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ORIGINAL ARTICLE

Nitric oxide affects cisplatin cytotoxicity oppositely in A2780 and A2780-CDDP cells via the connexin32/gap junction

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Abstract

Chemoresistance is a main obstacle in ovarian cancer therapy and new treatment strategies and further information regarding the mechanism of the medication cisplatin are urgently needed. Nitric oxide has a critical role in modulating the activity of chemotherapeutic drugs. Our previous work showed that connexin32 contributed to cisplatin resistance. However, whether nitric oxide is involved in connexin32-mediated cisplatin resistance remains unknown. In this study, using A2780 and A2780 cisplatin-resistant cells, we found that *S*-nitroso-*N*-acetyl-penicillamine, a nitric oxide donor, attenuated cisplatin toxicity by decreasing gap junctions in A2780 cells. Enhancement of gap junctions using retinoic acid reversed the effects of *S*-nitroso-*N*-acetyl-penicillamine on cisplatin toxicity. In A2780 cisplatin-resistant cells, however, *S*-nitroso-*N*-acetyl-penicillamine enhanced cisplatin toxicity by decreasing connexin32 expression. Downregulation of connexin32 expression by small interfering RNA exacerbated the effects of *S*-nitroso-*N*-acetyl-penicillamine on cisplatin cytotoxicity and upregulation of connexin32 expression by pcDNA transfection reversed the effects of *S*-nitroso-*N*-acetyl-penicillamine on cisplatin cytotoxicity. Our study suggests for the first time that combining cisplatin with nitric oxide in clinical therapies for ovarian cancer should be avoided before cisplatin resistance emerges. The present study provides a productive area of further study for increasing the efficacy of cisplatin by combining cisplatin with the specific inhibitors or enhancers of nitric oxide in clinical treatment.

KEYWORDS
cisplatin resistance, connexin32, gap junction, ovarian cancer, *S*-nitroso-*N*-acetyl-penicillamine

Abbreviations: CDDP, cisplatin; CTR1, copper transporter 1; Cx32, connexin32; EGFR, epidermal growth factor receptor; GJIC, gap junctional intercellular communication; NO, nitric oxide; NOS, nitric oxide synthase; RA, retinoic acid; SNAP, *S*-nitroso-*N*-acetyl-penicillamine.

Fan and Zheng contributed equally to this work.

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1 | INTRODUCTION

Ovarian cancer is the leading cause of death among patients with gynecological cancers.[1] Cisplatin (CDDP), the most common and effective platinum-based anticancer drug, is widely used in the chemotherapeutic treatment of ovarian cancer.[2] However, acquired resistance and adverse reactions are 2 major factors for the failure of CDDP in the treatment of ovarian cancer.[3,4] CDDP resistance primarily occurs due to decreased intracellular CDDP accumulation and decreased sensitivity to CDDP-induced apoptosis.[5,6] Therefore, more studies on the mechanisms of CDDP resistance and more effective approaches to subvert cellular resistance pathways are needed to improve the efficacy of cancer therapy.

Nitric oxide (NO), an endogenously synthesized primary messenger produced by the enzyme nitric oxide synthase (NOS), acts as an important regulator in signaling processes.[7,8] Upregulation of inducible nitric oxide synthase (iNOS) or exposure of cancer cells to high levels of NO can prevent tumor cell growth.[9-11] In contrast, evidence continues to mount that iNOS expression is associated with an aggressive tumor phenotype.[12] Moreover, there is no consensus on the effect of NO on chemotherapeutic drugs, including CDDP. For example, Chanvorachote and colleagues found that NO enhanced CDDP-induced apoptosis in lung cancer cells through inhibition of Bcl-2 ubiquitination,[13] while NO conferred cisplatin resistance in human lung cancer cells.[14] These conflicting results suggest a dual role for NO in CDDP cytotoxicity, however the underlying molecular mechanisms have not yet been defined.

Gap junctions (GJ) are formed from 2 hemichannels that are localized on sites of cell-cell contact and that consist of 6 connexins (Cx). GJs allow the direct exchange of molecules, including metabolic precursors, second messengers, nutrients, and ions (relative molecular mass up to 1 kDa).[15] Our previous studies have shown that GJs enhance CDDP cytotoxicity by increasing copper transporter 1 (CTR1)-mediated platinum uptake in tumor cells[16]; CDDP and oxaliplatin counteract their cytotoxic efficacy by direct inhibition of GJ intercellular communication and reduction of Cx expression.[17] These results suggested that inhibition of GJ compromised the effectiveness of CDDP/oxaliplatin and that chemoresistance of CDDP may be related to GJ/Cx.

