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# The gold complex auranofin sensitizes platinum resistant epithelial ovarian cancer cells to cisplatin

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ABSTRACT

Although numerous drugs have been tested to treat ovarian cancer (OC), survival rates remain low as there has been no major improvement from platinum (Pt)–based therapy and there is a high rate of Pt resistance in these tumors. Following several rounds of chemotherapy, OC cells develop Pt-resistance by increasing DNA repair and antioxidant defense mechanisms. This study aimed to design a treatment to combat recurrent stages of OC by repurposing the anti-rheumatic gold complex auranofin (AF). We demonstrate that AF enhances the efficacy of cisplatin (CDDP) in Pt-resistant epithelial OC (EOC) cells. The drug combination enhanced mitochondrial-dependent apoptosis, PARP cleavage, DNA damage, and ROS overproduction. These results suggest the potential to combine AF with CDDP as a strategy to improve CDDP sensitivity in recurrent EOCs.

## 1. Introduction

The survival rate for ovarian cancer (OC) patients is only 30–40 %, largely due to chemotherapy resistance [1]. The current treatment with cytoreductive surgery followed by platinum (Pt)-based chemotherapy with cisplatin (CDDP), was developed in the late 1970s [2,3] and approved by the FDA to treat advanced EOC and testicular cancer in 1978 (revisited in Ref. [4]). CDDP induces DNA damage via the formation of intrastrand and interstrand DNA crosslinks [4,5]. Other cellular targets include the mitochondria through the accumulation of reactive oxygen species (ROS), and the plasma membrane through changes in membrane fluidity [4]. Mechanisms of CDDP resistance acquired by cancer cells include increased DNA damage repair and reduced ROS levels via increased abundance of the antioxidant glutathione (GSH) [4]. Consequently, 80 % of patients with EOC will experience disease recurrence, with a tumor that is resistant to CDDP [4,6]. This highlights the need for a therapeutic agent to be coupled with CDDP as a second-line therapy to increase progression-free survival for this fatal disease.

Auranofin (AF), a gold complex approved in 1985 for rheumatoid arthritis, elicits cytotoxic effects in various cancers, including some subtypes of EOC [7,8]. We previously demonstrated the potent cytotoxicity of AF against the most frequent histological subtype of EOC, high-grade serous ovarian cancer (HGSOC) [9], in which AF targets the antioxidant enzyme, thioredoxin reductase (TrxR), and disrupts the mitochondrial membrane potential ultimately triggering cell death [10].

AF and CDDP share some cytotoxic mechanisms including overproduction of ROS [4,11–13], suggesting that combining them may enhance cytotoxicity. This combination has been studied on urothelial carcinoma [14] and small-cell lung cancer (SCLC) [15], in which AF increased the therapeutic effect of CDDP through cell cycle arrest and ROS accumulation [15], suggesting that combining these drugs would also enhance cytotoxicity in EOC cells. We explored this hypothesis in two models of CDDP-resistant EOC cells.

We provide evidence of synergistic cytotoxicity when combining AF and CDDP in Pt-resistant EOC cells, involving intrinsic caspase-dependent apoptosis, DNA damage, mitochondrial membrane depolarization, and oxidative stress. Combining these two metal-derived drugs was better than each one alone in triggering cell death in EOC cells.

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**Abbreviations**

| | |
|---|---|
| **EOC** | Epithelial ovarian cancer |
| **HGSOC** | High-grade serous ovarian cancer |
| **OC** | Ovarian cancer |
| **TrxR** | Thioredoxin reductase |
| **AF** | Auranofin |
| **CDDP** | Cisplatin |
| **CI** | Combination index |
| **ROS** | Reactive oxygen species |
| **NAC** | N-acetyl-L-cysteine |
| **Pt** | Platinum |

## 2. Materials and methods

### 2.1. Reagents and cell lines

TOV112D cells, isolated from a 42-year-old patient, are resistant to clinically achievable concentrations of CDDP, and were classified as a dedifferentiated endometrioid ovarian carcinoma based on extensive transcriptional profiling [16,17]. IGROV-1/CP is a cell line derived from IGROV-1 cells which had acquired resistance to CDDP by prolonged exposure in culture [18]; the original IGROV-1 cells were isolated from a grade 3 solid primary ovarian tumor in a 47-year-old patient [19] with a diagnosis of clear-cell ovarian carcinoma [16,20]. The cell lines were cultured in RPMI 1640 media (Gibco™, Hampton, New Hampshire, USA) [10]. The drugs used were auranofin (AF; A6733, Sigma Chemical Co., St. Louis, MO, USA), *cis*-diammineplatinum (II) dichloride (cisplatin [CDDP], P4394, Millipore, Burlington, Massachusetts, USA), and N-acetyl-L-cysteine (NAC) (A7250, Sigma-Aldrich, St. Louis, Missouri, USA).

### 2.2. Measuring IC50s and combination indexes

The concentration of CDDP that kills 50 % of cells (IC50) with or without AF was determined based on cellular viability. IGROV-1/CP or TOV112D were incubated with increasing concentrations of CDDP for 3 h followed by 72-h exposure to 2 μM AF or drug-free media. The viability was measured using the Guava Muse® cell viability reagent (MCH600103, Luminex, Austin, TX, USA) [10]. The combination index (CI), which signifies the drug interaction between CDDP and AF, was calculated using the Chou and Talalay method [21]; CI $>$ 1 means antagonism, a CI of 0 means no interaction, a CI of 1 means addition, and a CI $<$ 1 means synergism.

