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Association of docetaxel/paclitaxel with irradiation in ovarian carcinoma cell lines in bidimensional (sulforhodamine B assay) and tridimensional (spheroids) cultures

Geneviève Griffon-Etienne,[1,3] Jean-Louis Merlin[1] and Christian Marchal[2]

[1]Laboratoire de Recherche en Oncologie and [2]Service de Radiothérapie, Centre Alexis Vautrin, 54511 Vandœuvre-les-Nancy Cedex, France. Tel: (+33) 83 59 85 06; Fax: (+33) 83 44 60 71. [3]Present address: Massachusetts General Hospital, Harvard Medical School, Department of Radiation Oncology, Boston, MA 02114, USA.

The association of taxoid derivatives (paclitaxel and docetaxel) with irradiation was evaluated in ovarian carcinoma cell lines (A2780 and CAVEOC-2) using the multicellular tumor spheroids (MTS) tridimensional model and compared to the conventional bidimensional model. The radiosensitivity parameters were the surviving fraction at 2 Gy, and α calculated using the linear-quadratic model for monolayer culture, the residual/control volume ratios at 2 Gy (RSV_2) and doses inducing 50% decrease in MTS number (SCD_{50}) calculated for spheroids. In A2780 monolayer culture, the combination was synergistic for paclitaxel and additive for docetaxel. In spheroids, both compounds induced a decrease in RSV_2 and SCD_{50} in the two cell lines, and their combination with radiation was additive. Therefore, the radiosensitizing effect of taxoid derivatives was not constant in ovarian cell lines. The different results achieved in monolayer culture and in spheroids may suggest higher drug incorporation and fixation through the multiple cell layers of the spheroids than in monolayers.

Key words: Docetaxel, irradiation, multicellular tumor spheroid, ovarian tumor, paclitaxel, sulforhodamine B.

Introduction

Ionizing radiation treatments represent one of the main therapeutic approaches against cancers, particularly solid tumors. The possibility of using chemotherapeutic agents to selectively enhance radiation response in tumors is an appealing approach to improve the results of cancer treatment. The ideal drug for this therapeutic strategy would have potent independent anticancer action as well as the ability to sensitize radioresistant tumor cells to the letal effects of ionizing radiation. About 20 years ago, a novel antitumor agent, paclitaxel (Taxol®), was shown to be a mitotic spindle poison isolated from the bark of the Pacific yew, *Taxus brevifolia*.[1]

In 1981, Rhône-Poulenc and the Institut de Chimie des Substances Naturelles (Gif sur Yvette, France) concluded a cooperative research agreement about natural products extracted from yews. This collaboration led to the discovery of docetaxel (Taxotere®) (RP 56976; NSC 628503; *N*-debenzoyl-*N*-terbutoxycarbonyl-10-deacetyl taxol) in 1986.[2,3] It was obtained by hemisynthesis from a non-cytotoxic precursor, 10-deacetyl baccatin III, extracted from the needles of the European yew, *Taxus baccata*. The mechanism of antitumor action of this product is shown identical to that of paclitaxel,[2,4] but, in comparison with paclitaxel, docetaxel was found more active as a tubulin assembly promoter and as a microtubule stabilizer, and 2- to 5-fold more potent as an inhibitor of microtubule depolymerization.[5,6] These differences in potency were also observed at the cellular level[5] and *in vivo*.[6]

The unique mechanism of action consisted in binding of docetaxel and paclitaxel tightly to the β-tubulin subunit of microtubules thus preventing their depolymerization. One consequence of the effects of paclitaxel on microtubules is that proliferating cells treated with paclitaxel are blocked in the G_2 or M phases of the cell cycle.[7] This ability to arrest cells in G_2 and M makes paclitaxel a potential radiosensitizer because these phases are more radiosensitive.[8] In the clinical trials performed so far, paclitaxel has shown particularly encouraging activity against advanced ovarian cancer.[9]

Several studies reported the mechanisms of interaction between paclitaxel and radiation in cell lines,[10–13] and particularly in ovarian tumors,[11–13] and between docetaxel and radiation in human

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Correspondence to J-L Merlin

leukemia HL-60[14] and in HeLa cells.[15] Tishler *et al.*[16] reported using astrocytoma cells that paclitaxel-treated cells exhibited an enhancement of radiosensitivity only when irradiated in G_2/M. However, other studies have shown no sensitization on human cervical carcinoma cells when paclitaxel-treated cells were irradiated.[17] These evaluations were mostly performed using clonogenic assays. In this paper, we report the evaluation of interaction between taxoid derivatives (docetaxel and paclitaxel) and irradiation in two human ovarian tumour cell lines using the sulforhodamine B (SRB) assay and multicellular tumor spheroids (MTS) growth inhibition assay.

The SRB test quantifies indirectly viable cells by measuring total protein content,[18] and appeared to be more sensitive than the tetrazolium assay with a better linearity with cell number and higher reproducibility.[19,20]

MTS of human ovarian cells constitutes a useful model of solid tumor, *in vitro*.[21] Growing in three dimensions with central necrosis, spheroids have several other features in common with solid tumors, such as heterogeneity in cellular kinetics and the presence of hypoxic cells.[22]

In the present work, we evaluated the effectiveness of a combined treatment using taxoid derivatives and irradiation in ovarian adenocarcinoma cells growing as tridimensional spheroids in comparison with cells growing as monolayers.