Existing data strongly suggest that there is reciprocal regulation between NO and Cx. Cx-based channels are necessary for NO to cross the plasma membrane and GJ provides a rapid and efficient pathway for NO transfer.[18] Conversely, exogenously applied or endogenously produced NO could alter GJ function or Cx expression.[19,20]

Our recent studies have shown that Cx32 expression is significantly upregulated in cervical cancer tissues and that Cx32 is distributed mainly around the cytoplasm and nucleus, this distribution could inhibit apoptosis by modulating EGFR expression.[21-23] Moreover, we have found that Cx32 expression was significantly increased and clustered in the cytoplasm of A2780-CDDP cells,[24] indicating that Cx32 may be associated with the chemoresistance of CDDP. Nevertheless, if NO regulates CDDP cytotoxicity by regulating GJ/Cx32 remains unknown. In this study, we investigated the effect of SNAP (a class donor of NO) on the toxicity of CDDP in A2780 cisplatin-sensitive cells and A2780 cisplatin-resistant cells and explored the underlying mechanism.

2 | MATERIALS AND METHODS

2.1 | Cell lines and cell culture

The ovarian cancer A2780 cell line was purchased from ATCC and A2780 CDDP-resistant cells (A2780-CDDP) were obtained as described previously.[24] Cells were maintained in DMEM supplemented with 10% FBS and grown at 37°C in a 5% CO_2 in air atmosphere. To maintain the resistance, A2780-CDDP cells were cultured in complete medium containing 1 μg/mL CDDP (Sigma-Aldrich).

2.2 | Nitrite assay

We evaluated the amount of nitrite released in A2780 cells and A2780-CDDP cells following treatment with SNAP for 48 h. Nitrite accumulation was detected using a total NO assay kit (Beyotime Institute of Biotechnology). Total NO production in the cell lysates was determined by measuring the concentrations of nitrate and nitrite, stable metabolites of NO. Absorbance of each sample was determined at 540 nm.[25]

2.3 | Cell proliferation assay

The effect of SNAP (Sigma-Aldrich) on cell proliferation of A2780 and A2780-CDDP cells was measured using the Cell Counting Kit-8 (CCK-8) assay. Briefly, cells were trypsinized and seeded into 96-well plates at a density of 5×10^3 cells per well. CDDP was then added at various final concentrations. After 48 h of incubation, 10 μL of CCK-8 was added to 90 μL of normal medium until visual color conversion occurred. Finally, a microplate reader (BioTek) was used to determine absorbance at 490 nm.

2.4 | Membrane protein extraction and western blotting

Membrane protein and cytoplasmic protein were extracted using a Mem-PER™ Plus Membrane Protein Extraction kit (Thermo Fisher Scientific). Total proteins were harvested from

the cells, separated by SDS-PAGE, and transferred onto PVDF membranes (Millipore). The membranes were blocked with 5% nonfat milk in TBST for 1 h at room temperature, followed by incubation with the appropriate primary antibodies overnight at 4°C. Monoclonal antibodies against Cx32 (1:1000; Sigma-Aldrich), cleaved-caspase 3 (1:1000; Cell Signaling Technology), Na-K-ATPase (1:1000; Cell Signaling Technology) and β-tubulin (1:10 000; Sigma-Aldrich) were used. Then, membranes were incubated with the relevant secondary antibody (Cell Signaling Technology) for 1 h at room temperature and then washed 3 times with TBST . Immunoreactive bands were visualized using the Chemiluminescent HRP Substrate Kit (Millipore) and the bands were quantified using ImageJ software.

2.5 | Parachute dye coupling assay

Molecular permeability of GJs was measured using the parachute dye coupling assay.[26] Briefly, cells were grown to 80%-90% confluency in 12-well plates and donor cells were preloaded with Calcein-AM (Invitrogen) and CM-DiI (Invitrogen) and incubated at 37°C for 30 min away from light. Unincorporated dye was removed by 4 consecutive washes with PBS. Donor cells were trypsinized and seeded onto the receiving cells at a concentration of 500 cells/mL and then incubated for 4 h at 37°C. Signal intensity was observed using a fluorescence microscope (Olympus IX71). The average number of receiver cells (green fluorescence) around every donor cell (both green and red fluorescence) was recorded as an index of GJ function.

2.6 | Hoechst 33258 staining and apoptosis analysis

Cells were fixed with 4% formaldehyde for 20 min, permeabilized with a solution containing 1% BSA and 0.5% Triton X-100 for 15 min, stained with 0.1 μg/mL Hoechst 33258 at 37°C for 15 min in the dark, and washed with PBS. The stained cells were observed using a fluorescence microscope (Olympus IX71). Apoptosis was assessed using flow cytometry and an Annexin V-FITC apoptosis detection kit (Biotool). Expo32 Software (Beckman XL) was used to identify apoptotic cells. The ratio of early apoptotic cells was compared with that of the controls for each experiment.

