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# Paclitaxel, Carboplatin and 1,25-D3 Inhibit Proliferation of Ovarian Cancer Cells *In Vitro*

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**Abstract.** *Background/Aim: The combination of paclitaxel and carboplatin is the standard chemotherapy for ovarian cancer. Previous studies have implied that vitamin D (1,25-D3) may have growth inhibitory effects in ovarian cancer. This study aimed to investigate the effect of paclitaxel, carboplatin and 1,25-D3 on the growth of ovarian cancer cells in vitro, based on the hypothesis that 1,25-D3 might potentiate the effect of paclitaxel and/or carboplatin. Materials and Methods: Three non-commercial ovarian carcinoma cell lines UT-OV-1(mucinous), UT-OV-3B (serous) and UT-OV-4 (endometrioid) were exposed to different concentrations of 1,25-D3, paclitaxel and carboplatin, respectively. The cell viability was measured using a Crystal violet assay kit. The cellular vitamin D receptor (VDR) mRNA levels were measured by qRT-PCR using the LightCycler equipment. Results: The growth-inhibitory effect of the combination of paclitaxel and carboplatin was 56% in UT-OV-1, 33% in UT-OV-3B and 47% in UT-OV-4 cells. Single 1,25-D3 (10 μM) inhibited the growth of UT-OV-3B and UT-OV-4 by 23% and 28%, respectively, whereas no effect was seen in UT-OV-1 cells. These results are in line with the finding that the expression of VDR was high in UT-OV-3B and UT-OV-4, but very low in UT-OV-1. The combination of 1,25-D3, paclitaxel and carboplatin resulted in 61%, 46% and 58% growth reduction in UT-OV-1, UT-OV-3B and UT-OV-4 cells, respectively. The additive effect of 1,25-D3 was 21% in UT-OV-4, 20% in UT-OV-3B and 12% in UT-OV-1 cell line. Conclusion: The results imply that combining 1,25-D3 with paclitaxel and carboplatin may potentiate their growth inhibitory effect on ovarian cancer cells with high VDR expression.*



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*Key Words:* Vitamin D, 1,25-D3, ovarian cancer, *in vitro*, paclitaxel, carboplatin, growth inhibition, VDR.

In 2018 there were approximately 300,000 new ovarian cancer cases worldwide. The estimated incidence was 6.6 and mortality 3.9 per 100,000 women per year. (1). Ovarian cancer is the most lethal malignancy of the female reproductive system. Surgery combined with cytotoxic therapy leads to favorable clinical response in up to 80% of patients but the majority of patient relapse (2). The overall survival (OS) has marginally increased in the past decades despite advances in chemotherapeutic agents, targeted therapy, and more radical surgery (3). However, complete resection of all visible disease has been shown to significantly improve outcome and OS (4-6).

Calcitriol (1,25-dihydroxycholecalciferol, 1,25-D3) is the hormonally active form of vitamin D. It is well known as an important regulator of calcium synthesis and bone metabolism (7-9). 1,25-D3 attaches to the vitamin D receptor (VDR) in the cell nucleus and this complex interacts with retinoid X receptor (RXR) resulting in the regulation of the activity of vitamin D-responsive genes. By turning these genes on or off, the complex helps to control calcium and phosphate absorption and growth regulatory processes in the cell (10-11). 1,25-D3 induces $G_0$-$G_1$ cell-cycle arrest and thereby stops cell proliferation, triggers VDR-mediated cell death, inhibits angiogenesis, and promotes differentiation in many cancer cells *in vitro* and *in vivo* (12-17). The presence

of functional VDR receptor in tumors and cancer cell lines might thus represent a target for cancer therapy. Disabled VDR activity leads to 1,25-D3 insensitivity, loss of anti-proliferative effects, increase in oxidative DNA damage and accelerated tumorigenesis (10-11).

Many different cell types, including normal and malignant ovarian cells, contain VDR and express vitamin D metabolizing enzymes 1α-hydroxylase and 24-hydroxylase to synthesize and degrade active 1,25-D3 locally (18-22). VDR-polymorphisms have been indicated to correlate with a higher risk of ovarian cancer (23). This links vitamin D status with cellular anti-tumor actions, and vitamin D status may be a modulator of cancer progression in persons living with cancer (24-25). In 2016 a large Mendelian randomization study suggested that genetically low major circulating form of vitamin D (25(OH)D) plasma levels are associated with a higher incidence of ovarian cancer (26). Moreover, higher concentrations of 25(OH)D seemed to be associated with longer survival rates at the case-control study of 1,600 women diagnosed with epithelial ovarian cancer (27). Also, a meta-analysis of individual cohort studies found a tendency between low circulating 25(OH)D and ovarian cancer incidence although this finding was not statistically significant (28).

