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RESEARCH ARTICLE

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Sanguinarine enhances cisplatin sensitivity via glutathione depletion in cisplatin-resistant ovarian cancer (A2780) cells

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Abstract

Ovarian cancer is considered as one of the most lethal gynecological cancers, and cisplatin-based therapy has an important role as the first-line option for chemotherapy. Resistance to chemotherapy is the main obstacle against successful cancer chemotherapy with cisplatin. Therefore, identifying potent compositions and molecules with fewer side-effects is a big challenge to overcome cisplatin resistance. In this study, we investigated the possible mechanism and potency of sanguinarine, a plant-derived alkaloid, in human cisplatin-resistant ovarian cancer (A2780/R) cells. The effect of sanguinarine on cytotoxicity of cisplatin was determined by MTT assay. Apoptosis-inducing effect of sanguinarine alone and in combination with cisplatin was evaluated by annexin V/PI assay and DAPI staining. Intracellular glutathione (GSH) content was quantitated using GSH assay kit after treatment with sanguinarine. Results indicated that sanguinarine enhances the sensitivity of A2780/R cells to cisplatin. Apoptosis-inducing effect of cisplatin was also enhanced when combined with sanguinarine. Furthermore, sanguinarine reduced intracellular GSH content in a dose-dependent but not time-dependent manner. These findings suggest that sanguinarine could reverse cisplatin resistance in A2780/R cells through GSH reduction. Therefore, sanguinarine can be used as one of the potent adjuvants for ovarian cancer chemotherapy.

KEYWORDS

cisplatin resistance, glutathione, ovarian cancer, sanguinarine

1 | INTRODUCTION

Ovarian cancer is considered as one of the most lethal cancers of the women. Standard treatment procedures for ovarian cancer include surgery followed by chemotherapy, and cisplatin is considered as the first-line chemotherapy option (Itamochi & Kigawa, 2012; Oronsky et al., 2017). Despite the favorable initial response, most patients develop resistance after several times of exposure to cisplatin. Therefore, patients suffering from ovarian cancer have a very poor five-year survival rate (Eckstein, 2011; Markman & Bookman, 2000). Multidrug resistance (MDR) against chemotherapeutics can be developed with various mechanisms such as overexpression of drug efflux proteins, reduced drug uptake, enhanced activity of DNA repair pathways, alteration of target molecules, alteration of apoptotic pathways, and inactivation of drugs by glutathione, and other detoxifying agents (Shafiei-Irannejad, Samadi, Salehi, et al., 2018; Shafiei-Irannejad, Samadi,

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Yousefi, et al., 2018). Among them, enhanced drug inactivation by glutathione (GSH) has been shown to be the main mechanism for resistance against cisplatin (Nikounezhad, Nakhjavani, & Shirazi, 2017). When cisplatin enters inside the cells, it forms active electrophilic metabolites by aquation hydrolysis which can react with various intracellular molecules such as DNA (Perez, 1998). However, GSH can conjugate with these active metabolites and GSH–cisplatin complex is pumped out of the cell by the glutathione-S-conjugate (GS-X) export pump which prevents them from reaching to target molecules, and as a result, resistance to cisplatin will occur (Meijer, Mulder, Hospers, Uges, & De Vries, 1990; Zhang, Li, & Yu, 2017). Therefore, targeting intracellular GSH can be considered as a promising strategy for successful chemotherapy with cisplatin.

Previous studies have shown that sanguinarine, a plant-derived alkaloid, can reduce intracellular GSH content (Debiton, Madelmont, Legault, & Barthomeuf, 2003; Ulrichová et al., 2001). Sanguinarine belongs to the family of benzophenanthridine alkaloids and have shown several biological properties including anti-inflammatory, antibacterial, and antifungal activities. In addition to that, anticancer effects of sanguinarine have been reported in different cancers with multiple pathways (Matkar, Wrischnik, & Hellmann-Blumberg, 2008; Park et al., 2010). Ulrichova and colleagues have shown that sanguinarine decreases intracellular GSH content in hepatocytes (Ulrichová et al., 2001). In another study, Debiton and colleagues showed GSH depletion in L-929 murine cells after treatment with sanguinarine (Debiton et al., 2003). Since GSH content is associated with resistance to cisplatin, it seems that the natural compound, sanguinarine, can reverse GSH-associated resistance in cisplatin-resistant cells. Therefore, in the current study we investigate the possible effects of sanguinarine on cytotoxicity of cisplatin, apoptosis-inducing effects of cisplatin, and intracellular GSH levels in cisplatin-resistant ovarian cancer (A2780/R) cells.

2 | METHODS AND MATERIALS

Cisplatin and sanguinarine were obtained from Cayman, USA. N-Acetylcysteine (NAC) was obtained from Avicenna Laboratories Inc, Iran. 4,6-Diamidino-2-phenylindole (DAPI) and 3-(4,5-dimethylthiazol)-2, 5-diphenyltetrazolium bromide (MTT) were obtained from Sigma Co, USA. Fetal bovine serum (FBS), RPMI-1640, and trypsin-EDTA were purchased from Gibco, USA. Human sensitive (A2780/S) and cisplatin-resistant ovarian cancer cells (A2780/R) were kind gifts from FH. Shirazi, Pharmaceutical Sciences Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Annexin V/Propidium iodide apoptosis kit was purchased from MabTag GmbH, Germany. GSH (reduced glutathione) assay kit was obtained from ZellBio GmbH, Germany. This study was carried out under the approved protocols with the ethical committee of Urmia University of Medical Sciences (IR.UMSU.REC.1397.254).