2.7 | Immunofluorescence staining

Cells were fixed for 20 min in 4% paraformaldehyde at room temperature and permeabilized with a solution containing 1% BSA and 0.5% Triton X-100 for 15 min. After 1 h of blocking in 10% normal goat serum, cells were incubated overnight at 4°C with a primary antibody against Cx32 (1:200; Sigma-Aldrich), followed by incubation for 1 h at room temperature in the dark with a FITC-conjugated goat anti-mouse secondary antibody (1:400; Invitrogen). Hoechst 33258 and Alexa Fluor 488 Phalloidin (Cell Signaling Technology) were used to stain the nucleus and cytoarchitecture, respectively. Then, cells were observed using a confocal microscope.

2.8 | DNA plasmid transfection and siRNA interference

Cells were seeded onto 6-well plates, grown to 80% confluency and then transfected with Cx32-expressing plasmid (Genecopoeia) using Lipofectamine 2000 (Invitrogen) and following the manufacturer's instructions. For siRNA interference, cells were grown to 30%-50% confluency, and nonspecific siRNA or targeted siRNA (RiboBio) were transfected into cells using Lipofectamine 2000, and in accordance with the manufacturer's instructions. After 48 h, siRNA interference was detected by western blotting. The sequences for siRNAs targeting Cx32 (siCx32) are as follows: siCx32_1, 5′-CACCAACAACACATAGAAA-3′; siCx32_2, 5′-GCATCTGCATTATCCTCAA-3′; siCx32_3, 5′-GCCTC TCACCTGAATACAA-3′.

2.9 | Statistical analysis

All experiments were repeated at least 3 times. The data were analyzed statistically using one-way ANOVA and Student *t* test with GraphPad Prism 6.0 software. A *P*-value < .05 was considered to indicate a statistically significant difference.

3 | RESULTS

3.1 | The A2780-CDDP cell line was established and the amount of NO was detected

A2780-CDDP cells were established using a method described previously.[24] Initially, the IC_{50} of A2780 cells and A2780-CDDP cells was 3.99 ± 0.39 μg/mL and 21.72 ± 2.05 μg/mL, respectively, as determined by CCK-8 assay (Figure 1A). The resistance index (RI) of A2780-CDDP cells was 5.4, suggesting that the cisplatin-resistant ovarian cancer cell line was successful established. Next the cytotoxic effects of SNAP on A2780 and A2780-CDDP cells were examined using the CCK-8 assay. Results showed that SNAP exhibited no cytotoxicity toward both A2780 and A2780-CDDP cells at concentrations below 100 μmol/L (Figure 1B). SNAP (100 μmol/L, 48 h) produced the maximum dose of NO (Figure 1C) and produced NO in a time-dependent manner on A2780 and A2780-CDDP cells (Figure 1D). Therefore, SNAP (100 μmol/L, 48 h) was used for subsequent experiments.