Material and methods

Chemicals

Paclitaxel (Taxol®, NSC 125973; Sigma, St Quentin Fallavier, France) and docetaxel (Taxotere®, RP 56976, NSC 628503; Rhône-Poulenc Rorer SA, Vitry-sur-Seine, France via Dr MC Bissery) were received as a powder. Stock solutions (10 mM) were prepared in ethanol. Dilutions were prepared in sterilized water extemporaneously before each experiment. The final concentration of ethanol never exceeded 0.5%.

Cell lines

Two human ovarian cell lines were used. The A2780 cell line was provided generously by Professor EA De Bruijn (Laboratory of Cancer Research and Clinical Oncology, University of Wilrijk, Belgium). The CAVEOC-2 cell line was established and characterized in our laboratory, derived from a human ovarian endometrioid adenocarcinoma.[23] These cell lines were continuously maintained as monolayer cultures in RPMI 1640 medium (Gibco, Eragny, France) supplemented with 10% inactivated fetal calf serum (Dutscher, Brumath, France), penicillin (500 UI/ml), gentamycin (5 μg/ml), hydrocortisone (0.5 mg/ml), transferrin (2.5 μg/ml), epidermal growth factor (10 μg/ml) and glutamine (0.3 mg/ml). Cell culture were passaged every 7 days to ensure exponential growth.

Irradiation procedure

All irradiations were performed using a Theratron 780C ^{60}Co unit (Theratronics, Ottawa, Canada) operating at 1.25 MeV. Cells and spheroids were irradiated at a dose rate of 5 Gy/min at room temperature. Doses ranged from 0 to 8 Gy (0, 2, 4, 6 and 8 Gy).

Analysis of dose–response curves

Dose–response curves of the two cell lines obtained from the SRB test were fitted using the linear-quadratic model

$$SF = e^{-\alpha D - \beta D^2}$$

From this equation, the parameters α and the surviving fraction (SF) at 2 Gy (SF_2)[24] were deduced.

Cytotoxicity was evaluated from the SRB test and the concentration of taxoid required to inhibit 50% of cellular growth (IC_{50}) was calculated according to the median-effect principle.[25]

SRB assay

The SRB assay was performed according to the method of Skehan *et al.*[18] as already described.[19] The cells were harvested from exponential phase culture by trypsinization, counted and plated in 96-well microtiter plates. The optimal seeding densities of each cell line were determined to ensure adequate absorbance readings in control wells during a 14 day assay. An initial concentration of 200 cells/well was found suitable for the two cell lines. All experiments were performed with exponentially growing cells treated with paclitaxel or docetaxel for 72 h before irradiation. The duration of exposure of cells to taxoids was chosen according to the doubling time of these cell lines, so that all cells could divide at least once. Taxoid concentrations

used corresponded to concentrations which inhibited 10% (IC_{10}), 20% (IC_{20}) and 30% (IC_{30}) of cellular growth.

After 72 h exposure, culture medium was removed from the wells in order to eliminate the drugs and replaced by fresh medium. The cells were then irradiated. Following an additional 10 day incubation, the effect of taxoid/radiation association was estimated by performing the SRB assay. Briefly, the cells were washed with PBS and fixed by means of protein precipitation with trichloroacetic acid at 4°C for 1 h. After washing with tap water, the cells were stained with SRB solution (Aldrich-Chimie, St Quentin Fallavier, France). Protein bound stain was solubilized with unbuffered Tris base [tris (hydroxymethyl) aminomethane] (Merck, Darmstadt, Germany). The optical density was read at 540 nm on a Titertek Multiskan MCC/340 microplate reader (Flow Laboratories, Les Ulis, France).

Each dose level was plated in triplicate, experiments were repeated three times. Results are expressed as relative absorbance as compared with untreated controls. Drug concentration (nM) inhibiting the cellular growth by 10, 20, 30 and 50% was calculated using the median-effect principle.[25]

MTS

A2780 and CAVEOC-2 cell lines were plated according to the modified method of Yuhas *et al.*[26] and described by Griffon *et al.*[21] Briefly, six-well plates, coated with 0.5% agarose (Prolabo, Paris, France) in order to inhibit attachment to the bottom of the wells, received 2 ml of cellular suspension containing 10^4 cells/ml and were incubated at 37°C, in 5% CO_2. At 2–3 days later, spheroids with a diameter of approximately 300 μm developed and were individually seeded in 24-well plates coated with 0.5% agarose and treated. The taxoid concentrations used were 0.01, 0.1 and 1 nM. Each concentration corresponded to a growth inhibition of 10–40% of control spheroids. After 72 h incubation, drugs were eliminated by removing the culture medium and spheroids were irradiated and incubated at 37°C, in 5% CO_2. The growth of MTS was determined by measuring, twice per week, the diameter which was converted into volume, assuming spherical geometry ($V = 1/6\pi D^3$).

Each experiment was repeated in triplicate and at least eight spheroids used for each drug dilution. Taxoids associated with radiation were tested concurrently.

Sixteen days after treatment, the residual/control MTS individual volume ratio after 2 Gy irradiation (RSV_2) was calculated mathematically from the fitted dose–response curves constructed by plotting the normalized ratio of $V_{control}/V_{irradiated}$ versus the irradiation dose.[21] MTS were considered as 'cured' when they 'shrunk' within 16 days after irradiation. The proportion of 'cured' MTS was calculated as the fraction of 'shrunk' spheroids relative to the total number of spheroids originally present in the treated population.[21] Then, spheroids control curves were plotted and the dose required to reduce the number by 50% as compared with the control (SCD_{50}) according to Stuschke *et al.* and Schwachöfer *et al.*[27,28] was calculated mathematically.