2.1 | Cell culture

A2780/S and A2780/R cells were cultured in RPMI-1640 medium containing 10% FBS, 100 unit/ml penicillin, and 100 mg/ml streptomycin and maintained in 37°C incubator with 50% CO_2. To maintain the resistance phenotype, A2780/R cells received 2.5 μM cisplatin in each culture which was removed from the medium one week prior to each experiment.

2.2 | Cell viability assay

To determine the cytotoxicity of various compounds, MTT assay was performed according to previous methods (Azimi et al., 2018). Briefly, A2780/S and A2780/R cells at the density of 10^4 cells/well were cultured in 96-well plates and incubated for 24 hr. Then, different concentrations of cisplatin with or without sanguinarine were added to the wells. After 48 hr, cells received fresh medium containing 500 μg/ml MTT. Following 4-hr incubation, 200 μl dimethyl sulfoxide replaced the MTT containing medium to solve the purple formazan crystals and incubated for further 30 min. Finally, the absorbances of all wells were measured at 570 nm using an ELISA plate reader (State Fax, USA). Cell viability and half-maximal inhibitory concentration (IC_{50}) of each treatment were also calculated. Moreover, to calculate the resistance fold (RF), the IC_{50} of A2780/R cells in each treatment was divided to IC_{50} of wild-type parental cells.

2.3 | Combination effect analysis

The coefficient of drug interaction (CDI) was determined according to the Chou–Talalay method to investigate the drug interaction between sanguinarine and cisplatin (Chou & Talalay, 1984). CDI was calculated with the following formula: $CDI = AB/A \times B$, where AB is the viability of cells treated with combination of sanguinarine and cisplatin, and A and B are the viability of cells treated with sanguinarine and cisplatin, respectively. According to this method, $CDI = 1$, $CDI > 1$, and $CDI < 1$ indicates additive, antagonistic, and synergistic effects, respectively.

2.4 | Apoptosis analysis

To evaluate the apoptosis-inducing effects of sanguinarine, flow cytometeric analysis of apoptosis was performed. 5×10^5 of A2780/R cells were seeded into each well of six-well plates and maintained in 37°C incubator for 24 hr. Afterward, cells were exposed to medium containing sanguinarine (1 μM),

cisplatin (11 μM), sanguinarine (1 μM) + cisplatin (11 μM), and sanguinarine (1 μM) + cisplatin (11 μM) + NAC (1 mM). Following 48 hr, cells were trypsinized, PBS washed, and resuspended in 100 μl annexin V binding buffer. Then, 5 μl of annexin V/FITC and 5 μl of propidium iodide were added and incubated at dark for 15 min. Finally, cells were centrifuged and resuspended in 200 μl of annexin V binding buffer and flow cytometeric analysis of apoptosis was measured using Partec GmbH (Munster, Germany).

2.5 | DAPI staining

DAPI staining was performed for visualization of the fragmented and condensed nuclei of the cells in different treated groups. 4×10^4 of A2780/R cells were cultured onto the glass coverslips placed in each well of 6-well plates and incubated at 37°C for overnight. After 24 hr, cells received fresh medium containing sanguinarine (1 μM), cisplatin (11 μM), and sanguinarine (1 μM) + cisplatin (11 μM). Control group received only fresh medium. After 48 hr, cells were PBS washed following by fixation with 4 wt% paraformaldehyde for 15 min. After another step of washing, the permeability of the cells was increased by addition of Triton X-100 (0.1% w/v) for 10 min. Afterward, cells were washed again and stained with 0.3 μg/ml DAPI. Following 15-min incubation at dark, the excess stain was removed by washing and the nuclei of the cells were observed with fluorescent microscope (Nikon Eclipse Ts2R, Japan).

2.6 | Measurement of intracellular GSH content

GSH assay kit (ZellBio, Germany) was used for quantification of intracellular GSH content. In brief, 5×10^5 of cells were cultured into each well of 6-well plates and incubated overnight at 37°C. Then, cells were treated with or without sanguinarine in the presence or absence of NAC for the indicated times. After each time, cells were harvested and washed with PBS and centrifuged at 270 *g* for 5 min. Then, 100 μl cell lysis buffer was added to the cell pellet and incubated on ice for 20 min followed by centrifugation at 20,200 *g* for 10 min. The supernatant of each sample was used for GSH assay using the manufacturer's protocol.

2.7 | Statistical analysis

All data were presented as mean ± *SD* of three independent experiments. GraphPad Prism software 6.01 was used for data analysis and construction of graphs. One-way analysis of variance (ANOVA) with Bonferroni's and Sidak's tests was performed to explain the significance between groups. *p*-Values lesser than .05 were considered as significant.

3 | RESULTS

3.1 | Characterization of A2780/S and A2780/R cells

MTT assay was performed to determine the viability of A2780/S and A2780/R cells following treatment with various concentrations of cisplatin. As seen in Figure 1a, A2780/S cells exhibited more toxicity to cisplatin compared with A2780/R cells, indicating the resistance phenotype in A2780/R cells. To further confirm the resistance phenotype, basal intracellular GSH content was measured and compared in A2780/R and A2780/S cells. Results showed that resistant cells have significantly higher amounts of GSH in comparison with parental sensitive cells (Figure 1b). As previously reported, cisplatin resistance in tumor cells is associated with