### 2.3. Annexin-V staining

IGROV-1/CP and TOV112D were treated with 10 μM CDDP for 3 h followed by 72-h incubation in the presence or absence of 2 μM or 0.5 μM AF, respectively. Cells were stained with Annexin-V and 7-AAD (Luminex), in which positive Annexin-V staining indicates early apoptosis and Annexin-V/7-AAD double positive staining signifies late apoptosis [10].

### 2.4. Cell cycle analysis

IGROV-1/CP cells and TOV112D cells were treated with 10 μM CDDP for 3 h followed by a 72-h treatment with 2 μM or 0.5 μM AF, respectively. DNA content histograms were generated by microcytometry as previously described [10,22].

### 2.5. Analysis of the mitochondrial membrane potential

IGROV-1/CP cells were treated with 15 μM CDDP for 3 h followed by a 72-h exposure to 2 μM AF, whereas the TOV112D cells were treated with 10 μM CDDP for 3 h followed by a 72-h exposure to 0.5 μM AF. The cells were stained with the cationic lipophilic dye, Mito-Potential (Luminex), followed by 7-AAD as previously reported [10].

### 2.6. Protein expression measurement

IGROV-1/CP and TOV112D cells were treated with 10 μM CDDP alone for 3 h, and 2 μM or 0.5 μM AF for 72 h, respectively, or the combination of CDDP for 3 h and AF for 72 h. The protein lysates were extracted and quantified [10]. The primary antibodies used were monoclonal anti-beta actin clone AC-15 (A5442, Sigma), polyclonal anti-PARP (9542S, Cell Signaling Technology, Danvers, MA, USA), monoclonal anti-phospho H2AX (Ser139) (9718, Cell Signaling), poly-clonal anti-caspase-3 (9662, Cell Signaling), monoclonal anti-caspase-9 (9508, Cell Signaling), and monoclonal anti-caspase-7 (D2Q3L1; 12827S, Cell Signaling). Secondary antibodies used were goat anti-rabbit IgG (H + L) HRP conjugate (1706515, BioRad Laboratories Inc., Hercules, CA, USA) and goat anti-mouse IgG (H + L) HRP conjugate (1706516, BioRad).

### 2.7. Treatment with the caspase-inhibitor z-DEVD-fmk

IGROV-1/CP and TOV112D cells were exposed for 3 h to 10 μM CDDP and for 48 h to 2 μM AF or 0.5 μM AF, respectively, in the presence or absence of 100 μM z-DEVD-fmk (S7312, Selleck Chemicals, Houston, USA), an irreversible caspase-3 inhibitor, which also potently inhibits caspase-6, -7, -8, and -10 (reviewed in Ref. [23]). The cell viability was assessed using the Guava Muse® cell analyzer (Millipore, Hayward, CA, USA).

### 2.8. Measurement of intracellular levels of reactive oxygen species

IGROV-1/CP cells were treated for 3 h with 15 μM CDDP followed by 72-h exposure to 2 μM AF, whereas TOV112D cells were exposed to 10 μM CDDP for 3 h followed by 48-h exposure to 0.5 μM AF. Superoxide levels were detected using a total intracellular oxidative stress assay [10].

### 2.9. Treatment with antioxidant NAC

To determine whether the cytotoxicity induced by the combination of AF and CDDP was ROS-dependent, IGROV-1/CP and TOV112D cells were treated with 10 μM CDDP for 3 h and 2 μM AF or 0.5 μM AF, respectively for 48 h in the presence or absence of 5 mM N-acetyl cysteine (NAC) (Sigma). Cell viability data was determined using the Guava Muse® cell analyzer (Millipore).

### 2.10. Statistics

For tests involving western blot analysis, experiments were repeated at least twice with a similar outcome. All other data represent triplicate experiments and are expressed as the mean ± SEM. Differences were considered statistically significant if $p < 0.05$. GraphPad Prism 10 (GraphPad Software, La Jolla, CA, USA) was used for multiple comparison analysis of data using one-way ANOVA followed by Tukey's multiple comparison test. IC50s were compared using a non-linear regression curve fit.

## 3. Results

### 3.1. AF re-sensitizes different subtypes of EOC cells to CDDP

In IGROV-1/CP cells, the IC50 of CDDP was 200 ± 23.4 μM (n = 3), which decreased to the clinically achievable concentration of 17 ± 3.12 μM (n = 3; $p < 0.001$) following the addition of AF (Fig. 1A). In TOV112D cells, the IC50 for CDDP was 80 ± 23.6 μM (n = 3), which decreased to 25 ± 1.1 μM (n = 3; $p < 0.001$) in the presence of AF (Fig. 1A). Furthermore, the interaction between the various concentrations of CDDP and the fixed concentration of AF in both cell lines was pharmacologically synergistic, with CI values below 1 (Fig. 1B).

### 3.2. The cytotoxicity of AF and CDDP in EOC occurs via apoptosis

The cytotoxic combination of AF and CDDP was associated with an increase in the early marker of apoptotic cell death, Annexin-V (Fig. 2A). CDDP alone was able to induce apoptosis in both types of EOC cells with a more pronounced effect in IGROV-1/CP cells. While AF had no effect alone, apoptosis was notably enhanced in both cell types when it was combined with CDDP (Fig. 2A). The drug combination also caused an accumulation of hypodiploid DNA content in both IGROV-1/CP cells and TOV112D cells (Fig. 2B) further suggesting apoptotic cell death [24].

