Antitumor Activity of Dehydroxymethylepoxyquinomicin (DHMEQ) in Monotherapy and Combination with Cisplatin in the SKOV-3 Ovarian Cancer Model

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Abstract: The objective of this study was to evaluate the antitumor activity of DHMEQ as monotherapy and in combination with cisplatin in a human ovarian cancer xenograft model. Cisplatin was used as a comparator. To create the xenograft model, human ovarian cancer cells (SKOV-3 line) were subcutaneously implanted into immunodeficient mice. The study was conducted on female SCID Beige C.B-17 Cg-Prkdcscid Lystbg/Crl mice. Antitumor activity was determined by comparing tumor growth inhibition (TGI) in the treatment groups to that in the control group. Results showed that daily intraperitoneal administration of DHMEQ at a dose of 14 mg/kg following a single intraperitoneal dose of cisplatin at 4 mg/kg reduced tumor growth in the SKOV-3 cell line xenograft model.

Keywords: DHMEQ, xenograft, SKOV-3, tumor growth inhibition, antitumor activity, cisplatin.

INTRODUCTION

Ovarian cancer (OC) is one of the most dangerous types of gynecological malignancies [1, 2]. Annually, more than 200,000 cases of OC are diagnosed worldwide, with some reports indicating up to 295,000 cases [3, 4]. It is projected that the incidence of ovarian cancer will significantly increase by 2040 [5]. Statistical data from 1990 to 2017 show that the mortality rate from ovarian cancer has increased by 842% [6]. In the structure of oncological diseases among the female population of Russia, ovarian cancer accounts for 44%, which is one-third of all gynecological cancers [7]. About 30% of all women diagnosed with ovarian cancer die within the first year after diagnosis. This situation arises because the symptoms of this pathology in the early stage are imperceptible [8]. Despite extensive research in oncology, there are currently limited methods for early-stage diagnosis of ovarian cancer [9, 10]. Interval or primary cytoreductive surgery and combined therapy with platinum and taxane drugs play a crucial role in OC treatment [11-13]. The response rate to first-line therapy is about 80-90%, but most patients subsequently experience recurrence and develop resistance to therapy, resulting in a 5-year survival rate of less than 35% [7]. Therefore, the search for new effective drugs and combinations for the treatment of OC is an urgent task in modern oncology [14]. The aim of this study is to evaluate the antitumor activity of the combination of dehydroxymethyl epoxychinomicin (DHMEQ) and cisplatin in an ovarian cancer model *in vivo*. Previous studies have also provided data on the acute toxicity of DHMEQ with a single intraperitoneal injection in mice [15-17]. Cisplatin was chosen as the comparator and for combination studies, as it is one of the most frequently used drugs for treating ovarian cancer, including disseminated forms, and is also used in hyperthermic intraperitoneal chemotherapy (HIPEC) [18, 19].

MATERIALS AND METHODS

Animals

Immunodeficient mice are a convenient model for studying the antitumor activity of drugs. The absence of immunity allows human cancer cells to be transplanted, increasing the predictive reliability of the activity of the studied substances. Female SCID Beige C.B-17 Cg-Prkdcscid Lystbg/Crl mice (Charles River), aged 13 weeks at the start of the study, were used. Air in the laboratory is triple-filtered, with the final stage through HEPA filters. The animals were housed under controlled environmental conditions (22-26°C and 30-70% relative humidity). Temperature and humidity were monitored using a computerized system. The rooms

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were maintained on a 12-hour light/dark cycle with 10-15 air changes per hour. Immunodeficient mice were kept in individually ventilated cages (1285L type, Techniplast, 4-8 mice per cage) connected to a TouchSLIMLinePlus air conditioning system (Sealsafe system from Techniplast). The cages were mechanically and chemically cleaned and autoclaved before use. "Rehofix MK-2000" (Rettenmeyer-Rus LLC) was used as bedding. The animals had free access to water and food. The mice were fed a complete diet for laboratory animals, JL Rat and Mouse Auto 6F-Ovals, cat. No. 5K67 (LabDiet). The food was autoclaved and provided ad libitum in the feed recess of the steel mesh cage cover. Filtered tap water was autoclaved and provided ad libitum in standard autoclaved drinking bottles with steel nozzles. Each animal was assigned an individual number marked on the tail with a special marker. Cages were labeled with the following information: protocol number, species, sex, number of animals, start and end of the experiment, responsible staff member, drug name, date, and method of administration. Bedding was replaced twice a week, and water bottles were changed every two days. The animal rooms were cleaned daily. The adaptation period before the experiment was over two weeks, during which the animals were inspected daily. No abnormalities were found.