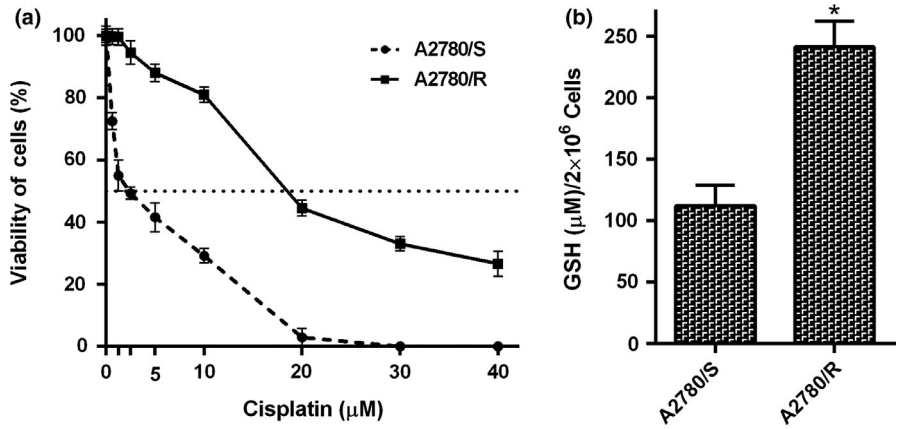

FIGURE 1 Depicting resistance phenotype in A2780/R cells. (a) The viability of A2780/S and A2780/R cells after treatment with different concentrations of cisplatin for 48 hr was assessed by MTT assay. Each point represents the mean ± *SD* of three independent experiments. (b) Basal GSH content was measured in both A2780/S and A2780/R cells using GSH assay kit. Each point represents the mean ± *SD* of three independent experiments. *Significance at $p < .05$

intracellular GSH levels (Britten, Green, & Warenius, 1992; Jamali et al., 2015; Nikounezhad et al., 2017).

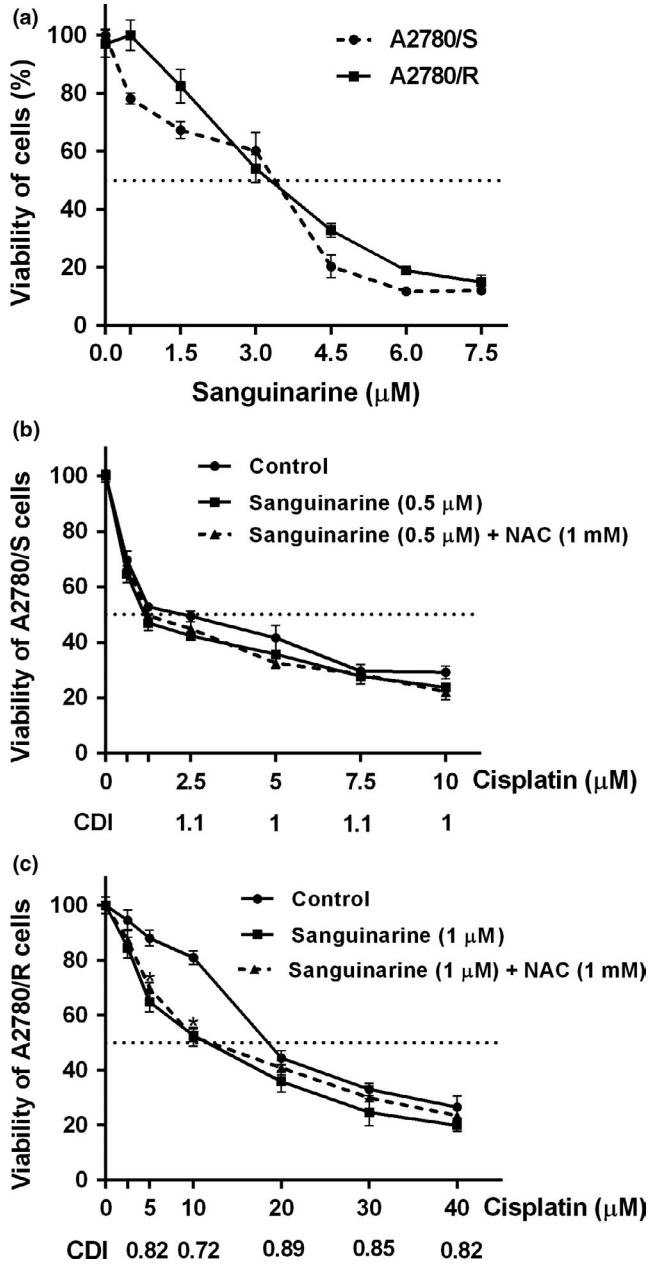

FIGURE 2 The cytotoxicity of sanguinarine and cisplatin in A2780/S and A2780/R cells. (a) The cytotoxicity of sanguinarine in A780/S and A2780/R cells for 48 hr was determined with MTT assay. Each point represents the mean ± *SD* of three independent experiments. (b, c) A780/S and A2780/R cells were treated with 0.5, 1 μM sanguinarine, respectively, in combination with different concentrations of cisplatin in the presence or absence of 1 mM NAC (N-acetylcysteine) in both cells for 48 hr. Cell viability was determined by MTT assay. Each point represents the mean ± *SD* of three independent experiments. *Significance at $p < .05$

3.2 | Effects of sanguinarine on viability of ovarian cancer cells

A2780/S and A2780/R cells were exposed to various concentrations of sanguinarine (0.5–7.5 μM) for 48 hr, and the viability of cells was determined with MTT assay. As seen in Figure 2a, the IC_{50} of sanguinarine in A2780/R and A2780/S cells was 2.36 and 3.05 μM, respectively. The toxic effects of sanguinarine in A2780/S cells start from 1 μM and in A2780/R cells start from 1.5 μM. Therefore, a safe concentration of 0.5 μM for A2780/S cells and 1 μM for A2780/R cells was chosen and combined with different concentrations of cisplatin in both cells (Figure 2b, c). Shift to left in the cytotoxicity profile of cisplatin in A2780/R cells following treatment with sanguinarine indicates that sanguinarine could successfully enhance the toxicity of cisplatin in these cells. Treatment with sanguinarine significantly decreased the IC_{50} and RF values in A2780/R cells, indicating that sanguinarine reversed cisplatin resistance in resistant ovarian cancer cells (Table 1). Furthermore, CDI analysis indicated that the combination of sanguinarine and cisplatin was synergistic since the CDI values were lesser than 1 among all concentrations (Figure 2c). Addition of 1mM NAC to the combination of sanguinarine and cisplatin could not reverse the cytotoxic effects. In A2780/S cells, sanguinarine could also enhance the toxicity of cisplatin, although this effect was lower in comparison to A2780/R cells.

