RESEARCH ARTICLE

Synergistic Anticancer Effects of Aluminum Oxide Nanoparticle (Al2O3NPs) Combined with Doxorubicin in Inhibiting SK-OV-3 Ovarian Cancer Cell Proliferation

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ABSTRACT

Ovarian cancer, particularly aggressive subtypes like SK-OV-3, remains a significant challenge due to limited treatment options, necessitating the development of more effective therapeutic strategies. Aluminum oxide nanoparticle (Al2O3NPs) have demonstrated potential in cancer therapy because of their unique properties, including enhanced drug delivery and cytotoxic effects. This study investigates the synergistic anticancer effects of aluminum nanoparticles combined with doxorubicin on SK-OV-3 ovarian cancer cells. The effectiveness of the combination treatment was assessed by evaluating cell viability using the MTT assay. Oxidative stress was quantified by measuring reactive oxygen species (ROS) levels with the DCFH reagent, while lipid peroxidation (LPO) was analyzed by detecting malondialdehyde (MDA) levels. The antioxidant capacity was measured by assessing glutathione (GSH) levels. The results indicated that the combination of Al2O3NPs and doxorubicin significantly reduced SK-OV-3 cell viability, suggesting potent anticancer effects. Additionally, the treatment induced oxidative stress, as evidenced by increased ROS production, elevated MDA levels, and decreased GSH levels. These findings demonstrate that aluminum nanoparticles, in combination with doxorubicin, exert a synergistic anticancer effect by promoting oxidative stress and inhibiting the proliferation of SK-OV-3 cells. These findings underscore the potential of Al2O3NPs as an effective therapeutic modality in ovarian cancer treatment, particularly for aggressive subtypes like SK-OV-3.

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INTRODUCTION

Ovarian cancer, particularly high-grade serous subtypes such as SK-OV-3, is one of the most lethal gynecologic malignancies, ranking as the fifth leading cause of cancer-related mortality among women worldwide(1). The aggressive nature of ovarian cancer is often attributed to late-stage diagnosis, intrinsic chemoresistance, and its complex interaction with the endocrine system(2). Hormonal influences, including the roles of estrogen, progesterone, and gonadotropins, are believed to contribute to the proliferation and progression of ovarian cancer, making the endocrine system a critical area of focus for therapeutic interventions(3). Despite advancements in surgery and chemotherapy, the five-year survival rate for advanced-stage ovarian cancer remains below 30%, emphasizing the urgent need for novel therapeutic strategies(4). Systemic therapies, such as tyrosine kinase inhibitors and immune checkpoint inhibitors, have shown promise but are often hindered by treatment resistance, adverse effects, and high costs(5).

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Nanotechnology has emerged as a transformative platform for cancer therapy, enabling targeted delivery of chemotherapeutic agents and enhancing therapeutic efficacy while minimizing systemic toxicity(6). Among the various nanomaterials explored, Aluminum oxide nanoparticle (Al2O3NPs), have garnered significant attention due to their unique physicochemical properties, including high thermal and electrical conductivity, oxidative reactivity, and the ability to penetrate cellular membranes(7-9). These properties allow aluminum nanoparticles to interact with intracellular biomolecules, generate reactive oxygen species (ROS), and disrupt cellular homeostasis. Moreover, Al2O3NPs possess intrinsic cytotoxic potential, which can be exploited to sensitize cancer cells to chemotherapeutic agents(10).

Doxorubicin, a widely used anthracycline chemotherapeutic agent, exerts its anticancer effects through multiple mechanisms, including DNA intercalation, inhibition of topoisomerase II, and generation of ROS(11). These mechanisms collectively lead to DNA damage, cell cycle arrest, and apoptosis. However, the clinical utility of doxorubicin is often limited by dose-dependent cardiotoxicity, systemic side effects, and the development of multidrug resistance in tumor cells(12). Integrating doxorubicin with nanomaterials such as Al2O3NPs offers a potential strategy to mitigate these limitations. Al2O3NPs can serve as drug carriers, facilitating targeted delivery to cancer cells, and can also amplify the oxidative stress induced by doxorubicin, thereby enhancing its cytotoxicity(13).