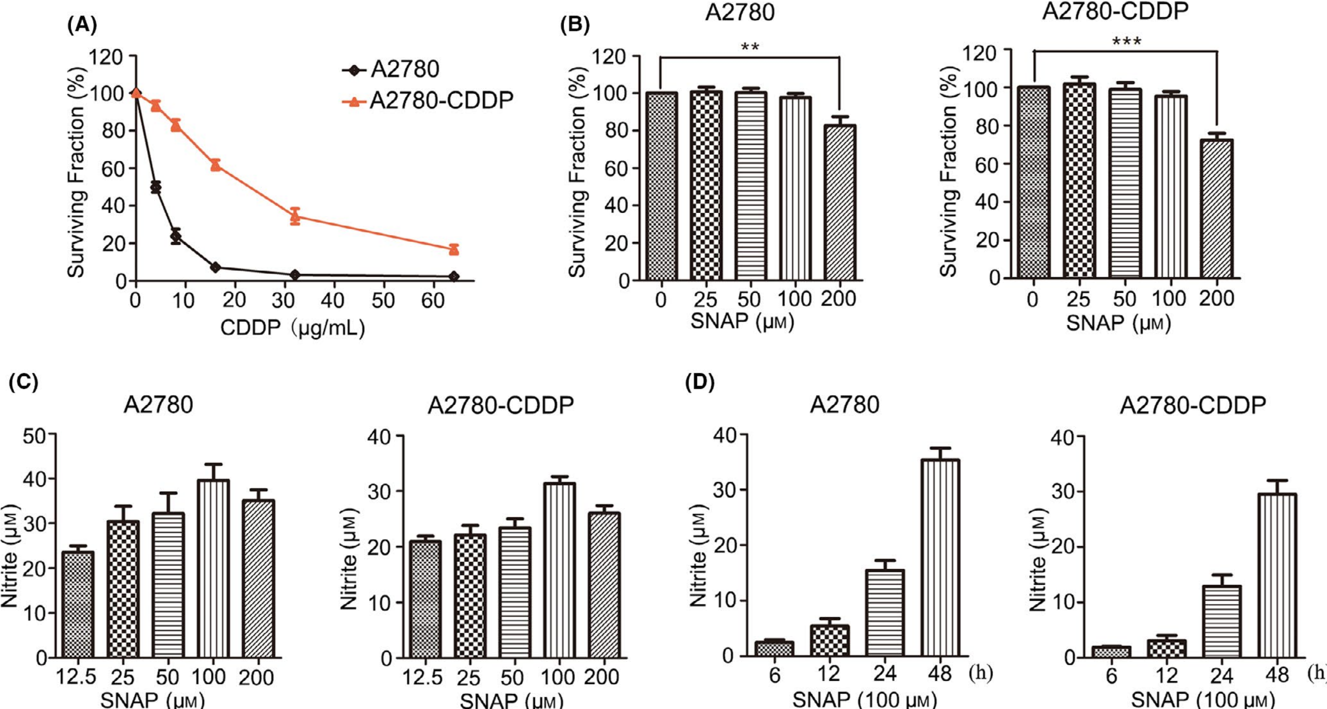

FIGURE 1 The A2780-CDDP (cisplatin) cell line was established and the amount of nitric oxide (NO) was detected. A, A2780 and A2780-CDDP cells were treated with CDDP gradient concentrations (0, 4, 8, 16, 32 and 64 μg/mL) for 48 h and IC_{50} values were determined by Cell Counting Kit-8 (CCK-8) assay (n = 5). B, Cytotoxic effect of *S*-nitroso-*N*-acetyl-penicillamine (SNAP) was assessed by CCK-8 assay in A2780 and A2780-CDDP cells (n = 4). C, D, Amount of NO produced by SNAP was detected at different concentrations and time points in A2780 and A2780-CDDP cells (n = 4). Values are expressed as the mean ± SEM; $^{**}P < .01$, $^{***}P < .001$

3.2 | NO decreased CDDP cytotoxicity by decreasing GJ function in A2780 cells

Then, we determined whether NO induced by SNAP influenced the cytotoxicity of CDDP in A2780 cells. Cells were exposed to gradient concentrations (0, 4, 8, 16, 32 and 64 μg/mL) of CDDP for 48 h either with (100 μmol/L SNAP) or without SNAP. As shown in Figure 2(A), SNAP significantly counteracted the cytotoxicity of CDDP (8 μg/mL or 16 μg/mL) in A2780 cells, and the IC_{50} value was increased from 3.99 ± 0.39 μg/mL to 6.60 ± 1.0 μg/mL.

Next, we explored the mechanism by which NO decreased CDDP cytotoxicity in ovarian cancer cells. As shown in Figure 2(B), co-treatment with SNAP (100 μmol/L) and CDDP (4 μg/mL) for 48 h markedly reduced the function of GJs in A2780 cells. This raised the question whether NO decreased CDDP cytotoxicity via GJs. The results of the CCK-8 assay showed that CDDP (4 μg/mL, 48 h) exerted significant cytotoxicity and that co-treatment with SNAP increased the growth of the surviving fraction. However, cytoprotection was reversed by enhancement of GJs through preincubation with RA (50 μmol/L, 4 h; Figure 2C). Moreover, nuclear morphological changes were assessed using fluorescence microscopy and early apoptosis rate of cells was examined using flow cytometry. Similarly, as shown in Figure 2(D,E), noticeable apoptosis of A2780 cells was induced by CDDP (4 μg/mL, 48 h), but was significantly suppressed by co-treatment with CDDP and SNAP. Furthermore, enhancement of GJs by RA preincubation (50 μmol/L, 4 h) in A2780 cells markedly reversed the anti-apoptotic effect of SNAP. Our previous work revealed that RA increased the GJIC of A2780 cells.[24] Together, these results indicated that NO decreased the cytotoxic activity of CDDP by decreasing GJ function in A2780 cells.