Treatment

DHMEQ (synthesized by TechnoChem CO. LTD., Tokyo, Japan) was dissolved in 100% DMSO. Stock solutions of DHMEQ in 100% DMSO were stored for no more than two weeks at +4°C in a dark place, away from direct sunlight. Cisplatin (Cisplatin-LENS, produced by Veropharm, Russia) was administered intraperitoneally at a volume of 8 mL/kg, as the concentration of cisplatin in the factory packaging was 0.5 mg/mL. For the first administration of DHMEQ with cisplatin, the DHMEQ stock solution was diluted with the cisplatin solution to a concentration of 175 mg/mL and administered at a volume of 8 mL/kg (the final DMSO concentration in the solution was 0.31%). According to our own data the LD50 value for DHMEQ with a single intraperitoneal injection in female mice was 176,82±35,61 mg/kg, classifying DHMEQ as a Class III toxicity compound (moderately toxic) according to Russian government standard 12.1.007-76 and maximum tolerated dose for female SCID Beige mice (C.B-17 Cg-Prkdcscid Lystbg/Crl, Charles River) is 4 mg/kg. For groups receiving DHMEQ at doses of 7 and 14 mg/kg for the first administration, solutions with concentrations of 175 and 87.5 mg/mL were prepared and administered at a volume of 8 mL/kg. The control group received the solvent at 8 mL/kg. The second and subsequent administrations were performed at a volume of 5 mL/kg. Working solutions of DHMEQ in DMSO and saline were prepared with DHMEQ concentrations of 28 (for a dose of 14 mg/kg) and 14 (for a dose of 7 mg/kg) mg/mL (the final DMSO concentration in the solution was 0.5%). The drug was administered intraperitoneally to mice at 5 mL/kg. The control group received the solvent at 5 mL/kg. Working solutions of DHMEQ were prepared immediately before intraperitoneal administration and were not stored.

Tumor Measurements and Antitumor Activity

Human ovarian cancer SKOV-3 cells were cultured in RPMI-1640 medium (PanEco; C330p) containing 10% FBS (HyClone; SV30160.03), 2 mM sodium glutamine (PanEco; F032), 1x sodium pyruvate (PanEco; F023), 1x non-essential amino acids (PanEco; F115/50), 1x penicillin-streptomycin (PanEco; A065). Cells were thawed, cultured in T175 flasks, and passaged every 3-4 days. For subcutaneous injection, cells were washed in α-MEM medium (PanEco; C180p) without serum, counted, and centrifuged at 900 rpm (R rotor 20.4 cm). Cells were resuspended in α-MEM medium at <10°C to a final concentration of 50 million/mL. Matrigel (Corning® Matrigel #354234) was added to the cell suspension to a final concentration of 25 million/mL. The distribution of animals into experimental groups is detailed in Table **1**. The cell suspension was injected subcutaneously along the spine (right shoulder blade area) at 0.2 mL (5 million cells) per mouse. The injection site was shaved and disinfected with AHD-2000. Main Measured Parameters: Subcutaneous tumor size was measured twice weekly. Tumor volume was calculated using the formula: Volume=π/6 × L × W^2, where L is the largest diameter and W is the smallest. Measurements were taken with calipers. Antitumor activity was determined by comparing tumor growth inhibition (TGI) in the treatment groups to the control group. TGI was calculated using: TGI=(C−T)×100/C, where C is the average tumor size in the control group and T is the tumor size in the experimental group. TGI was assessed on days 14 and 18 of the study. Animal health and behavior were monitored, and lethality was recorded. Body weight was measured twice weekly. Average tumor sizes at the end of the study were used for statistical comparisons of tumor growth inhibition. The Mann-Whitney test was used for intergroup comparisons (GraphPad Prism). Statistical significance was set at ≤ 0.05. All animals were euthanized by CO_2

Table 1: Animal Groups in the Experiment

Group Number	Drug	Number of Animals	Dose (mg/kg)	Injection Volume (mL/kg)	Number of Injections
1	Control/Solvent	6	0	5	14
2	Cisplatin/Solvent	7	4	8/5	Single/14
3	DHMEQ	6	7	5	14
4	DHMEQ	7	14	5	14
5	Cisplatin/DHMEQ	7	4/14	8/5	Single/14

inhalation following European Commission recommendations [20].