3.3 | Effect of sanguinarine on apoptosis induction in A2780/R cells by cisplatin

To determine the effect of sanguinarine treatment on cisplatin-induced apoptosis in A2780/R cells, flow cytometeric analysis of apoptosis was performed after staining with annexin V/PI. As seen in Figure 3, combination of sanguinarine and cisplatin significantly enhanced the migration of A2780/R cells to apoptotic regions in comparison with each compound alone (Figure 3). When NAC was added to this combination, no substantial change was observed in the rate of apoptosis. DAPI staining was also performed further to flow cytometery to observe the nuclei of the cells after treatment with sanguinarine and cisplatin. The morphology of chromatin and integrity of nucleus is a good indicator of healthy and apoptotic cells. Fluorescence microscopy images were shown in Figure 4. As observed, control group that only received fresh medium did not show any change in nuclei as they were homogenously stained. Furthermore, single treatment with sanguinarine or cisplatin could only lead to chromatin condensation in comparison with control group, while combination of sanguinarine and cisplatin led to apoptosis in A2780/R cells as confirmed by chromatin condensation and fragmentation. Moreover, the number of cells was decreased in combination treatment group, indicating that this combination prevented the proliferation of cells.

TABLE 1 Effect of treatment with sanguinarine on cisplatin cytotoxicity in A2780/S and A2780/R cells

	Cisplatin		Sanguinarine 1 μM + Cisplatin	
Cell lines	**IC_{50} (μM)**	**RF**	**IC_{50} (μM)**	**RF**
A2780/S	2.14 ± 0.51	–	1.43 ± 0.21	–
A2780/R	19.7 ± 1.53	9.2	10.93 ± 1.22	5.1[a]

Note: The viability of cells was determined following treatment with different concentrations of cisplatin in the presence or absence of 1 μM sanguinarine. Each value represents the mean ± *SD* of three independent experiments. RF values were calculated by dividing the IC_{50} of resistant cells in each treatment to the IC_{50} of sensitive cells.

[a]Significance at $p < .05$.

3.4 | Intracellular GSH content

Effects of sanguinarine on intracellular GSH content of A2780/R cells following treatment with different concentrations of sanguinarine and different time intervals were determined using a GSH assay kit. When sanguinarine 1 μM was added to the medium of A2780/R cells, 27% of cellular GSH was decreased after 30 min (Figure 5a). After 1, 3, and 6 hr, no significant change was observed in GSH levels in comparison with 30 min, indicating that GSH depletion following sanguinarine treatment was very fast and mostly occurred in primary minutes. When cells were exposed to different concentrations of sanguinarine, a dose-dependent decline was observed in cellular GSH content (Figure 5b). At all concentrations, addition of 1 mM NAC had no significant change on GSH levels in comparison with cells receiving only sanguinarine.

4 | DISCUSSION

Multidrug resistance is the main barrier against successful cancer chemotherapy. Several mechanisms and pathways are involved in MDR development, but the main mechanism for resistance to cisplatin is enhanced drug inactivation by cellular GSH (Kawahara et al., 2019; Meijer et al., 1990; Nikounezhad et al., 2017; Perez, 1998). GSH, an important intracellular antioxidant defense, conjugates to active electrophilic cisplatin inside the cells and inactivates it before reaching to DNA. Lida and coworkers in 2001 investigated the effect of GSH depletion on cisplatin resistant in colonic cancer cells. They targeted GSH synthesis by a hammerhead ribozyme against the rate limiting γ-glutamylcysteine synthetase enzyme in GSH synthesis. They found that reduction in GSH levels sensitized colonic cancer cells to cisplatin (Iida et al., 2001). In a very recent study performed by Kawahara and colleagues, targeting an enzyme involved in sulfur metabolism (cystathionine β-synthase), by exogenous carbon monoxide (CO) enhanced the sensitivity of ovarian cancer cells to cisplatin through intracellular GSH reduction (Kawahara et al., 2019). Therefore, targeting intracellular GSH can be a promising approach to overcome cisplatin resistance. Recently, plant-derived compounds have gained especial interest among researchers for the treatment of different human diseases due to their natural origin and lower toxicity.

Sanguinarine, a naturally occurring alkaloid, has shown anticancer effects in several studies further to its anti-inflammatory and antibacterial effects. Sanguinarine exerts its anticancer properties by targeting multiple pathways and molecules inside the cells such as VEGF, Akt, p38, p27, cyclin D1, XIAP, Bid, and Bcl-2 (Kalogris et al., 2014; Kim et al., 2008; Xu et al., 2013). Sanguinarine was also capable of reducing intracellular GSH content in various types of tumor cells (Debiton et al., 2003; Jang et al., 2009; Ulrichová et al., 2001). As mentioned, GSH is the major contributor in cisplatin resistance. Therefore, in the present study we investigated the influence of sanguinarine on resistance to cisplatin in cisplatin-resistant ovarian cancer (A2780/R) cells.