In a previous *in vitro* study, we demonstrated that 1,25-D3 combined with paclitaxel and carboplatin, inhibits endometrial cancer cell growth and may even enhance the cytotoxic effect of these agents (29). Ovarian cancer is clinically well known to be sensitive to the combination of paclitaxel and carboplatin (3), which has also been demonstrated *in vitro* (30-32). Previous *in vitro* studies have also implied that 1,25-D3 has a growth inhibitory effect on ovarian cancer cells (22, 33, 34). To our knowledge, however, there are no preclinical studies examining the effect of simultaneous use of paclitaxel, carboplatin and 1,25-D3 in ovarian cancer cells. The aim of the present study was to test the growth inhibitory effect of these both as single agents and in combination on ovarian cancer cell lines *in vitro*.

## Materials and Methods

*Cell lines*. Five non-commercial ovarian cancer cell lines (UT-OV-1, UT-OV-2, UT-OV-3A, UT-OV-3B and UT-OV-4) were used in this study. The cell lines were established at the University of Turku, Turku, Finland (35). The cell lines were originally derived from primary or metastatic stage III-IV epithelial ovarian carcinomas. UT-OV-1 was mucinous, UT-OV-2 and UT-OV-4 were endometrioid and UT-OV-3A and -3B were serous cystadenocarcinomas (35).

*Cell cultures and treatments*. The cells were cultured as described previously (31). The cells were sub-cultured weekly and maintained in a logarithmic phase in 75 $cm^2$ culture flasks in Dulbecco's modified Eagle minimal essential medium (DMEM), containing 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, 1% glutamine and 1% non-essential amino acids at 37°C in a humidified atmosphere supplemented with 5% $CO_2$. All cell lines were tested for mycoplasma contamination.

Paclitaxel (Hospira® 6mg/ml, UK) and carboplatin (Accord® 10 mg/ml, UK) were purchased from the Pharmacy of the Tampere University Hospital. 1,25-D3 was purchased from Sigma-Aldrich (St. Louis, MO, USA). Paclitaxel was initially diluted in 0.9% sodium chloride to give a 0.1 mM concentration. For each experiment final dilutions of 1-10 nM paclitaxel were prepared in DMEM. Carboplatin was diluted in sterile water to get a stock solution of 100 μg/ml and the final concentrations used were 0.1-50 μg/ml. 1,25-D3 was dissolved in 95% ethanol and diluted in DMEM to get final concentrations between 10 nM and 10 μM. All final dilutions were prepared immediately before use. The concentrations of drugs were based on previous reports as well as pharmacological relevance (29,30,32,36).

*VDR expression*. The VDR mRNA levels in UT-OV-1, UT-OV-2, UT-OV-3A, UT-OV-3B and UT-OV-4 cells were measured by quantitative reverse transcription polymerase chain reaction (qRT-PCR) using the LightCycler equipment (Roche, Mannheim, Germany). Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) and was reverse transcribed with SuperScript™ First-Strand Synthesis System for RT–PCR (Invitrogen, Carlsbad, CA, USA), as described elsewhere (29). qRT-PCR was performed using 20 μM gene specific primers (Sigma-Aldrich, sense 5' ATCGGCATGATGAAGGAGTT 3', antisense 5' TGCTCCTCAGACAGCTTGG 3') and 10 μM UPL probe (probe number #12). The PCR program included the following steps: 10 min denaturation at 95˚C followed by 45 cycles of 10 s denaturation at 95˚C, 30 s annealing at 60˚C and 1 s elongation at 72˚C. The expression levels were normalized using glyceraldehyde-3-phosphate dehydrogenase (GAPD) housekeeping gene.

*Cell growth assays*. The cells were plated on 96-well plates at 40,000 cells/well (UT-OV-1), at 70,000 cells/well (UT-OV-3B) and at 60,000 cells/well (UT-OV-4) for each experiment, respectively. The number of cells plated per well was adjusted according to the plating efficiency of each cell line. Cells were allowed to adhere overnight and then exposed to indicated concentrations of paclitaxel, carboplatin, and 1,25-D3 alone or in combination for three days. The cell viability was measured using the Crystal violet assay kit (Abcam, Germany). The O.D. of the crystal violet staining was measured at 590 nm and is directly proportional to the cell biomass. All measurements were performed in six replicates and the individual experiments were repeated three times.

Statistical analyses were carried out using the IBM SPSS Statistics for Windows, Version 22.0. Armonk, IBM Corp. Released 2013, NY: IBM Corp. Independent sample *t*-test were used to compare differences between treatments to investigate the effect of paclitaxel, carboplatin and 1.25-D3.