### 3.3. The cytotoxicity caused by the combination AF/CDDP is associated with intrinsic apoptosis, PARP cleavage, and DNA damage

To further confirm that apoptosis induced by the combination AF/CDDP in IGROV-1/CP and TOV112D cells is mediated by the mitochondrial pathway, we assessed the cleavage of the initiator caspase-9 from the 49 kDa (kDa) pro-caspase form into 39 and 37 kDa fragments. Caspase-9 was activated by the combination AF/CDDP. Interestingly, the cleavage of pro-caspase-9 was also induced by CDDP alone in both cell types (Fig. 3A).

Caspases-3 and -7 are executioner caspases in the apoptotic pathway of programmed cell death [25]. In the IGROV-1/CP cells, CDDP induced a slight cleavage of caspases-3 and -7 that was enhanced by the addition of AF (Fig. 3A). To further document the induction of apoptosis by the drug combination, we measured the cleavage of poly ADP-ribose polymerase-1 (PARP), a nuclear enzyme that is heavily involved in DNA repair [26]. PARP can be cleaved into two fragments by caspases-3 and -7, which inactivates its role in the DNA repair pathway [26]. In IGROV-1/CP, PARP cleavage was highly evident only when cells were exposed to both AF and CDDP (Fig. 3A). In contrast, PARP cleavage was apparent in TOV112D cells following treatment with either AF or CDDP alone; but it was enhanced by the drug combination (Fig. 3A).

Early events in the DNA damage response, including the cleavage of PARP, also involve the formation of the DNA damage marker, γ H2AX which is thought to increase DNA accessibility by DNA repair enzymes [27]. In IGROV-1/CP cells, both AF and CDDP increased the expression of γH2AX, and this was greatly enhanced by the drug combination (Fig. 3A). In the TOV112D cells, AF alone had little effect on γH2AX

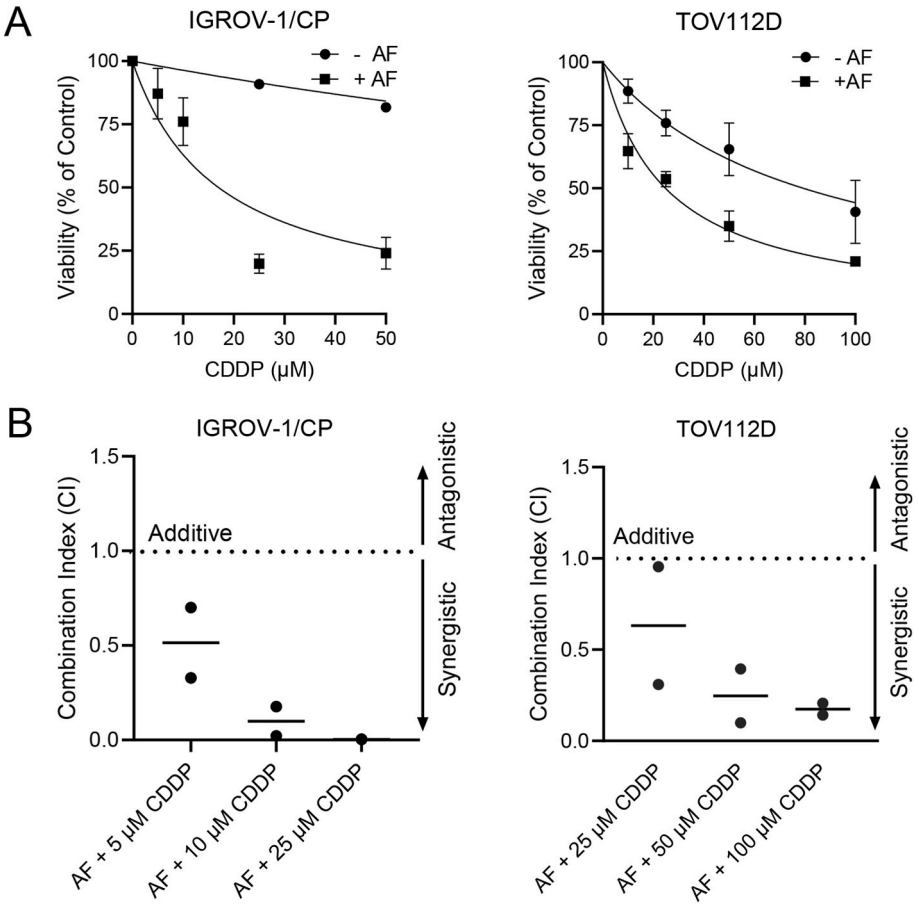


**Fig. 1.** Effect of AF on the toxicity of CDDP in IGROV-1/CP and TOV112D cells. Cells were treated with increased concentrations of CDDP for 3 h followed or not by a fixed concentration (2 μM) of AF for 72 h. The viability was determined using microcytometry. Panel A shows a decrease in the IC50s of CDDP in both cell lines following the addition of AF. Panel B displays the synergistic interaction between AF and CDDP (CI < 1) in the two cell lines.