Our results showed that sanguinarine decreased the RF and IC_{50} values of cisplatin in A2780/R cells. At concentrations ranging 0.5–1.5 μM, sanguinarine did not show significant cytotoxicity in A2780/R cells. Therefore, the concentration of 1 μM was chosen as a safe concentration to minimize the toxic effects of sanguinarine itself and combined with various concentrations of cisplatin. We observed that treatment with sanguinarine 1 μM significantly enhanced the cytotoxicity of cisplatin in A2780/R cells as confirmed by reduced IC_{50} and RF values. The addition of thiol-reducing agent, NAC, could not reverse the cytotoxic effects of combination treatment. In sensitive wild-type ovarian cancer (A2780/S) cells, the concentration of 0.5 μM was chosen as a safe concentration based on the results of MTT assay. Sanguinarine could also decrease the IC_{50} of cisplatin in A2780/S cells, but this effect was much lower in comparison with resistant cell types. Cisplatin is a widely used anticancer drug for human malignancies, and however, cisplatin-based chemotherapy is accompanied by sever adverse effects on healthy organs and tissues such as nephrotoxicity and cardiotoxicity which have a direct association with drug concentrations (El-Awady, Moustafa, Abo-Elmatty, & Radwan, 2011; Pabla & Dong, 2008). Thus, reducing the effective concentration of cisplatin is a favorable target in chemotherapy. To achieve this goal, one strategy is to use cisplatin with safe compounds. As observed in results, combination therapy with sanguinarine significantly reduced the IC_{50} of cisplatin in A278/R cells, suggesting that sanguinarine can be considered as a potent compound for improving adverse toxic effects of cisplatin, with no change on its efficiency.

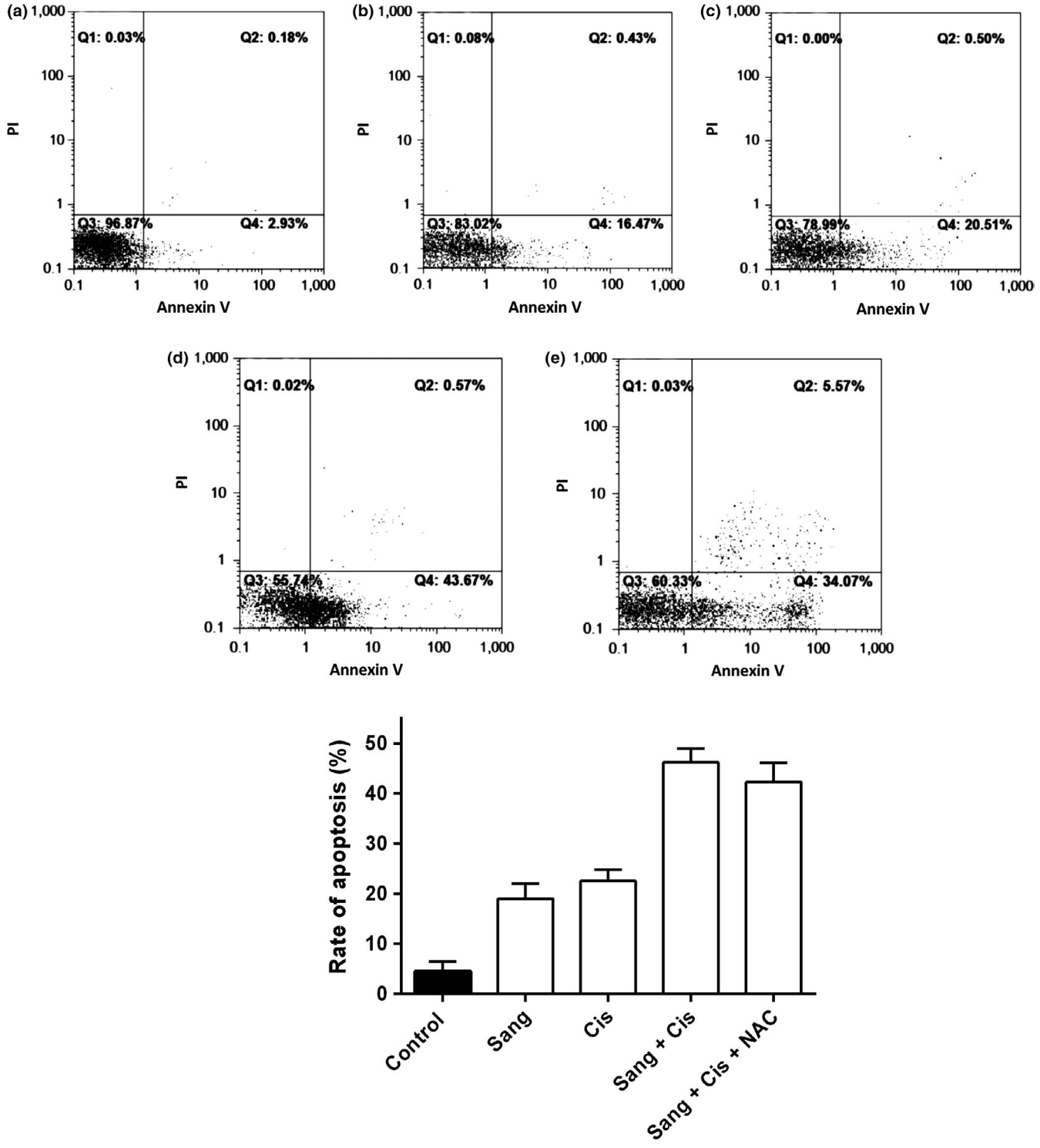

FIGURE 3 Flow cytometeric analysis of apoptosis in A2780/R cells following 48-hr treatment with: (a) fresh medium (Control), (b) sanguinarine (1 μM), (c) cisplatin (10 μM), (d) sanguinarine (1 μM) + cisplatin (10 μM), and (e) sanguinarine (1 μM) + cisplatin (10 μM) + NAC (*N*-acetylcysteine; 1 mM)

To further analyze the effect of sanguinarine on cisplatin cytotoxicity, flow cytometeric analysis of apoptosis and DAPI staining was performed to evaluate the apoptosis-inducing effect of sanguinarine in combination with cisplatin in A2780/R cells. Results of flow cytometery analysis indicated that combining sanguinarine with cisplatin enhanced the migration of cells to apoptotic regions more than each compound alone. NAC could not decrease the apoptosis caused by sanguinarine and cisplatin. Same results were observed in DAPI staining in which the nuclei of the cells in combination group showed more fragmented DNA compared with cells receiving each compound alone and untreated control group.