## Results

Before evaluating the effect of the chemotherapeutic drugs and 1,25-D3, five ovarian carcinoma cell lines were tested for the expression of VDR. VDR-expression was highest in the UT-OV-3B and UT-OV-4 cell lines (Figure 1). UT-OV-1 also expressed VDR to some extent. The expression of VDR in the UT-OV-2 and UT-OV-3A cell lines was very low (Figure 1). Hence, the subsequent experiments were performed using only three cell lines: UT-OV-1, OT-OV-3B and UT-OV-4.

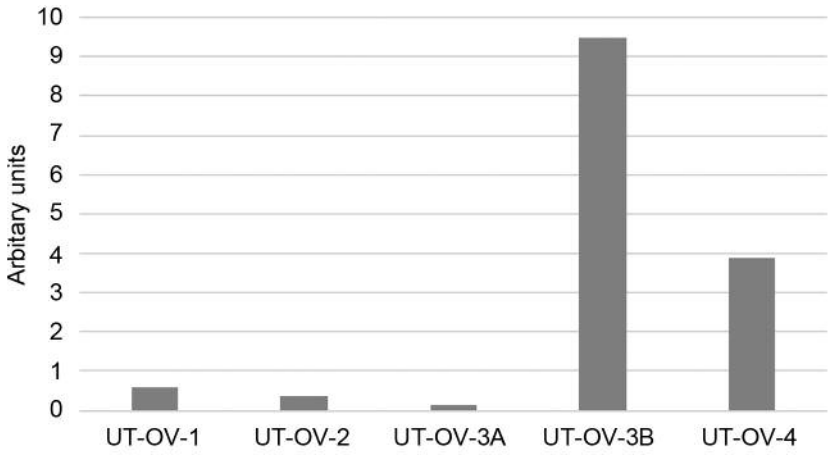


Figure 1. *Vitamin D receptor (VDR) expression in UT-OV-1, UT-OV-2, UT-OV-3A, UT-OV-3B and UT-OV-4 ovarian cancer cell lines.*

The ovarian carcinoma cell lines were first exposed to different concentrations of 1,25-D3 (10 nM, 50 nM, 100 nM, 1 μM and 10 μM) to determine the estimated drug concentration which causes 25% growth inhibition ($IC_{25}$) in cancer cell lines expressing VDR. All three cell lines responded poorly to the concentrations of 10 to 100 nM of 1,25-D3. In UT-OV-1 cells, the growth inhibition was even negligible with the higher doses of 1,25-D3: 3% with 1 μM and 4% with 10 μM of 1,25-D3, respectively, when compared to untreated controls. (Figure 2). In UT-OV-3B cells, the corresponding growth inhibitory percentages were 13% and 26%, and in UT-OV-4 cells 12% and 32%, respectively (Figure 2). Based on these results, the dose of 10 μM of 1,25-D3 was chosen for the next experiments where the effects of the combinations of the cytotoxic agents and 1,25-D3 were evaluated.

Similarly, the cells were cultured with different concentrations of paclitaxel (1 nM, 5 nM and 10 nM) and carboplatin (5 μg/ml, 10 μg/ml and 50 μg/ml) to get the estimated drug concentration causing approximately 25% inhibition of cell growth ($IC_{25}$). In UT-OV-1 cell line, the $IC_{25}$ concentration of carboplatin was 10 μg/ml and for paclitaxel 5 nM (Figure 3). In UT-OV-3B and UT-OV-4, the corresponding $IC_{25}$ values for carboplatin were 50 μg/ml and 10 μg/ml and for paclitaxel 5 nM and 1 nM, respectively (Figure 3).

Subsequently, each cell line was treated with the chosen concentrations of paclitaxel, carboplatin and 1,25-D3 as single agents as well as with the different combinations of the drugs to assess if there are any synergistic or additive effects between the drugs (Table I, Figure 4).

The growth inhibition in UT-OV-1 cell line was 48% with paclitaxel alone (5nM), 29% with carboplatin alone (10 μg/ml) and 56% with the combination of the two. The cytotoxic effect of the combination of the two drugs was more effective than single paclitaxel or single carboplatin (both $p<0.001$). Single 10 μM 1,25-D3 inhibited the cell growth by 3%. The growth inhibitory effect of the combinations of paclitaxel and 1,25-D3 and of carboplatin and 1,25-D3 were 50% and 36% ($p=0.172$ and $p=0.028$), respectively. Thus, the combination of the single agents and 1,25-D3 brought a minor significant effect with carboplatin and 1,25-D3. The most effective was the combination of these three drugs with 61% growth inhibition which achieved statistical significance when compared to the control, to the combination of paclitaxel and carboplatin and to the single paclitaxel ($p<0.001$). An additive effect of 1,25-D3 with the combination of paclitaxel and carboplatin was demonstrated.