Cytotoxicity of two taxoids was evaluated from the concentration of agent required to reduce 50% of spheroids (SCC_{50}) which was calculated in the same way as SCD_{50}.

The surviving fraction at 2 Gy and α linear-quadratic parameter values were calculated from control data spheroids (SF_{2sph}, α_{sph}) after treatment according to Stuschke *et al.*[28]

Analysis of drug/radiation interactions

Combined effects of taxoids and radiation were analyzed from isobolograms where an additivity envelope was plotted.[29,30]

Statistical analysis

Data obtained from irradiated cells with or without taxoids were analyzed using the Mann–Whitney's *U*-test.

Data obtained from cells irradiated with paclitaxel and with docetaxel as well as RSV_2, SF_{2sph} and α parameters was analyzed using Wilcoxon's test.

The correlation between SCD_{50} and RSV_2 parameters values was analyzed using Spearman's rank test.

A significance level of $p < 0.05$ was used throughout.

Results

Dose–response curves for taxoids

Dose–reponse curves for cells in monolayer to paclitaxel and docetaxel are illustrated in Figure 1. IC_{50} values of paclitaxel and docetaxel tested against A2780 cell line were 5.51 ($\pm$ 2.12) and 1.77

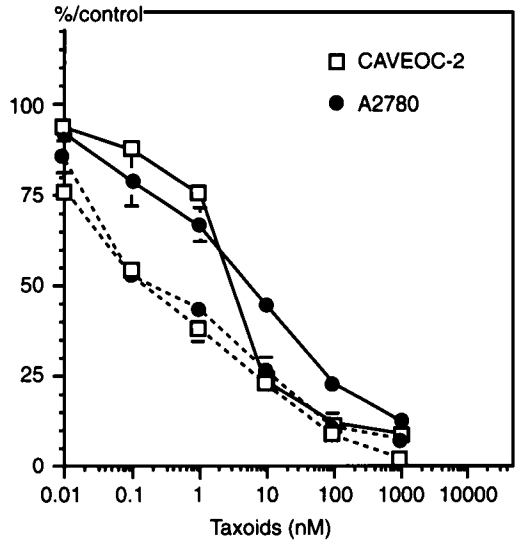

Figure 1. Taxoid responsiveness of A2780 and CAVEOC-2 human ovarian tumor cell lines: paclitaxel (solid line) or docetaxel (broken line). Taxoids were added to asynchronous cell populations for 72 h. Error bars represent SEM.

(± 0.20) nM, respectively. Those tested against CAVEOC-2 cell line were 3.33 (± 0.26) and 0.68 (± 0.28) nM, respectively. Docetaxel was found to be more potent than paclitaxel. Docetaxel was 3- to 5-fold more active than paclitaxel.

Dose–response curves for spheroids as a function of concentration of each taxoid were plotted 16 days after treatment and are illustrated in Figure 2.

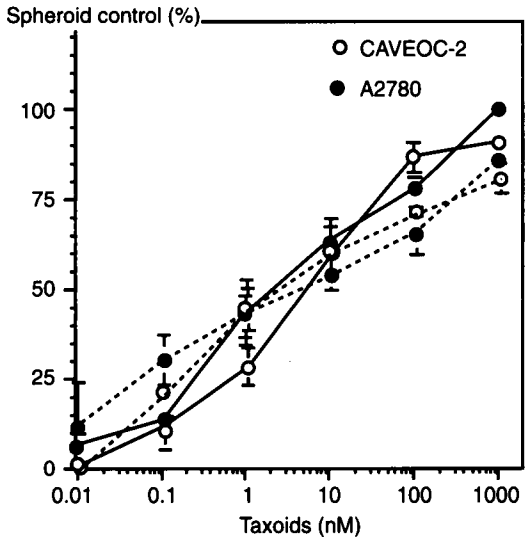

Figure 2. Taxoid responsiveness of MTS obtained from A2780 and CAVEOC-2 cell lines: paclitaxel (solid line) or docetaxel (broken line). Taxoids were added to spheroid populations for 72 h. Error bars represent SEM.

Concentration values of paclitaxel and docetaxel required to reduce 50% of spheroids from A2780 cells (SCC_{50}) were 3.28 (± 0.49) and 0.59 (± 0.32) nM, respectively. Those of spheroids from CAVEOC-2 cells were 1.51 (± 0.19) and 0.70 (± 0.36) nM, respectively. Docetaxel was again 3- to 5-fold more active than paclitaxel. However, no correlation was observed between IC_{50} and SCC_{50}.

Dose–response curves for graded doses of radiation combined with three fixed concentrations of docetaxel or paclitaxel

Figure 3 illustrates dose–response curves of A2780 and CAVEOC-2 cell lines in monolayers treated with three different concentrations of paclitaxel or docetaxel (IC_{10}, IC_{20}, and IC_{30}) associated with radiation. From these curves, CAVEOC-2 cells were not radiosensitized by taxoids, conversely to A2780 cells. This observation was confirmed by the radiosensitivity parameters (SF_2 and α) reported in Table 1. The comparison of these parameters values in the CAVEOC-2 cell line obtained with or without taxoids did not show any statistical difference, conversely to A2780 cells ($p < 0.05$). While the linear component of the survival curve, α, was increased by taxoids approximately 25 times and the surviving fraction at 2 Gy decreased by 32% with taxoids in A2780 cells, the values of radiosensitivity parameters in the CAVEOC-2 cell line remained almost unchanged as compared to those obtained with radiation alone.

