Carboplatin–Paclitaxel- and Carboplatin–Docetaxel-Induced Cytotoxic Effect in Epithelial Ovarian Carcinoma In Vitro

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BACKGROUND. The combination of paclitaxel and cisplatin is standard for patients with newly diagnosed epithelial ovarian carcinoma. The role of another taxane, docetaxel, currently is being studied. Due to its milder nonhematologic toxicity carboplatin increasingly is being substituted for cisplatin in taxane-based combinations. The purpose of this study was to compare the combination of carboplatin-paclitaxel with carboplatin-docetaxel in ovarian carcinoma in vitro, and to assess the type of interaction, if any.

METHODS. Sensitivity to carboplatin and the concomitant use of a taxane and carboplatin was studied in 4 ovarian carcinoma cell lines using the 96-well plate clonogenic assay. Chemosensitivity was expressed as the IC50 value (i.e., the drug concentration causing 50% inhibition of clonogenic survival). IC50 values were obtained from dose-response curves after fitting the data to the linear quadratic equation. Synergism was studied by the area under the survival curve ratios (AUC ratios), obtained by numeric integration. The AUC ratio and the surviving fraction (SF) value after the administration of taxane alone were compared using the Student *t* test for paired data.

RESULTS. The IC50 values for carboplatin were between 0.5-1.6 μg/mL; there was only a 3.2-fold difference between individual cell lines. Carboplatin administered concomitantly with a taxane had either an additive or supra-additive growth inhibitory effect on all four ovarian carcinoma cell lines. A supra-additive effect occurred after simultaneous exposure of the cells to carboplatin at all tested paclitaxel concentrations in three of four cell lines (UT-OC-3, UT-OC-5, and SK-OV-3). The carboplatin-docetaxel combination had a supra-additive effect at the two highest docetaxel concentrations in two cell lines (UT-OC-4 and UT-OC-5) and at the highest docetaxel concentration in the other two cell lines (UT-OC-3 and SK-OV-3).

CONCLUSIONS. Carboplatin has a synergistic effect when used with paclitaxel or docetaxel. A supra-additive effect is achieved with a wider range of paclitaxel concentrations than docetaxel concentrations. ***Cancer*** **1999;86:2066–73.**
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In two randomized trials a combination of paclitaxel and cisplatin has been found to be superior to a combination of cisplatin and cyclophosphamide in the treatment of ovarian carcinoma.[1,2] The paclitaxel and cisplatin combination is associated with vomiting, alopecia, and neuropathy, which are the most common nonhematologic side effects.[1,3] Because carboplatin causes significantly fewer of

TABLE 1
Passages and Plating Efficiency of Four Epithelial Ovarian Carcinoma Cell Lines and the Sensitivity of these Cell Lines to Cisplatin, Carboplatin, Paclitaxel, and Docetaxel (Expressed as IC50 Values, Corresponding to a Drug Concentration Causing 50% Inhibition of Clonogenic Survival)

	Passages	Plating efficiency	Carboplatin IC50 ± SD (μg/mL)	Paclitaxel[26] IC50 ± SD (nM)	Docetaxel[27] IC50 ± SD (nM)
UT-OC-3	15–25	0.08–0.13	0.5 ± 0.1	1.3 ± 0.1	1.0 ± 0.1
UT-OC-4	21–28	0.07–0.08	1.1 ± 0.2	1.0 ± 0.1	0.5 ± 0.1
UT-OC-5	17–27	0.08–0.14	0.8 ± 0.1	1.4 ± 0.1	1.2 ± 0.2
SK-OV-3	25–34	0.34–0.56	1.6 ± 0.1	3.4 ± 0.2	1.3 ± 0.2

IC50: causing 50% inhibition of clonogenic survival; SD: standard deviation.

these side effects,[4,5] it increasingly is being substituted for cisplatin in combination therapy.