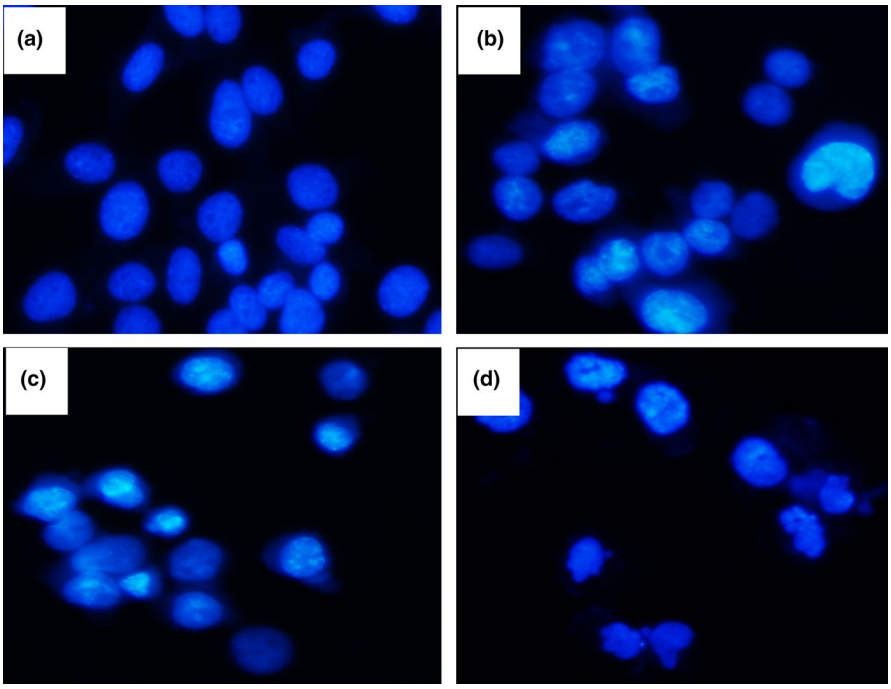

FIGURE 4 Fluorescence microscopy images of A2780/R cells stained with DAPI following 48-hr treatment with (a) fresh medium (Control), (b) sanguinarine (1 μM), (c) cisplatin (10 μM), (d) sanguinarine (1 μM) + cisplatin (10 μM)

As mentioned above, cellular GSH is the main mechanism for the development of cisplatin resistance. Cisplatin was used to establish the cisplatin-resistant ovarian tumor cells from parental A2780/S cells (Nikounezhad, Nakhjavani, & Shirazi, 2016). The resistant phenotype was confirmed by enhanced cisplatin IC_{50} and basal intracellular GSH content. To examine whether sanguinarine affected GSH levels inside the cells, intracellular GSH content was measured after treatment with sanguinarine at different concentrations and different time intervals. Results indicated that GSH reduction with sanguinarine occurs 30 min after treatment, and after 1, 3, and 6 hr, no significant change was observed in comparison with 30 min, indicating that GSH depletion by sanguinarine is a fast process. In addition, sanguinarine reduces GSH levels in a dose-dependent manner as higher concentrations showed significantly lower GSH levels. Addition of NAC, as a thiol-reducing agent, had no effect on GSH levels after treatment with sanguinarine. The failure in reversing GSH depletion by NAC indicates that GSH depletion occurs by mechanisms other than routine biological mechanisms. Debiton and colleagues in an in vitro experiment showed that sanguinarine directly reacts with GSH at pH 7.4 in a time- and concentration-dependent manner. They hypothesized that the reactive quaternary cationic form of sanguinarine at physiological pH is highly sensitive to nucleophilic attack by thiols in GSH molecule (Debiton et al., 2003). This is also in accordance with the findings of our study and justifies the early and rapid GSH depletion in our results.

As discussed earlier, GSH nullifies the active form of cisplatin which is pumped out of the cell before reaching

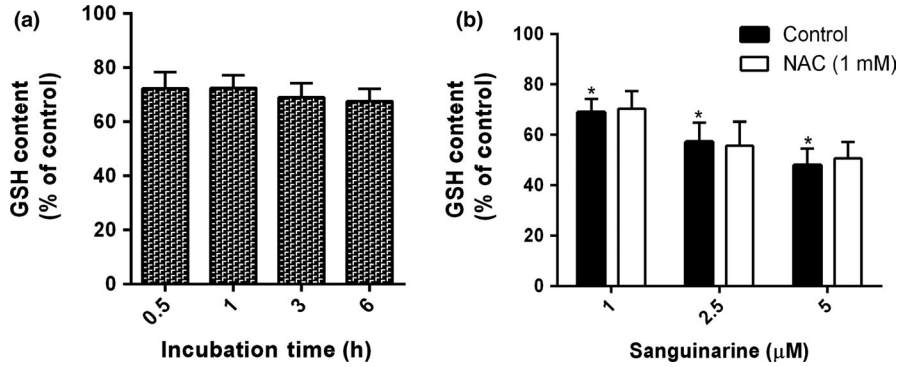

FIGURE 5 Intracellular GSH content following treatment with sanguinarine. (a) A2780/R cells were treated with sanguinarine 1 μM and intracellular GSH content was measured with GSH assay kit after 0.5, 1, 3, and 6 hr. Each point represents the mean ± *SD* of three independent experiments. *Significance at $p < .05$. (b) A2780/R cells were treated with sanguinarine (1, 2.5, and 5 μM) in the presence or absence of NAC (*N*-acetylcysteine) for 3 hr, and intracellular GSH content was measured using GSH assay kit. Each point represents the mean ± *SD* of three independent experiments. *Significance at $p < .05$

