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Cholesterol Dysregulation Enhances Viability and Migration in Ovarian Cancer Cells and Modulates Carboplatin Response

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Abstract

Background

Cholesterol metabolism plays a pivotal role in cancer progression and treatment resistance. This study investigates the effects of exogenous cholesterol on cell viability and migration, both as a monotherapy and in combination with carboplatin, in ovarian cancer cell lines representing disease progression: PEO1, PEO4, and PEO6.

Methods

PEO1, PEO4, and PEO6 cells were treated with 1 μg/mL or 5 μg/mL cholesterol, with or without carboplatin (100 μM or 200 μM). Cell viability was assessed with a luminescent adenosine triphosphate assay (CellTiter-Glo), and migration was measured using wound healing assays. Treatment groups were compared within each cell line by one-way analysis of variance.

Results

Cholesterol alone significantly enhanced viability in all three cell lines, with the most robust and consistent effects observed in PEO1 cells. Co-treatment with cholesterol and carboplatin further increased viability compared to carboplatin alone, suggesting a cholesterol-mediated protective effect, most prominent in PEO1 and PEO4. Cholesterol also promoted cell migration in all three lines, with a significant dose effect only in PEO4, with PEO1 showing the strongest enhancement. While carboplatin inhibited migration, cholesterol partially reversed this effect, particularly in PEO1, PEO4 and PEO6 lines who demonstrated modest recovery.

Conclusions

Exogenous cholesterol promotes ovarian cancer cell viability and migration and mitigates carboplatin-induced suppression in a cell line–specific manner. The most pronounced effects were seen in PEO1, suggesting a potential link between cholesterol dysregulation and chemoresistance. These findings underscore the importance of considering lipid metabolism in therapeutic strategies for ovarian cancer.

Background

In 2026, ovarian cancer (OC) ranks as the fifth leading cause of cancer mortality among women in the United States, contributing to 4.3% of all cancer-related female deaths. [1] The life-time risk for OC is approximately 1.4%[2], and the majority of cases are diagnosed at advanced stage due to lack of specific symptoms. The average 5-year relative survival rate for OC is 51%, ranging from 93% at early stage to 31% at advanced stage. Ovarian cancer is a heterogeneous group of malignancies subdivided into three

distinctive histological groups: epithelial ovarian cancer (EOC), germ cell tumors (GCT) and sex-cord stromal tumors (SCST) [3], with 90% of the cases being epithelial and the remaining 10% comprising non-epithelial tumors.

EOC can be further subdivided into five primary tumor cell histology subtypes: high-grade serous ovarian cancer (HGSOC), low-grade serous ovarian cancer (LGSOC), mucinous, endometrioid, and clear cell carcinomas. The most common subtype is HGSOC, accounting for close to 70% of all OC cases. [4]

A combination including platinum-based chemotherapy and cytoreductive surgery is the gold standard treatment for HGSOC. [3, 5]The median progression-free survival time for most patients is 18 to 24 months even though they are initially responsive to the chemotherapy [6]. One of the main platinum-based drugs used in the treatment of EOC is carboplatin, which induces DNA damage, dysfunction of mitochondria and activation of apoptotic pathways [7]. However, because most patients eventually develop chemoresistance and have a poor prognosis, an in-depth investigation of the underlying mechanisms of drug resistance is essential for improving clinical outcomes[8].

In the advanced stages of HGSOC, the development of malignant ascites, an accumulation of fluid in the peritoneal cavity, is observed in more than one third of ovarian cancer patients and most of the recurrent cases[9]. Ovarian cancer tends to metastasize to the omentum, the layer of adipose tissue covering the intraperitoneal organs. As a result, its malignant ascites forms part of a lipid-rich tumor microenvironment (TME) [9].

Shifts in lipid metabolism and the creation of the adipocyte-rich TME are involved in providing energy sources, irregular expression of critical enzymes, transport proteins, pro-inflammatory factors, and cellular receptors in cancer cells[10, 11]. This unique microenvironment is essential for OC tumorigenesis, tumor immune escape, chemoresistance, and recurrence[11].

Cholesterol is a lipophilic molecule and a critical component of cell membranes and plasma lipoproteins. Additionally, it is a precursor to many bioactive molecules such as bile acids, vitamin D and steroid hormones[12]. Transportation of cholesterol throughout the body is performed by lipoproteins in the blood due to its hydrophobic nature. Low-density lipoproteins (LDL) are responsible for providing cholesterol to peripheral cells and high-density lipoproteins (HDL) are responsible for collecting the cholesterol from periphery and transporting it to the liver [13].

One of the notable underlying issues resulting in tumor recurrence and chemoresistance in OC patients is cholesterol dysregulation. [14] These shifts in cholesterol metabolism are part of the bigger phenomenon in cancer cells called metabolic reprogramming. These adaptations are essential for promoting the fast and uncontrolled growth of cancer cells while supporting their survival[15].

In metabolic reprogramming, ovarian cancer cells lose the normal feedback that limits cholesterol production and uptake. *TP53*, mutated in almost all HGSOC [16], contributes to this: mutant *TP53* increases expression of HMG-CoA reductase, the rate-limiting enzyme of cholesterol synthesis [17], and

blocking this enzyme with lovastatin prevented the development of HGSOC precursor lesions and ovarian tumors in mice [18].

As HGSOC becomes platinum-resistant, cells shift from making cholesterol to taking it up. In cell lines derived from the same patients before and after resistance, resistant cells produced less cholesterol, expressed more of the low-density lipoprotein receptor (LDLR) and had more cholesterol in their membranes; removing lipids from the culture medium restored their sensitivity to cisplatin [19]. Consistently, reducing LDLR made ovarian cancer cells more sensitive to cisplatin; while increasing it made them less sensitive [20]. Membrane cholesterol may limit drug entry into the cell [14], although the role of the cholesterol exporter ABCA1 is unclear, as high ABCA1 expression has been linked to poorer survival in HGSOC [21]. Finally, cholesterol in malignant ascites reduced sensitivity to cisplatin and paclitaxel by increasing drug efflux pumps, and high ascitic cholesterol was associated with earlier recurrence [22].

Overall, this evidence highlights a potential combination of platinum-based chemotherapy drugs with therapeutic approaches to inhibit cholesterol absorption and biosynthesis for improving survival rate and treatment outcome in ovarian cancer patients.

Whether cholesterol alone protects HGSOC cells from carboplatin, and whether this protection changes as the disease progresses from a platinum-sensitive to a platinum-resistant state, remains unknown. Addressing this question required cells from the same patient at successive stages of treatment, so that differences in response reflect disease evolution rather than differences between patients.

This study investigates the impact of exogenous cholesterol on ovarian cancer cell viability, migration and response to carboplatin using a series of high-grade serous cell lines derived from the same patient at different stages of disease progression. These cell lines were collected after initial chemotherapy, at the onset of chemoresistance, and at a later stage following continued treatment – corresponding to PEO1, PEO4, and PEO6, respectively [23–25].

