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The Anti-Tumor Effects of Carboplatin, Progesterone, and Calcitriol in Endometrial Cancer Cells

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** Bu çalışma, ilk yazarın uzmanlık tezinden türetilmiştir.*

ABSTRACT
Aim: Platinum analogs and progesterone are used in the treatment of endometrial cancer. With calcitriol having anti-carcinogenic effects on many cancers, its effects on endometrial cancer are not clearly known. Therefore, we planned our study to compare the anti-tumor effects of carboplatin, progesterone, and calcitriol in endometrial cancer cell culture.
Material and Methods: Ishikawa cells were used in our study as endometrial cancer cell culture. A control group in which no medication was used, was compared with the study groups that received carboplatin, progesterone, and calcitriol. Anti-tumor effects of carboplatin, progesterone, and calcitriol were calculated by using the apoptosis and viability rates for each agent and its combinations (carboplatin + progesterone, carboplatin + calcitriol).
Results: The viability rates of all three agents were found significantly lower when they were compared alone or in combination with the control group ($p < 0.001$). When cell viability with carboplatin + progesterone combination was compared with carboplatin alone, no significant difference was observed ($p > 0.05$). However, when the cell viability levels of the carboplatin + calcitriol combination were compared with the carboplatin used alone, it was observed that calcitriol triggered the anti-tumor effects of carboplatin and increased cell death ($p = 0.001$).
Conclusion: It was observed that carboplatin, progesterone, and calcitriol dose and time-dependently increased their inhibitory effects on tumor cells. Besides, when calcitriol was used combined with carboplatin, it enhanced its lethal effects on cells. The use of carboplatin and calcitriol combined in endometrial cancer may be an option in the treatment of this cancer type with high mortality. Further studies are needed to support this opinion.
Key words: Endometrial cancer, carboplatin, progesterone, calcitriol.

INTRODUCTION

Endometrial cancer (EC) is known as the most common malignancy of the female genital system in developing countries (1). Nulliparity, menopause in older ages, obesity, polycystic ovary syndrome (PCOS), estrogen secreting tumors, estrogen treatment without combining with progesterone during the menopausal period, tamoxifen use, diabetes, and Hereditary Nonpolyposis Colon Cancer (HNPCC) are known risk factors for endometrial cancer.

Breastfeeding, oral contraceptives use, physical activity and smoking are among the protective factors against EC. The main complaint in 90% of patients with endometrial cancer is abnormal uterine bleeding and bleeding which is most commonly seen in the postmenopausal period (2; 3) . It has been reported that the leading causes of death because of endometrial cancer are delay in diagnosis as well as inadequate prediction of the prognosis of the disease and low response to chemotherapy and hormone therapy in advanced stages (4). Platinum analogs and progesterone are used in the treatment of endometrial cancer (5; 6; 7). Although calcitriol has anti-carcinogenic effects on many cancers, its effects on endometrial cancer are not clearly defined.

Carboplatin is a second-generation antineoplastic alkylating agent that contains platinum and is used in the treatment of several tumors (EC, lung cancer, ovarian cancer) (5; 8). Carboplatin binds to the DNA in chromatin in tumor cells with its platinum content and causes the restructuring of the nucleosome structure of the DNA. Although initially, platinum seems to be adapted to the nucleosome structure, this condition has been demonstrated to prevent tumor cells from dividing, growing, and multiplying by causing DNA damage. Carboplatin slows the development and spread of tumor cells in the body by this mechanism, (5; 8).

Progesterone affects both epithelial and stromal compartments in the endometrium. Studies in mice revealed that the progesterone receptor (PR) PR-A modulated the antiproliferative effects of progesterone in the uterus and PR-B, and it was found that cell growth was stimulated in the absence of PR-A. Medroxyprogesterone acetate (MPA) and megestrol acetate are recommended for patients as conservative treatment options who are not candidates for surgery (7).