In the UT-OV-3B cell line, the cytotoxic effect of single 5 nM paclitaxel was 44% and that of single 50 μg/ml carboplatin 29%. The cytotoxicity of the combination of the two drugs was 33%. Surprisingly, single paclitaxel was thus more effective than the combination with carboplatin 44% *vs*. 33% ($p<0.001$). Single 10 μM 1,25-D3 inhibited the cell growth by 23%. Single paclitaxel was more efficient than the combination of paclitaxel and 1,25-D3, 44% *vs*. 39%. Thus, no additive or synergistic effect was achieved over single paclitaxel. The growth inhibition seen with the combination of carboplatin and 1,25-D3 was 35% (compared to single 1,25D-3 $p<0.001$). The additive effect with this drug combination was shown and was statistically significant. The cytotoxic effect of the combination of the three drugs (paclitaxel, carboplatin and 1,25-D3) was 46%. Only a minor additive effect was achieved with this combination over single paclitaxel (44%) and it was statistically significant ($p=0.021$).

In the UT-OV-4 cell line, single 1 nM paclitaxel was more efficient than single 10 μg/ml carboplatin but the combination of two drugs was the most efficient. The growth

inhibition was 36% with paclitaxel and 47% with the combination of paclitaxel and carboplatin ($p<0.001$). The corresponding percentage for single carboplatin was 30% and the difference compared to the combination of these two was significant ($p<0.001$). Single 1,25-D3 (10 μM) inhibited the cell growth by 28%. A dose of 10 μM of 1,25-D3 combined with carboplatin 10 μg/ml or with 1 nM paclitaxel was equal with the combination of carboplatin and paclitaxel (cytotoxicity of 47%, 46% and 47%) (all $p<0.001$ when compared to the control). The suppression of the cell growth in comparison to the control was 58% with the combination of the three drugs: paclitaxel, carboplatin and 1,25-D3. The additive effect of 1,25-D3 was 21% when comparing the combination of three drugs to the combination of two drugs: paclitaxel and carboplatin. This difference was statistically significant ($p=0.001$).

## Discussion

Our preliminary results showed that 1,25-D3 inhibits ovarian cancer cell growth *in vitro* as a single agent, but also in combination with paclitaxel and carboplatin. The most efficient combination was 1,25-D3, paclitaxel and carboplatin together in all three cell lines. The anti-tumor effect was 61% in UT-OV-1, 46% in UT-OV-3B and 58% in UT-OV-4 cell line. The corresponding numbers for the combination of paclitaxel and carboplatin were 56%, 33% and 47%, respectively.

The additive effect of 1,25-D3 was 21% in UT-OV-4, 20% in UT-OV-3B and 12% in UT-OV-1 cell line. Furthermore, the combination of carboplatin or paclitaxel with 1,25-D3 was equal with the combination of paclitaxel and carboplatin in UT-OV-3B and UT-OV-4 cell lines. In the UT-OV-1 cell line the combination of paclitaxel and carboplatin was the most efficient when compared to the other two-drug combinations. Surprisingly, in the UT-OV-3B cell line (serous cystadenocarcinoma) single-agent paclitaxel was more effective than the combination of paclitaxel and carboplatin and almost as effective as the combination of the three drugs (44% *vs*. 46%). These data are, however, in line with our previous study examining endometrial cancer cell lines (29). Adding carboplatin did not provide any additive or synergistic effect. This is strange because serous cystadenocarcinoma is generally sensitive for carboplatin. UT-OV-3B cell line was stage III or IV but it might have been low-grade serous carcinoma which is more often platinum resistant. It is also possible that the concentrations we used were suboptimal. Fanning *et al*. (37) compared cytotoxicity concentrations of cisplatin and carboplatin and in their study the $IC_{50}$ value for carboplatin was as high as 490 μg/ml with exposure periods 1, 4, 24 and 48 h. They used the commercial OVCAR-3 cell line. For a specific patient after the dose of 350 mg/m$^2$ carboplatin, the peak plasma level was 123 μM (45 μg/ml) (38) which is almost the same concentration we used in the UT-OV-3B cell line. As this is a small *in vitro* study our results need to be confirmed by aid of *in vivo* epithelial ovarian cancer (EOC) models. However, we used non-commercial well-established cell lines, which should closer resemble the original tumors than the commercial ones, and at least additive anti-tumor effect was seen in all but one of the cell lines.