Methods

Cell lines and culture conditions

The PEO1, PEO4 and PEO6 cell lines were established by Langdon and colleagues from the malignant ascites of a single patient with high-grade serous ovarian carcinoma at successive stages of disease [23] : PEO1 after initial platinum-based chemotherapy, while the disease was still platinum-sensitive, PEO4 at relapse after the onset of platinum resistance, and PEO6 at a later stage following continued treatment [24]. The cell lines were obtained from Dr. Toshiyasu Taniguchi (Fred Hutchinson Cancer Center, Seattle, Washington, United States) with the written consent of Dr. Simon P. Langdon (Edinburgh Cancer Research Centre, Edinburgh, United Kingdom). Human ovarian cancer cell lines PEO1, PEO4, and PEO6 were cultured in RPMI-1640 medium (Wisent, Saint-Jean-Baptiste, Quebec, Canada; catalogue number 350-007-CL) supplemented with 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

(HEPES; Corning, Corning, New York, United States), 2 mM dipeptide glutamine (GlutaGro, Corning), 1 mM sodium pyruvate (Corning), 10 µg/mL insulin (Gibco, Grand Island, New York, United States), 23.8 mM sodium bicarbonate (MilliporeSigma, St-Louis, Missouri, United States), 10% fetal bovine serum (FBS) (Wisent, catalogue number 080–450) and 1% penicillin-streptomycin (100 IU/mL penicillin and 100 µg/mL streptomycin; Wisent, catalogue number 450-201-EL). Cells were maintained at 37°C in a humidified atmosphere with 5% CO_2.

Wound Healing Assay

Cells were seeded into 6-well plates at a density sufficient to reach full confluence within 24 hours. Upon reaching confluence, a sterile 200 µL pipette tip was used to create a straight-line scratch ("wound") across the monolayer in each well. The wells were gently washed with phosphate-buffered saline (PBS; Wisent, catalogue number 311-010-CL) to remove detached cells and debris, followed by treatment with various compounds as described in the next section.

Treatment Conditions

Cells were treated with the following agents: Cholesterol at 1 µg/mL and 5 µg/mL; carboplatin at 100 µM and 200 µM; combination treatments: 1 µg/mL cholesterol + 100 µM carboplatin, 1 µg/mL cholesterol + 200 µM carboplatin, 5 µg/mL cholesterol + 100 µM carboplatin, 5 µg/mL cholesterol + 200 µM carboplatin. Images of the wound area were taken at 0 hours (baseline) and again at 48 hours for PEO1, and 72 hours for PEO4 and PEO6 using an inverted phase-contrast microscope. Wound closure was assessed by comparing the wound area at 0 hours and post-treatment time points.

Cell Viability Assay

Cell viability following treatment was assessed using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega, Madison, Wisconsin, United States; catalogue number G7570), which quantifies adenosine triphosphate as an indicator of metabolically active cells, according to the manufacturer’s protocol. Cells were plated in opaque white-walled 96-well plates, allowed to attach for 24 hours, and treated as described above. After the indicated incubation period (48 or 72 hours depending on the cell line), a volume of CellTiter-Glo reagent equal to the volume of medium was added to each well, plates were mixed on an orbital shaker for 2 minutes and incubated at room temperature for 10 minutes to stabilize the signal. Luminescence was measured using a plate reader. Luminescence values were normalized to untreated controls.

Statistical Analysis

All experiments were performed as three independent biological replicates. Data are expressed as mean ± standard deviation (SD). Statistical analyses were carried out using GraphPad Prism version 8.4.3 for

Windows (GraphPad Software, Boston, Massachusetts, United States). One-way analysis of variance (ANOVA) was used to assess differences between treatment groups. A p-value < 0.05 was considered statistically significant.

Results

Cholesterol's Effect on Migration

PEO1

In the scratch assay, treatment with cholesterol alone significantly enhanced wound closure in PEO1 cells at both concentrations (Fig. 1A and 1B). Specifically, 1 µg/mL and 5 µg/mL cholesterol treatments resulted in increased migration compared to control ($p = 0.0298$ and $p = 0.0191$, respectively) (Additional file 1: Table S1), indicating a pro-migratory effect, with no significant difference between the two doses ($p = 0.7132$). In contrast, carboplatin alone reduced migration in PEO1 cells, with significant inhibition observed at both 100 µM ($p = 0.0409$) and 200 µM ($p = 0.0012$), the latter producing a pronounced cytotoxic effect. When cholesterol was co-administered with carboplatin, no significant changes in migration were noted at 100 µM carboplatin, regardless of cholesterol dose ($p = 0.7220$ and $p = 0.7463$ for 1 µg/mL and 5 µg/mL, respectively). At 200 µM carboplatin, wound closure with 1 µg/mL cholesterol remained lower than control ($p = 0.0354$), whereas with 5 µg/mL cholesterol it did not differ significantly from control ($p = 0.8258$). Further comparisons revealed that both 1 µg/mL and 5 µg/mL cholesterol significantly enhanced migration when added to carboplatin-treated cells, with p-values ranging from 0.0271 to 0.0001 across conditions (carboplatin 100 µM plus 1 µg/mL or 5 µg/mL cholesterol: $p = 0.0271$ and $p = 0.0111$; carboplatin 200 µM plus 1 µg/mL or 5 µg/mL cholesterol: $p = 0.0023$ and $p = 0.0001$). Notably, the combination of carboplatin and cholesterol still resulted in lower migration compared to cholesterol alone ($p = 0.0036$, $p = 0.0001$, $p = 0.0007$ and $p = 0.0014$ for carboplatin 100 µM plus 1 µg/mL, 100 µM plus 5 µg/mL, 200 µM plus 1 µg/mL and 200 µM plus 5 µg/mL, respectively), though significantly higher than carboplatin alone. These findings indicate that cholesterol promotes migration in PEO1 cells and can partially counteract carboplatin-induced migration inhibition.

(A) Representative phase-contrast images of the wound at 0 and 48 hours in PEO1 cells treated with cholesterol (1 µg/mL or 5 µg/mL), carboplatin (100 µM or 200 µM), or their combinations. (B) Quantification of wound closure at 48 hours using ImageJ, expressed as relative wound opening (fold change); higher values indicate greater wound closure. Data are presented as mean ± standard deviation of three independent biological replicates. Statistical comparisons are provided in Additional file 1: Table S1.

PEO4

In PEO4 cells, cholesterol alone increased migration only at the higher concentration (Fig. 2A and 2B), with 5 µg/mL reaching statistical significance compared to control ($p = 0.0296$) (Additional file 1: Table S1), while 1 µg/mL had no significant effect ($p = 0.8857$). Treatment with carboplatin alone significantly

inhibited migration at both 100 μM (p = 0.0060) and 200 μM (p = 0.0049), reflecting a dose-dependent cytotoxic effect. Co-treatment of cholesterol with 100 μM carboplatin did not significantly reverse this inhibition (p = 0.5810 for 1 μg/mL; p = 0.0849 for 5 μg/mL), and similarly, no protective effect was observed at 200 μM carboplatin (p = 0.3598 and p = 0.9758 for 1 μg/mL and 5 μg/mL cholesterol, respectively). However, when comparing cholesterol plus carboplatin to carboplatin alone, significant increases in migration were observed for both cholesterol doses at 100 μM carboplatin (p = 0.0005 and p = 0.0007) and at 200 μM (p = 0.0032 and p = 0.0010), suggesting a mitigating effect of cholesterol on carboplatin-induced migration inhibition. Additionally, direct comparison of 5 μg/mL vs 1 μg/mL cholesterol showed a significant enhancement in wound closure (p = 0.0021), confirming the dose-responsiveness in PEO4. Notably, only the highest-dose combination (200 μM carboplatin + 5 μg/mL cholesterol) produced a significant increase in migration when compared to cholesterol alone (p = 0.0013); the other combinations did not differ from cholesterol alone (p = 0.2561, p = 0.5834 and p = 0.1726 for carboplatin 100 μM plus 1 μg/mL, 100 μM plus 5 μg/mL and 200 μM plus 1 μg/mL, respectively). Collectively, these findings suggest that in PEO4 cells, cholesterol alone modestly enhances migration, and more importantly, it can significantly restore migration when co-administered with carboplatin, particularly at higher doses and when compared directly to carboplatin treatment alone.