Some favorable evidence has been found in studies on endometrial pathologies such as endometriosis and EC, related to the antiproliferative and anti-carcinogenic effects of calcitriol [1,25-dihydroxyvitamin D3 (1,25OH) 2D3]. In 1996, Yabushita et al. demonstrated that in the RL-95-2 EC cell line, growth in cells showing VDR(vitamin d reveptor) expression could be inhibited in a dose-dependent manner following VD treatment (cells were treated with 50 nM calcitriol for 6 days and cell growth was inhibited 44%) (5). Besides, in cases with cancer, high amounts of VD in the circulation have been reported to reduce the risk of developing certain types of cancer (breast, colorectal, gastric, hematologic, head, and neck, kidney, lung, ovary, pancreas, liver, prostate and skin). Nevertheless, the relationship between

EC and VD is still controversial. Therefore, a study that evaluates the molecular basis of the local effect of VD in EC would be very valuable (9). Chemotherapy alone or combination with radiotherapy is mostly used as an adjuvant treatment in patients with advanced stage EC. Besides, they can be used as a primary treatment method in surgically unfavorable cases. There are studies supporting the success of combined treatment in reducing the risk of death and recurrence in high-risk EC (10). However, current treatment options may be inadequate for endometrial cancer.

To the best of the authors' knowledge, any studies in the literature showing the efficacy of carboplatin, progesterone, and calcitriol combinations on endometrial cancer cells do not exist. In the current study, we aimed to investigate the antitumor effects of carboplatin used as chemotherapeutic, progesterone which exists in hormonotherapy, and calcitriol (1,25-dihydroxy cholecalciferol), effects of which has only been shown in experimental studies yet, in endometrial tissue.

MATERIAL AND METHODS

The study was carried out in the University Science and Technology Center (BİLTEM) laboratory of our institution after ethical approval was obtained by the local ethics committee Endometrial cancer Ishikawa cell cultures were used in the study. Progesterone (Sigma®P-8783), calcitriol (Calderol®), carboplatin (150 mg / 15 ml), Cell-APO PercentageApoptosisAssay kit (Biocolor Life Science) were used as study kits. For each agent and its combinations, 10,000 Ishikawa cells per well in a 96-well plate were evaluated after 24-48-72 hours using a light microscope and an automatic cell counting device. The study was repeated on 3 different days with 8 samples with the same concentration of substances.

Determination of Cell Viability

Tetrazolium MTT (3- (4,5-dimethyl thiazolyl-2) -2,5-diphenyltetrazolium bromide) forms the purple-colored formazan by being reduced by dehydrogenase enzyme activity in metabolically active cells. The absorbance of this compound was measured and analyzed with a spectrophotometer. In a 96-well plate, 10,000 Ishikawa cells per well were inoculated in 100 µl of Growth Medium Minimum Essential Medium Eagle Medium (MEM). Cells were kept in an incubator containing 5% CO2 at 37 °C for 24 hours. At the end of 24 hours, the medium was aspirated and 100 µl of serum-free materials prepared in the same medium were added to the wells. The medium in the wells was aspirated 24, 48, and 72 hours later, and the MTT

solution (prepared as 0.5 mg/ml in serum-free medium) was added to the wells at 100 µl. The formation of formazan becomes visible at the bottom of the wells after the plates are kept in the incubator at 37 ° C, 5% CO2 (between 2-4 hours). The solution in the wells was carefully taken at the end of 2 hours, and 200 µl DMSO (dimethyl sulfoxide) was added to dissolve the formed formazan and was shaken in a microplate shaker for 15 minutes at room temperature in order to dissolve the formazan and provide homogeneous distribution. At the end of the process, absorbance readings were performed at 570 nm in the microplate reader, and the effect of the substances used in the study on the viability of Ishikawa cells was calculated with the following formula.

Cell viability was calculated by the following equation: Cell viability (%) = (absorbance value of treated group/absorbance value of control group) x100. The concentrations (mM) that killed more than half of the cells at the end of 24 hours were determined as the highest dose for the drugs we used. Subsequent doses were gradually reduced, and the minimum effective doses that were least likely to damage living cells were determined.

Detecting Cells Apoptosis

Commercial Biolocor Apo Percentage apoptosis kit was used to detect apoptosis in Ishikawa cells. In this method, cells undergoing apoptosis take the dye in the reagents, and a pink-purple color is formed. The absorbance values were recorded to calculate the apoptosis rates.