The relationship between ovarian cancer and oxidative stress is particularly notable. Elevated ROS levels can induce lipid peroxidation, protein oxidation, and DNA damage, leading to apoptosis. However, ovarian cancer cells, including SK-OV-3, often upregulate antioxidant defense systems, such as GSH, to maintain redox homeostasis and promote survival(14). However, cancer cells often upregulate antioxidant systems, such as GSH and superoxide dismutase, to counteract oxidative stress and maintain redox balance(15). The combined use of Al2O3NPs and doxorubicin holds the potential to overwhelm these defenses by simultaneously increasing ROS production and depleting antioxidant reserves, thereby inducing oxidative stress-mediated cell death(16).

In this study, we investigate the synergistic anticancer effects of aluminum nanoparticles in combination with doxorubicin on SK-OV-3 ovarian cancer cells. We evaluate cell viability, ROS production, lipid peroxidation, and GSH levels to elucidate the mechanisms underlying these effects. By integrating nanotechnology with conventional chemotherapy, this research aims to provide new insights into the development of effective therapeutic strategies for ovarian cancer and explore the potential of aluminum nanoparticles as a novel adjuvant in targeting endocrine-related cancer pathways.

MATERIALS AND METHODS

Chemicals

Aluminum oxide nanoparticle (Al2O3NPs) from a US Research Nanomaterials Inc. / size 30–10 nm and purity of 99.99% with its device specifications was obtained from the domestic representative of Pishgaman Nano Materials Iranian Company in Mashhad. Doxorubicin hydrochloride was obtained from Pfizer (New York, NY, USA). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin, trypsin-EDTA, and phosphate-buffered saline (PBS) were supplied by Gibco (Thermo Fisher Scientific, Waltham, MA, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent, 2',7'-dichlorofluorescin diacetate (DCFH-DA), thiobarbituric acid (TBA), and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) were purchased from Sigma-Aldrich. All other chemicals were of analytical grade and obtained from standard suppliers.

Cell Culture

SK-OV-3 ovarian cancer cells were obtained from the National Cell Bank of Iran (Pasteur Institute, Tehran, Iran). Cells were cultured in DMEM high glucose culture medium supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin. Cultures were maintained in a humidified incubator at 37°C with 5% CO_2. Cells were subculture every 3–4 days to maintain exponential growth and used between passages 5 and 15 for all experiments(17, 18).

Treatment Protocol

SK-OV-3 cells were seeded in 96-well plates at a density of 1×10^4 cells per well and allowed to attach overnight. Cells were treated with various concentrations of Al2O3 (10, 15, 20, 25 and 30 μM) in combination with doxorubicin (10 μg/mL) for

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Table 1. Information about aluminum oxide nanoparticles(Al2O3NPs)

Al2O3 Nanoparticles Dispersion in IPA / 2-Propanol Inspection Report (COA) Standard									
Appearance	Crystal Structure and Type	PH value	Original particle size	Assay Al2O3	Solvent	Iron	Arsenic	Pb	Al2O3 Purity
Translucent liquid	Gamma	6-8	15nm	≥10%	90% of 2-Propanol / IPA	≤23ppm	≤1.5ppm	≤2ppm	99.99%