The combination of paclitaxel and cisplatin currently is being compared with the combination of paclitaxel and carboplatin in three randomized trials. According to preliminary results these combinations have comparable efficacy[6,7] but, surprisingly, Grade 1-2 neuropathy has been detected in 70% of patients in both arms.[6] In a German study Grade 2-3 neuropathy occurred in 26% of the patients receiving the paclitaxel and carboplatin combination in which paclitaxel was infused over the course of 3 hours.[7]

Docetaxel, which is derived from the needles of the European yew tree (Taxus baccata), is semisynthetic compound structurally related to paclitaxel. Docetaxel has a longer residence time, produces higher intracellular concentrations, and has a superior therapeutic index in vivo when compared with paclitaxel.[8–10] Docetaxel may cause a unique fluid retention syndrome usually characterized by peripheral edema and weight gain.[11,12] Premedication with corticosteroids delays the onset of this syndrome according to findings in a recent randomized trial. The feasibility of the cisplatin and docetaxel combination in the treatment of ovarian carcinoma has been tested in a multicenter trial, with an overall response rate of 66% among 61 evaluable patients.[13] Grade 2-3 neuropathy, which most likely was associated with cisplatin, occurred in 25% of the patients receiving this combination. Preliminary results regarding carboplatin and docetaxel-induced neurotoxicity in 141 patients have shown that significant neuropathy is uncommon, with Grade 2 neuropathy occurring in $< 6\%$ of the patients. Overall nonhematologic toxicities are rare, and the regimen appears to be tolerated better than the docetaxel and cisplatin combination.[14] Consequently, the combination of carboplatin and docetaxel currently is of interest as a potentially safer but equally effective combination as cisplatin and docetaxel.

In a previous in vitro study, we compared the cytotoxic effects of cisplatin and paclitaxel and cisplatin and docetaxel combinations in seven ovarian carcinoma cell lines.[15] On a molar basis, the combination of cisplatin and docetaxel was more cytotoxic than the combination of cisplatin and paclitaxel. In the current study we compare the combination of carboplatin and paclitaxel with that of carboplatin and docetaxel and assess the type of interaction between these agents. There is a lack of preclinical data regarding the simultaneous use of paclitaxel or docetaxel and platinum analogues and, to our knowledge, the efficacy of these combinations has not been tested previously in vitro.

MATERIALS AND METHODS

Cell Lines

Four ovarian epithelial carcinoma cell lines were tested (Table 1). The UT-OC-3 and UT-OC-5 cells are derived from serous cystadenocarcinoma and the UT-OC-4 cell line is of endometrioid type. The SK-OV-3 cell line[16] is epithelial in origin and was obtained from the American TypeCulture Collection (Rockville, MD). The UT-OC-5 cell line was derived from a metastatic omental tumor whereas the other cell lines were derived from primary tumors. The donors of the UT-OC-4 and UT-OC-5 cell lines had received pelvic radiotherapy for cervical carcinoma 30 and 5 years, respectively, before the diagnosis of ovarian carcinoma. The donors had not received any chemotherapeutic drugs before establishment of the cell lines. We recently characterized five UT-OC- cell lines by testing doubling times, DNA flow cytometry, and cytogenetic aberrations by comparative genomic hybridization. The UT-OC-4 and UT-OC-5 cell lines used in this study were found to have more DNA sequence copy number aberrations compared with the other cell lines

TABLE 2
Effects of Paclitaxel on Clonogenic Survival of Four Epithelial Ovarian Carcinoma Cell Lines. Paclitaxel was Used as a Single Agent and in Combination with Carboplatin

Cell line (IC50 for paclitaxel)	Paclitaxel concentration (nM)	Carboplatin concentration (μg/mL)	$S_{paclitaxel}$[a]	AUC ratio[b]	*P* value[c]	
UT-OC-3	0.6	0.1–1.0	0.95 ± 0.05	0.86 ± 0.06	0.034	(SA)
(1.3 nM)	0.8		0.87 ± 0.05	0.69 ± 0.07	0.00053	(SA)
	1.0		0.70 ± 0.03	0.50 ± 0.06	0.00019	(SA)
UT-OC-4	0.4	0.5–1.5	0.92 ± 0.05	0.89 ± 0.06	0.44	(A)
(1.0 nM)	0.6		0.84 ± 0.08	0.78 ± 0.05	0.22	(A)
	0.8		0.75 ± 0.04	0.71 ± 0.14	0.14	(A)
UT-OC-5	0.6	0.5–1.5	0.97 ± 0.04	0.91 ± 0.04	0.0096	(SA)
(1.4 nM)	0.8		0.87 ± 0.05	0.75 ± 0.04	0.00011	(SA)
	1.0		0.67 ± 0.06	0.53 ± 0.04	0.000082	(SA)
SK-OV-3	1.5	1.0–2.0	1.00 ± 0.08	0.89 ± 0.03	0.0096	(SA)
(3.4 nM)	2.0		0.98 ± 0.07	0.81 ± 0.03	0.00082	(SA)
	3.0		0.74 ± 0.06	0.63 ± 0.03	0.0055	(SA)