Dose–response curves of MTS

Dose–response curves of MTS of two cell lines were similar whatever the taxoid (Figure 4). From these curves defined by a logarithmic equation, values of the residual volume of spheroids at 2 Gy (RSV_2) were calculated and are reported in Table 2. The comparison between these values obtained with the paclitaxel/irradiation and docetaxel/irradiation associations did not reveal any statistical difference whatever the taxoid concentration used. This was in agreement with results obtained with the SRB assay in monolayer cultures.

In all cases, taxoids induced a decrease of the RSV_2 parameter. In the A2780 cell line, RSV_2 was decreased progressively by 40–80% as a function to paclitaxel concentration and by 46–83% as a function to docetaxel concentration. In the CAVEOC-2

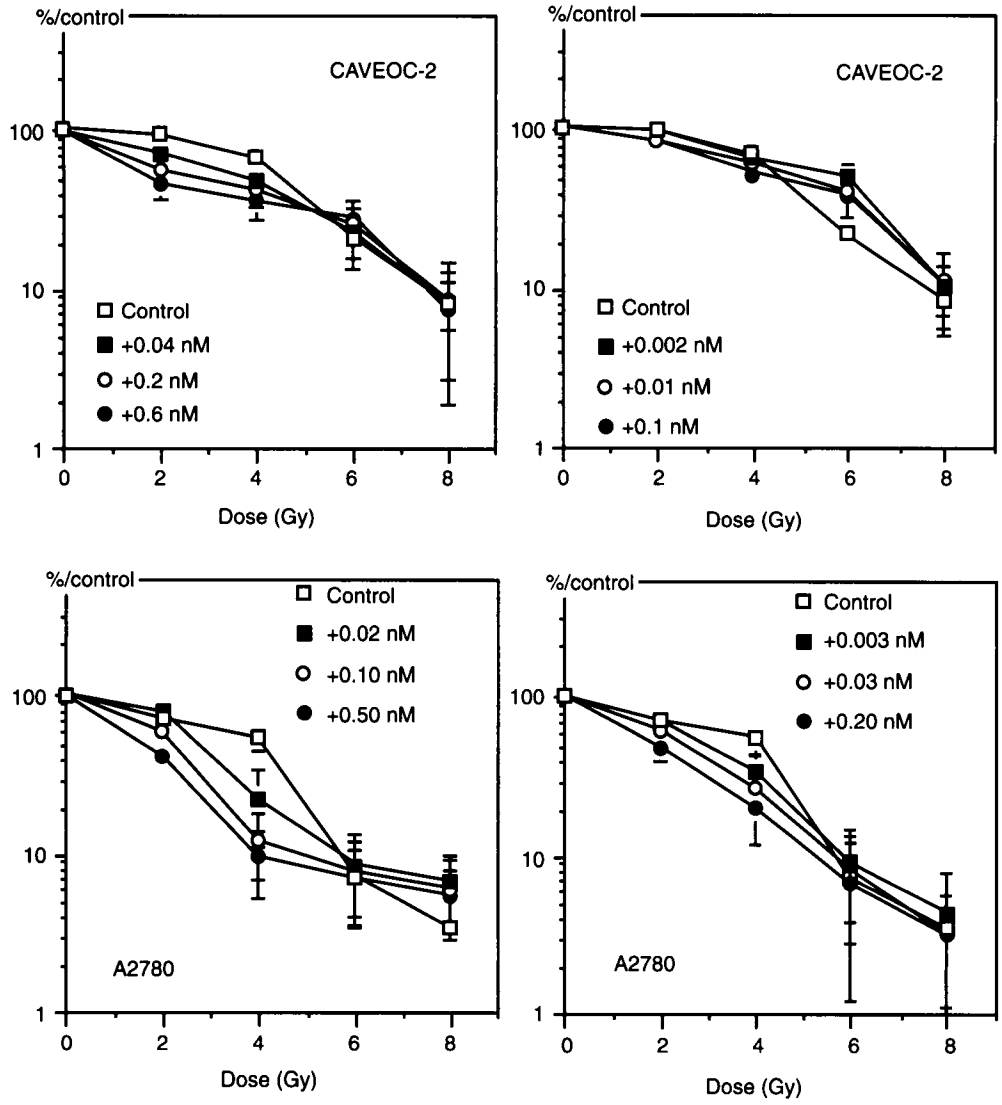

Figure 3. Dose–response curves of CAVEOC-2 and A2780 cell lines in monolayers irradiated after treatment for 72 h in the absence or presence of taxoids. For each concentration of taxoid, values were calculated as compared to non-irradiated control. Each point represents the mean (± SEM) of three wells and each assay was performed in triplicate.

cell line, the reduction of RSV_2 by taxoids was less important by 34–55% with paclitaxel and by 37–47% with docetaxel. However, a statistical difference ($p < 0.05$) was observed between parameters obtained from irradiated spheroids without taxoids and those irradiated after exposure to taxoids.

In monolayer culture, no statistical difference was documented in CAVEOC-2 cells.