3.3 | SNAP enhanced the turnover of Cx32 from the cytomembrane to cytoplasm in A2780 cells

Co-treatment with SNAP and CDDP markedly reduced GJ function in A2780 cells. Western blotting was performed to investigate whether Cx32 was affected. As shown in Figure 3(A), SNAP did not affect Cx32 expression in A2780 cells. Our previous studies demonstrated that the cytoplasmic distribution of Cx32 decreases chemotherapy-induced apoptosis. Therefore, we asked whether SNAP had an effect on transport of Cx32 from the cytomembrane to the cytoplasm. Results of immunofluorescence staining showed that, while Cx32 was primarily localized in the cytomembrane in the control group, co-treatment with SNAP and CDDP caused an accumulation of Cx32 in the cytoplasm (Figure 3B). Western blotting results also showed that cytoplasmic Cx32 levels were increased significantly and that Cx32 levels in the cytomembrane were decreased significantly in the presence of SNAP and CDDP, when compared with the control group (Figure 3C,D). These results suggested that NO promoted the turnover of Cx32 from the cytomembrane to the cytoplasm in A2780 cells.

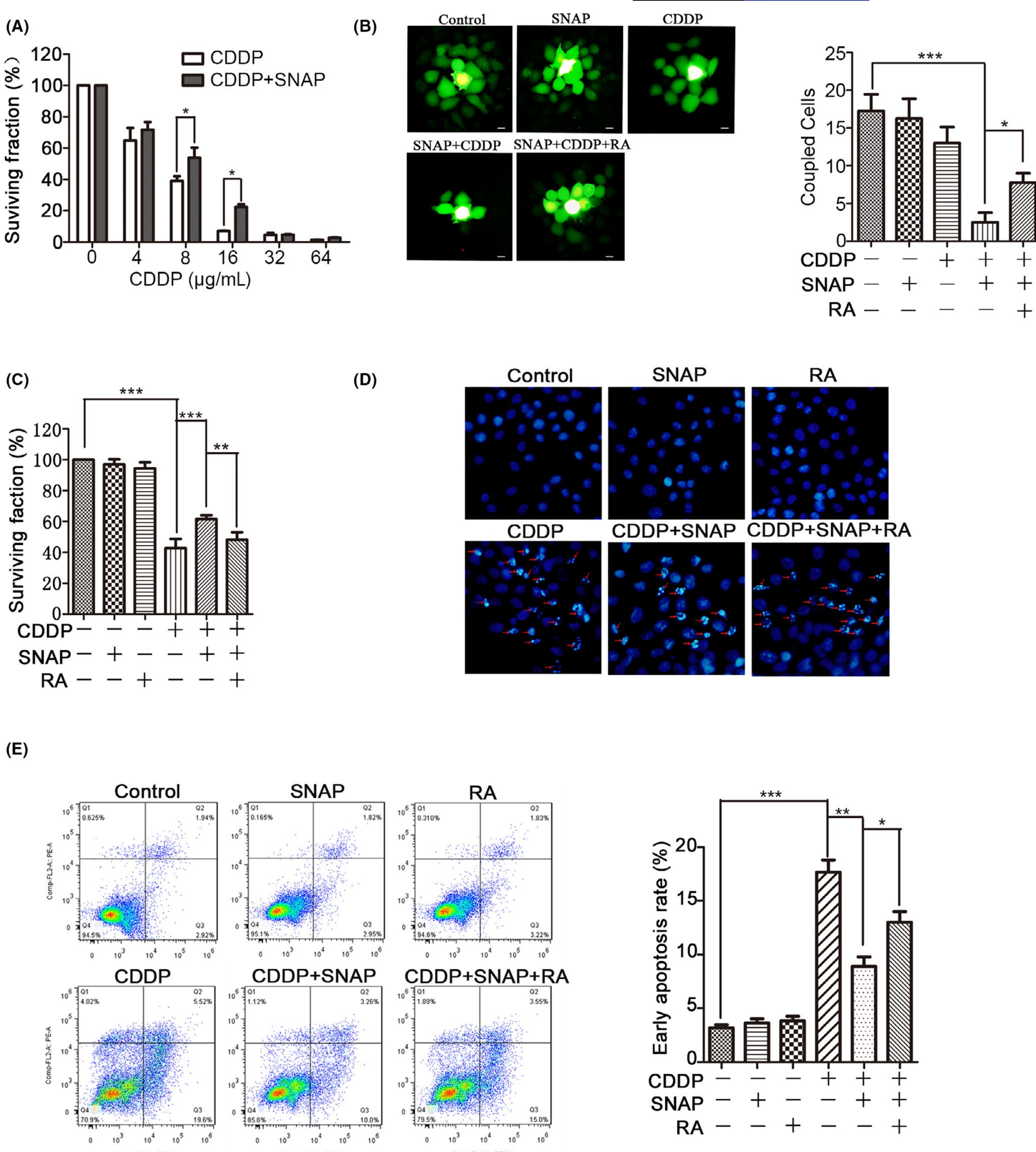

FIGURE 2 Nitric oxide (NO) decreased cisplatin (CDDP) cytotoxicity by decreasing gap junction (GJ) function in A2780 cells. A, Cell Counting Kit-8 (CCK-8) assay was performed to determine the effects of *S*-nitroso-*N*-acetyl-penicillamine (SNAP) on the cytotoxicity of CDDP in A2780 cells (n = 4). B, Parachute assay was used to determine effect of SNAP on GJ function in A2780 cells. Scale bars represent 20 μm (n = 4). C, CCK-8 assay was performed to determine the effects of SNAP and retinoic acid (RA) on the cytotoxic activity of CDDP in A2780 cells (n = 4). D, Morphological changes of apoptosis in nuclei were detected by Hoechst 33258 staining (n = 4). E, Apoptosis of A2780 cells was analyzed by flow cytometry (n = 4). Values are expressed as the mean ± SEM; **P* < .05, ***P* < .01; ****P* < .001