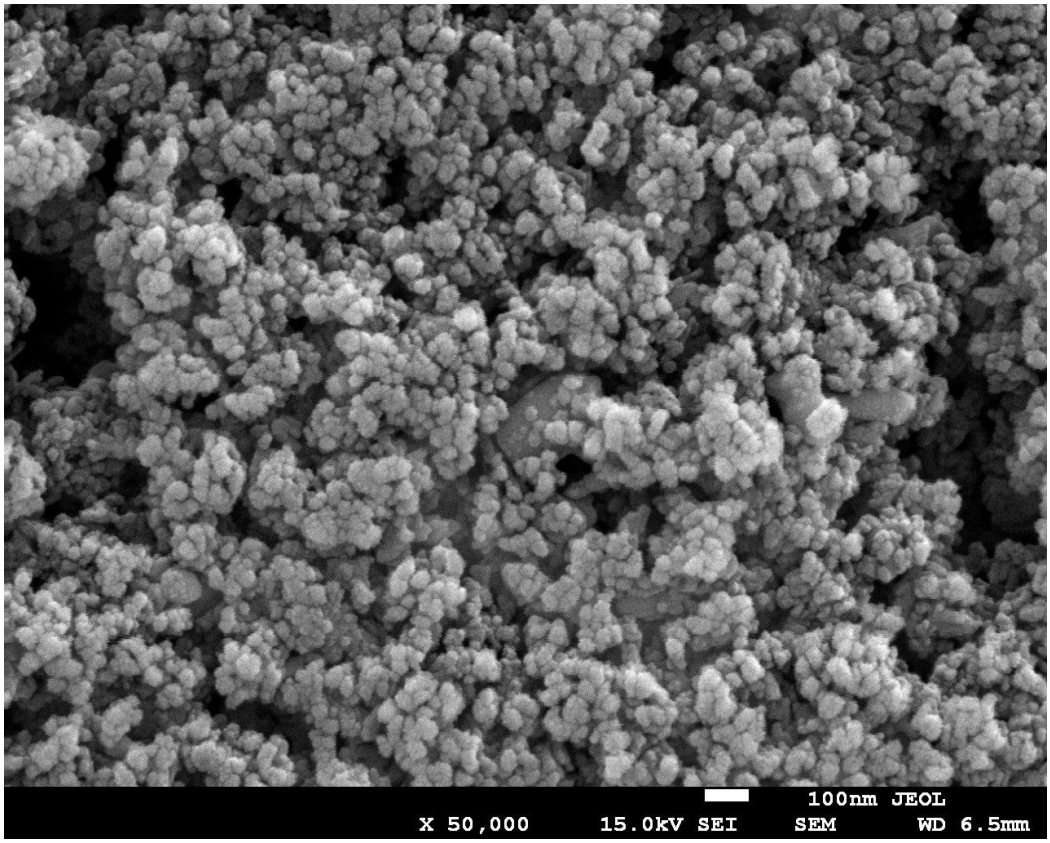

Fig. 1. The Fig. of Al2O3NPs

24 hours. Untreated cells served as controls.

Cell Viability Assay

The cytotoxic effects of treatments on SK-OV-3 cells were assessed using the MTT assay. After treatment, 20 µL of MTT reagent (5 mg/mL) was added to each well and incubated for 4 hours at 37°C. Formazan crystals formed during the reaction were dissolved in 150 µL of dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm using a microplate reader (BioTek, Winooski, VT, USA). The percentage of cell viability was calculated relative to untreated controls(19, 20).

Measurement of Reactive Oxygen Species (ROS)

Intracellular ROS levels were measured using the DCFH-DA assay. Cells were incubated with DCFH-DA (10 µM) for 30 minutes at 37°C, followed by treatment with aluminum nanoparticles and doxorubicin. Fluorescence intensity was measured using a fluorescence microplate reader (excitation: 485 nm; emission: 530 nm). Results were expressed as a fold increase in ROS levels compared to untreated controls(21, 22).

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Lipid Peroxidation Assay

Lipid peroxidation was quantified by measuring malondialdehyde (MDA) levels using the thiobarbituric acid-reactive substances (TBARS) assay. Cell lysates were mixed with TBA reagent and incubated at 95°C for 30 minutes. After cooling, samples were centrifuged at 10,000 × g for 10 minutes, and the supernatant absorbance was measured at 532 nm. MDA levels were expressed in nmol/mg protein(23, 24).

Glutathione (GSH) Assay

GSH levels were determined using the DTNB reagent. Cell lysates were prepared, and 50 µL of each sample was mixed with 50 µL of DTNB reagent (10 mM) in PBS. Absorbance was measured at 412 nm, and GSH levels were calculated using a standard curve(25, 26).