The UT-OV-1 cell line was mucinous cystadenocarcinoma and UT-OV-4 endometrioid cystadenocarcinoma. Both cell lines have been earlier shown to be sensitive to paclitaxel,

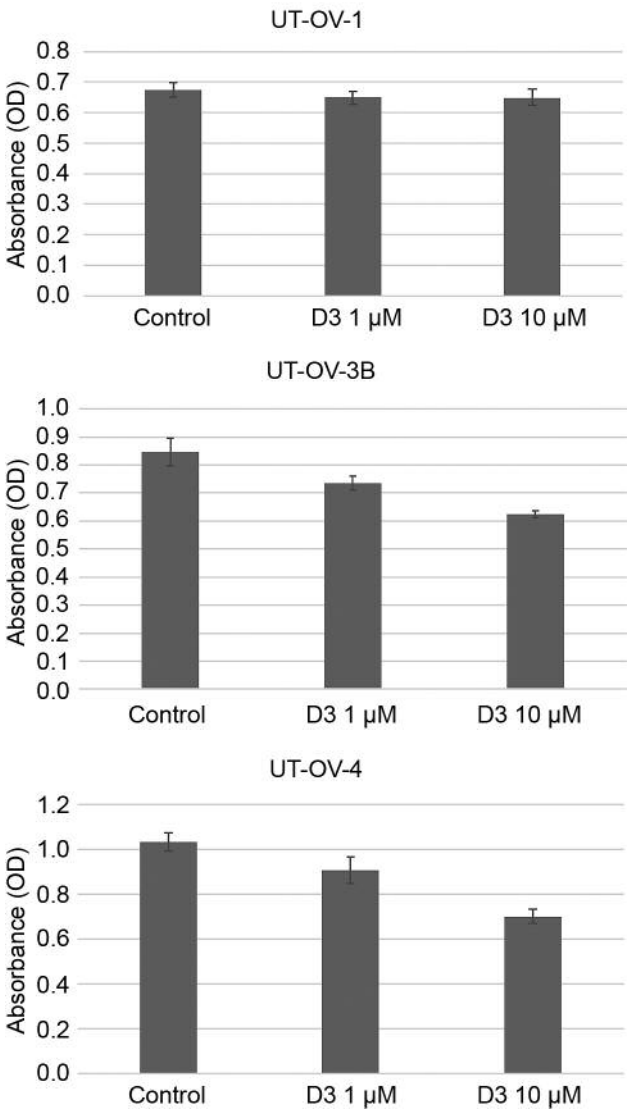


Figure 2. *Effect of cytotoxic agents on UT-OV-1, UT-OV-3B and UT-OV-4 ovarian cancer cell growth. The cells were treated for three days with indicated drugs and optical density (OD) was measured at 590 nM. OD is directly proportional to cell biomass. Mean and SD of six replicates are shown. P: Paclitaxel, Ca: carboplatin.*