(A) Representative phase-contrast images of the wound at 0 and 72 hours in PEO4 cells treated with cholesterol (1 μg/mL or 5 μg/mL), carboplatin (100 μM or 200 μM), or their combinations. (B) Quantification of wound closure at 72 hours using ImageJ, expressed as relative wound opening (fold change); higher values indicate greater wound closure. Data are presented as mean ± standard deviation of three independent biological replicates. Statistical comparisons are provided in Additional file 1: Table S1.

PEO6

In PEO6 cells, cholesterol alone significantly promoted wound closure (Fig. 3A and 3B), with both 1 μg/mL and 5 μg/mL treatments showing statistically significant increases in migration compared to control (p = 0.0166 and p = 0.0025, respectively) (Additional file 1: Table S1). Treatment with carboplatin alone inhibited migration, with a non-significant trend observed at 100 μM (p = 0.0961) and a highly significant reduction at 200 μM (p = 0.0007), indicating a strong cytotoxic effect at the higher concentration. Co-treatment with cholesterol and 100 μM carboplatin did not yield any significant changes in migration (p = 0.6975 and p = 0.4636 for 1 μg/mL and 5 μg/mL, respectively). While 5 μg/mL cholesterol combined with 200 μM carboplatin showed a borderline increase in migration (p = 0.0476), the effect was less robust than in other cell lines. The combination of 1 μg/mL cholesterol with 200 μM carboplatin did not significantly change migration compared to control (p = 0.2739). In contrast, when compared directly to carboplatin alone, both 1 μg/mL and 5 μg/mL cholesterol significantly improved migration at 200 μM (p = 0.0003 and p = 0.0020, respectively), but not at 100 μM (p = 0.2561 and p = 0.0915), suggesting a protective effect of cholesterol against carboplatin-induced migration inhibition. Additionally, the combination of 100 μM carboplatin and 1 μg/mL cholesterol significantly enhanced migration relative to 1 μg/mL cholesterol alone (p = 0.0378), further supporting the pro-migratory role of

cholesterol under stress conditions. The combination of 200 μM carboplatin and 1 μg/mL cholesterol also differed from 1 μg/mL cholesterol alone (p = 0.0078), whereas the 5 μg/mL combinations did not differ from 5 μg/mL cholesterol alone (p = 0.0938 at 100 μM and p = 0.6089 at 200 μM). No significant differences were observed when comparing 5 μg/mL to 1 μg/mL cholesterol (p = 0.9497). These results indicate that PEO6 cells are responsive to cholesterol-induced migration, particularly in the context of high-dose carboplatin, although the effect is less pronounced than in PEO1 or PEO4.

(A) Representative phase-contrast images of the wound at 0 and 72 hours in PEO6 cells treated with cholesterol (1 μg/mL or 5 μg/mL), carboplatin (100 μM or 200 μM), or their combinations. (B) Quantification of wound closure at 72 hours using ImageJ, expressed as relative wound opening (fold change); higher values indicate greater wound closure. Data are presented as mean ± standard deviation of three independent biological replicates. Statistical comparisons are provided in Additional file 1: Table S1.

Cholesterol's Effect on Viability

PEO1

To determine the impact of cholesterol on PEO1 cell viability, cells were treated with cholesterol (1 μg/mL and 5 μg/mL), either alone or in combination with carboplatin. Cell viability outcomes are illustrated in (Fig. 4), with corresponding statistical significance values detailed in (Additional file 1: Table S2).

Treatment with cholesterol alone resulted in a significant increase in cell viability compared to control (1 μg/mL: $p = 0.0060$; 5 μg/mL: $p = 0.0039$), indicating a pro-survival effect of cholesterol on PEO1 cells. The difference between the two cholesterol doses was not statistically significant ($p = 0.0576$), suggesting a possible non-linear dose-response at these concentrations.

Carboplatin alone (100 μM or 200 μM) significantly reduced cell viability compared to control ($p = 0.0311$ and $p = 0.0006$, respectively), confirming its cytotoxicity. Notably, co-treatment with cholesterol (either 1 μg/mL or 5 μg/mL) attenuated the cytotoxic effects of carboplatin, resulting in significantly higher viability than carboplatin alone (e.g., carbo100 μM + 1 μg/mL vs carbo100 μM: p = 0.0172; carbo100 μM + 5 μg/mL vs carbo100 μM: p < 0.0001; carbo200 μM + 1 μg/mL vs carbo200 μM: $p = 0.0008$; carbo200 μM + 5 μg/mL vs carbo200 μM: $p = 0.0002$). Viability with carboplatin 100 μM plus cholesterol was higher than control (p = 0.0017 and p = 0.0007 for 1 μg/mL and 5 μg/mL), as was viability with carboplatin 200 μM plus 1 μg/mL cholesterol (p = 0.0347), whereas viability with carboplatin 200 μM plus 5 μg/mL cholesterol was lower than control (p = 0.3337). Compared with cholesterol alone at the same concentrations, viability was higher with carboplatin 100 μM plus 5 μg/mL than with 5 μg/mL alone (p = 0.0174), lower with carboplatin 200 μM plus 1 μg/mL than with 1 μg/mL alone (p = 0.0029) and with carboplatin 200 μM plus 5 μg/mL than with 5 μg/mL alone (p = 0.0003), and did not differ with carboplatin 100 μM plus 1 μg/mL from 1 μg/mL alone (p = 0.6232). The two cholesterol doses did not differ when combined with carboplatin (p = 0.0721 at 100 μM and p = 0.3803 at 200 μM).

PEO1 cells were treated with cholesterol (1 µg/mL or 5 µg/mL), carboplatin (100 µM or 200 µM), or their combinations. Viability was measured at 48 hours with the CellTiter-Glo luminescent cell viability assay and expressed as fold change relative to untreated control. Data are presented as mean ± standard deviation of three independent biological replicates. Statistical comparisons are provided in Additional file 1: Table S2.

PEO4

To investigate the role of cholesterol in modulating PEO4 cell viability, cells were treated with cholesterol alone (1 µg/mL and 5 µg/mL) and in combination with carboplatin. Cell viability was evaluated and is presented in (Fig. 5), with statistical comparisons as per Additional file 1: Table S2.

Treatment with cholesterol alone led to a marked increase in cell viability compared to untreated control (1 µg/mL: $p = 0.0198$; 5 µg/mL: $p = 0.0020$). The effect was dose-dependent, with 5 µg/mL cholesterol showing a more pronounced increase in viability than 1 µg/mL ($p = 0.0053$), consistent with a pro-survival role of cholesterol in PEO4 cells.

Carboplatin alone significantly reduced PEO4 cell viability, at 200 µM ($p = 0.0030$), whereas the reduction at 100 µM did not reach significance ($p = 0.5156$) (Additional file 1: Table S2). However, co-treatment with cholesterol partially rescued cell viability. For instance, carbo100 µM + 1 µg/mL and carbo100 µM + 5 µg/mL both showed significantly higher viability compared to carboplatin alone ($p = 0.0172$ and $p < 0.0001$, respectively), suggesting that cholesterol attenuates the cytotoxic effects of carboplatin. However, the two cholesterol doses did not differ significantly when combined with carboplatin ($p = 0.0721$ at 100 µM and $p = 0.3803$ at 200 µM).

