

EFFECTS OF DOCETAXEL IN COMBINATION WITH RADIATION ON HUMAN HEAD AND NECK CANCER CELLS (ZMK-1) AND CERVICAL SQUAMOUS CELL CARCINOMA CELLS (CASKI)

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The purpose of this study was to determine, as we did for paclitaxel, the cytotoxic and radiosensitizing potential of docetaxel in human head and neck cancer cells (ZMK-1), and in cervical squamous cell carcinoma cells (CaSki). ZMK-1 cells were incubated with docetaxel for 3, 9 or 24 hr before irradiation and 24 hr after irradiation. CaSki cells were incubated with docetaxel 24 hr before and after irradiation. For ZMK-1 cells, the docetaxel concentrations (0.7, 0.7 and 0.35 nM) were determined to obtain approximately equivalent cell survival at the different incubation times (3, 9 and 24 hr, respectively). For CaSki cells, the necessary concentration of docetaxel was 0.07 nM. Radiation doses were given from 0 to 7 Gy. Cell survival was measured by a standard clonogenic assay after a 9-day incubation. Flow cytometry was used to measure the capacity of docetaxel to accumulate cells in the G2/M phase of the cell cycle. We observed a weak accumulation of cells in the G2/M phase for the ZMK-1 cells and a pronounced accumulation for CaSki cells. For docetaxel incubation before irradiation, the isoeffect enhancement ratios for ZMK-1 cells determined at the 37% survival level were 1.18, 2.01, and 2.40 for pre-incubation at 3, 9 and 24 hr, respectively; for CaSki cells the ratio was 1.44. For a docetaxel incubation of 24 hr after irradiation, the isoeffect enhancement ratios determined at the 37% survival level were 1.54 and 1.17 for the ZMK-1, and CaSki cells, respectively. A radiosensitizing effect of docetaxel could be demonstrated unambiguously in the two cell lines used. In contrast to our previously published results with paclitaxel, docetaxel seems to be a better radiosensitizer than paclitaxel.

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Key words: radiotherapy; docetaxel; radiosensitisation; SCC-cell lines

Paclitaxel (PAC) and docetaxel (DOC) are the prototypes of a new class of microtubule-targeting diterpenoids referred to as taxanes. PAC was first isolated from the bark of the Pacific yew *Taxus brevifolia*. DOC, a hemisynthetic analogue of PAC, was prepared using English yew *Taxus baccata* needle extracts. PAC and DOC are cytotoxic against proliferating mammalian cells *in vitro*. PAC binds specifically to microtubules, alters their dynamics, promotes the reorganization of the microtubular network into bundles or asters and stabilizes microtubules against disruption by various agents. DOC produces similar effects, yet it appears more potent than PAC on a molar basis (reviewed in ref. 1).

In clinical trials PAC and DOC have already been proved to have potent antineoplastic activity against a variety of advanced solid human tumors,² including an encouraging cytotoxic activity of DOC in first- and second-line therapy for patients with head and neck tumors. The mode of action of the taxoids suggests an accumulation of cells in the radiosensitive G2/M-phase of the cell cycle and consequently a cell cycle-specific radiosensitization. Phase I/II trials reported on the feasibility and high response rates of combined DOC (weekly application) and radiation treatment, with mucositis and neutropenia being the most relevant side effects. Severe cutaneous, neurologic and pulmonary toxicity were also dose limiting. The maximal tolerated weekly dose remains undefined.³

The results of numerous reports on *in vitro* radiosensitization by PAC, involving solid as well as hematopoietic tumor cell lines, remained inconsistent. Only a few publications have compared the effectiveness of DOC and PAC.^{4–6}

We reported on the radiosensitizing as well as the radioprotective effects of PAC using gynecological and head and neck tumor cell lines.^{7,8} The effects were dependent on the concentrations applied, as well as the timing schedule of PAC and irradiation. The aim of the present study was to determine the cytotoxic and radiosensitizing effect of DOC in two squamous cell carcinoma cell lines, ZMK-1, and CaSki. Furthermore, the results were compared with the effects of PAC in the same cell lines by using the same experimental methods published previously (Pradier et al., 1999).

MATERIAL AND METHODS

Cell culture and cell lines

The CaSki cervical squamous cell carcinoma cell line was obtained from the Tumor Bank of the Deutsches Krebsforschungszentrum, Heidelberg, Germany. The ZMK-1 cell line was established from a probe of a squamous cell carcinoma of the gingival mucosa; the tumor had not been treated before. ZMK-1 cells carry a p53 mutation, CaSki cells are known to contain wild-type p53 gene sequences but no wild-type p53 protein, due to an accelerated protein degradation by the human papillomavirus-16 or-18E6 oncoprotein. The doubling times for both cell lines are close to 24 hr, and their plating efficiencies are higher than 80%.