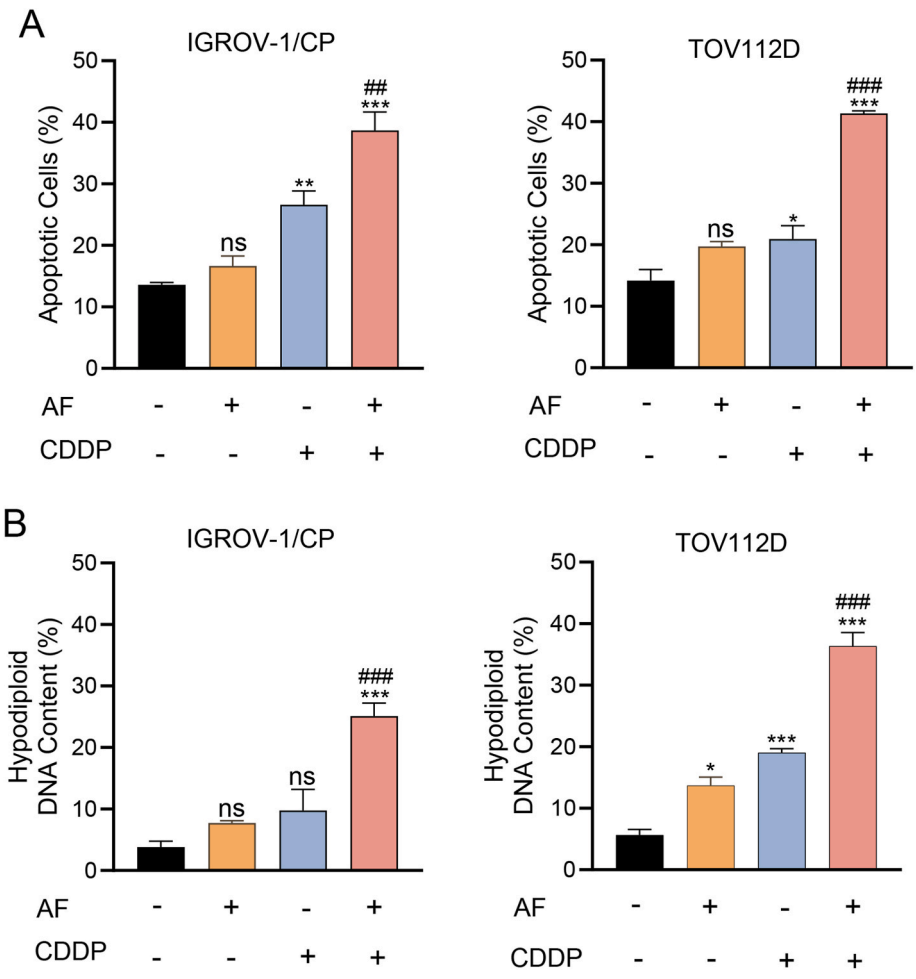


**Fig. 2.** Effect of AF/CDDP combination on the induction of apoptosis in IGROV-1/CP and TOV112D cells. Cells were treated with 10 μM CDDP for 3 h followed by a 72-h exposure to 2 μM or 0.5 μM AF and stained either with Annexin-V and 7-AAD to determine apoptosis (Panel A) or propidium iodide (PI) for DNA content (Panel B). *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ when compared to vehicle. ##$p < 0.01$ and ###$p < 0.001$ when compared to AF- or CDDP-treated cells.

expression, but there was a notable enhancement by exposure to CDDP and by both drugs in combination (Fig. 3A).

Since we observed the induction of mitochondrial-dependent apoptosis via caspase-9 cleavage by the AF/CDDP combination, we decided to explore the state of the mitochondria in response to the treatment. We found distinctive changes in the mitochondrial membrane potential of IGROV-1/CP and TOV112D cells following exposure to AF/CDDP when compared to each drug alone (Fig. 3B). The mitochondrial membrane was depolarized slightly by CDDP and more dramatically by the addition of AF to CDDP in IGROV-1/CP cells. In TOV112D cells, there was a slight depolarization of the mitochondrial membrane caused by AF, more so by CDDP, and even greater enhancement by the combination AF/CDDP (Fig. 3B).

Finally, we observed that the cytotoxicity induced by AF and CDDP in both types of EOC cells was partially yet significantly reversed in the presence of z-DEVD-fmk, an irreversible inhibitor of caspase 3 [25] (Fig. 3C).

### 3.4. AF and CDDP increase oxidative stress in EOC cells

We hypothesized that the disruption of the mitochondrial membrane potential elicited by the AF/CDDP combination was likely induced by ROS accumulation. To determine whether these two metals together act as pro-oxidants we measured the total intracellular levels of ROS in response to AF, CDDP, or the combination of both drugs. In IGROV-1/CP cells, there was a slight induction of ROS by AF, and a greater induction by CDDP, which did not increase further with the addition of AF; in TOV112D cells the drug combination produced a higher ROS accumulation than either drug alone (Fig. 4A).

To determine if the cytotoxicity of AF/CDDP is dependent on ROS production, we treated the EOC cells with these drugs in the presence of the antioxidant NAC. NAC minimized death from the drug combination (Fig. 4B). In addition, NAC was able to reverse in part the cell deterioration induced by AF/CDDP in IGROV-1/CP and TOV112D cells (Fig. 4C).

## 4. Discussion

The present study focused on the interaction between the gold complex AF and the DNA damaging agent CDDP in Pt-resistant EOC cells. We demonstrated a synergistic increase in cytotoxicity against two EOC cell types when AF was combined with CDDP [16,18]. Despite the large difference in sensitivity to CDDP between the two cell lines, the addition of AF significantly decreased the IC50s for CDDP in both types of EOC cells to the range of ~20 μM, which is within clinically achievable concentrations [28]. While a similar effect has been reported in small-cell lung carcinoma cells (SCLC) [15] and urothelial carcinoma cells [29], our study is the first to document that AF can restore Pt sensitivity in Pt-resistant EOCs, establishing the basis for potential success of this combination in treating this fatal disease.