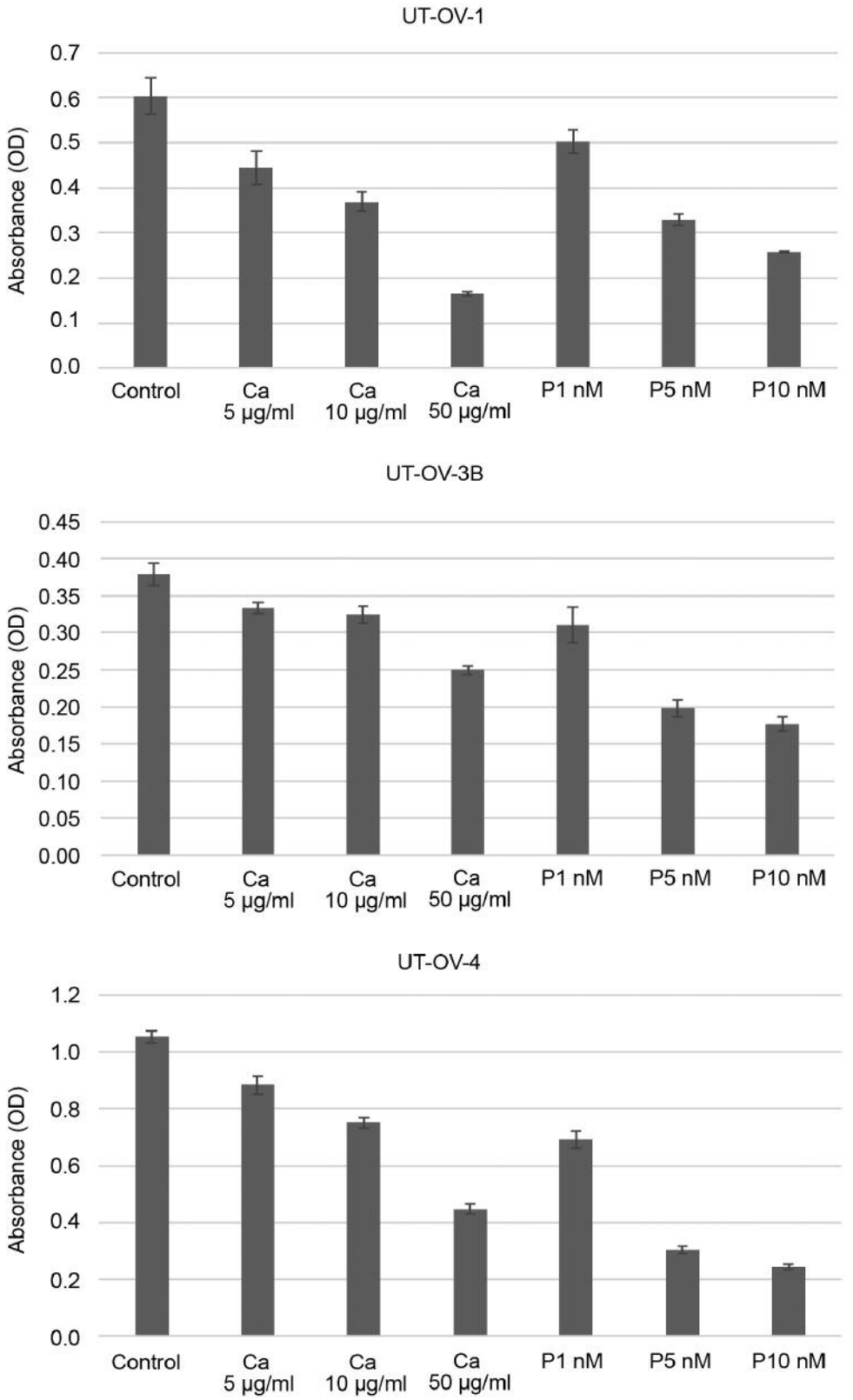


Figure 3. *Effect of various concentrations of 1,25-D3 on UT-OV-1, UT-OV-3B and UT-OV-4 ovarian cancer cell growth. The cells were treated for three days with indicated concentrations of 1,25-D3 and optical density (OD) was measured at 590 nM. OD is directly proportional to the cell biomass. Mean and SD of six replicates is shown. D3: 1,25-D3.*

Table I. *The percentage of cytotoxicity of single agents and of different combination of drugs in three different ovarian carcinoma cell lines (UT-OV-1, UT-OV-3B, UT-OV-4). P: Paclitaxel; Ca: carboplatin; D3: 1,25-D3.*

| Cytotoxicity | | UT-OV-4 | UT-OV-3B | UT-OV-1 |
|---|---|---|---|---|
| D3 | 10 μM | 28% | 23% | 3% |
| P | 1/5/5 nM | 36% | 44% | 48% |
| Ca | 10/50/10 μg/ml | 30% | 29% | 29% |
| P + Ca | | | 47% | 33% |
| 56% | | | | |
| P + D3 | | 46% | 39% | 50% |
| Ca + D3 | | 47% | 35% | 36% |
| P + Ca + D3 | | 58% | 46% | 61% |

carboplatin and cisplatin (31). Although this finding is in line with our results, the sensitivity of the UT-OV-1 is quite surprising, as mucinous ovarian cancer is generally considered resistant to platinum-based chemotherapy (39-40). Moreover, mucinous ovarian cancer cell lines have been found to have intrinsic platinum resistance (41). Because of our contrasting result, we confirmed the mucinous character of the UT-OV-1 by immunohistochemistry. The tumor cells were positive for CDX2, CEA, CK7 and p53, which are features of ovarian mucinous adenocarcinoma, intestinal type (42, 43). This discrepancy might be due to the availability of better diagnostic tools which has led to improved histopathological diagnosis of different types of mucinous ovarian cancer. Mucinous ovarian cancer has also several common pathological and molecular features with gastrointestinal tumors and it has been recently reviewed that altered mucin expression indicates chemoresistance in breast and colon cancer (44), but no data regarding the effect of altered mucin expression has to our knowledge been reported in ovarian cancer.