This protective effect was similarly observed with 200 µM carboplatin. Cells treated with carbo200 µM + 1 µg/mL and carbo200 µM + 5 µg/mL cholesterol exhibited higher viability than those treated with carboplatin alone ($p = 0.0008$ and $p = 0.0002$, respectively), indicating a consistent mitigating effect of cholesterol across both concentrations of chemotherapy. Compared with control, viability was higher only with carboplatin 100 µM plus 5 µg/mL cholesterol ($p = 0.0040$), and not with carboplatin 100 µM plus 1 µg/mL ($p = 0.0719$), carboplatin 200 µM plus 1 µg/mL ($p = 0.0514$) or carboplatin 200 µM plus 5 µg/mL ($p = 0.0600$). Compared with cholesterol alone, viability was lower with carboplatin 100 µM plus 5 µg/mL ($p = 0.0174$), carboplatin 200 µM plus 1 µg/mL ($p = 0.0029$) and carboplatin 200 µM plus 5 µg/mL ($p = 0.0003$), but not with carboplatin 100 µM plus 1 µg/mL ($p = 0.6232$).

PEO4 cells were treated with cholesterol (1 µg/mL or 5 µg/mL), carboplatin (100 µM or 200 µM), or their combinations. Viability was measured at 72 hours with the CellTiter-Glo luminescent cell viability assay and expressed as fold change relative to untreated control. Data are presented as mean ± standard deviation of three independent biological replicates. Statistical comparisons are provided in Additional file 1: Table S2.

PEO6

To determine the influence of cholesterol on PEO6 cell viability, cells were treated with cholesterol (1 µg/mL and 5 µg/mL), carboplatin, and their combinations. The viability outcomes are shown in Fig. 6, with relevant statistical values detailed in Additional file 1: Table S2.

Treatment with cholesterol alone resulted in a modest but statistically significant increase in viability relative to the untreated control group (1 µg/mL: $p = 0.0386$; 5 µg/mL: $p = 0.0079$). The increase was dose-responsive, with 5 µg/mL cholesterol inducing a slightly higher viability than 1 µg/mL ($p = 0.0161$), suggesting a mild pro-survival effect of cholesterol in PEO6 cells.

Carboplatin alone (both 100 µM and 200 µM) significantly decreased PEO6 cell viability (100 µM: $p = 0.0161$; 200 µM: $p = 0.0029$), confirming its cytotoxic effect. However, co-treatment with cholesterol partially reversed this reduction. In particular, carbo100 µM + 1 µg/mL and carbo100 µM + 5 µg/mL cholesterol showed statistically higher viability compared to carboplatin alone ($p = 0.0108$ and $p < 0.0001$, respectively), although the difference between the two doses was not significant at 100 µM ($p = 0.1527$). Similarly, viability in carbo200 µM+cholesterol groups was increased compared to carbo200 µM alone ($p = 0.0475$ and $p = 0.0122$ for 1 µg/mL and 5 µg/mL, respectively), indicating that cholesterol attenuates carboplatin cytotoxicity in PEO6 cells. Viability with carboplatin 200 µM plus cholesterol was also higher with 5 µg/mL than with 1 µg/mL ($p = 0.0205$). Compared with control, viability was higher with carboplatin 100 µM plus 5 µg/mL ($p = 0.0034$), lower with carboplatin 200 µM plus 1 µg/mL ($p = 0.0022$) and carboplatin 200 µM plus 5 µg/mL ($p = 0.0194$), and not different with carboplatin 100 µM plus 1 µg/mL ($p = 0.1491$). Compared with cholesterol alone, viability was lower with carboplatin 200 µM plus 1 µg/mL ($p < 0.0001$) and 5 µg/mL ($p = 0.0004$), but not with carboplatin 100 µM plus 1 µg/mL ($p = 0.8387$) or 5 µg/mL ($p = 0.3506$).

PEO6 cells were treated with cholesterol (1 µg/mL or 5 µg/mL), carboplatin (100 µM or 200 µM), or their combinations. Viability was measured at 72 hours with the CellTiter-Glo luminescent cell viability assay and expressed as fold change relative to untreated control. Data are presented as mean ± standard deviation of three independent biological replicates. Statistical comparisons are provided in Additional file 1: Table S2.

Discussion

Cholesterol alone increased cell migration in all three ovarian cancer cell lines (Additional file 1: Table S1), although the concentration required differed between lines. In PEO1 and PEO6, wound closure increased at both 1 µg/mL and 5 µg/mL cholesterol, with no further increase at the higher concentration. In PEO4, wound closure increased only at 5 µg/mL, suggesting that this line requires a higher cholesterol concentration to respond. As migration is an early step in peritoneal dissemination, these findings support a pro-tumor role for cholesterol in ovarian cancer, consistent with the dysregulation of cholesterol metabolism described in this disease and the enrichment of cholesterol in malignant ascites [14, 22, 26]. Because cholesterol also increased viability, part of the increase in wound closure may reflect proliferation rather than migration alone.

Carboplatin alone reduced migration in all three lines at 200 μM, and in PEO1 and PEO4 at 100 μM. At 100 μM carboplatin, adding cholesterol brought wound closure in PEO1 and PEO4 back to a level that was not significantly different from untreated control cells. In PEO6, carboplatin 100 μM alone did not significantly reduce migration, so an effect of cholesterol could not be assessed at this dose. At 200 μM carboplatin, migration was significantly reduced in all three cell lines. Adding either concentration of cholesterol increased wound closure compared with carboplatin alone in all three lines. When compared with untreated control cells, the result depended on the cholesterol concentration. With 5 μg/mL cholesterol, wound closure was not significantly different from control in PEO1 and PEO4 and was higher than control in PEO6 ($p = 0.0476$). With 1 μg/mL cholesterol, wound closure was not significantly different from control in PEO4 and PEO6, but remained lower than control in PEO1 ($p = 0.0354$).

Cholesterol increased viability compared with carboplatin alone in all three cell lines at both carboplatin concentrations. At 200 μM carboplatin, recovery of viability was greatest in PEO1 and smaller in PEO4 and PEO6. Taken together, these results suggest that cells collected earlier in the disease course respond more strongly to exogenous cholesterol, whereas the later platinum-resistant line may already rely on intrinsic adaptations in cholesterol handling, such as increased low-density lipoprotein receptor expression or reduced efflux through ATP-binding cassette transporter A1 [14, 26, 27].

These findings fit with clinical and translational data linking peritoneal lipids to ovarian cancer outcomes. Cholesterol levels are higher in malignant ascites than in benign ascites, and high ascitic cholesterol has been associated with shorter recurrence-free survival in patients with ovarian cancer, in part through activation of liver X receptors and upregulation of drug efflux pumps [22]. Lipidomic profiling has shown that lipid levels in ascites correlate with those in plasma, and that lipoprotein receptor expression differs between ovarian cancer and benign tumors, further implicating lipid uptake in ovarian cancer biology [28]. Omental adipocytes also transfer lipids directly to ovarian cancer cells and promote their migration and growth [29].