The combination of AF and CDDP strongly induced apoptosis through the intrinsic pathway in both cell lines, enhancing the cleavage

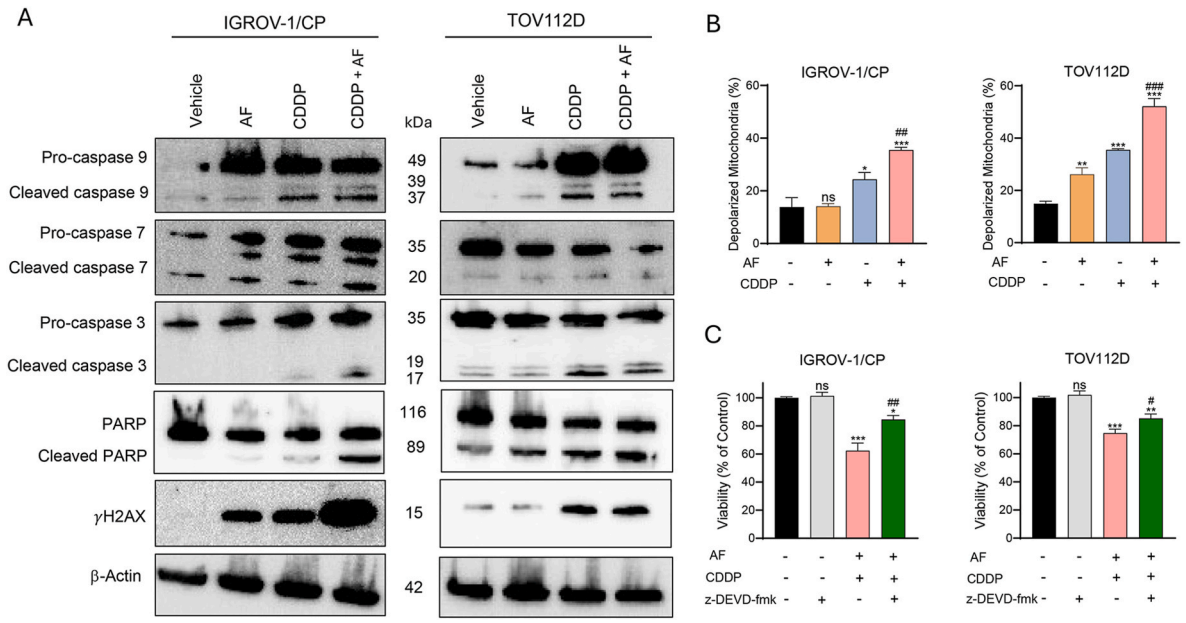


**Fig. 3.** A) Effect of CDDP (3 h), AF (72 h), or their combination on the cleavage of PARP, caspases-9, -7, and -3, and on the expression of $\gamma$ H2AX as detected by western blotting. $\beta$-actin was used as a protein loading control. B) AF/CDDP combination increases mitochondrial membrane depolarization in comparison to each drug alone. *p < 0.05, **p < 0.01, and ***p < 0.001 compared to vehicle; ###p < 0.001 when compared to each drug separately. C) The caspase-3 inhibitor z-DEVD-fmk partially rescues the cells from AF/CDDP-induced cytotoxicity following 48 h treatment; *p < 0.05, **p < 0.01, ***p < 0.001 when compared to vehicle. #p < 0.05 and ##p < 0.01 when compared to AF/CDDP treatment.