Statistical Analysis

All experiments were performed in triplicate, and results are expressed as mean ± standard deviation (SD). Statistical analysis was conducted using GraphPad Prism v8.0 (GraphPad Software, San Diego, CA, USA). Differences between groups were assessed using one-way ANOVA followed by Tukey's post hoc test. A p-value <0.05 was considered statistically significant.

RESULTS

Effect of Al2O3NPs and Doxorubicin on Ovarian Cancer Cell Viability

The cytotoxic effects of Al2O3NPs in combination with doxorubicin on ovarian cancer cells (SK-OV-3) were evaluated using the MTT assay. As shown in figure 1 Al2O3NPs at concentrations of 10, 15, 20, 25 and 30 µM, combined with doxorubicin (10 µg/mL), resulted in a dose-dependent reduction in cell viability after 24 hours of treatment. The combination treatment showed a synergistic effect, with a greater inhibitory effect compared to either agent alone ($p<0.05$).

Induction of Oxidative Stress

Reactive oxygen species (ROS) levels were measured to assess the oxidative stress induced

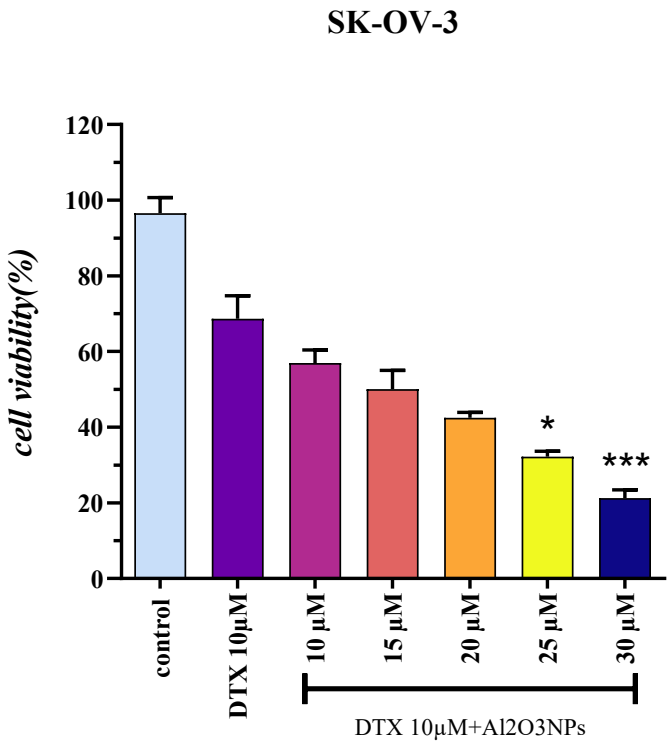

Fig. 1. Dose-dependent reduction in SK-OV-3 ovarian cancer cell viability after 24-hour treatment with Al2O3NPs at concentrations of 10, 15, 20, 25, and 30 µM, in combination with 10 µg/mL doxorubicin. The combination therapy exhibits a synergistic effect, significantly enhancing cytotoxicity compared to individual treatments. * ($p<0.05$) and *** ($p<0.001$) versus DTX group.