Clinically, statins, which lower cholesterol by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase, have been associated with improved survival in ovarian cancer. In a meta-analysis restricted to studies without immortal time bias, statin use was associated with better survival (pooled hazard ratio 0.76, 95% confidence interval 0.68 to 0.85) [30]. In a population-based cohort of 4,207 patients from British Columbia, post-diagnosis statin use was associated with better survival, including in serous cancers (adjusted hazard ratio 0.80, 95% confidence interval 0.67 to 0.96) [31]. However, a Danish nationwide cohort of 4,419 patients found no significant association between post-diagnostic statin use and ovarian cancer-specific mortality (hazard ratio 0.90, 95% confidence interval 0.76 to 1.08) [32]. Our finding that cholesterol attenuates carboplatin cytotoxicity offers a possible biological explanation for these associations and supports testing statins or cholesterol uptake inhibitors in combination with platinum chemotherapy in preclinical models and prospective trials.

This study has limitations. Experiments were performed in two-dimensional monolayers of three cell lines derived from a single patient, which limits generalizability. Treatments were given in serum-

containing medium, which already provides lipoproteins, and intracellular cholesterol content and uptake were not measured, therefore the effects observed cannot yet be attributed to a specific mechanism. Wound closure reflects both migration and proliferation, and these were not separated. Future work should quantify intracellular and membrane cholesterol, assess the expression of the low-density lipoprotein receptor, sterol regulatory element-binding protein 2 and ATP-binding cassette transporter A1, test whether statins or cholesterol uptake inhibitors reverse the protective effect of cholesterol, and confirm these findings in three-dimensional and patient-derived models.

Conclusions

Exogenous cholesterol increased the viability and migration of PEO1, PEO4 and PEO6 ovarian cancer cells, which represent successive stages of disease in a single patient with high-grade serous ovarian carcinoma. When combined with carboplatin, cholesterol attenuated carboplatin-induced loss of viability in all three cell lines at both carboplatin concentrations and attenuated the inhibition of migration in all three lines at the higher carboplatin concentration. This protection was partial, as cholesterol reduced but did not abolish the effects of carboplatin. These findings identify cholesterol availability as a potential modifier of platinum response in high-grade serous ovarian carcinoma. Given the high cholesterol content of malignant ascites and the wide availability of cholesterol-lowering drugs, mechanistic studies and testing of statins or cholesterol uptake inhibitors in combination with platinum chemotherapy in three-dimensional and patient-derived models are warranted

Abbreviations

ABCA1
ATP-binding cassette transporter A1
ANOVA
analysis of variance
EOC
epithelial ovarian cancer
FBS
fetal bovine serum
GCT
germ cell tumor
HDL
high-density lipoprotein
HEPES
4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HGSOC
high-grade serous ovarian carcinoma
LDL
low-density lipoprotein

LDLR
low-density lipoprotein receptor
LGSOC
low-grade serous ovarian carcinoma
OC
ovarian cancer
PBS
phosphate-buffered saline
SCST
sex-cord stromal tumor
SD
standard deviation
TME
tumor microenvironment

Declarations

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Not applicable.

Consent for publication:

Not applicable.

Competing interests:

Authors declare that they have no competing interests.

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Author Contribution

Z.Q., S.O.A.L., B.T-C., I.B., G.L. S.O.A.L. inception, Z.Q. Y.B., L.X., experiments, analysis and redaction, I.B., G.L., X.Z. and S.O.A.L. reviewed and edited the manuscript ; S.O.A.L supervision.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

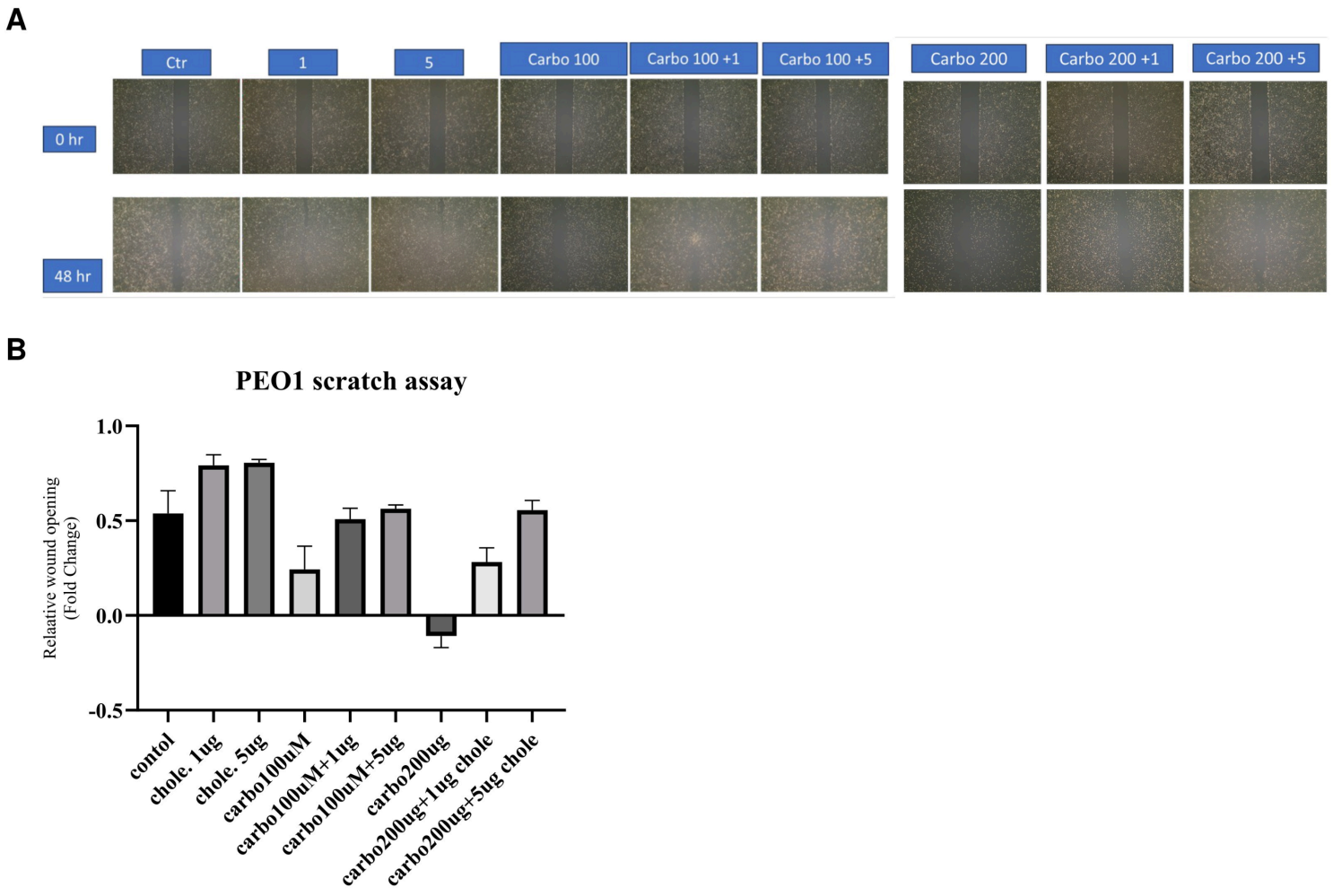


Figure 1

Effect of cholesterol and carboplatin on wound closure in PEO1 cells

(A) Representative phase-contrast images of the wound at 0 and 48 hours in PEO1 cells treated with cholesterol (1 μg/mL or 5 μg/mL), carboplatin (100 μM or 200 μM), or their combinations. (B) Quantification of wound closure at 48 hours using ImageJ, expressed as relative wound opening (fold change); higher values indicate greater wound closure. Data are presented as mean ± standard deviation of three independent biological replicates. Statistical comparisons are provided in Additional file 1: Table S1.