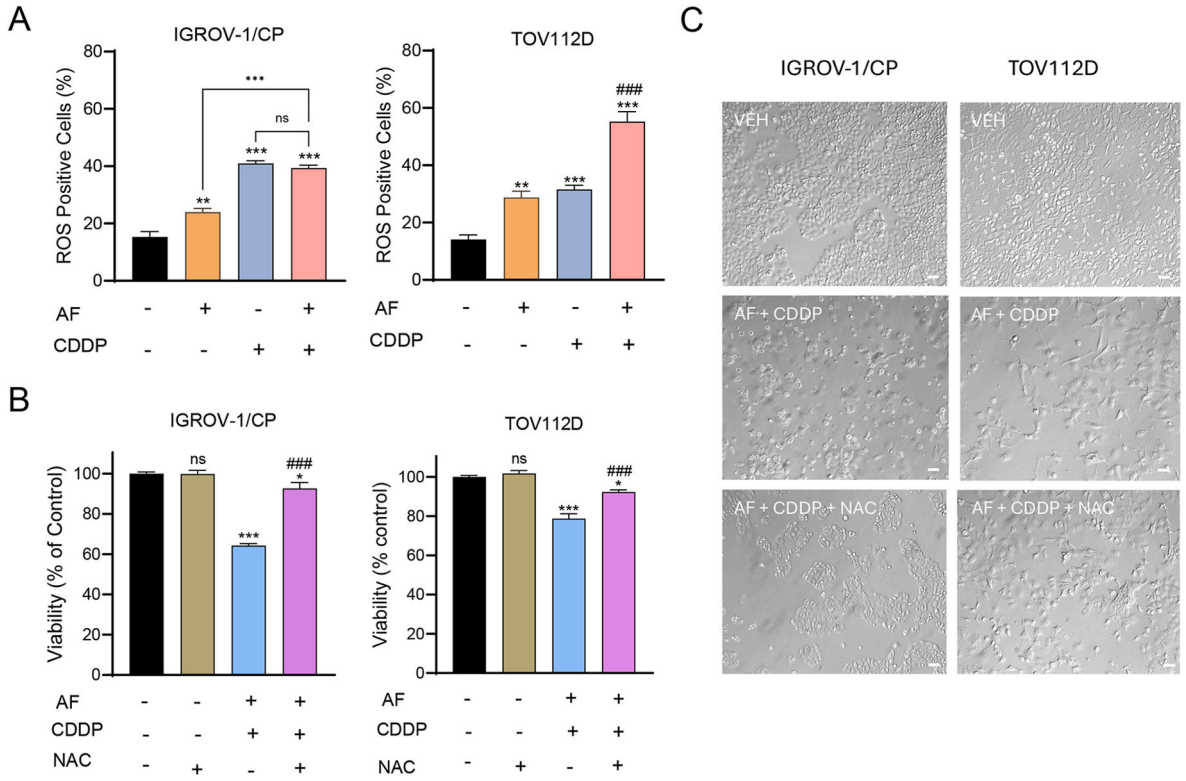


**Fig. 4.** A) Effect of AF, CDDP, or their combination on the production of ROS. Cells were treated with 15 μM CDDP for 3 h and 2 μM AF for 72 h in IGROV-1/CP cells, and 10 μM CDDP for 48 with 0.5 μM AF for 48 h in TOV112D cells. **p < 0.01 and ***p < 0.001 when compared to vehicle. ###p < 0.001 when compared to AF or CDDP alone. B) Protection of AF/CDDP-induced cytotoxicity in the presence of 5 mM NAC for 48 h *p < 0.05 and ***p < 0.001 compared to vehicle. ###p < 0.001 when compared to AF/CDDP. C) Phase-contrast images obtained after 48 h incubation with the indicated drugs. VEH, vehicle; AF, auranofin; CDDP, cisplatin; NAC, N-acetyl-L-cysteine. Scale bars = 50 μm.

of caspase-9 and the executioner caspases-3 and -7 [25]. The partial dependence of the combined cytotoxicity on caspase-3 activation has not been reported in previous studies on this drug combination [14,15].

PARP cleavage was also enhanced by combining AF with CDDP, particularly in the more Pt-resistant IGROV-1/CP cells, and a similar effect has been reported in SCLC cells [15]. DNA damage was increased by CDDP in both EOC cell lines, indicated by the upregulation of γH2AX and the damage was augmented by AF in IGROV-1/CP cells but not TOV112D.

The AF/CDDP combination induced mitochondrial membrane depolarization in both types of tumor cells, with a more pronounced effect in the TOV112D cells which are somewhat more sensitive to Pt. Similarly, others reported less mitochondrial membrane depolarization by CDDP in Pt-resistant OVCAR-8 cells compared to sensitive OVCAR-3 and OVCAR-4 cells [30].

Disruption of the mitochondrial membrane potential can be caused by mitochondrial ROS accumulation [4,31,32]. We previously reported that AF acts as a pro-oxidant by inhibiting the TrxR antioxidant system in HGSOC cells [10], an effect also observed in other cancers [33–35]. Similarly, CDDP generates ROS through mitochondrial damage and electron transport chain impairment [4] in lung and prostate cancer cells [36]. The AF/CDDP combination enhances ROS production in TOV112D cells, but in IGROV-1/CP cells, ROS production seems to be primarily driven by CDDP, possibly due to upregulated antioxidant defences [37]. Notably, the cytotoxicity of AF/CDDP is ROS-dependent, as viability was rescued by the ROS scavenger, NAC (Fig. 4B), which can replenish GSH or scavenge oxidant species [38].

In conclusion, we have documented a synergistic cytotoxic interaction between AF and CDDP in Pt-resistant EOC cells that is associated with intrinsic caspase-dependent apoptosis, DNA damage, and ROS accumulation (Supplementary Fig. 1). These results indicate that AF can enhance Pt sensitivity in EOC cells, a strategy that can potentially overcome the acquired Pt resistance that commonly occurs in recurrent ovarian cancer.

## CRediT authorship contribution statement

**Farah H. Abdalbari:** Writing – original draft, Methodology, Investigation, Formal analysis. **Benjamin N. Forgie:** Writing – review & editing, Investigation. **Edith Zorychta:** Writing – review & editing. **Alicia A. Goyeneche:** Writing – review & editing, Supervision, Methodology. **Abu Shadat M. Noman:** Writing – review & editing, Funding acquisition. **Carlos M. Telleria:** Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2025.101996.

## Data availability

Data will be made available on request.