Statistical Methods

Statistical analyses were performed by IBM (International Business Machines) SPSS (Statistical Package for the Social Sciences) statistical package (version 25). The distribution of numerical data groups was evaluated by Shapiro Wilk's test. The Levene test was used to evaluate the distribution of the homogeneity of the groups. To compare multiple groups, the F values (an indicator of homogenization of the distribution) or Welch values (an indicator of the non-homogeneity of the distribution) were used for the statistics. Dunnett T3 method was used for Welch-Test, and Tukey HSD method was used for F-Test for comparison of paired groups. P values <0.05 were considered statistically significant for all tests.

RESULTS

The substance concentration used for the control medium and the mediums of progesterone, carboplatin, calcitriol, the viability, and apoptosis rates are shown below.

Viability Level Results

1.1 Carboplatin

The effect of carboplatin on the viability of Ishikawa cells is shown in Table 1.

Table 1. The effect of carboplatin on cell viability of Ishikawa cells

Carboplatin consentration (0.3-20.0 mM)	**24th hour % cell death**	**48th hour % cell death**	**72nd hour % cell death**
Control (CM)	4.38	0.68	8.7
0.3	20.27	40.12	98.25
0.6	21.62	97.26	All cells died
3.0	86.15	99.18	All cells died
6.0	95.74	All cells died	All cells died
20.0	98.61	All cells died	All cells died
Carboplatin concentration (0.035-3.5mM)	**24th hour % cell death**	**48th hour % cell death**	**72nd hour % cell death**
0.035	16.99	5.13	25.96
0.14	17.46	22.6	63.06
0.7	68.06	77.08	All cells died

1.4	89.46	All cells died	All cells died
2.0	91.48	All cells died	All cells died
3.5	94.28	All cells died	All cells died

MTT absorbance results of Carboplatin	
Carboplatin concentration (mM)	24th hour (mean ± SD)
Control (GM)	0.555 ± 0.094
Control (CM)	0.533 ± 0.051
0.035	0.469 ± 0.031^{a}
0.14	0.467 ± 0.086^{b}
0.7	0.211 ± 0.047^{c}
1.4	0.103 ± 0.015^{d}
2.0	0.093 ± 0.083^{e}
3.5	0.078 ± 0.013^{f}

CM: Control Medium GM: Growth Medium SD: Standard Deviation, MTT: [(3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide)

$P^{a,b}<0.0001$ when compared with c,d,e,f

$p^{c}<0.0001$ when compared with GM,CM, a, b

$p^{c}<0.005$ when compared with d

$p^{c}<0.003$ when compared with e

$p^{c}<0.001$ f ile kıyaslandığında

$p^{d}<0.0001$ when compared with GM, CM, a,b

$p^{d}<0.005$ when compared with c

$p^e<0.0001$ when compared with GM, CM, a, b

$p^e<0.003$ when compared with c

$p^f<0.0001$ when compared with GM,CM,a,b

$p^f<0.001$ when compared with c

As it can be understood from **Table 1**, When cell viability was evaluated, it was observed that there was no difference between GM and CM that is used as solvent control. At the end of 24 hours, the 3.0 mM concentration was determined as the highest dose that killed more than half of the cells. The IC50 value of carboplatin at the end of 24 hours was determined as 0.5 mM. A significant difference in cell viability was detected between all carboplatin concentrations and both GM and CM ($p < 0.05$). Although there were no significant differences between 1.4, 2.0, and 3.5 mM concentrations ($p > 0.05$), it was observed that as the dose of carboplatin increased, its lethal power on Ishikawa cells increased and the number of living cells in the environment decreased.