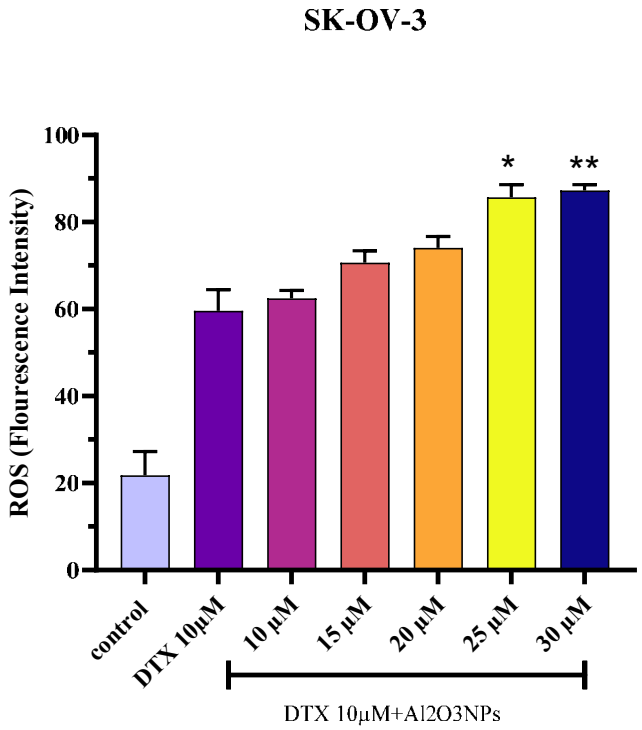

Fig. 2. Dose-dependent increase in reactive oxygen species (ROS) production in SK-OV-3 ovarian cancer cells after 24-hour treatment with Al2O3NPs at concentrations of 10, 15, 20, 25, and 30 µM, in combination with 10 µg/mL doxorubicin. The combination treatment significantly elevates ROS levels compared to individual treatments, ($p<0.05$), indicating a synergistic effect. * ($p<0.05$) and **($p<0.01$) versus DTX group.

by Al2O3NPs in combination with doxorubicin. Treatment with Al2O3NPs (10, 15, 20, 25and 30 µM) in combination with doxorubicin (10 µg/mL) caused a significant increase in ROS production, with ROS levels elevated compared to DTX alone ($p<0.01$), indicating a synergistic effect.

Lipid Peroxidation

Malondialdehyde (MDA) levels, a marker of lipid peroxidation, were significantly elevated in ovarian cancer cells (SK-OV-3) treated with Al2O3NPs in combination with doxorubicin. Treatment with Al2O3NPs (10, 15, 20,25 and 30 µM) and doxorubicin (10 µg/mL) resulted in significant increase in MDA levels compared to individual treatments ($p<0.01$), indicating enhanced oxidative damage to cellular membranes.

Depletion of Intracellular Glutathione (GSH)

Intracellular glutathione (GSH) levels were significantly reduced following treatment with Al2O3NPs in combination with doxorubicin. Al2O3NPs (10, 15, 20, 25 and 30 µM) combined with doxorubicin (10 µg/mL) caused significant reduction in GSH levels compared to individual treatments ($p<0.01$), highlighting the role of oxidative stress in the cytotoxic mechanism.

Synergistic Cytotoxicity Through Oxidative Stress Pathways

The findings collectively demonstrate that the combination of Al2O3NPs and doxorubicin exerts synergistic cytotoxic effects on HepG2 cells. This synergy is likely mediated through enhanced oxidative stress, as evidenced by increased ROS generation, lipid peroxidation, and GSH depletion.

DISCUSSION

This study evaluated the synergistic anticancer effects of aluminum nanoparticles in combination with doxorubicin on ovarian cancer cells. The results demonstrate that this combination significantly enhances cytotoxicity compared to individual treatments, primarily through

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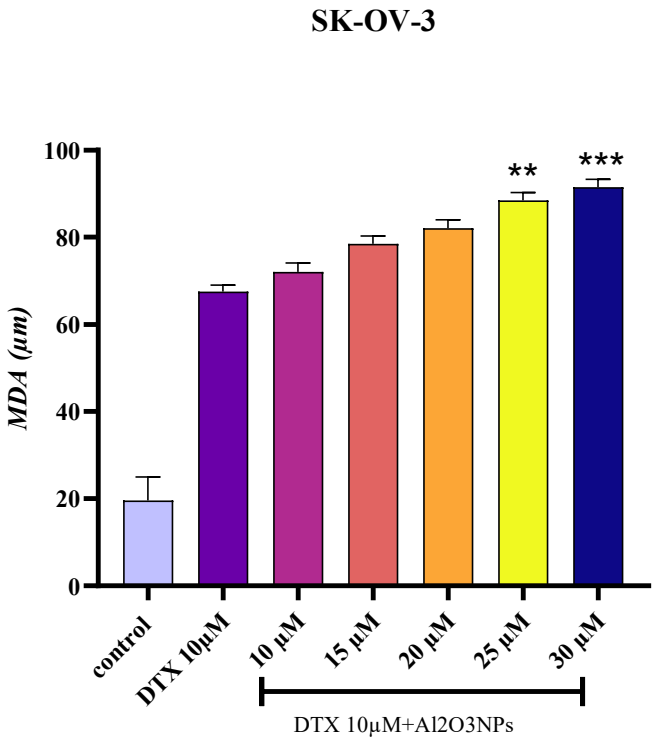

