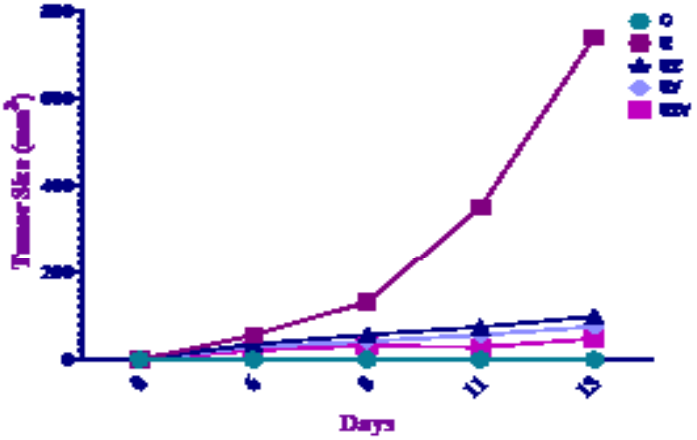

Fig. 1. Effect of irradiated tumor cell lysate vaccine and or ZnONPs on tumor size (mm³).