IC50: causing 50% inhibition of clonogenic survival; AUC: area under the survival curve; SA: supra-additive effect; A: additive effect.
[a] Clonogenic survival after incubation in the indicated concentration.
[b] Ratio between the area under the survival curve (AUC) for carboplatin plus paclitaxel divided by the AUC for carboplatin alone.
[c] *P* values according to the Student *t* test.

but no other obvious differences were detected. In histologic evaluation the original tumors were classified as ovarian endometrioid carcinoma and cystadenocarcinoma.[17] Therefore these cell lines can be considered to be representative of epithelial ovarian carcinoma.

Cell Culture

Prior to the experiments, the cells were kept in logarithmic growth in T25 culture flasks by passing weekly in Dulbecco modified Eagle minimal essential medium containing 2 mM L-glutamine, 1% nonessential amino acids, 100 U/mL^{-1} streptomycin, 100 U/mL^{-1} penicillin, and 10% fetal bovine serum (FBS). Cells in midlogarithmic growth (40–60% confluence) were used for the experiments and fed with fresh medium on the day before plating.

Drug Preparation

Carboplatin was dissolved in the growth medium to obtain a stock solution of 100 μg/mL and sterilized by pressing the solution through a 0.22-μm filter. The final dilutions were made immediately before use, and new stock solutions were made for each experiment. Paclitaxel initially was dissolved in 0.9% sodium chloride to achieve a solution of 0.1 mM. Stock solutions were prepared in Ham F-12 medium containing 10% FBS to obtain a solution of 100 nM and stored at −40 °C. A dose of 807.9 mg docetaxel was diluted in 1 mL of ethanol to achieve a stock solution of 0.1 mM and stored at −40 °C. These solutions were diluted further with sterile water to achieve a solution of 100 nM immediately before each experiment. Pure drug was used in all experiments, at concentrations noted in Tables 2 and 3. The four carboplatin concentrations that ultimately were used in the experiments were selected from previously determined dose-response curves. The taxane concentrations used concomitantly with carboplatin were based on pilot series conducted before the experiments.

Clonogenic Assay

The 96-well plate clonogenic assay based on limiting dilutions was used. The assay has been described in detail previously.[18,19] A minimum of three experiments including duplicate plates were performed for each cell line. The cells were harvested with trypsin-ethylenediamine tetraacetic acid to obtain a single-cell suspension, counted, and diluted in Ham F-12 medium containing 15% FBS. The number of cells plated per well was adjusted according to the plating efficiency (PE) of the cell line. Approximately 120 μL of stock solution containing 4167 cells mL^{-1} was diluted in 25 mL of growth medium; a concentration of 2 cells per well was achieved by applying 100 μL of this cell suspension to each well. After 24 hours, 100 μL of the growth medium containing the desired concentrations of carboplatin was added.

TABLE 3
Effects of Docetaxel on Clonogenic Survival of Four Epithelial Ovarian Carcinoma Cell Lines. Docetaxel was Used as a Single Agent and in Combination with Carboplatin

Cell line (IC50 for docetaxel)	Docetaxel concentration (nM)	Carboplatin concentration (μg/mL)	$S_{docetaxel}$[a]	AUC ratio[b]	*P* value[c]	
UT-OC-3	0.2	0.1–1.0	0.86 ± 0.07	0.87 ± 0.06	0.83	(A)
(1.0 nM)	0.5		0.75 ± 0.06	0.69 ± 0.05	0.088	(A)
	0.8		0.67 ± 0.08	0.57 ± 0.04	0.038	(SA)
UT-OC-4	0.2	0.5–1.5	0.91 ± 0.06	0.87 ± 0.02	0.27	(A)
(0.5 nM)	0.3		0.89 ± 0.06	0.76 ± 0.03	0.0019	(SA)
	0.4		0.72 ± 0.05	0.50 ± 0.04	0.000081	(SA)
UT-OC-5	0.2	0.5–1.5	0.89 ± 0.04	0.87 ± 0.02	0.30	(A)
(1.2 nM)	0.4		0.76 ± 0.04	0.69 ± 0.04	0.013	(SA)
	0.6		0.63 ± 0.04	0.52 ± 0.04	0.0008	(SA)
SK-OV-3	0.6	1.0–2.0	0.94 ± 0.05	0.81 ± 0.03	0.00071	(SA)
(1.3 nM)	0.8		0.72 ± 0.04	0.69 ± 0.03	0.22	(A)
	1.0		0.58 ± 0.03	0.51 ± 0.03	0.0085	(SA)

IC50: causing 50% inhibition of clonogenic survival; AUC: area under the survival curve; A: additive effect; SA: supra-additive effect.