However, there is both experimental and clinical evidence that the platinum resistance of mucinous ovarian cancer is not universal. Ricci *et al*. (45) reported the molecular, metabolic and pharmacological characterization of two patient derived xenografts (PDXs) #164 and #182 from mucinous ovarian carcinomas. PDX#164 was derived from a Stage IV tumor and PDX#182 from a Stage I tumor. PDX#164 was found to be moderately sensitive to both platinum and paclitaxel while PDX#182 was not.

In retrospective clinical series response rates have varied between 13-60% to first-line carboplatin and paclitaxel chemotherapy among women with mucinous ovarian cancer (46). The biological actions of 1,25-D3 are mediated by VDR, mostly *via* genomic pathways. The expression of VDR by cancer cells is required for the anti-proliferative effects of 1,25-D3 *in vitro* (10). However, there is evidence that VDR expression or function may become aberrant during cancer development because of altered target gene regulation, overexpression of 1,25-D3 24-hydroxylase (catabolizing enzyme of 1,25-D3) or deregulation of pathways downstream of VDR (such as apoptosis) (25). Peng *et al*. (47) also reported that the antiproliferative effects of 1,25-D3 and its analogs and intact VDR-signaling machinery are dependent on a positive estrogen receptor status in breast cancer cell lines. On the other hand, it seems that in ovarian cancer there is no correlation with positive estrogen receptor status and 1,25-D3 actions (19). In our study three of five ovarian carcinoma cell lines were VDR-positive. Of note is that UT-OV-3B and UT-OV-4 strongly expressed VDR, while UT-OV-1, UT-OV-2 and UT-OV-3A did not. The explanation for the different actions of 1,25-D3 might be that VDR activity was disabled as described earlier.

Earlier studies support the present findings and the role of 1,25-D3 in cancer therapy. 1,25-D3 induces GADD45-gene and causes cell-cycle arrest at the G2/M transition in ovarian cancer cells (48). Furthermore, in a variety of studies a synthetic vitamin D analog (EB1809) has shown antiproliferative efficacy in ovarian tumors and tumor cells through induction of cell death, cell-cycle arrest, differentiation and inhibition of angiogenesis (15, 17, 22, 49). Lungchukiet *et al*. (50) demonstrated that 1,25-D3 suppresses epithelial ovarian cancer invasion into omentum *in vitro* and in an animal model through VDR-expression. In addition, 1,25-D3 has been found to be a potent inhibitor of ovarian cancer cell growth *in vitro* and increase the antiproliferative ability of carboplatin by altering the cell cycle and enhancing apoptosis (33). Liu *et al*. (51) found that 1,25-D3 delays the progression of ovarian cancer by increasing the expression of VDR and E-cadherin and decreasing β-catenin *in vitro* and *in vivo* in mouse models. This is in concordance with our results and provides evidence on the use of 1,25-D3 as a potential therapeutic agent for ovarian cancer.

The authors are not aware of any studies comparing preclinically the effect of single-agents and the combination of 1,25-D3 and paclitaxel and carboplatin in ovarian cancer. The cytotoxic ability of single agents of 1,25-D3, paclitaxel and carboplatin has been evaluated in a wide panel of tumor types *in vitro* and *in vivo*, also in ovarian carcinoma (30-32, 34, 49, 52). Rodriquez *et al*. (53) examined the progestin/vitamin D combination in ovarian cancer cells and whether progestins inhibit the CYP24A1 enzyme. They demonstrated that theire combination synergistically reduced cell viability and induced apoptosis. Moreover, progestins inhibited CYP24A1, thus extending 1,25-D3 activity. Ly *et al*. (54) showed similar synergistic result with liarozole (an inhibitor of retinoic acid and aromatase) and 1,25-D3 in the aggressive prostate cancer cell line DU145. They suggested that this might be due to inhibition of 24-hydroxylase