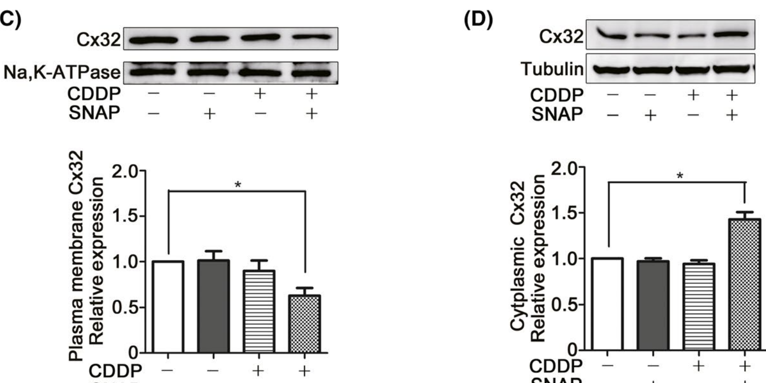

FIGURE 3 Turnover of cytomembrane-localized connexin32 (Cx32) after *S*-nitroso-*N*-acetyl-penicillamine (SNAP) and cisplatin (CDDP) treatment in A2780 cells. A, Protein level of Cx32 remained unchanged by incubation with CDDP and SNAP in A2780 cells (n = 4). B, Cells were stained with Hoechst 33258 (blue nuclear), anti-Cx32 antibody (red), and Alexa Fluor 488 Phalloidin (green) respectively. Images were obtained under a ×63 oil objective lens. Scale bars represent 10 μm (n = 3). C, D, Redistribution of Cx32 in the cytomembrane and cytoplasm was determined by western blot (n = 3). Na^+-K^+-ATPase and tubulin were used as a loading control for membrane protein and cytoplasmic proteins respectively. Values are expressed as the mean ± SEM; $^*P < .05$

3.4 | NO enhanced the cytotoxic activity of CDDP while decreased Cx32 protein level in A2780-CDDP cells

A2780-CDDP cells were exposed to CDDP (0, 4, 8, 16, 32 and 64 μg/mL) for 48 h combined with SNAP (100 μmol/L) or not. As shown in Figure 4(A), SNAP enhanced significantly the cytotoxicity of CDDP (8 μg/mL, 16 μg/mL or 32 μg/mL) in A2780-CDDP cells, and the IC_{50} value decreased from 21.72 ± 2.05 μg/mL to 11.80 ± 1.32 μg/mL. As shown in Figure 4(B), SNAP could significantly increase apoptosis, as detected by morphological changes in the nucleus (Figure 4B). These data indicated that NO induced by SNAP enhanced the cytotoxicity of CDDP in A2780-CDDP cells.

The effects of SNAP (100 μmol/L, 48 h) and CDDP (22 μg/ml, 48 h) on GJ function in A2780-CDDP cells was determined. As shown in Figure 4(C), both SNAP and CDDP had no obvious effect on GJ function. Interestingly, combined treatment with SNAP (100 μmol/L) and CDDP (22 μg/mL) for 48 h decreased Cx32 expression in A2780-CDDP cells significantly (Figure 4D).