[a] Clonogenic survival after incubation in the indicated concentration.

[b] Ratio between the area under the survival curve (AUC) for carboplatin plus docetaxel, divided by the AUC for carboplatin alone.

[c] *P* values are according to the Student *t* test.

For studying the concomitant use of taxanes and carboplatin, the desired concentrations of paclitaxel or docetaxel and 120 μL of stock solution containing 4,167 cells/mL^{-1} were diluted in 25 mL of growth medium. A concentration of 2 cells per well was achieved by applying 100 μL of this stock solution per each well of the 96-well plate. The desired carboplatin concentrations along with the same paclitaxel and docetaxel concentrations as those of the day before were added to each well after the plates had been incubated for 24 hours. All drugs were retained in the plates throughout the whole incubation period. The plates were incubated at 37 °C with 5% CO_2 for 4 weeks, after which the number of wells containing coherent, living colonies, comprised of ≥ 32 cells, was counted using an inverted phase-contrast microscope.

Data Analysis

PE was calculated by the formula PE = -ln (number of negative wells/total number of wells)/number of cells plated per well.[20] Fraction survival data were fitted to the linear quadratic model $F = \exp[-(\alpha D + BD^2)]$ and a microcomputer program was used to obtain the area under the curve (AUC) by numeric integration. The simultaneous effects of carboplatin and paclitaxel or docetaxel were determined as the ratio between the AUC for carboplatin plus paclitaxel or docetaxel, divided by the AUC for carboplatin alone. This AUC ratio was compared with the surviving fraction (SF) after the indicated concentration of paclitaxel or docetaxel alone. If the AUC ratio was significantly smaller than the corresponding SF value, a supra-additive effect was detected. The AUC ratio and the SF value were compared using the Student *t* test for paired data. Drug sensitivities were compared by IC50 values (i.e., 50% clonogenic inhibition of the surviving fraction), obtained from the fitted dose-response curves. Linear regression analysis was used to estimate the correlation between the growth inhibitory effect of carboplatin, cisplatin, paclitaxel, and docetaxel.

RESULTS

The carboplatin IC50 values for four epithelial ovarian carcinoma cell lines varied between 0.5 and 1.6 μg/mL (1.35–4.31 μM), and the mean IC50 value was 1.0 μg/mL (Table 1). The difference in the IC50 values between individual cell lines was 3.2-fold. The type of interaction after simultaneous use of taxanes and carboplatin and the statistical significance of supra-additivity are presented in Tables 2 and 3. The fitted survival curves for the four cell lines after concomitant exposure to paclitaxel or docetaxel and carboplatin are shown in Figure 1. All paclitaxel concentrations caused a supra-additive effect in the UT-OC-3, UT-OC-5, and SK-OV-3 cell lines. In the UT-OC-4 cells there was an additive effect at all tested paclitaxel