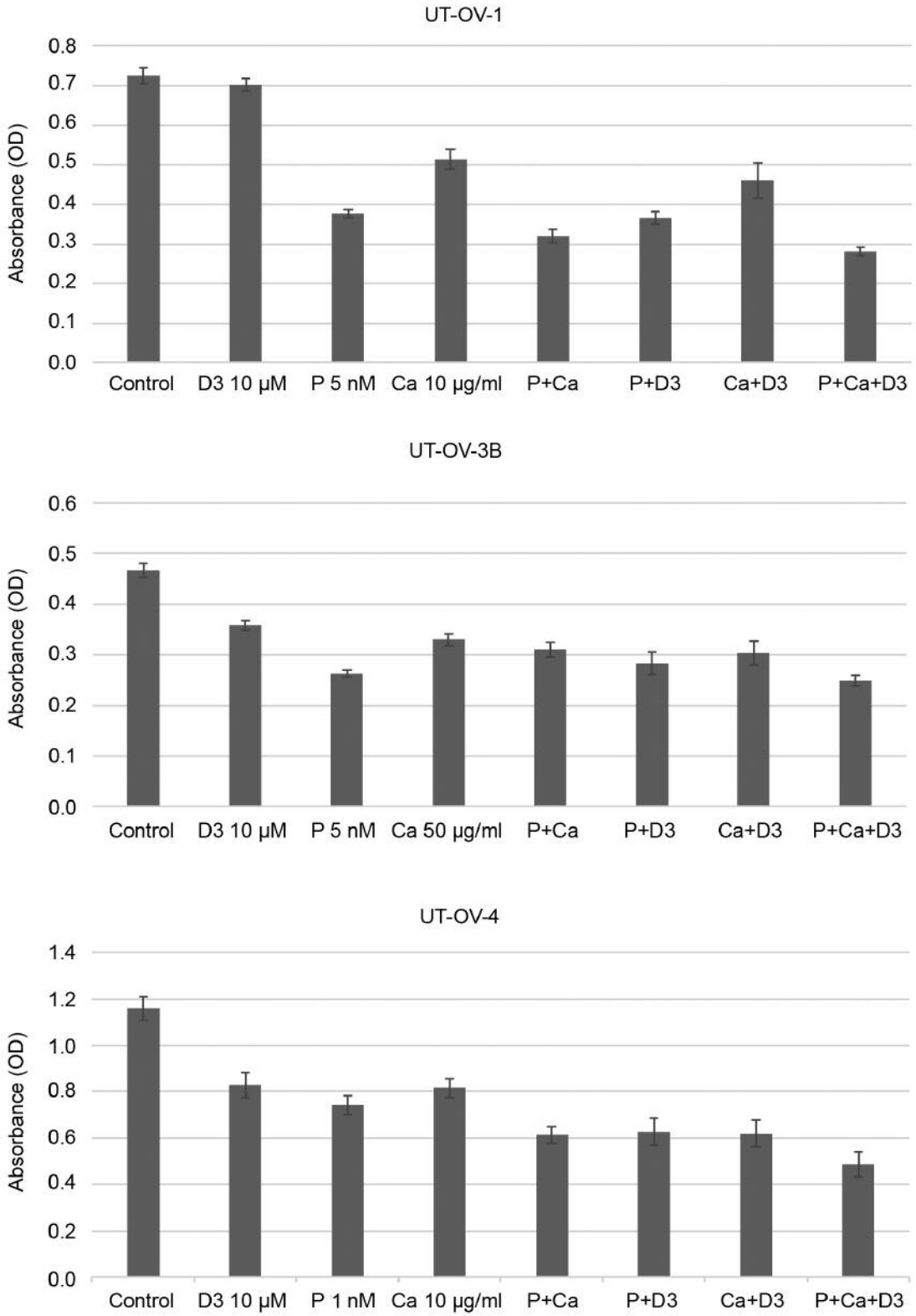


Figure 4. *Cell growth inhibition in UT-OV-1, UT-OV-3B and UT-OV-4 ovarian cancer cells with different combinations of cytotoxic agents and 1,25-D3. The cells were treated for three days with indicated drugs and optical density (OD) was measured at 590 nM. OD is directly proportional to the cell biomass. Mean and SD of six replicates are shown. P: Paclitaxel, Ca: carboplatin, D3: 1,25-D3.*

activity, leading to increased 1,25-D3 half-life and VDR up-regulation. Previous data suggest that the addition of 1,25-D3 to multiple chemotherapy regimens increases the activity of treatments and potentially leads to a better response rate to the regimens.

In conclusion, we demonstrated using three ovarian carcinoma cell lines that 1,25-D3 has growth inhibitory effect on VDR-expressing cell lines both alone and combined with paclitaxel and carboplatin. Further *in vitro* and *in vivo* studies are warranted for evaluation of the anticancer efficacy and the role of 1,25-D3 in relation to other ovarian cancer treatment modalities in the tumor microenvironment and in developed EOC models.

## Conflicts of Interest

The Authors declare no conflicts of interest.

## Authors' Contributions

Dr. Tea Kuittinen wrote the first draft of the manuscript, formed the figures and assisted the laboratory personnel at Prof. Kallioniemi's laboratory in the cell culture experiments. Dr. Päivi Rovio presented the original idea and reviewed the manuscript. Ms. Tiina Luukkaala performed the statistical analysis. Dr. Marita Laurila performed immunostainings. Prof. Seija Grenman provided the cell lines, established originally in her laboratory. Prof. Johanna Mäenpää, the senior author, and Prof. Anne Kallioniemi supervised the study, and revised the manuscript.

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