Spheroid control curves

Dose–effect curves representing the relative number of cured MTS 16 days after irradiation alone or after 72 h exposure with taxoids were plotted (Figure 5). As observed for the dose–response curves, spheroid control curves of each cell line were similar for both taxoids. The dose required to reduce the spheroids number by 50% compared with the control (SCD_{50}) is reported in Table 2. In all cases, taxoids induced a decrease in SCD_{50}. In A2780 cells, from 46 to 72% with paclitaxel and from 58 to 78% with docetaxel related to the concentration. In CAVEOC-2 cells, SCD_{50} was decreased from 5 to 34% with paclitaxel and from 8 to 32% with docetaxel. A significant correlation was found between SCD_{50} and RSV_2 (paclitaxel: $p < 0.01$, $r = 0.740$; docetaxel: $p < 0.01$, $r = 0.799$) which means that tumor control

Table 1. Radiosensitivity parameters, SF_2 and α, calculated from response–dose curves of A2780 and CAVEOC-1 cell lines in monolayers (SRB assay): cells irradiated after treatment without (control) or with paclitaxel or docetaxel at three different concentrations (IC_{10}, IC_{20} and IC_{30})

	SF_2				α (Gy^{-1})			
	Control	IC_{10}[a]	IC_{20}	IC_{30}	Control	IC_{10}	IC_{20}	IC_{30}
Paclitaxel								
A2780	0.75 (± 0.02)[b]	0.52 (± 0.08)	0.50 (± 0.10)	0.46 (± 0.09)	0.01 (± 0.01)	0.299 (± 0.090)	0.352 (± 0.100)	0.369 (± 0.100)
CAVEOC-2	0.85 (± 0.03)	0.81 (± 0.02)	0.72 (± 0.07)	0.73 (± 0.04)	0.027 (± 0.020)	0.04 (± 0.02)	0.110 (± 0.070)	0.190 (± 0.080)
Docetaxel								
A2780	0.75 (± 0.02)	0.62 (± 0.06)	0.54 (± 0.06)	0.42 (± 0.08)	0.01 (± 0.01)	0.157 (± 0.006)	0.258 (± 0.040)	0.399 (± 0.100)
CAVEOC-2	0.85 (± 0.03)	0.89 (± 0.06)	0.85 (± 0.02)	0.79 (± 0.04)	0.027 (± 0.020)	0.0	0.011 (± 0.010)	0.03 (± 0.04)

[a]Concentrations (nM) of taxoid derivate associated with radiation: for paclitaxel: IC_{10}: 0.02 (A2780); 0.04 (CAVEOC-2); IC_{20}: 0.10 (A2780); 0.20 (CAVEOC-2); IC_{30}: 0.50 (A2780); 0.60 (CAVEOC-1); for docetaxel; IC_{10}: 0.003 (A2780); 0.002 (CAVEOC-2); IC_{20}: 0.03 (A2780); 0.01 (CAVEOC-2); IC_{30}: 0.20 (A2780); 0.10 (CAVEOC-2).
[b]Mean ± SEM.

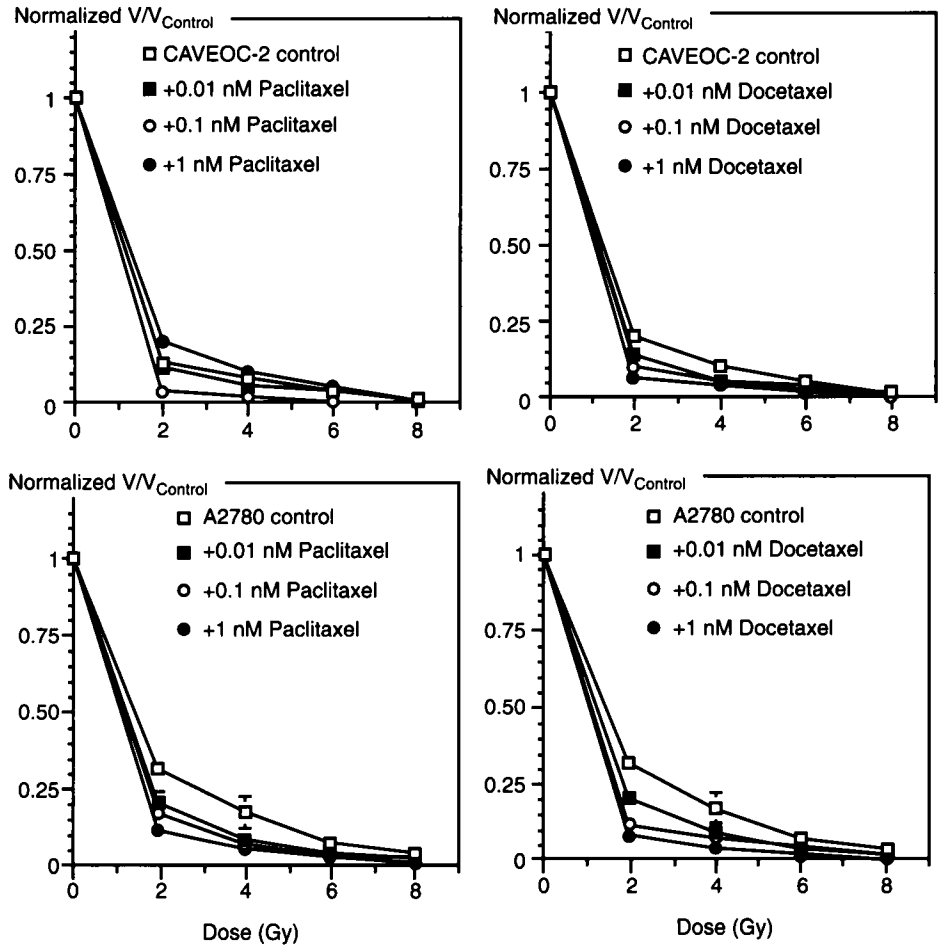

Figure 4. Dose–response curves of spheroids from A2780 and CAVEOC-2 cell lines 16 days after irradiation alone or associated with paclitaxel or docetaxel at three different concentrations (0.01, 0.1 and 1 nM). Each point represents the mean (± SEM) of three experiments.