Table 2. The effect of progesterone on cell viability of Ishikawa cells

Progesterone concentration (µM)	**24th hour % cell death**	**48th hour % cell death**	**72nd hour % cell death**
Control	99.52	83.56	91.17
10	29.46	12.6	7.59
50	29.42	3.57	4.23
100	12.81	1.79	All cells died
200	8.68	All cells died	All cells died
300	4.94	All cells died	All cells died
Progesterone	**24th hour % cell**	**48th hour % cell**	**72nd hour % cell**

concentration (µM)	**death**	**death**	**death**
Control	0.48	16.45	8.83
10	70.54	87.4	92.41
50	70.58	96.43	95.78
100	87.19	98.21	All cells died
200	91.32	All cells died	All cells died
300	95.06	All cells died	All cells died

MTT absorbance results of Progesterone	
Progesterone concentration (µM)	24th hour (mean ± SD)
Control (GM)	1.43 ± 0.16
Control (CM)	1.423 ± 0.066
10	0.457 ± 0.078^{a}
50	0.456 ± 0.042^{b}
100	0.227 ± 0.035^{c}
200	0.17 ± 0.014^{d}
300	0.118 ± 0.013^{e}

CM: Control Medium GM: Growth Medium SD: Standard Deviation, MTT: [(3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide)

$P^{a}<0.0001$ when compared with GM, CM, c,d,e

$P^{b}<0.0001$ when compared with GM,CM,c,d,e

$p^{c}<0.0001$ when compared with GM, CM, a,b,e

$p^{c}<0.03$ when compared with d

p^d<0.0001 when compared with GM, CM, a,b,e,

p^e<0.0001 when compared with GM, CM, a, b,c,d

As reported in Table 2, in terms of cell viability, it was observed that there was no difference between GM and CM used as solution control (p> 0.05). The IC_{50} value of progesterone at the end of 24 hours is reported as 10.34 μM. There were significant differences regarding the cell viability between all progesterone concentrations and both GM and CM (p <0.05). Significant differences were observed between all other concentrations, except for the difference between progesterone concentrations of 10-50 μM. It can be understood from the graph that as the dose of progesterone increased, its lethal power on Ishikawa cells increased and the number of alive cells decreased.

Table 3. The effect of calcitriol on cell viability of Ishikawa cells

Calcitriol concentration (nM)	**24th hour % cell death**	**48th hour % cell death**	**72nd hour % cell death**
Control	99.52	83.56	90.91
10	96.15	91.3	73.02
50	28.77	22.66	15.79
100	26.56	7.02	8.06
150	17.88	4.93	5.06
200	11.72	3.0	3.41
Calcitriol concentration (nM)	**24th hour % cell death**	**48th hour % cell death**	**72nd hour % cell death**
Control	0.48	16.44	9.08
10	3.85	8.7	26.98

50	71.23	77.34	84.21
100	73.44	92.98	91.93
150	82.12	95.07	94.94
200	88.29	97	96.59

MTT absorbance results of Calcitriol	
Calcitriol concentration (nM)	24th hour (mean ± SD)
Control (GM)	1.43 ± 0.16
Control (CM)	1.423 ± 0.066
10	1.38 ± 0.097^{a}
50	0.447 ± 0.065^{b}
100	0.416 ± 0.043^{c}
150	0.296 ± 0.026^{d}
200	0.211 ± 0.032^{e}

CM: Control Medium GM: Growth Medium SD: Standard Deviation,, MTT: [(3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide)

$P^{a}<0.0001$ when compared with b, c,d,e

$P^{b}<0.0001$ when compared with GM, CM, a, e

$P^{b}<0.003$ when compared with c

$p^{c}<0.0001$ when compared with GM, CM, a,d,e

$p^{d}<0.0001$ when compared with GM, CM, a,c

$p^{d}<0.003$ when compared with b

p^{d}<0.001 when compared with e

p^{e}<0.0001 when compared with GM, CM, a, b,c

p^{e}<0.001 when compared with d

As represented in Table 3, no difference was observed between GM used as control and CM used as solvent control. This condition does not change for the 48th and 72nd hours. When 10 nM calcitriol concentration was compared with GM and CM at every hour, no difference was observed between them (p> 0.05). Cell viability was observed to decrease dramatically after 10 nM concentration in every hour, it was reported that cell viability decreased linearly depending on the concentration and the difference between concentrations was statistically significant (p <0.05). The IC_{50} value of calcitriol after 24 hours was reported as 28.04 nM.