RESULTS AND DISCUSSION

Throughout the experiment, no significant clinical abnormalities were observed in animal behavior. One animal in the 14 mg/kg DHMEQ group died after the 13th administration, which is consistent with the observation that higher doses of DHMEQ can result in toxicity, as reported in other studies. No other fatalities occurred. The animals remained active with normal appetite and water consumption. DHMEQ and cisplatin administration did not significantly affect body weight (Figure **1**), which aligns with previous research indicating that DHMEQ has a relatively mild impact on systemic toxicity compared to other chemotherapeutic agents.

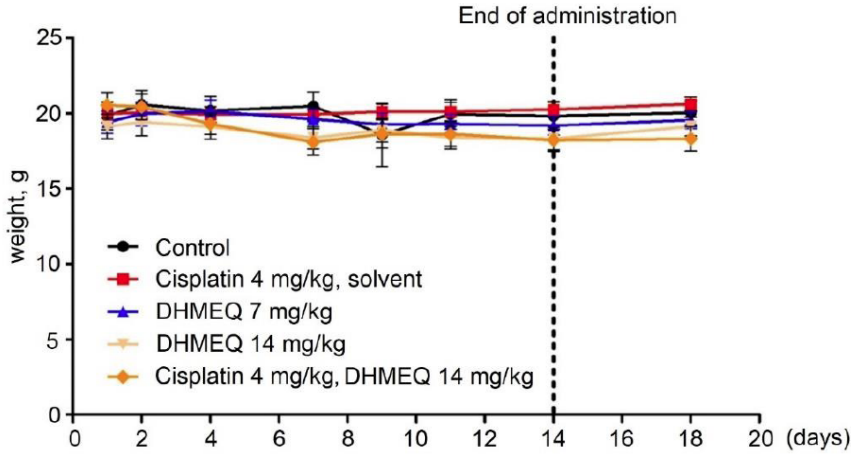

Figure 1: Effect of DHMEQ and Cisplatin on Animal Body Weight in the SKOV-3 Xenograft Model.

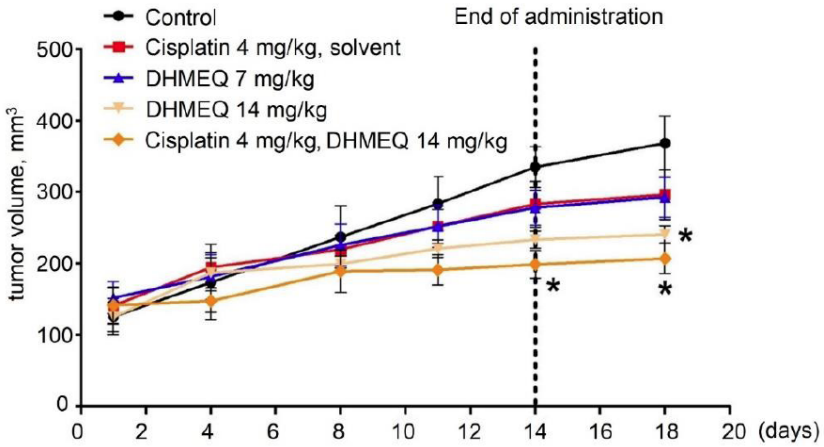

Figure 2: Effect of DHMEQ and Cisplatin on Tumor Growth in the SKOV-3 Xenograft Model (*p<0.05 compared to Control group).

The effects of the investigated compounds on tumor growth are illustrated in Figure **2**. These findings are in line with earlier studies that demonstrated the antitumor efficacy of DHMEQ, particularly at higher doses, in various cancer models.

In the control group, tumor size steadily increased. Tumors in animals receiving daily DHMEQ at 7 mg/kg or a single cisplatin dose at 4 mg/kg also grew over time, showing no significant difference from the control group. This lack of significant tumor inhibition at lower doses of DHMEQ is consistent with previous studies that suggest a dose-dependent response in the antitumor activity of DHMEQ. Daily DHMEQ at 14 mg/kg, alone or after a single cisplatin dose, significantly slowed tumor growth, which is supported by earlier findings showing that higher doses of DHMEQ have enhanced therapeutic effects. This effect persisted four days post-treatment, further corroborating the sustained antitumor activity observed in other studies with DHMEQ.

On days 14 and 18 (Figures **3-6**), DHMEQ at 14 mg/kg, alone or after cisplatin, significantly reduced tumor size and increased TGI. This is consistent with literature indicating that combining DHMEQ with other chemotherapeutic agents like cisplatin can potentiate antitumor effects, as seen in other xenograft models.