Table 2. Radiosensitivity parameters, RSV_2 and SCD_{50}, calculated from dose–response or spheroid control curves of A2780 and CAVEOC-2 spheroids after irradiation alone (control) or treated for 72 h with paclitaxel or docetaxel

	RSV_2[a]				SCD_{50}[b] (Gy)			
	Control	C1[c]	C2	C3	Control	C1	C2	C3
Paclitaxel								
A2780	0.30 (± 0.06)[d]	0.18 (± 0.02)	0.10 (± 0.04)	0.06 (± 0.06)	4.46 (± 2.77)	2.37 (± 0.67)	1.86 (± 0.11)	1.25 (± 0.67)
CAVEOC-2	0.38 (± 0.03)	0.25 (± 0.03)	0.21 (± 0.05)	0.17 (± 0.06)	3.90 (± 1.90)	3.70 (± 1.15)	2.96 (± 0.56)	2.58 (± 0.39)
Docetaxel								
A2780	0.30 (± 0.06)	0.16 (± 0.03)	0.05 (± 0.03)	0.05 (± 0.03)	4.46 (± 2.77)	1.85 (± 0.36)	1.06 (± 0.36)	0.96 (± 0.25)
CAVEOC-2	0.38 (± 0.03)	0.24 (± 0.04)	0.20 (± 0.04)	0.20 (± 0.11)	3.90 (± 1.90)	3.57 (± 0.80)	2.93 (± 0.26)	2.64 (± 0.56)

[a]Residual spheroid volume at 2 Gy.
[b]Dose required to reduce the spheroid number by 50% as compared with untreated control.
[c]C1: 0.01 nM, C2: 0.1 nM, C3: 1 nM.
[d]Standard deviation.

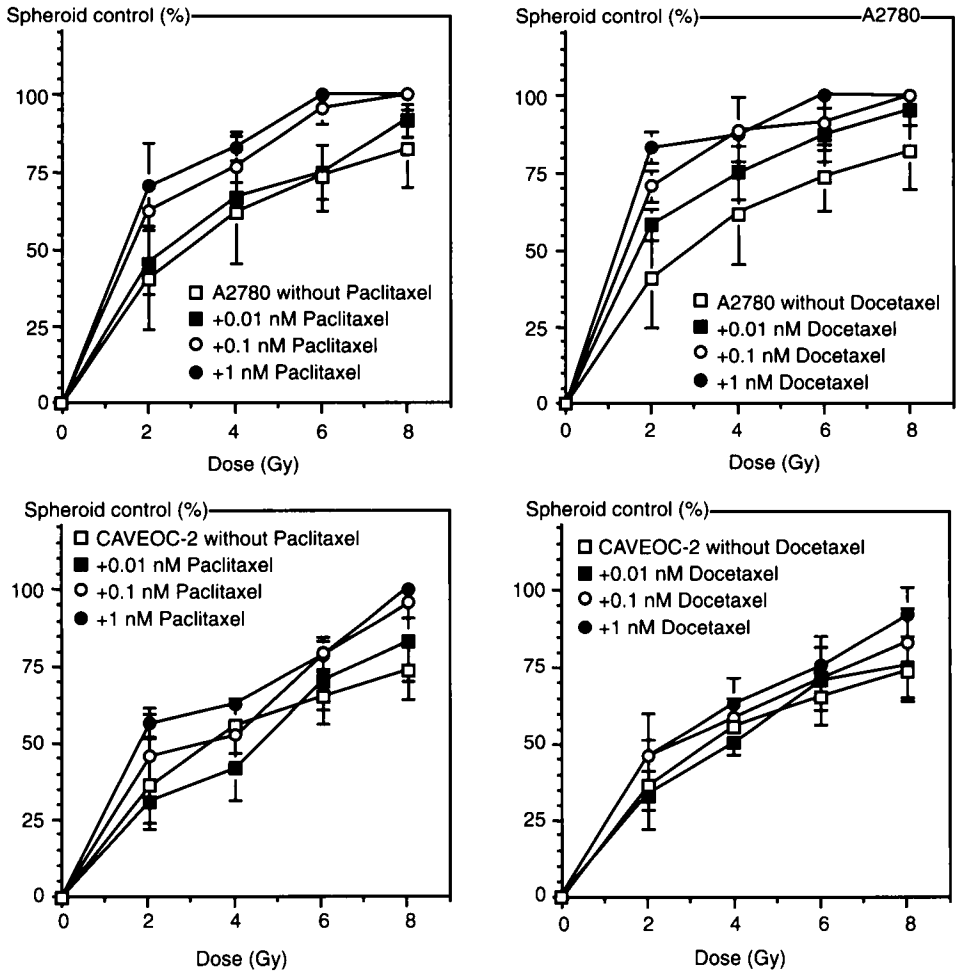

Figure 5. Spheroid control curves of A2780 and CAVEOC-2 cell lines 16 days after irradiation alone or associated with paclitaxel or docetaxel at three different concentrations (0.01, 0.1, 1 nM). Each point represents the mean (± SEM) of three experiments.

(SCD_{50}) was correlated with volume regression (RSV_2).