Fig. 3. Malondialdehyde (MDA) levels in SK-OV-3 ovarian cancer cells after 24-hour treatment with Al2O3NPs at concentrations of 10, 15, 20, 25, and 30 µM, both alone and in combination with 10 µg/mL doxorubicin. The combination treatment significantly increases MDA levels compared to individual treatments ($p<0.01$), indicating enhanced oxidative damage to cellular membranes. ** ($p<0.01$) and ***($p<0.001$) versus DTX group.

mechanisms involving oxidative stress, disruption of redox homeostasis, and potentiation of doxorubicin's effects. These findings underscore the potential of Al2O3NPs as effective co-therapeutic agents in ovarian cancer treatment, with significant mechanistic insights into their action.

The synergistic effects observed in SKOV-3 cells are likely driven by several interrelated mechanisms. Al2O3NPs are known to induce significant oxidative stress by generating ROS due to their high surface activity and potential for electron transfer reactions. In this study, treatment with doxorubicin combination with Al2O3NPs at increasing concentrations (10–30 µM) led to a dose-dependent increase in ROS production, as evidenced by elevated MDA levels. The depletion of GSH, a critical antioxidant, further confirmed the inability of cancer cells to neutralize the surge in ROS. These oxidative changes compromise cellular structures, including lipids, proteins, and nucleic acids, ultimately leading to apoptotic cell death.

Doxorubicin exerts its anticancer effects through multiple mechanisms, including DNA intercalation, inhibition of topoisomerase II, and induction of ROS. In SKOV-3 cells, the combination of Al2O3NPs and doxorubicin amplified ROS production and oxidative damage beyond levels achieved with either treatment alone(11, 27). This is consistent with the hypothesis that nanoparticles enhance the redox imbalance within cancer cells, sensitizing them to chemotherapy. The synergistic increase in oxidative stress likely overwhelmed the cellular repair systems, driving apoptosis through intrinsic pathways(12, 28).

Mitochondrial dysfunction represents another key mechanism contributing to the observed cytotoxicity. Excessive ROS can damage mitochondrial membranes, disrupt the electron transport chain, and trigger the release of pro-apoptotic factors such as cytochrome c(29, 30). This, in turn, activates caspase-mediated apoptotic pathways(31, 32). Although mitochondrial parameters were not directly assessed in this study, the marked reduction in cell viability and enhanced ROS generation strongly suggest a mitochondrial component.