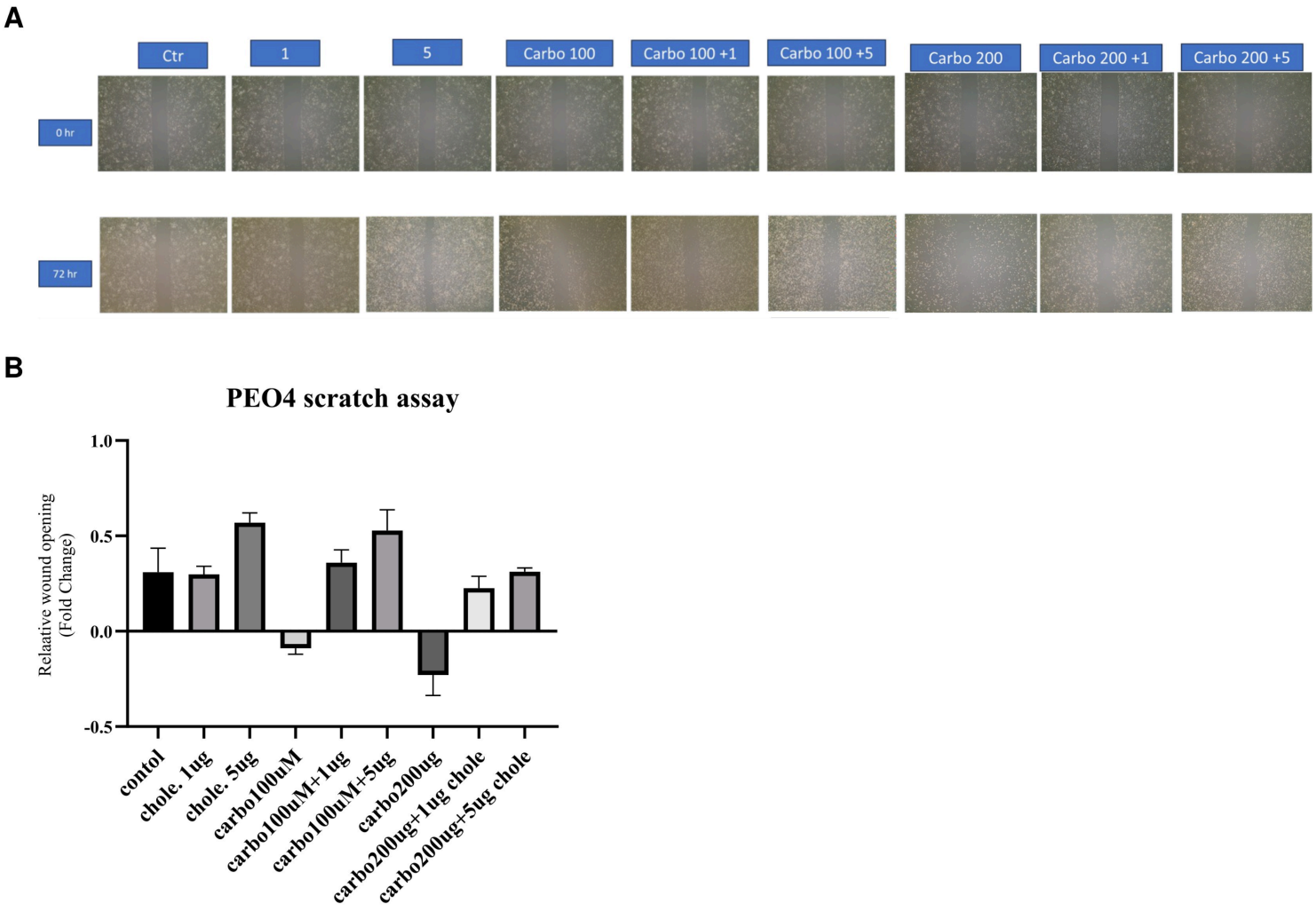


Figure 2

Effect of cholesterol and carboplatin on wound closure in PEO4 cells

(A) Representative phase-contrast images of the wound at 0 and 72 hours in PEO4 cells treated with cholesterol (1 μg/mL or 5 μg/mL), carboplatin (100 μM or 200 μM), or their combinations. (B) Quantification of wound closure at 72 hours using ImageJ, expressed as relative wound opening (fold change); higher values indicate greater wound closure. Data are presented as mean ± standard deviation of three independent biological replicates. Statistical comparisons are provided in Additional file 1: Table S1.