Table 4. 24th-hour MTT absorbance results of all agents and their combinations

Drugs	**24th hour (mean ± SD)**
Control (GM)	2.017 ± 0.23^{a}
Carboplatin	0.731 ± 0.135^{b}
Progesterone	1.147 ± 0.094^{c}
Calcitriol	1.008 ± 0.156^{d}
Carboplatin + Progesterone	0.649 ± 0.128^{e}
Carboplatin + Calcitriol	0.405 ± 0.066^{f}

MTT: [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) GM: Growth Medium

SD: Standard Deviation

P^{a}<0.0001 when compared with b, c,d,e, f

P^{b}<0.0001 when compared with a,c

P^{b}<0.01 when compared with d

P^{b}<0.001 when compared with f

p^{c}<0.0001 when compared with a, b, d, e

p^{e}<0.0001 when compared with a,c, d

p^{f}<0.0001 when compared with a, c, d

p^{f}<0.001 when compared with b

As summarized in Table 4, the data obtained are in accordance with our 24-hour MTT results obtained using different concentrations of all three drugs. It was reported that the absorbance values of all three compounds alone and in their combinations were statistically significantly lower when compared to the GM as the control group ($p < 0.001$). When the absorbance of the carboplatin + progesterone combination was compared with the carboplatin levels alone, it was observed that the difference between them was not significant. However, when the absorbance levels of the combination of carboplatin + calcitriol were compared with the absorbance values of carboplatin levels alone, it was observed that calcitriol triggered the anti-tumor effects of carboplatin and increased cell death ($p = 0.001$).

Results of Apoptosis

It was observed that all three agents triggered apoptosis in cells, carboplatin dose-dependently increased cell apoptosis, and progesterone and calcitriol triggered necrosis in cells with increasing doses. Besides, when drug combinations were evaluated, no difference was found between apoptosis levels (Figure 1).

Figure 1. Apoptosis levels of cells after IC_{50} doses were applied

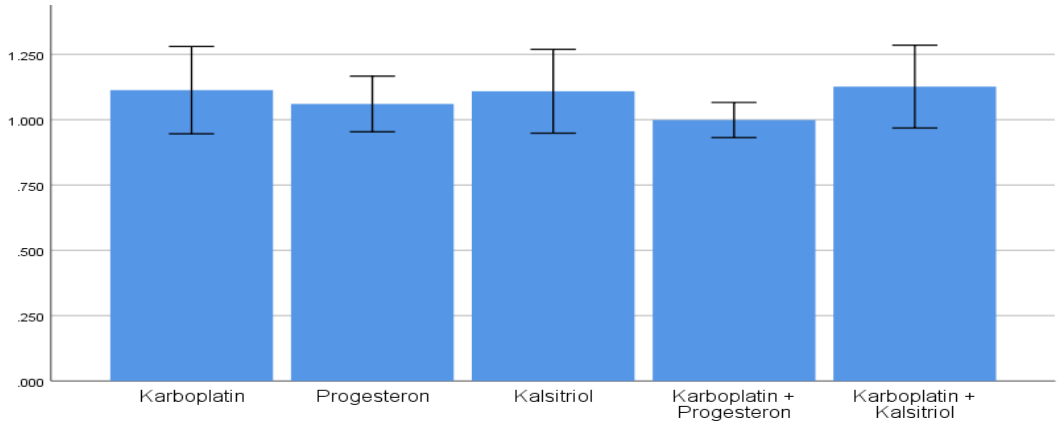

Table 5. Apoptosis results of Carboplatin

Carboplatin concentration (mM)	**48th hour (mean ± SD)**
0.35	0.131 ± 0.014^{a}
0.14	0.333 ± 0.051^{b}
0.7	0.447 ± 0.094^{c}
1.4	0.585 ± 0.106^{d}
2.0	0.671 ± 0.052^{e}
3.0	0.678 ± 0.073^{f}

SD: Standard Deviation

$P^{a}<0.0001$ when compared with b,c,d,e,f

$p^{b}<0.0001$ when compared with a, d,e,f

$p^{c}<0.0001$ when compared with a, e, f

$p^{d}<0.0001$ when compared with a,b

$p^{e}<0.0001$ when compared with a, b,c

$p^{f}<0.0001$ when compared with a,b,c

Table 5 represents the increase in the number of apoptotic cells with increasing carboplatin doses. The difference between the mean values obtained at each concentration was found to be statistically significant.