to its target molecule, DNA (Yu, Yang, Zhang, He, & Ren, 2015; Zhang et al., 2017). To further explore the molecular mechanism of sanguinarine effects on cisplatin resistance in ovarian cancer cells, it would be better to analyze the levels of active platinum and rate of DNA damage after treatment with sanguinarine (Wan et al., 2018), but due to limitations in this study it has not been determined. Therefore, this issue is suggested to be clarified in the future studies.

To conclude, our results indicated that treatment with sanguinarine can increase the sensitivity of A2780/R cells to cytotoxic effects of sanguinarine. The apoptosis-inducing effects of cisplatin were also enhanced after combination treatment with sanguinarine. Sanguinarine reversed cisplatin resistance in A2780/R cells through intracellular GSH depletion. However, more studies are needed to investigate the effects of sanguinarine on other mechanisms involved in MDR such as expression of drug efflux transporters.

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CONFLICT OF INTEREST

Regarding the publication of this article, financial, and/or otherwise, the authors declare that they have no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Azimi, A., Majidinia, M., Shafiei-Irannejad, V., Jahanban-Esfahlan, R., Ahmadi, Y., Karimian, A., … Yousefi, B. (2018). Suppression of p53R2 gene expression with specific siRNA sensitizes HepG2 cells to doxorubicin. *Gene*, *642*, 249–255. https://doi.org/10.1016/j.gene.2017.11.008

Britten, R. A., Green, J. A., & Warenius, H. M. (1992). Cellular glutathione (GSH) and glutathione S-transferase (GST) activity in human ovarian tumor biopsies following exposure to alkylating agents. *International Journal of Radiation Oncology Biology Physics*, *24*(3), 527–531. https://doi.org/10.1016/0360-3016(92)91069-Y

Chou, T.-C., & Talalay, P. (1984). Quantitative analysis of dose-effect relationships: The combined effects of multiple drugs or enzyme inhibitors. *Advances in Enzyme Regulation*, *22*, 27–55. https://doi.org/10.1016/0065-2571(84)90007-4

Debiton, E., Madelmont, J.-C., Legault, J., & Barthomeuf, C. (2003). Sanguinarine-induced apoptosis is associated with an early and severe cellular glutathione depletion. *Cancer Chemotherapy and Pharmacology*, *51*(6), 474–482.

Eckstein, N. (2011). Platinum resistance in breast and ovarian cancer cell lines. *Journal of Experimental & Clinical Cancer Research*, *30*(1), 1. https://doi.org/10.1186/1756-9966-30-91

El-Awady, E.-S.-E., Moustafa, Y. M., Abo-Elmatty, D. M., & Radwan, A. (2011). Cisplatin-induced cardiotoxicity: Mechanisms and cardioprotective strategies. *European Journal of Pharmacology*, *650*(1), 335–341. https://doi.org/10.1016/j.ejphar.2010.09.085

Iida, T., Kijima, H., Urata, Y., Goto, S., Ihara, Y., Oka, M., … Kondo, T. (2001). Hammerhead ribozyme against γ-glutamylcysteine synthetase sensitizes human colonic cancer cells to cisplatin by down-regulating both the glutathione synthesis and the expression of multidrug resistance proteins. *Cancer Gene Therapy*, *8*(10), 803. https://doi.org/10.1038/sj.cgt.7700371

Itamochi, H., & Kigawa, J. (2012). Clinical trials and future potential of targeted therapy for ovarian cancer. *International Journal of Clinical Oncology*, *17*(5), 430–440. https://doi.org/10.1007/s10147-012-0459-8

Jamali, B., Nakhjavani, M., Hosseinzadeh, L., Amidi, S., Nikounezhad, N., & Shirazi, F. H. (2015). Intracellular GSH alterations and its relationship to level of resistance following exposure to cisplatin in cancer cells. *Iranian Journal of Pharmaceutical Research: IJPR*, *14*(2), 513.

Jang, B.-C., Park, J.-G., Song, D.-K., Baek, W.-K., Yoo, S. K., Jung, K.-H., … Suh, S.-I. (2009). Sanguinarine induces apoptosis in A549 human lung cancer cells primarily via cellular glutathione depletion. *Toxicology in Vitro*, *23*(2), 281–287. https://doi.org/10.1016/j.tiv.2008.12.013

Kalogris, C., Garulli, C., Pietrella, L., Gambini, V., Pucciarelli, S., Lucci, C., … Amici, A. (2014). Sanguinarine suppresses basal-like breast cancer growth through dihydrofolate reductase inhibition. *Biochemical Pharmacology*, *90*(3), 226–234. https://doi.org/10.1016/j.bcp.2014.05.014