Daily intraperitoneal DHMEQ at 14 mg/kg, following a single cisplatin dose at 4 mg/kg, significantly reduced tumor growth rates in the SKOV-3 xenograft model. This result aligns with other studies that have shown the enhanced efficacy of DHMEQ when used in combination with cisplatin, suggesting a synergistic effect that could be leveraged in future therapeutic strategies. If no other studies related to this specific combination and dosage are available in the literature,

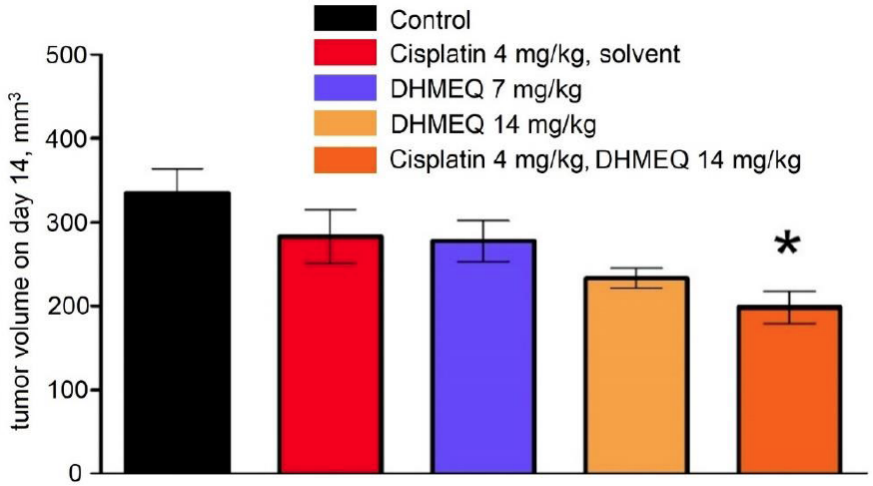

Figure 3: Tumor Volume on Day 14 (*p<0.05 compared to Control group).

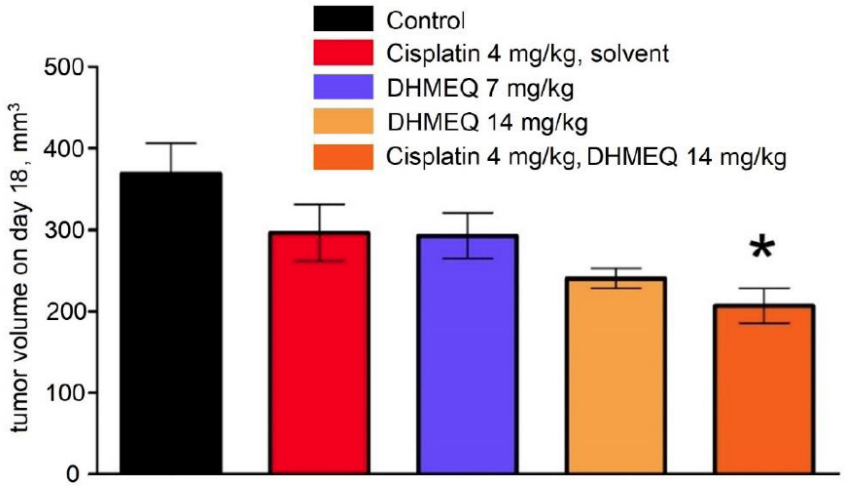

Figure 4: Tumor Volume on Day 18 (*p<0.05 compared to Control group).

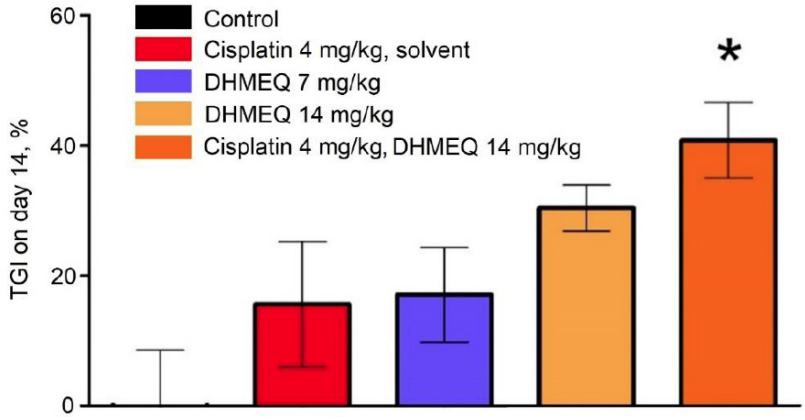

Figure 5: Tumor Growth Inhibition (TGI) on Day 14 (*p<0.05 compared to Control group).