SF_{2sph} and α_{sph} parameters

Values of SF_{2sph} and α_{sph} were calculated from controlled spheroids of each cell line and are reported in Table 3. By comparing RSV_2 and SF_{2sph} parameters, no statistical difference was observed in the cases of association ($p = 0.062$).

Analysis of interaction

For a given iso-effect level (surviving fraction), the enhancement ratio by the combined treatment for cells in both spheroids and monolayers was calculated from Figures 3 and 5, respectively. The enhancement ratio was defined as the ratio of a concentration of paclitaxel or docetaxel alone for a given level of surviving fraction to that irradiation dose combined with a fixed concentration of each taxoid that would give the same surviving fraction. The results are described in Table 4 and showed globally that taxoids and radiation interacted additively.

Discussion

The data presented here demonstrated that the radiosensitizing effect of paclitaxel and docetaxel was not constant in the two ovarian cell lines. As no study was reported concerning docetaxel with irradiation associations in ovarian tumors, we compared our data with results obtained with paclitaxel.

Several authors[10–17,31] reported that paclitaxel is thought to act as a radiosensitizer through its ability to block cells in mitosis (G_2/M), which is the most radiosensitive phase of the cycle.[8] However, the production of a G_2/M block by taxoid derivatives does not automatically confer an increase sensitivity to radiation,[11,12] as we observed with CAVEOC-2

Table 3. Radiosensitivity parameters, SF_{2sph} and α_{sph}, calculated from spheroid control curves of A2780 and CAVEOC-1 cell lines: cells irradiated after exposure to paclitaxel or docetaxel at three different concentrations (IC_{10}, IC_{20} and IC_{30})

	SF_{2sph}				α_{sph} (Gy^{-1})			
	Control	IC_{10}[a]	IC_{20}	IC_{30}	Control	IC_{10}	IC_{20}	IC_{30}
Paclitaxel								
A2780	0.44 (± 0.01)[b]	0.36 (± 0.08)	0.13 (± 0.08)	0.12 (± 0.08)	0.41 (± 0.13)	0.50 (± 0.05)	0.85 (± 0.13)	0.91 (± 0.11)
CAVEOC-2	0.44 (± 0.06)	0.35 (± 0.11)	0.37 (± 0.14)	0.20 (± 0.15)	0.40 (± 0.06)	0.39 (± 0.35)	0.74 (± 0.26)	0.74 (± 0.28)
Docetaxel								
A2780	0.44 (± 0.11)	0.36 (± 0.11)	0.04 (± 0.01)	0.05 (± 0.00)	0.41 (± 0.13)	—	1.00 (± 0.00)	1.00 (± 0.00)
CAVEOC-2	0.44 (± 0.06)	0.40 (± 0.08)	0.33 (± 0.11)	0.25 (± 0.01)	0.40 (± 0.06)	0.45 (± 0.10)	0.55 (± 0.17)	0.66 (± 0.01)

[a]Concentrations (nM) of taxoid derivate associated with radiation: for paclitaxel: IC_{10}: 0.02 (A2780); 0.04 (CAVEOC-2); IC_{20}: 0.10 (A2780); 0.20 (CAVEOC-2); IC_{30}: 0.50 (A2780); 0.60 (CAVEOC-1); for docetaxel; IC_{10}: 0.003 (A2780); 0.002 (CAVEOC-2); IC_{20}: 0.03 (A2780); 0.01 (CAVEOC-2); IC_{30}: 0.20 (A2780); 0.10 (CAVEOC-2).
[b]Mean ± SEM.

Table 4. Analysis of interactions encountered in associating taxoids exposure for 72 h followed by irradiation in A2780 and CAVEOC-2 ovarian cell lines

	Paclitaxel		Docetaxel	
	Monolayer	Spheroids	Monolayer	Spheroids
A2780	supra-additive	additive	IC_{10}: supra-additive IC_{20}: additive IC_{30}: additive	additive
CAVEOC-2	additive	additive	additive	additive

cells in monolayer culture where no significant difference was observed between radiosensitivity parameters calculated from irradiated cells with or without taxoid derivates. Few studies have been published concerning radiopotentiation of ovarian adenocarcinomas by taxoids. Nevertheless, Steren *et al.*[13] verified the radiosensitivity effect of paclitaxel in three ovarian cell lines using the ATP bioluminescence assay. Our results are consistent with those of Liebman *et al.*[11,12] who analyzed four different cell lines among which one was not radiosensitized to paclitaxel. In the same way, Geard and Jones[32] observed no radiosensitization effect in a cervix cell line and observed that paclitaxel blocked the cells in G_2/M as well as in G_1 phase and suggested that the effects of this compound on relatively radioresistant phases of the cell cycle would explain the lack of radiation sensitization in this cell line. Consequently, in addition to cell cycle perturbation induced by paclitaxel, other different mechanisms that would allow us to potentiate the response to radiation and modality of radiosensitization by paclitaxel are not clearly known. Recently, Milas *et al.*[33] showed by determining the effects of the treatments in murine mammalian cells *in vivo* by tumor growth delay and the radiation dose required to control 50% of the tumors (TCD_{50}) that paclitaxel was a potent enhancer of tumor radioresponse and that its effect was mediated by reoxygenation of hypoxic tumor cells.