Table 6. Apoptosis results of Progesterone

Progesterone concentration (mM)	**48th hour (mean ± SD)**
10	0.456 ± 0.059^{a}
50	0.284 ± 0.047^{b}
100	0.279 ± 0.043^{c}
200	0.201 ± 0.026^{d}
300	0.167 ± 0.035^{e}

SD: Standard Deviation

$P^{a}<0.0001$ when compared with b,c,d,e

$p^{b}<0.0001$ when compared with a, e

$p^{b}<0.002$ when compared with d

$p^{c}<0.0001$ when compared with a, e

$p^{c}<0.002$ when compared with d

$p^{d}<0.0001$ when compared with a

$p^{d}<0.002$ when compared with b,c

$p^{e}<0.0001$ when compared with a, b,c

In Table 6, it is observed that apoptosis in cells increased with the increasing progesterone doses. In addition, with the presence of fewer cells in the environment due to the increase in the undergoing necrosis, it is observed that amount of absorbance obtained decreased. The difference between the mean values obtained at each concentration was found to be statistically significant.

Table 7. Apoptosis results of calcitriol

Calcitriol concentration (nM)	**48th hour (mean ± SD)**
10	0.545 ± 0.087^{a}
50	0.298 ± 0.037^{b}
100	0.307 ± 0.132^{c}
150	0.188 ± 0.008^{d}
200	0.144 ± 0.010^{e}

SD: Standard Deviation

$P^{a}<0.0001$ when compared with b,c,d,e

$p^{b}<0.0001$ when compared with a, e

$p^{b}<0.013$ when compared with d

$p^{c}<0.0001$ when compared with a, e

$p^{c}<0.006$ when compared with d

$p^{d}<0.0001$ when compared with a

$p^{d}<0.013$ when compared with b

$p^{d}<0.006$ when compared with c

$p^{e}<0.0001$ when compared with a, b,c

In Table 7, it is presented that apoptosis in cells increased with the increasing doses of calcitriol. In addition, because of fewer cells being present in the environment with the increase in the number of cells having necrosis, it is observed that the amount of absorbance obtained decreased. The difference between the mean values obtained at each concentration and the difference between them was found to be statistically significant.

DISCUSSION

Today with limited treatment options, endometrium cancer is known as a cancer type that is still open to new treatment alternatives. In the literature, cell culture studies on this subject are very limited. There are studies in the literature investigating the efficacy of both carboplatin, progesterone, and progesterone in combination with calcitriol, however, to the best of the authors' knowledge, there are not any studies evaluating the combinations of carboplatin with calcitriol, carboplatin, and progesterone. Therefore, the results of this may contribute to the literature for the treatment of endometrial cancer.

Regarding the effect of carboplatin on cancer cells, it is known that DNA base pairs attach to alkyl groups, leading DNA repairing enzymes to break down the DNA. Besides, these agents may cause mutations by preventing DNA synthesis by forming cross-links between nucleotides in DNA. In a study investigating the effects of carboplatin and cisplatin agents on endometrial cancer cells, carboplatin was used in eight different endometrial cancer cells at doses of 0.05-2.5 μg / ml (highest dose 67μM). They found the highest IC_{50} value was 1.2 μg / ml and the lowest as 0.1 μg / ml. They reported the mean IC_{50} value for carboplatin was 0.5 μg / ml (1.34 μM) (11). In another cell culture study, the sensitivity of carboplatin (for doses of 0.1 μg / ml, 0.5 μg / ml, 5 μg / ml, 10 μg / ml, and 20 μg / ml), (1,25OH) $_2D_3$ and paclitaxel was analyzed in two different endometrial adenocarcinoma cells. They reported that the IC_{50} value for cancer cells in the first cell culture was between 0.5-1 μg / ml (1.34-2.68 μM), while the IC_{50} value for cancer cells in the second cell culture was 0.1-1 μg / ml (0.268-2.68 μM) (12). These aforementioned limited studies in the literature and our current study were performed at 0.3, 0.6, 3.0, 6.0, and 20.0 mM doses of carboplatin for endometrial cancer cells and the IC_{50} value was reported as 0.5 mM. This data reveals how high the cell-killing potential is even at low doses. Furthermore, according to the results of this study, it can be concluded that as the carboplatin dose increases, more tumor cells die.