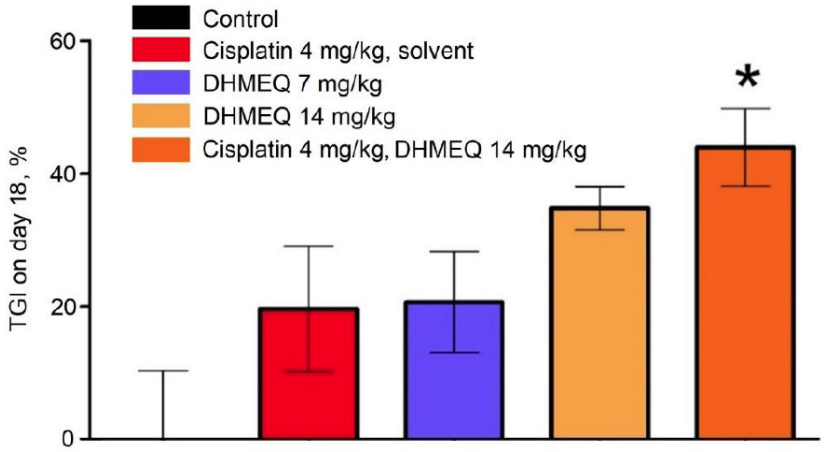

Figure 6: Tumor Growth Inhibition (TGI) on Day 18 (*p<0.05 compared to Control group).

this finding would represent a novel contribution to the field, and further investigation would be warranted to confirm these results.

CONCLUSION

This study provides compelling evidence that daily intraperitoneal administration of DHMEQ at a high dose of 14 mg/kg, both as a monotherapy and in combination with a single intraperitoneal dose of cisplatin at 4 mg/kg, significantly inhibits tumor growth in the SKOV-3 xenograft model of human ovarian cancer. The findings reveal that the combination of DHMEQ and cisplatin leads to a more pronounced tumor growth inhibition (TGI) compared to DHMEQ alone, particularly on days 14 and 18, suggesting a synergistic interaction between these two agents. This result aligns with previous studies that have demonstrated the potentiation of anticancer effects when combining DHMEQ with other chemotherapeutic agents, such as doxorubicin and paclitaxel, which also work by inducing apoptosis and inhibiting NF-κB signaling pathways.

The significant reduction in tumor growth observed with the high-dose combination treatment underscores the potential therapeutic advantage of this regimen. Cisplatin, a platinum-based chemotherapy drug, is known for its ability to induce DNA cross-linking, leading to apoptosis in rapidly dividing cancer cells. However, the efficacy of cisplatin can be limited by the activation of survival pathways, including those mediated by NF-κB, which contributes to chemoresistance. DHMEQ, an NF-κB inhibitor, can sensitize cancer cells to cisplatin by blocking these survival signals, thereby enhancing the overall anticancer effect. This is particularly important in the context of ovarian cancer, where resistance to chemotherapy remains a significant clinical challenge.

In contrast, the study also highlights that a single intraperitoneal administration of cisplatin at 4 mg/kg or a lower dose of DHMEQ at 7 mg/kg, even when combined with cisplatin, did not significantly inhibit tumor growth. This observation is consistent with the dose-dependent nature of many anticancer agents, where lower doses may not achieve sufficient plasma

concentrations to exert a robust therapeutic effect. Furthermore, it suggests that the efficacy of DHMEQ as an anticancer agent is highly dependent on achieving a threshold dose, below which its ability to inhibit NF-κB and induce apoptosis is markedly reduced.

The findings from this study suggest that a higher dose of DHMEQ, particularly in combination with cisplatin, offers a more effective strategy for inhibiting tumor growth in ovarian cancer models. This has potential implications for developing new therapeutic protocols that could enhance the efficacy of existing chemotherapies while minimizing the risk of resistance. Future studies should explore the long-term effects of such combination therapies, including their impact on survival rates and potential side effects, to better understand the clinical applicability of these findings. Additionally, it would be valuable to investigate the molecular mechanisms underlying the observed synergistic effects, which could lead to the identification of new therapeutic targets and strategies for overcoming drug resistance in ovarian cancer.

In summary, the study underscores the importance of dose optimization in cancer therapy and provides a strong rationale for further investigation of DHMEQ, particularly in combination with cisplatin, as a potential treatment strategy for ovarian cancer. The promising results from this preclinical model suggest that such combinations could be translated into clinical practice, potentially improving outcomes for patients with resistant or recurrent ovarian cancer.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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