3.5 | SNAP enhanced the cytotoxic activity of CDDP by decreasing Cx32 expression in A2780-CDDP cells

Next, we explored the mechanism by which SNAP increased CDDP cytotoxicity in A2780-CDDP cells. To explore the role of Cx32, Cx32 expression was notably knocked down by transfection of cells with Cx32 siRNAs. Western blot assay showed that Cx32 siRNA3 markedly inhibited Cx32 expression in A2780-CDDP cells (Figure 5A), therefore Cx32 siRNA3 was chosen for subsequent experiments. Knockdown of Cx32 by siRNA further exacerbated the surviving fraction and upregulated the incidence of apoptosis in A2780-CDDP cells co-treated with SNAP and CDDP, as determined by CCK-8 assay, flow cytometry, and western blot respectively (Figure 5B-D). In contrast, as shown in Figure 5(E), Cx32 expression in cells was notably enhanced by transfection with pcDNA Cx32. The results of the CCK-8 assay showed that overexpression of Cx32 obviously decreased CDDP cytotoxicity in A2780-CDDP cells. Taken together, these results indicated that NO enhanced the cytotoxic activity of CDDP by decreasing Cx32 expression in A2780-CDDP cells.

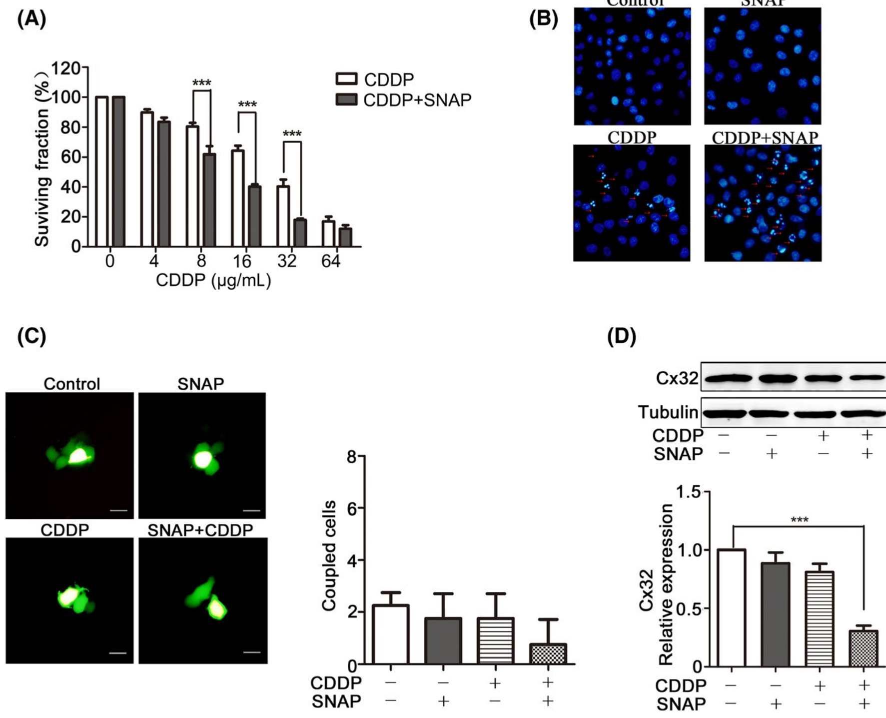

FIGURE 4 Effects of nitric oxide (NO) on the cytotoxic activity of cisplatin (CDDP) in A2780-CDDP cells. A, Effect of *S*-nitroso-*N*-acetyl-penicillamine (SNAP) on the cytotoxic activity of CDDP in A2780-CDDP cells (n = 5). B, SNAP could significantly increase apoptosis, as detected by Hoechst 33258 staining (n = 4). C, Parachute assay was used to determine the effect of SNAP on gap junction (GJ) function in A2780 cells. Scale bars represent 20 μm (n = 4). D, Co-treatment with SNAP and CDDP significantly decreased the expression of connexin32 (Cx32) in A2780-CDDP cells (n = 4). Values are expressed as the mean ± SEM. ***$P < .001$

4 | DISCUSSION

Platinum compounds are first-line drugs for standard ovarian cancer chemotherapy. However, resistance to platinum drugs remains a major obstacle to treatment success.[27] To prevent resistance to platinum drugs, such as CDDP, in ovarian cancer, further studies are needed on the mechanisms of CDDP resistance and on novel therapeutic strategies. In this study, we found that the NO donor SNAP had different effects on CDDP toxicity in cisplatin-sensitive A2780 cells and in cisplatin-resistant A2780-CDDP cells. SNAP attenuated CDDP toxicity by decreasing GJ function in A2780 cells, while SNAP enhanced CDDP toxicity by decreasing Cx32 expression in A2780-CDDP cells.