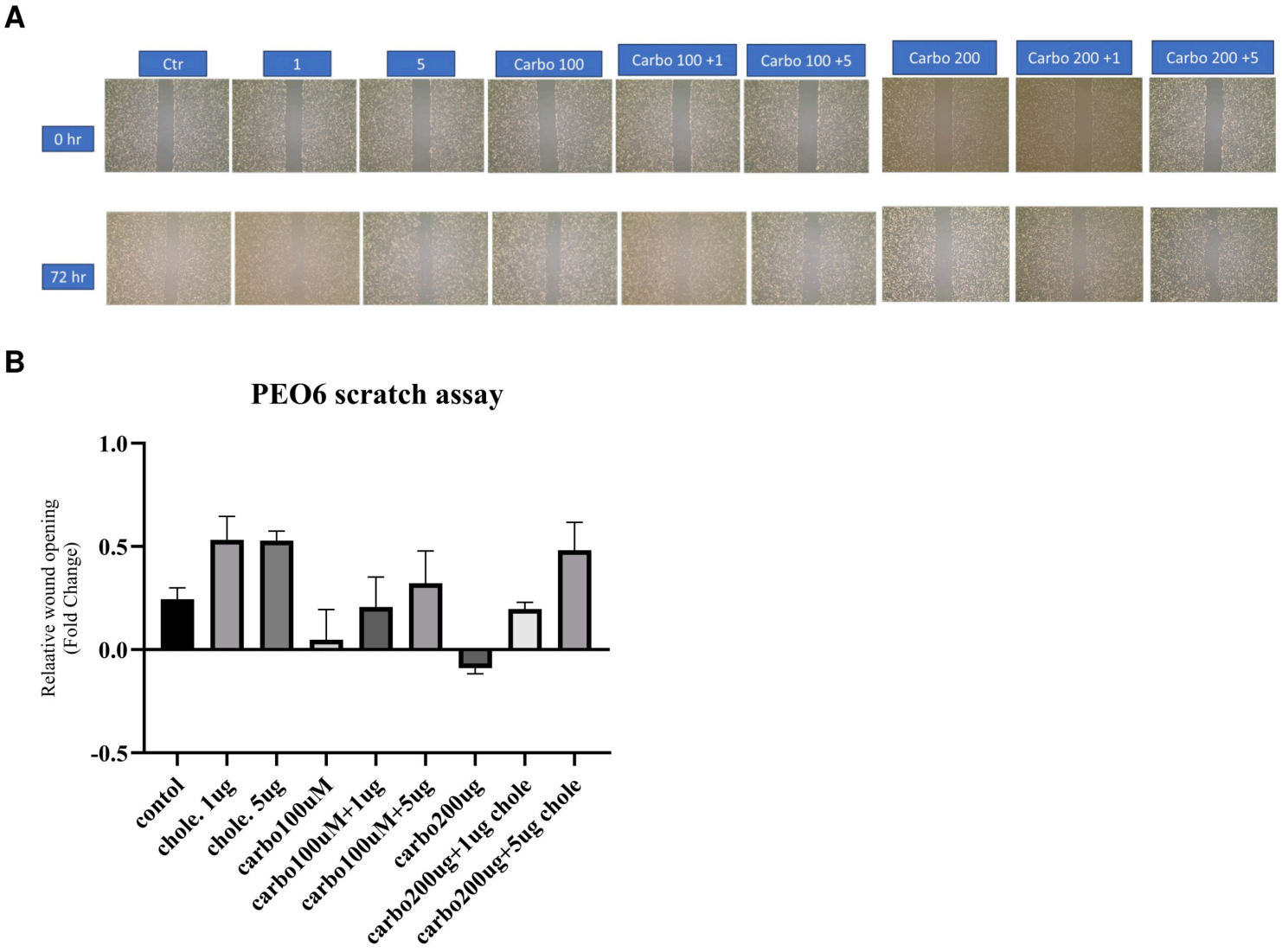


Figure 3

Effect of cholesterol and carboplatin on wound closure in PEO6 cells

(A) Representative phase-contrast images of the wound at 0 and 72 hours in PEO6 cells treated with cholesterol (1 μg/mL or 5 μg/mL), carboplatin (100 μM or 200 μM), or their combinations. (B) Quantification of wound closure at 72 hours using ImageJ, expressed as relative wound opening (fold change); higher values indicate greater wound closure. Data are presented as mean ± standard deviation of three independent biological replicates. Statistical comparisons are provided in Additional file 1: Table S1.

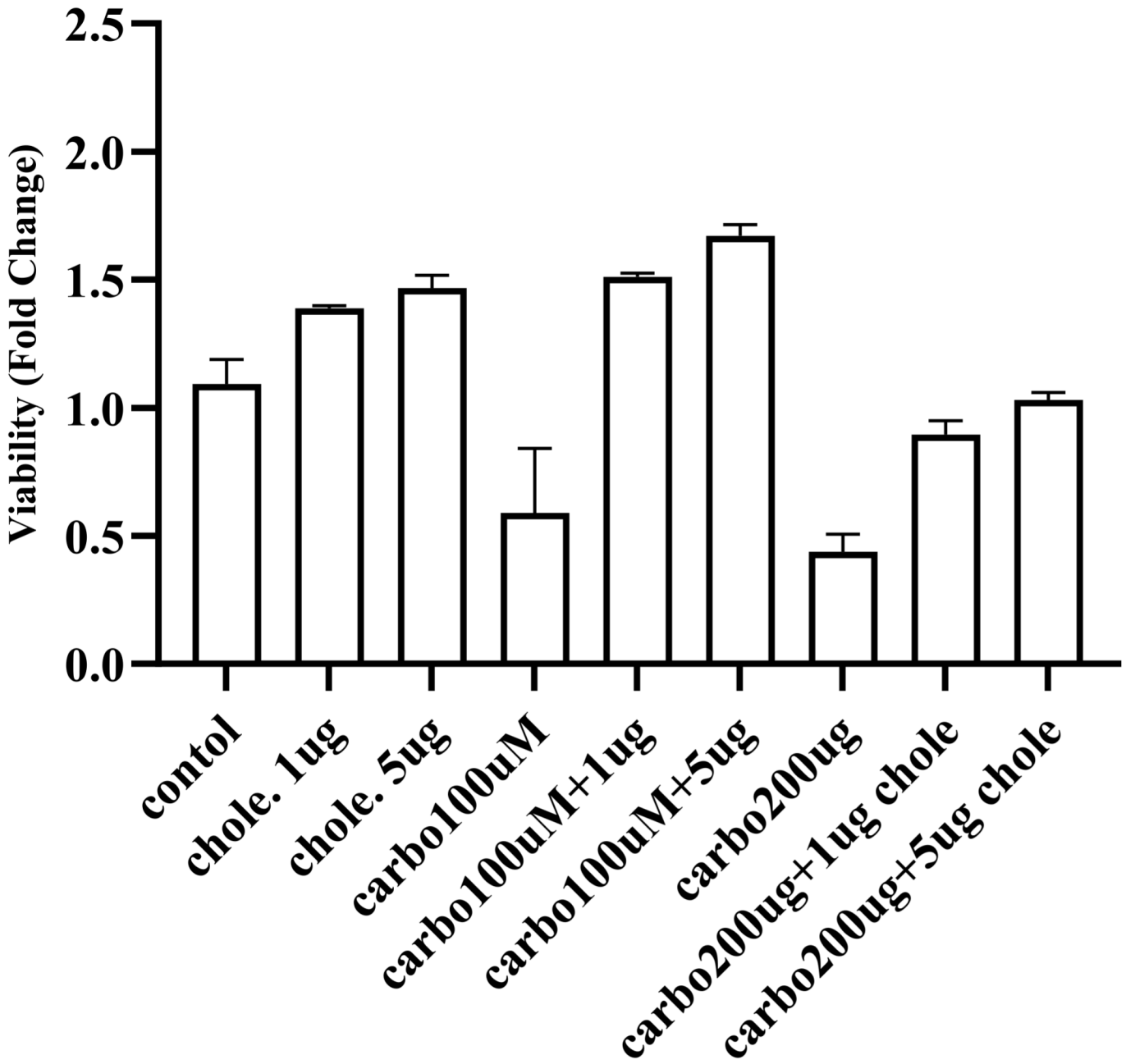


Figure 4

Effect of cholesterol and carboplatin on viability of PEO1 cells

PEO1 cells were treated with cholesterol (1 µg/mL or 5 µg/mL), carboplatin (100 µM or 200 µM), or their combinations. Viability was measured at 48 hours with the CellTiter-Glo luminescent cell viability assay and expressed as fold change relative to untreated control. Data are presented as mean ± standard

deviation of three independent biological replicates. Statistical comparisons are provided in Additional file 1: Table S2.