## References

[1] L. Wang, X. Wang, X. Zhu, L. Zhong, Q. Jiang, Y. Wang, Q. Tang, Q. Li, C. Zhang, H. Wang, D. Zou, Drug resistance in ovarian cancer: from mechanism to clinical trial, Mol. Cancer 23 (2024) 66, https://doi.org/10.1186/s12943-024-01967-3.
[2] M.P. Garrido, A.N. Fredes, L. Lobos-González, M. Valenzuela-Valderrama, D. B. Vera, C. Romero, Current treatments and new possible complementary therapies for epithelial ovarian cancer, Biomedicines 10 (2021), https://doi.org/10.3390/biomedicines10010077.
[3] M.A. Lisio, L. Fu, A. Goyeneche, Z.H. Gao, C. Telleria, High-grade serous ovarian cancer: basic sciences, clinical and therapeutic standpoints, Int. J. Mol. Sci. 20 (2019), https://doi.org/10.3390/ijms20040952.
[4] B.N. Forgie, R. Prakash, C.M. Telleria, Revisiting the anti-cancer toxicity of clinically approved platinating derivatives, Int. J. Mol. Sci. 23 (2022), https://doi.org/10.3390/ijms232315410.
[5] L. Kelland, The resurgence of platinum-based cancer chemotherapy, Nat. Rev. Cancer 7 (2007) 573–584, https://doi.org/10.1038/nrc2167.
[6] S. Pignata, S. C Cecere, A. Du Bois, P. Harter, F. Heitz, Treatment of recurrent ovarian cancer, Ann. Oncol. 28 (2017) viii51–viii56, https://doi.org/10.1093/annonc/mdx441.
[7] M.E. Suarez-Almazor, C.H. Spooner, E. Belseck, B. Shea, Auranofin versus placebo in rheumatoid arthritis, Cochrane Database Syst. Rev. 2000 (2000) Cd002048, https://doi.org/10.1002/14651858.Cd002048.
[8] F.H. Abdalbari, C.M. Telleria, The gold complex auranofin: new perspectives for cancer therapy, Discov. Oncol. 12 (2021) 42, https://doi.org/10.1007/s12672-021-00439-0.
[9] E.N. Kotnik, M.M. Mullen, N.C. Spies, T. Li, M. Inkman, J. Zhang, F. Martins-Rodrigues, I.S. Hagemann, C.K. McCourt, P.H. Thaker, A.R. Hagemann, M. A. Powell, D.G. Mutch, D. Khabele, G.D. Longmore, E.R. Mardis, C.A. Maher, C. A. Miller, K.C. Fuh, Genetic characterization of primary and metastatic high-grade serous ovarian cancer tumors reveals distinct features associated with survival, Commun. Biol. 6 (2023) 688, https://doi.org/10.1038/s42003-023-05026-3.
[10] F.H. Abdalbari, E. Martinez-Jaramillo, B.N. Forgie, E. Tran, E. Zorychta, A. A. Goyeneche, S. Sabri, C.M. Telleria, Auranofin induces lethality driven by reactive oxygen species in high-grade serous ovarian cancer cells, Cancers 15 (2023) 5136.
[11] X. Zhang, K. Selvaraju, A.A. Saei, P. D'Arcy, R.A. Zubarev, E.S.J. Arnér, S. Linder, Repurposing of auranofin: thioredoxin reductase remains a primary target of the drug, Biochimie 162 (2019) 46–54, https://doi.org/10.1016/j.biochi.2019.03.015.
[12] H.H. Jang, Regulation of protein degradation by proteasomes in cancer, J. Cancer Prev. 23 (2018) 153–161, https://doi.org/10.15430/jcp.2018.23.4.153.
[13] G.R. Tundo, D. Sbardella, C. Ciaccio, S. De Pascali, V. Campanella, P. Cozza, U. Tarantino, M. Coletta, F.P. Fanizzi, S. Marini, Effect of cisplatin on proteasome activity, J. Inorg. Biochem. 153 (2015) 253–258, https://doi.org/10.1016/j.jinorgbio.2015.08.027.
[14] S.Y. Chen, C.N. Chao, H.Y. Huang, C.Y. Fang, Auranofin induces urothelial carcinoma cell death via reactive oxygen species production and synergy with cisplatin, Oncol. Lett. 23 (2022) 61, https://doi.org/10.3892/ol.2021.13179.
[15] X. Liu, W. Wang, Y. Yin, M. Li, H. Li, H. Xiang, A. Xu, X. Mei, B. Hong, W. Lin, A high-throughput drug screen identifies auranofin as a potential sensitizer of cisplatin in small cell lung cancer, Invest. N. Drugs 37 (2019) 1166–1176, https://doi.org/10.1007/s10637-019-00750-2.
[16] B.M. Barnes, L. Nelson, A. Tighe, G.J. Burghel, I.H. Lin, S. Desai, J.C. McGrail, R. D. Morgan, S.S. Taylor, Distinct transcriptional programs stratify ovarian cancer cell lines into the five major histological subtypes, Genome Med. 13 (2021) 140, https://doi.org/10.1186/s13073-021-00952-5.
[17] A.N. Karnezis, S.Y. Chen, C. Chow, W. Yang, W.P.D. Hendricks, P. Ramos, N. Briones, A.-M. Mes-Masson, T. Bosse, C.B. Gilks, J.M. Trent, B. Weissman, D. G. Huntsman, Y. Wang, Re-assigning the histologic identities of COV434 and TOV-112D ovarian cancer cell lines, Gynecol. Oncol. 160 (2021) 568–578, https://doi.org/10.1016/j.ygyno.2020.12.004.
[18] L. Cheng, W. Lu, B. Kulkarni, T. Pejovic, X. Yan, J.H. Chiang, L. Hood, K. Odunsi, B. Lin, Analysis of chemotherapy response programs in ovarian cancers by the next-generation sequencing technologies, Gynecol. Oncol. 117 (2010) 159–169, https://doi.org/10.1016/j.ygyno.2010.01.041.
[19] J. Benard, J. Da Silva, M.C. De Blois, P. Boyer, P. Duvillard, E. Chiric, G. Riou, Characterization of a human ovarian adenocarcinoma line, IGROV1, in tissue culture and in nude mice, Cancer Res. 45 (1985) 4970–4979.
[20] L. Li, J. Liu, W. Yu, X. Lou, B. Huang, B. Lin, Deep transcriptome profiling of ovarian cancer cells using next-generation sequencing approach, in: A. Malek, O. Tchernitsa (Eds.), Ovarian Cancer: Methods and Protocols, Humana Press, Totowa, NJ, 2013, pp. 139–169.
[21] T.C. Chou, P. Talalay, Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors, Adv. Enzym. Regul. 22 (1984) 27–55, https://doi.org/10.1016/0065-2571(84)90007-4.
[22] M.R. Subeha, A.A. Goyeneche, P. Bustamante, M.A. Lisio, J.V. Burnier, C. M. Telleria, Nelfinavir induces cytotoxicity towards high-grade serous ovarian cancer cells, involving induction of the unfolded protein response, modulation of protein synthesis, DNA damage, lysosomal impairment, and potentiation of toxicity caused by proteasome inhibition, Cancers (Basel) 14 (2021), https://doi.org/10.3390/cancers14010099.
[23] S. Dhani, Y. Zhao, B. Zhivotovsky, A long way to go: caspase inhibitors in clinical use, Cell Death Dis. 12 (2021) 949, https://doi.org/10.1038/s41419-021-04240-3.
[24] M. Kajstura, H.D. Halicka, J. Pryjma, Z. Darzynkiewicz, Discontinuous fragmentation of nuclear DNA during apoptosis revealed by discrete "sub-G1"