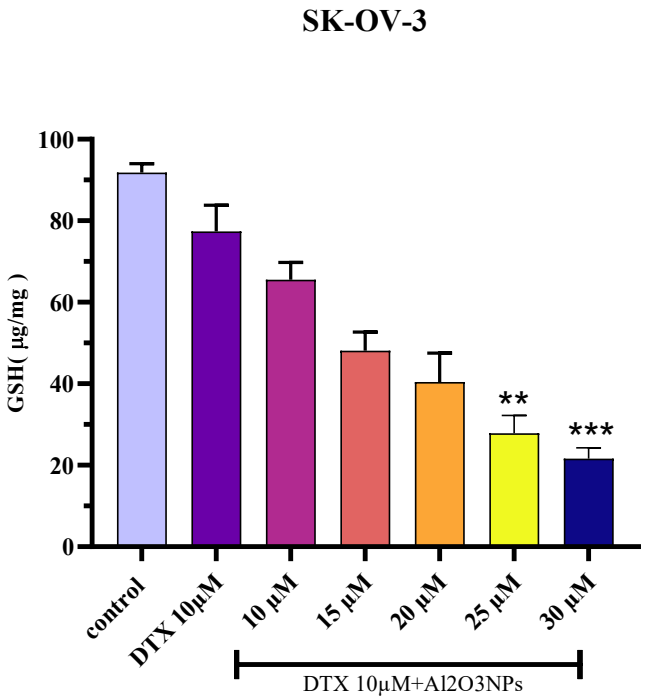

Fig. 4. Intracellular glutathione (GSH) levels in SK-OV-3 ovarian cancer cells after 24-hour treatment with Al2O3NPs at concentrations of 10, 15, 20, 25, and 30 µM, both alone and in combination with 10 µg/mL doxorubicin. The combination treatment significantly reduces GSH levels compared to individual treatments ($p<0.01$), highlighting the role of oxidative stress in the cytotoxic mechanism. ** ($p<0.01$) and ***($p<0.001$) versus DTX group

The findings of this study align with existing research on metal-based nanoparticles in cancer therapy. For instance, silver and gold nanoparticles have been shown to potentiate the effects of doxorubicin in various cancer models, including breast and liver cancers(33, 34). A recent study by Moawad et al. demonstrated that silver nanoparticles enhance doxorubicin-induced ROS production and apoptosis, a mechanism closely paralleling the effects observed with Al2O3NPs in the present study(35).

However, aluminum nanoparticles possess unique properties that may provide distinct advantages. Unlike inert nanoparticles, Al2O3NPs actively participate in redox cycling and Fenton-like reactions, generating robust oxidative stress(36). Additionally, their physicochemical characteristics, such as tunable size and surface charge, enhance cellular uptake and target specificity. Compared to other nanoparticles, Al2O3NPs may exert stronger oxidative effects, making them particularly suitable for redox-sensitive cancers like ovarian cancer(37, 38).

The synergistic effects of Al2O3NPs and doxorubicin observed in SKOV-3 cells hold significant promise for ovarian cancer therapy, a disease often associated with poor prognosis and chemoresistance. By combining Al2O3NPs with doxorubicin, it may be possible to lower the required dose of doxorubicin, reducing its systemic toxicity while maintaining or even enhancing therapeutic efficacy. Moreover, the ability of Al2O3NPs to amplify oxidative stress within cancer cells provides a potential strategy for overcoming chemoresistance, as oxidative pathways are critical vulnerabilities in resistant tumors.

The selective cytotoxicity of Al2O3NPs toward cancer cells, likely due to their elevated basal ROS levels, is another key advantage. Normal cells, with their more robust antioxidant defenses, are less susceptible to ALNP-induced oxidative stress, suggesting a favorable therapeutic index for this combination approach.

Study Limitations and Future Directions

While the findings are promising, several limitations warrant consideration. This study was conducted in vitro using SKOV-3 cells, and the

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results may not fully translate to in vivo conditions. Factors such as nanoparticle biodistribution, immune clearance, and systemic toxicity need to be evaluated in animal models. Additionally, the long-term safety of Al2O3NPs, particularly their potential to accumulate in tissues, should be thoroughly investigated.

Future research should focus on optimizing the physicochemical properties of Al2O3NPs to enhance their therapeutic efficacy. Surface functionalization with targeting ligands could improve their selectivity for ovarian cancer cells while minimizing off-target effects. Furthermore, studies exploring the role of Al2O3NPs in modulating the tumor microenvironment, including their effects on angiogenesis and immune responses, could provide valuable insights.

Another important avenue for future research involves evaluating the combination of Al2O3NPs with other chemotherapeutic agents or immunotherapies. The ability of Al2O3NPs to modulate redox-sensitive signaling pathways may synergize with emerging therapeutic strategies targeting the tumor's metabolic vulnerabilities.

CONCLUSION

This study highlights the synergistic anticancer effects of Al2O3NPs in combination with doxorubicin in ovarian cancer cells. Through mechanisms involving enhanced oxidative stress, depletion of antioxidant defenses, and potentiation of doxorubicin's cytotoxic effects, this combination strategy offers a promising approach for ovarian cancer treatment. These findings provide a strong foundation for the development of Al2O3NPs -based combination therapies, which could improve therapeutic outcomes while minimizing systemic toxicity. Further research is needed to validate these results in vivo and to optimize Al2O3NPs formulations for clinical application.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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