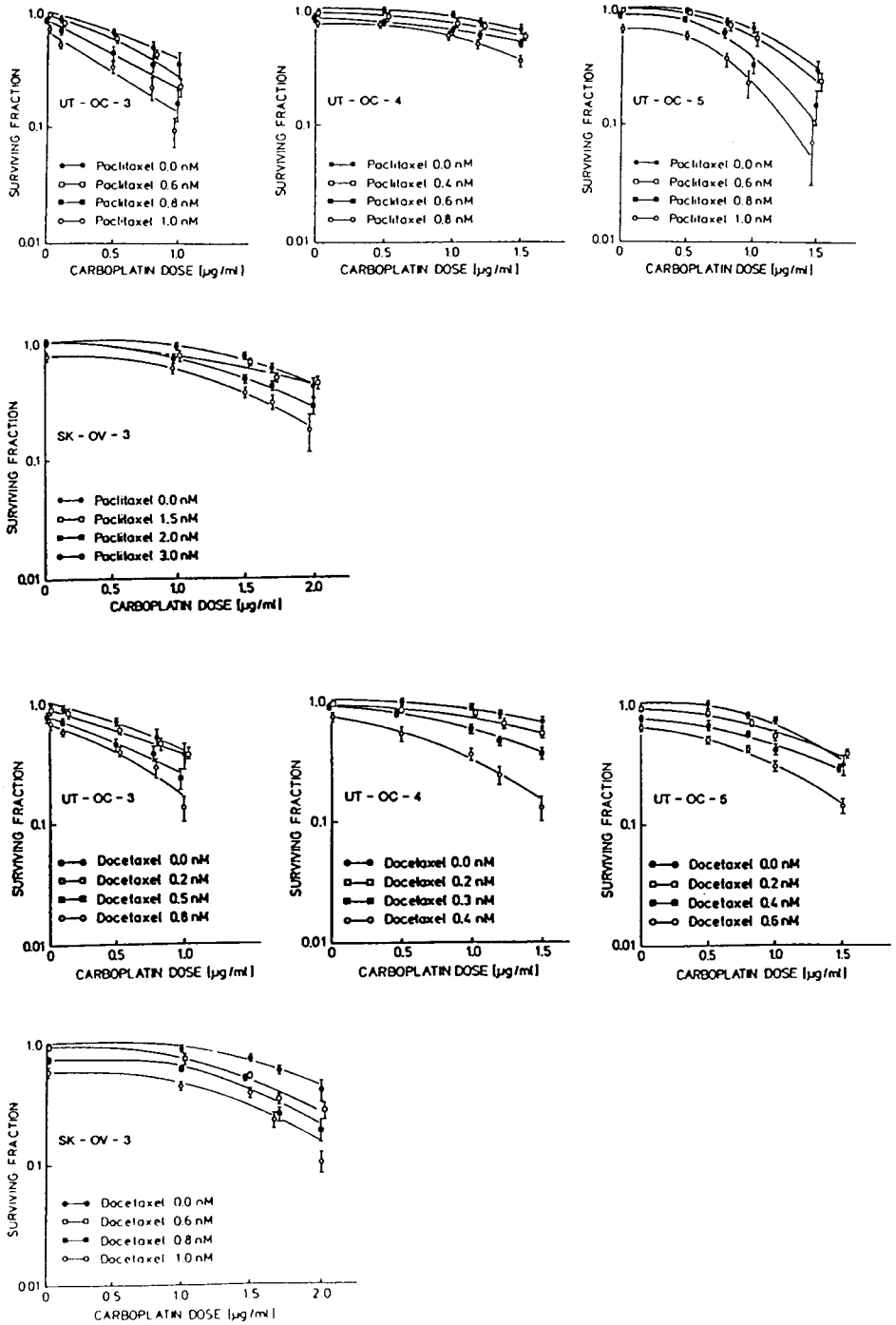

FIGURE 1. Effects of simultaneous use of carboplatin and paclitaxel or docetaxel. Fitted carboplatin curves for four ovarian carcinoma cell lines without paclitaxel or docetaxel and combined with the desired taxane concentrations are shown. The results are presented as the average of the actual data points and the bars show one standard deviation.

concentrations. In the UT-OC-3 and UT-OC-5 cell lines, which exhibited supra-additivity already at lower paclitaxel concentrations, increasing paclitaxel concentrations resulted in increased supra-additivity. Thus, in these cell lines the degree of supra-additivity correlated with the concentration of paclitaxel (data not shown). In the UT-OC-4 cells an additive growth inhibitory effect was observed at all tested paclitaxel concentrations but there was no supra-additive effect.

The effect of concomitant use of docetaxel and carboplatin is shown in Table 3. There was a supra-additive effect in all cell lines after simultaneous exposition to carboplatin and the highest docetaxel concentration. In the UT-OC-4 and UT-OC-5 cell lines a

supra-additive growth inhibitory effect was achieved, also at lower docetaxel concentrations, and it was concentration-dependent (data not shown). However, the lowest docetaxel concentration caused only a purely additive effect. The growth of the UT-OC-3 cells was inhibited in an additive fashion at 0.2 nM and 0.5 nM of docetaxel (P values of 0.83 and 0.088, respectively), corresponding to 20% and 50%, respectively, of the IC50 value. The SK-OV-3 cells showed a clear supra-additive effect when 0.6 nM or 1.0 nM of docetaxel was combined with carboplatin (P values of 0.00071 and 0.0085, respectively). In repeated experiments a 0.8-nM concentration of docetaxel combined with carboplatin caused only a purely additive growth inhibitory effect ($P = 0.22$) on these cells (Table 3).