Gap junctions are composed of Cx proteins, which are indispensable for cell homeostasis, growth, differentiation, and death. GJs and Cx (particularly Cx43) are widely thought to have anti-proliferative effects on a wide range of cancer cells.[28] Our previous studies showed that GJ could enhance CDDP cytotoxicity in different tumor cells[17,29,30] and another study also suggested that GJs improved CDDP resistance and that Cx43 reversed the resistance of A549 cells to CDDP by inhibiting epithelial-mesenchymal transition.[31] These results indicated that inhibition of GJs compromised the effectiveness of CDDP. In the present study, we found that SNAP could decrease CDDP cytotoxicity and that GJs were decreased or inhibited. However SNAP did not affect Cx32 expression, this also indicated that GJs could decrease CDDP cytotoxicity. We hypothesized that SNAP may alter the redistribution of Cx32. We used 2 methods to detect whether Cx32 was transported to the cytoplasm from the cytomembrane when cells are treated with SNAP and CDDP. We used Alexa Fluor 488 Phalloidin to stain cytoarchitecture; results of confocal microscopy and cytomembrane isolation were consistent, showing that co-treatment with SNAP and CDDP caused Cx32 to aggregate in the cytoplasm of A2780 cells.

However, the roles of GJs and Cx in carcinogenesis and drug resistance are complex and there have been no related studies on whether CDDP resistance is related to GJs/Cx in real drug-resistant cells. Therefore it will be necessary to establish A2780-CDDP cells for future study. Over the past 50 y, GJs have mostly been demonstrated to act as tumor suppressors as they have shown inhibitory effects on tumor growth.[28,32] However, recently, more studies have focused on the connexin protein itself.[33] Cx are reportedly able to regulate cell proliferation, migration, and apoptosis in a GJIC-independent manner. We found that Cx32 expression was increased in A2780-CDDP cells, but that GJs were decreased or inhibited. In A2780-CDDP cells, Cx32 accumulated in the cytoplasm,[24] indicating that cytoplasmic Cx32 may have an anti-apoptosis effect in a GJIC-independent manner. In the present study, we found that SNAP enhanced CDDP toxicity by decreasing Cx32 expression in A2780-CDDP cells. These results further confirmed the findings that cytoplasmic Cx32 may have an anti-apoptosis effect and that SNAP could decrease CDDP cytotoxicity by decreasing the expression of cytoplasmic Cx32.

Hemichannels are crucial in microenvironmental communication and cell function.[28] Interestingly, it has been reported that NO could increase the activity of hemichannels by regulating gating and permeability, which has been attributed to modification of cysteine residues located in the C-terminal region of Cx.[34] Therefore, changes in hemichannels might be related to the

FIGURE 5 *S*-Nitroso-*N*-acetyl-penicillamine (SNAP) enhanced the cytotoxic activity of cisplatin (CDDP) in A2780-CDDP cells via modulation of connexin32 (Cx32) expression. A, Expression levels of Cx32 were knocked down by Cx32 siRNA and were determined by western blot (n = 5). B, Effects of SNAP on the cytotoxicity of CDDP in A2780 cells were assessed by Cell Counting Kit-8 (CCK-8) assays following transfection with Cx32 siRNA3 (n = 4). C, Apoptosis of A2780-CDDP cells was analyzed by flow cytometry (n = 4). D, Expression levels of cleaved-caspase-3 were determined by western blot (n = 4). E, A2780-CDDP cells were transfected with pcDNA Cx32 and the expression levels of Cx32 were determined by western blot. Effects of SNAP on the cytotoxicity of CDDP in A2780-CDDP cells were assessed by CCK-8 assays following transfection with pcDNA Cx32 (n = 4). Values are expressed as the mean ± SEM; **P* < .05, ***P* < .01, ****P* < .001

different effects of cisplatin cytotoxicity and SNAP, but this needs further exploration.

There has been some related research on the relationship between NO and CDDP resistance, but results were conflicting. However, the role of SNAP in mediating CDDP cytotoxicity has not been compared previously in both cisplatin-sensitive cell lines and cisplatin-resistant cell lines and there have been no related studies on whether SNAP regulation of CDDP resistance is related to changes in Cx expression or GJ function. In this study, we provide a novel molecular mechanism that contributes to resistance to NO signaling in A2780-CDDP cells via modulation of Cx32 expression.

Our findings, for the first time, suggested that combining CDDP with SNAP in clinical therapies for ovarian cancer should be avoided before CDDP resistance emerges. This is a productive area for further study on increasing the efficacy of cisplatin in clinical treatment by combining CDDP with specific SNAP inhibitors or enhancers. Combination of CDDP with specific SNAP inhibitors may prevent the formation of CDDP resistance at initial stages, while combination of CDDP with specific SNAP enhancers could reverse CDDP resistance or prevent the development of drug resistance in ovarian cancers.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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