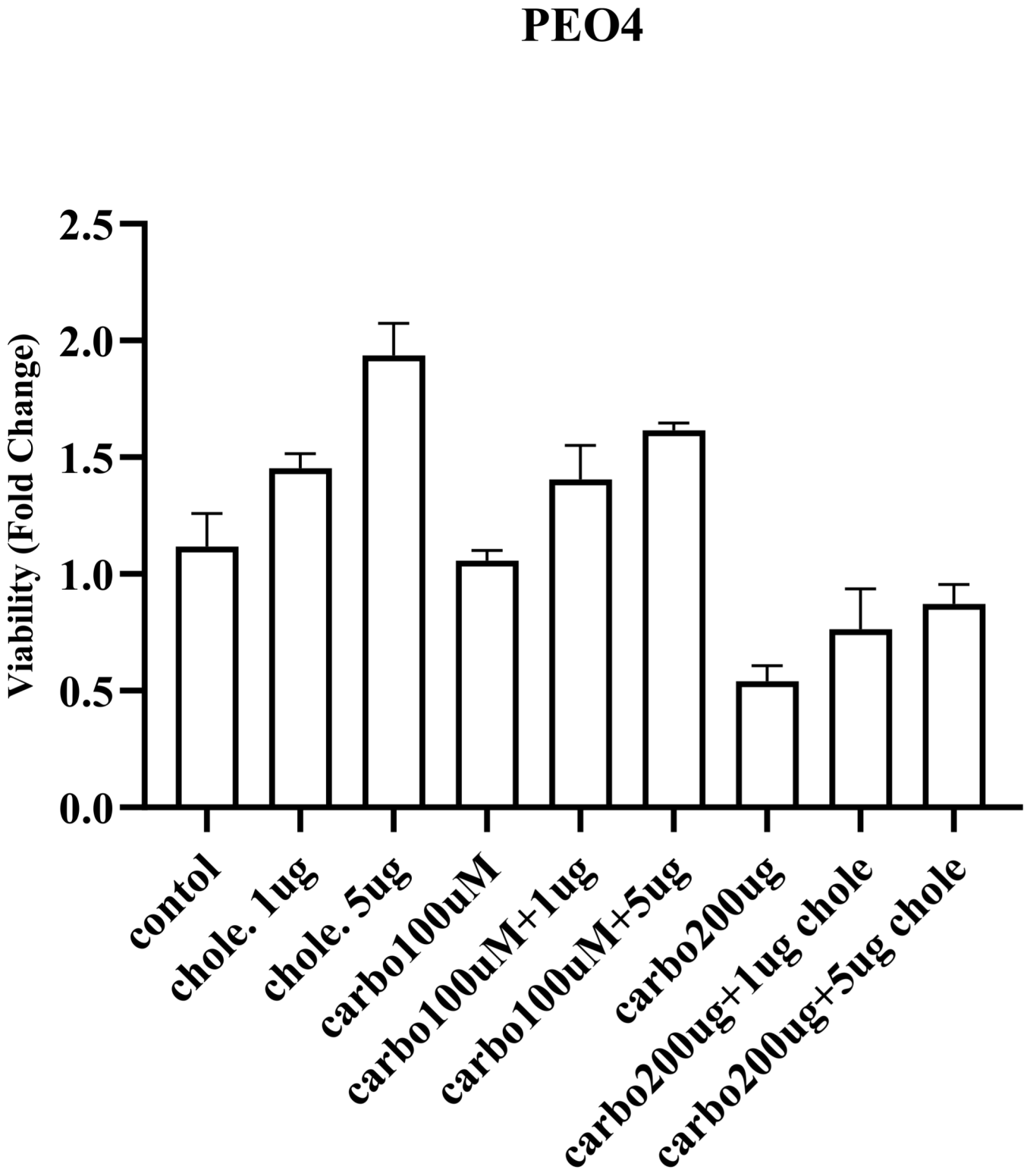


Figure 5

Effect of cholesterol and carboplatin on viability of PEO4 cells

PEO4 cells were treated with cholesterol (1 μg/mL or 5 μg/mL), carboplatin (100 μM or 200 μM), or their combinations. Viability was measured at 72 hours with the CellTiter-Glo luminescent cell viability assay and expressed as fold change relative to untreated control. Data are presented as mean ± standard deviation of three independent biological replicates. Statistical comparisons are provided in Additional file 1: Table S2.

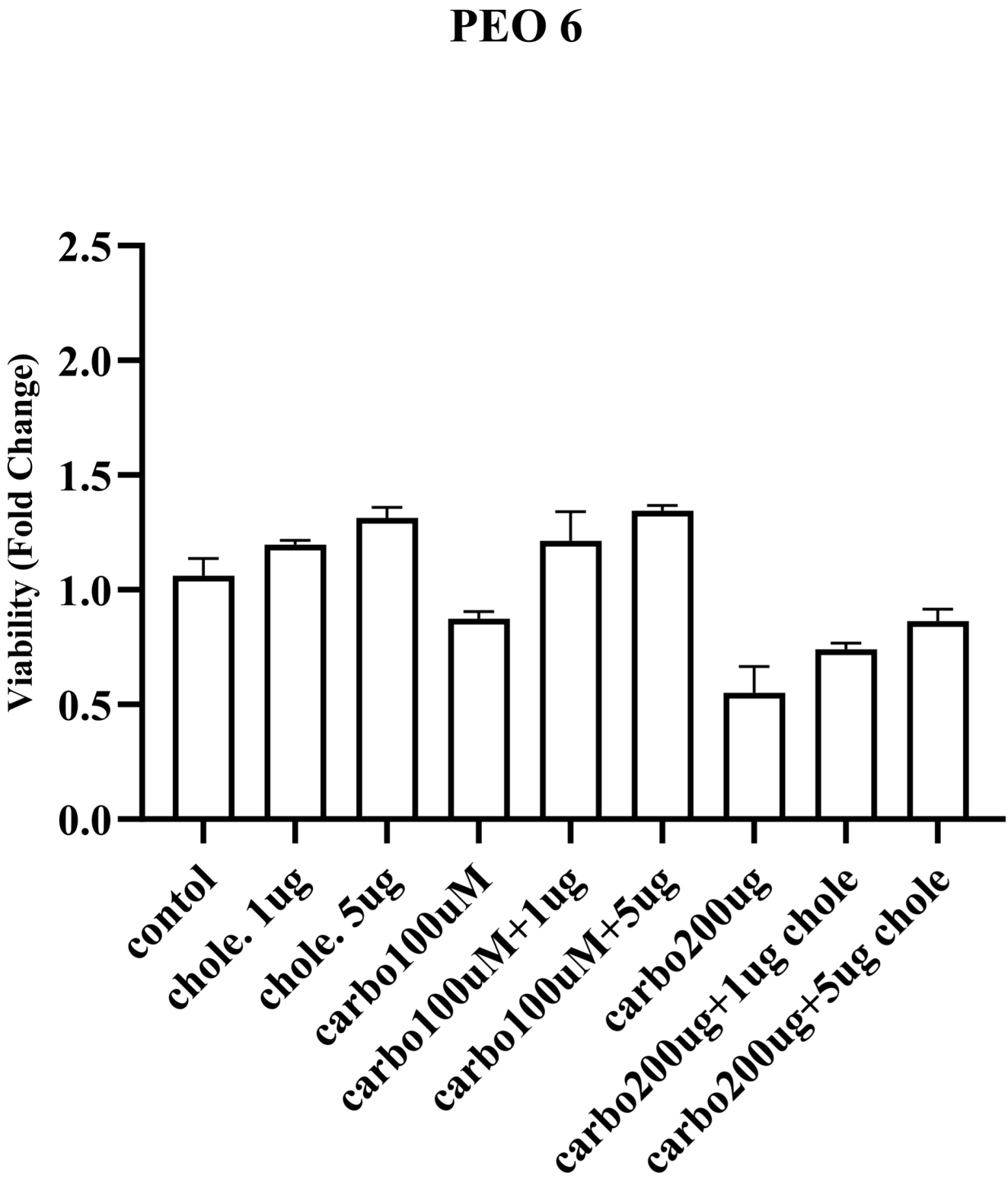


Figure 6

Effect of cholesterol and carboplatin on viability of PEO6 cells

PEO6 cells were treated with cholesterol (1 µg/mL or 5 µg/mL), carboplatin (100 µM or 200 µM), or their combinations. Viability was measured at 72 hours with the CellTiter-Glo luminescent cell viability assay and expressed as fold change relative to untreated control. Data are presented as mean ± standard deviation of three independent biological replicates. Statistical comparisons are provided in Additional file 1: Table S2.

Supplementary Files

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