DISCUSSION

Preclinical evidence suggests that the new member of the taxoid family, docetaxel, may be superior to paclitaxel on a molar basis. Docetaxel has been reported to be up to fivefold more potent in vitro than paclitaxel.[21,22] However, to our knowledge there is a lack of in vitro data regarding the growth inhibitory effect of docetaxel combined with other cytotoxic agents. Human breast carcinoma cell lines exposed to edatrexate prior to docetaxel demonstrated an additive or supra-additive growth inhibitory effect whereas it is interesting to note that the reverse schedule demonstrated antagonism.[23] Similarly, treatment with vinca alkaloids followed by docetaxel showed an additive or supra-additive effect in human lung carcinoma and mice melanoma cells, but antagonism occurred at the reverse schedule.[24] Docetaxel given simultaneously with carboplatin caused a synergistic growth inhibitory effect in two of three lung carcinoma cell lines.[25] In the current study we compared the cytotoxicity of carboplatin combined with paclitaxel and docetaxel in ovarian carcinoma in vitro. Both combinations had a pronounced cytotoxic effect but growth was inhibited supra-additively more often with the combination of carboplatin and paclitaxel.

We previously tested the cytotoxic effects of cisplatin, paclitaxel, and docetaxel in ovarian carcinoma in vitro.[26,27] The carboplatin sensitivities obtained in the current study were within the same range as those observed for endometrial carcinoma cell lines.[19] Because both of these studies have been performed with the same method, the results are comparable. Data obtained from the current study do not indicate that there is a positive association between the sensitivities of the taxanes and carboplatin, which is in agreement with previous results regarding nine ovarian carcinoma cell lines.[28]

In a recent study from our laboratory[15] the combination of docetaxel and cisplatin was more cytotoxic on a molar basis than the combination of paclitaxel and cisplatin. A supra-additive effect was found in four of seven cell lines after simultaneous exposure to cisplatin at all docetaxel concentrations tested and in two cell lines when cisplatin was used concomitantly with paclitaxel. The data obtained from the current study indicate that a supra-additive or additive effect also can be achieved with the concomitant use of carboplatin and a taxane. This is in agreement with the results obtained in a human lung carcinoma cell line. In that study[29] both simultaneous incubation as well as sequential exposure to paclitaxel followed by carboplatin were more cytotoxic than the sequence of carboplatin followed by paclitaxel. In the current study there was a supra-additive or additive effect at a wider range of paclitaxel concentrations than docetaxel concentrations. There already was a synergistic effect at lower paclitaxel concentrations, whereas only the two highest docetaxel concentrations caused a supra-additive effect when combined with carboplatin.

Data obtained from our recent[15] and current experiments suggest that the taxane concentrations required for a synergistic effect to emerge with platinum analogues are different. The taxanes have a completely distinct mechanism of action compared with the platinum analogues and there also are a number of differences between paclitaxel and docetaxel.[30,31]

Docetaxel is approximately twice as potent an inhibitor of microtubule depolymerization as paclitaxel.[21] In addition, cells subjected to docetaxel synthesize tubulin polymers that differ structurally from the tubulin polymers resulting from paclitaxel exposition.[30] Studies in P388 leukemia cells have shown that the half-life of efflux from the cells of docetaxel is three times longer than of paclitaxel.[31] Cisplatin and carboplatin differ with regard to the kinetics of interaction with DNA;[32,33] carboplatin is bound more slowly and to a lesser extent to DNA than cisplatin.[34] It is unlikely that microtubule stabilization affects the platination of DNA and that the interaction occurs at the level of membrane transport because platinum analogues mainly enter cells by passive diffusion.[35] Pretreatment with paclitaxel reduces the number of cells in the G_2/M-phase of the cell cycle and thus cells accumulate partly in the G_1 phase, in which cells are sensitive to the cytotoxic action of carboplatin and cisplatin.[36] Similarly, docetaxel induces G_2/M-phase block followed by DNA fragmentation.[37] Paclitaxel inhibits the repair of DNA damage by cisplatin in human ovarian carcinoma cells.[38] To our knowledge similar studies regarding docetaxel are not available, but the differ-

ences that occur after the simultaneous use of carboplatin or cisplatin and the two taxanes may be explained in part by differences in the ability of the taxanes to inhibit repair of DNA damage induced by the platinum analogue.

The results of the current study indicate that there is a synergistic effect of simultaneous exposure of ovarian carcinoma cell lines to carboplatin and a taxane and that the range of paclitaxel concentrations for this synergy to emerge is wider than that of docetaxel concentrations.

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