peaks on DNA content histograms, Cytometry A 71A (2007) 125–131, https://doi.org/10.1002/cyto.a.20357.
[25] J.G. Walsh, S.P. Cullen, C. Sheridan, A.U. Lüthi, C. Gerner, S.J. Martin, Executioner caspase-3 and caspase-7 are functionally distinct proteases, Proc. Natl. Acad. Sci. USA 105 (2008) 12815–12819, https://doi.org/10.1073/pnas.0707715105.
[26] M. Mashimo, M. Onishi, A. Uno, A. Tanimichi, A. Nobeyama, M. Mori, S. Yamada, S. Negi, X. Bu, J. Kato, J. Moss, N. Sanada, R. Kizu, T. Fujii, The 89-kDa PARP1 cleavage fragment serves as a cytoplasmic PAR carrier to induce AIF-mediated apoptosis, J. Biol. Chem. 296 (2021) 100046, https://doi.org/10.1074/jbc.RA120.014479.
[27] L.J. Mah, A. El-Osta, T.C. Karagiannis, γH2AX: a sensitive molecular marker of DNA damage and repair, Leukemia 24 (2010) 679–686, https://doi.org/10.1038/leu.2010.6.
[28] D.R. Liston, M. Davis, Clinically relevant concentrations of anticancer drugs: a guide for nonclinical studies, Clin. Cancer Res. 23 (2017) 3489–3498, https://doi.org/10.1158/1078-0432.CCR-16-3083.
[29] S.-Y. Chen, C.-N. Chao, H.-Y. Huang, C.-Y. Fang, Auranofin induces urothelial carcinoma cell death via reactive oxygen species production and synergy with cisplatin, Oncol. Lett. 23 (2022) 61, https://doi.org/10.3892/ol.2021.13179.
[30] M. Kleih, K. Böpple, M. Dong, A. Gaißler, S. Heine, M.A. Olayioye, W.E. Aulitzky, F. Essmann, Direct impact of cisplatin on mitochondria induces ROS production that dictates cell fate of ovarian cancer cells, Cell Death Dis. 10 (2019) 851, https://doi.org/10.1038/s41419-019-2081-4.
[31] A.V. Gyulkhandanyan, D.J. Allen, S. Mykhaylov, E. Lyubimov, H. Ni, J. Freedman, V. Leytin, Mitochondrial inner membrane depolarization as a marker of platelet apoptosis:disclosure of nonapoptotic membrane depolarization, Clin. Appl. Thromb. Hemost. 23 (2017) 139–147, https://doi.org/10.1177/1076029616665924.
[32] C. Fleury, B. Mignotte, J.-L. Vayssière, Mitochondrial reactive oxygen species in cell death signaling, Biochimie 84 (2002) 131–141, https://doi.org/10.1016/S0300-9084(02)01369-X.
[33] A. Sobhakumari, L. Love-Homan, E.V.M. Fletcher, S.M. Martin, A.D. Parsons, D. R. Spitz, C.M. Knudson, A.L. Simons, Susceptibility of human head and neck cancer cells to combined inhibition of glutathione and thioredoxin metabolism, PLoS One 7 (2012) e48175, https://doi.org/10.1371/journal.pone.0048175.
[34] B.M. Sutton, E. McGusty, D.T. Walz, M.J. DiMartino, Oral gold. Antiarthritic properties of alkylphosphinegold coordination complexes, J. Med. Chem. 15 (1972) 1095–1098, https://doi.org/10.1021/jm00281a001.
[35] D. Oommen, D. Yiannakis, A.N. Jha, BRCA1 deficiency increases the sensitivity of ovarian cancer cells to auranofin, Mutat. Res. Fund Mol. Mech. Mutagen 784–785 (2016) 8–15, https://doi.org/10.1016/j.mrfmmm.2015.11.002.
[36] R. Marullo, E. Werner, N. Degtyareva, B. Moore, G. Altavilla, S.S. Ramalingam, P. W. Doetsch, Cisplatin induces a mitochondrial-ROS response that contributes to cytotoxicity depending on mitochondrial redox status and bioenergetic functions, PLoS One 8 (2013) e81162, https://doi.org/10.1371/journal.pone.0081162.
[37] D. Trachootham, J. Alexandre, P. Huang, Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? Nat. Rev. Drug Discov. 8 (2009) 579–591, https://doi.org/10.1038/nrd2803.
[38] G. Aldini, A. Altomare, G. Baron, G. Vistoli, M. Carini, L. Borsani, F. Sergio, N-Acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why, Free Radic. Res. 52 (2018) 751–762, https://doi.org/10.1080/10715762.2018.1468564.