Progesterone is known as a highly effective agent in endometrial cancer both for prophylaxis and for treatment. Estradiol and progesterone act by playing an important role in the modulation of gene expression of Toll-Like Receptor. Calcitriol is known to have an antiproliferative effect in prostate, breast, colon, skin cancers, and leukemia cells through mechanisms that induce cell cycle arrest, apoptosis, and differentiation. Kavandi et al. used the progesterone dose of 25 μM and the calcitriol dose of 100 nM in their study, and they

collected the samples after treatment and used them for protein detection in western blot trials (13). The same group reported in their previous studies that they used appropriate treatment doses found in different endometrial cancer cell lines in cell viability and apoptosis trials (13). Nguyen et al. studied three different endometrial cancer cells named Ishikawa, HEC-1B, and RL-95. They used the progesterone dose between 12.5-200 μmol / L (12.5, 50, 100 and 200 μmol / L) and calcitriol dose between 50-400 nmol / L (50, 100, 200, and 400 nmol / L) and treated the cells for 72 hours and measured cell viability and caspase-3 enzyme, which is one of the best indicators of apoptosis, with the technique of Enzyme-Linked Immunosorbent Assay. Researchers reported that they tried to use the lowest effective dose to avoid the toxicity of high-dose progesterone that is seen in most studies. Also, they reported that they were careful to use the calcitriol at doses that would not cause hypercalcemia, since high-dose calcitriol may cause hypercalcemia in vivo (14). In our study, the IC_{50} value of progesterone at the end of 24 hours was reported as 10.34 μM. There was a significant difference in cell viability between all progesterone concentrations and both GM and CM ($p < 0.05$). Differences between all other concentrations were found to be significant, except for the difference between 10-50 μM progesterone concentrations. It was noted that, as the dose of progesterone increased, its lethal power on Ishikawa cells increased and the number of living cells in the environment decreased. This data revealed the high effectiveness of progesterone is even at low doses.

In our study, it was reported that calcitriol dramatically reduced cell viability after 10 nM concentration at every hour, cell viability decreased linearly related to the concentration, and the difference between concentrations was statistically significant. The IC_{50} value of calcitriol after 24 hours was reported as 28.04 nM. In our study, an increased effect on cell viability and an increase in cell death due to increased doses of progesterone and calcitriol were reported, as it was in the study of Nyugen et al. Accordingly, we may comment that calcitriol has a high potential of killing cells and that calcitriol may be beneficial in terms of survival and cure in treatment protocols.

In the literature, a study in which carboplatin was used together with other agents is not present. In this study, it was observed that the cell viability rates of all three agents alone and in their combination were significantly lower compared to the control group ($p < 0.001$). When the absorbance of carboplatin and progesterone combination was compared with carboplatin levels alone, it was observed that the difference between them was not significant.

However, when the absorbance levels of carboplatin and calcitriol combination were compared with the absorbance values of carboplatin levels alone, it was observed that calcitriol triggered the anti-tumor effects of carboplatin and increased cell death ($p = 0.001$). It is considered an important marker for the use of carboplatin and calcitriol together in the treatment. This suggests that combinations of two agents may increase the success rates of treatment with their inclusion in the treatment.

In our study, it was observed that all three combination treatments triggered apoptosis in cells, although no significant difference was detected between groups. Furthermore, it was observed that carboplatin increased apoptosis in cells dose-dependently, while progesterone and calcitriol triggered necrosis in cells with the increasing doses. Especially, more studies are needed to investigate the effects of these agents on apoptosis.

In conclusion, carboplatin, progesterone, and calcitriol may have inhibitory effects on endometrial tumor cells, depending on the dose and time. When calcitriol is applied to cells with carboplatin, it may increase its lethal effects on cells. All three substances can trigger apoptosis in cells. Using carboplatin, progesterone, and calcitriol together in endometrial cancer treatment may be a good option for this cancer type with high mortality. Further studies are needed to support these findings.

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