In monolayer cultures, using the linear-quadratic model of radiation survival curves, we are able to account for the ability of paclitaxel and docetaxel to radiosensitize cell lines. In each cell line, taxoids derivatives increased the α component of the radiation survival curve and decreased the fraction surviving at 2 Gy (SF_2). Some authors[12,34] reported that the mitotic cells presented a high α parameter. The effect of taxoids increased the value, so a cellular population presenting a low α parameter would be expected to be sensitized to radiation by taxoids. Conversely, cells with a high α parameter would acquire little additional radiation sensitivity after exposure to taxoids.[12] This suggestion is in agreement with our results: CAVEOC-2 cells which have a higher α ($0.03\ Gy^{-1}$) than A2780 cells ($0.01\ Gy^{-1}$) were less sensitized to radiation.

Liebmann *et al.*[11] concluded and suggested that these results could have two implications for clinical radiation therapy.[1] In tumors having a high α parameter such as melanomas, small cell lung cancers and some ovarian tumors, taxoids may not induce any radiosensitizing effect. Conversely, tumors having a low α parameter may be radiosensitized by taxoids.[2] The α parameter defines the intrinsic radiosensitivity at relatively low doses of radiation, consequently these results suggested that taxoids may be radiation sensitizers for radiation doses below 2 Gy which are within the range of dose normally used in clinics. In addition, radiosensitization was observed for taxoid concentrations which are within the range of plasma concentrations achieved in clinical situations.[15]

A2780 cells have shown a synergistic interaction between paclitaxel and radiation as already reported in human astrocytoma[16] and cervix carcinoma cells.[17]

In A2780 cells, with docetaxel, we only observed a synergistic interaction with radiation at low concentrations that became strictly additive as the concentration increased. This could be explained by the fact that the radiosensitizing effect can be only achieved if the cells are blocked in G_2/M phase but remain viable to be treated by radiotherapy. As already mentioned by Minarik and Hall[35] with paclitaxel in cervix carcinoma cells, high concentrations are able to induce not only G_2/M arrest but also cell death.

In CAVEOC-2 cells, no radiosensitization by taxoids has been observed, as already encountered by Stromberg *et al.*[15] in three human carcinoma cell lines with paclitaxel.

In MTS, although the culture modalities were quite different, in two cases, the radiosensitizing effects of paclitaxel and docetaxel were observed in A2780 cells. In CAVEOC-2 cells, whereas no significant difference was observed in monolayer culture, a significant difference was observed between RSV_2 parameters. This could be explained, in part, by higher drug incorporation and fixation through the multiple cell layers of the spheroids than in monolayers.[36,37] These phenomenon would then induce higher accumulation of cells in G_2/M phase. Milas *et al.*,[10] studying in murine mammalian cells *in vivo*, concluded that destroying cells blocked in the mitotic phase, an increase of apoptosis and tumor reoxygenation were mechanisms, in part, reponsible for tumor radiopotentiation by taxoids. In addition, another important factor could the presence of quiescent cells whose proportion depended on the spheroid size and which did not grow in monolayer culture.

MTS isobologram analyses gave different results than in monolayers. Indeed, paclitaxel as well as docetaxel induced an additive interaction in spheroids of the two cell lines whatever the drug concentration and the irradiation dose. This could support the hypothesis of a more important accumulation and fixation of taxoid derivatives in spheroids

than in monolayers. However, it was difficult to confirm this hypothesis without analyzing the intracellular level of paclitaxel and docetaxel in the inner and outer cell layers of spheroids.

Moreover, this evaluation of MTS confirms the power of the new parameter RSV_2 which correlates with the standard parameter SCD_{50} as well with paclitaxel/irradiation ($r = 0.749$) as docetaxel/irradiation associations ($r = 0.799$). We have reported this previously when evaluating the radiosensitivity of MTS obtained from human ovarian cancers,[21] which means that tumor regression kinetic (RSV_2) was related to tumor control (SCD_{50}). Whereas the surviving fraction at 2 Gy (SF_2) characterized the intrinsic radiosensitivity of cells,[24] the residual volume at 2 Gy evaluated the MTS radiosensitivity. SF_2 concerned single cells with or without capacity of proliferate after irradiation, RSV_2 concerned the cell population as a whole with intercellular communications.[38] However, in order to quantify the association between radiosensitivity of cell lines and radiation response of MTS, Stuschke *et al.*[28] deduced parameters values of SF_2 and α from spheroid control curves. Values of SF_{2sph} obtained from irradiation alone (44%) were lower than those obtained from the SRB assay (75–85%). Consequently, values of α_{sph} (0.41 Gy^{-1}) were higher than those obtained from the SRB assay (0.027–0.01 Gy^{-1}). A possible explanation for these discrepancies could be the different cell culture methods.

In conclusion, radiosensitization was not constantly induced by paclitaxel and docetaxel in human ovarian tumor cell lines whatever the culture technique used. Nevertheless, in monolayers, the radiosensitization appears to be largely due the ability of taxoids to increase the α parameter of the radiation survival curves of cells which are sensitive to the drugs. Cells which would be most sensitized to radiation by taxoids would have a low intrinsic α parameter. Further studies would be necessary in order to clear up mechanisms of radiopotentiation by taxoids, particularly in spheroids. The use of a tridimensional culture such as MTS would be appropriate for these studies because they simulate in part the conditions found in the peritoneum, where nodular structures similar to spheroids are found at advanced stages of growth of ovarian tumors.[39] In addition, MTS contain not only tumor cells, but also quiescent cells and extracellular matrix components which are not found in monolayer cultures. Therefore, this model could prove useful to investigate the consideration of taxoid derivatives and irradiation in other type of cancers.

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