Kawahara, B., Ramadoss, S., Chaudhuri, G., Janzen, C., Sen, S., & Mascharak, P. K. (2019). Carbon monoxide sensitizes cisplatin-resistant ovarian cancer cell lines toward cisplatin via attenuation of levels of glutathione and nuclear metallothionein. *Journal of Inorganic Biochemistry*, *191*, 29–39. https://doi.org/10.1016/j.jinorgbio.2018.11.003

Kim, S., Lee, T. J., Leem, J., Choi, K. S., Park, J. W., & Kwon, T. K. (2008). Sanguinarine-induced apoptosis: Generation of ROS, down-regulation of Bcl-2, c-FLIP, and synergy with TRAIL. *Journal of Cellular Biochemistry*, *104*(3), 895–907. https://doi.org/10.1002/jcb.21672

Markman, M., & Bookman, M. A. (2000). Second-line treatment of ovarian cancer. *The Oncologist*, *5*(1), 26–35. https://doi.org/10.1634/theoncologist.5-1-26

Matkar, S. S., Wrischnik, L. A., & Hellmann-Blumberg, U. (2008). Sanguinarine causes DNA damage and p53-independent cell death in human colon cancer cell lines. *Chemico-Biological Interactions*, *172*(1), 63–71. https://doi.org/10.1016/j.cbi.2007.12.006

Meijer, C., Mulder, N., Hospers, G., Uges, D., & De Vries, E. (1990). The role of glutathione in resistance to cisplatin in a human small cell lung cancer cell line. *British Journal of Cancer*, *62*(1), 72. https://doi.org/10.1038/bjc.1990.232

Nikounezhad, N., Nakhjavani, M., & Shirazi, F. H. (2016). Generation of cisplatin-resistant ovarian cancer cell lines. *Iranian Journal of Pharmaceutical Sciences*, *12*(1), 11–20.

Nikounezhad, N., Nakhjavani, M., & Shirazi, F. H. (2017). Cellular glutathione level does not predict ovarian cancer cells' resistance after initial or repeated exposure to cisplatin. *Journal of Experimental Therapeutics & Oncology*, *12*(1), 1–7.

Oronsky, B., Ray, C. M., Spira, A. I., Trepel, J. B., Carter, C. A., & Cottrill, H. M. (2017). A brief review of the management of platinum-resistant-platinum-refractory ovarian cancer. *Medical Oncology*, *34*(6), 103. https://doi.org/10.1007/s12032-017-0960-z

Pabla, N., & Dong, Z. (2008). Cisplatin nephrotoxicity: Mechanisms and renoprotective strategies. *Kidney International*, *73*(9), 994–1007. https://doi.org/10.1038/sj.ki.5002786

Park, H., Bergeron, E., Senta, H., Guillemette, K., Beauvais, S., Blouin, R., … Faucheux, N. (2010). Sanguinarine induces apoptosis of human osteosarcoma cells through the extrinsic and intrinsic pathways. *Biochemical and Biophysical Research Communications*, *399*(3), 446–451. https://doi.org/10.1016/j.bbrc.2010.07.114

Perez, R. (1998). Cellular and molecular determinants of cisplatin resistance. *European Journal of Cancer*, *34*(10), 1535–1542. https://doi.org/10.1016/S0959-8049(98)00227-5

Shafiei-Irannejad, V., Samadi, N., Salehi, R., Yousefi, B., Rahimi, M., Akbarzadeh, A., & Zarghami, N. (2018). Reversion of multidrug resistance by co-encapsulation of doxorubicin and metformin in poly (lactide-co-glycolide)-d-α-tocopheryl polyethylene glycol 1000 succinate nanoparticles. *Pharmaceutical Research*, *35*(6), 119. https://doi.org/10.1007/s11095-018-2404-7

Shafiei-Irannejad, V., Samadi, N., Yousefi, B., Salehi, R., Velaei, K., & Zarghami, N. (2018). Metformin enhances doxorubicin sensitivity via inhibition of doxorubicin efflux in P-gp-overexpressing MCF-7 cells. *Chemical Biology & Drug Design*, *91*(1), 269–276. https://doi.org/10.1111/cbdd.13078

Ulrichová, J., Dvořák, Z., Vičar, J., Lata, J., Smržová, J., Šedo, A., … m., (2001). Cytotoxicity of natural compounds in hepatocyte cell culture models: The case of quaternary benzo [c] phenanthridine alkaloids. *Toxicology Letters*, *125*(1–3), 125–132. https://doi.org/10.1016/S0378-4274(01)00430-1

Wan, B., Dai, L., Wang, L., Zhang, Y., Huang, H., Qian, G., & Yu, T. (2018). Knockdown of BRCA2 enhances cisplatin and cisplatin-induced autophagy in ovarian cancer cells. *Endocrine-Related Cancer*, *25*(1), 69–82. https://doi.org/10.1530/ERC-17-0261

Xu, J.-Y., Meng, Q.-H., Chong, Y., Jiao, Y., Zhao, L., Rosen, E. M., & Fan, S. (2013). Sanguinarine is a novel VEGF inhibitor involved in the suppression of angiogenesis and cell migration. *Molecular and Clinical Oncology*, *1*(2), 331–336. https://doi.org/10.3892/mco.2012.41

Yu, T., Yang, Y., Zhang, J., He, H., & Ren, X. (2015). Circumvention of cisplatin resistance in ovarian cancer by combination of cyclosporin A and low-intensity ultrasound. *European Journal of Pharmaceutics and Biopharmaceutics*, *91*, 103–110. https://doi.org/10.1016/j.ejpb.2015.02.003

Zhang, Y., Li, J., & Yu, T. (2017). Pharmacokinetic profiles of cancer sonochemotherapy. *Expert Opinion on Drug Delivery*, *14*(6), 745–753. https://doi.org/10.1080/17425247.2016.1232248

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