Both cell lines were cultured by standard methods and maintained as monolayers in minimum essential medium (MEM) supplemented with 10% FCS and 1% glutamine. Cells were incubated in a humidified air plus 5% CO₂ atmosphere at 37°C. All experiments were performed in exponentially growing cultures. Nunclon (Nunc, Roskilde, Denmark) 25 cm² culture flasks were used.

DOC treatment and irradiation

DOC (Taxotere®) was obtained from Rhône-Poulenc Rorer (Antony, France) and stored in frozen aliquots. Before use, it was thawed and diluted to the desired concentrations in the cell culture medium.

ZMK-1 cells were exposed to DOC for 3, 9 or 24 hr before irradiation or for 24 hr after irradiation. CaSki cells were exposed to DOC for 24 hr before irradiation, or for 24 hr after irradiation. Non-irradiated cultures, treated with DOC for the same incubation times, were used to normalize the survival curves for the effect of chemotherapy. In all experiments, drug treatment was stopped by a medium exchange. The DOC concentrations applied varied be-

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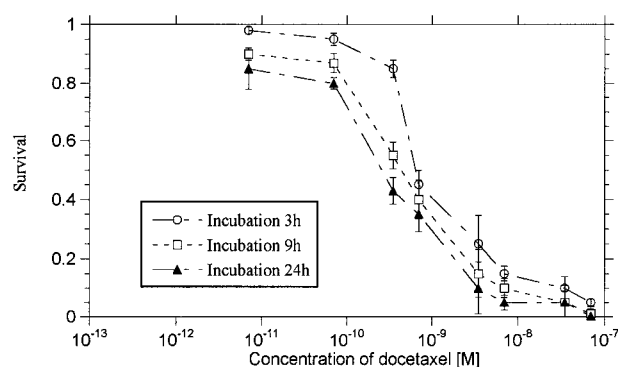


FIGURE 1 – Surviving ZMK-1 cells plotted as a function of docetaxel (DOC) concentration for different incubation times. The three curves represent the cytotoxic activity of DOC for 3, 9 and 24 hr incubations. Data represent mean values \pm SD. Error bars are standard deviations from three independent experiments.

tween 7×10^{-12} and 7×10^{-8} M, depending on the cell line used and the duration of the DOC incubation. To determine cytotoxicity, the DOC concentrations were first increased by 10^{-1} intervals and then further fractionated in the intervals with the steepest dose response in order to obtain a concentration-dependent survival curve as precise as possible.

Cells were irradiated with a Cobalt-60 source at a dose rate of 1.36 Gy/min. Radiation doses of 1 to 7 Gy were given as single doses. For each radiation dose DOC-treated cultures and non-treated control cultures were irradiated simultaneously. The cultures flasks were placed side by side on a Perspex plate (1 cm in diameter) to avoid an underdose in the bottom layer, as irradiation was delivered from the bottom of the flasks. Each culture flask was filled with 7 ml of medium, the medium level being approximately 3 mm above the cell layer.

Determination of cell survival

Cell survival was evaluated using a standard colony-forming assay. To measure clonogenic survival, cells were grown for 9 days, fixed with alcohol and stained with crystal violet. Colonies containing more than 50 cells were scored as survivors. Each experiment was performed at least three times, and each survival point was calculated from at least 12 single results.

To determine DOC cytotoxicity, 1,000 cells were plated in each of four culture flasks for each DOC concentration; 500 cells per culture flask were plated for the specific controls. To determine the radiation effects and the interaction of DOC and irradiation, 500 to 4,000 cells per culture flask were plated for low to high doses of radiation, respectively. All cells were allowed to attach for 24 hr; then the medium was removed, and fresh medium, or medium containing the desired DOC concentration was applied. At DOC concentrations at which no colonies were formed (zero survival), experiments were repeated with cell numbers up to 8,000 cells per culture flask. DOC survival data were plotted as functions of the DOC concentration, and the DOC concentrations that reduced the clonogenic survival to 40% to 50% of the untreated control were determined for each incubation time.

Radiation survival data were plotted as functions of the radiation dose, and sensitizer enhancement ratios (SERs) were calculated from the normalized survival curves at the 37% survival level using the KaleidaGraph software program (Synergy Software, Reading, CA, USA), version 3.08.

Isoologram analysis

DOC-radiation interactions were analyzed by constructing an envelope of additivity in an isoeffect plot, as described by Steel and Peckham⁹ and Kano *et al.*¹⁰ The additivity envelope was constructed

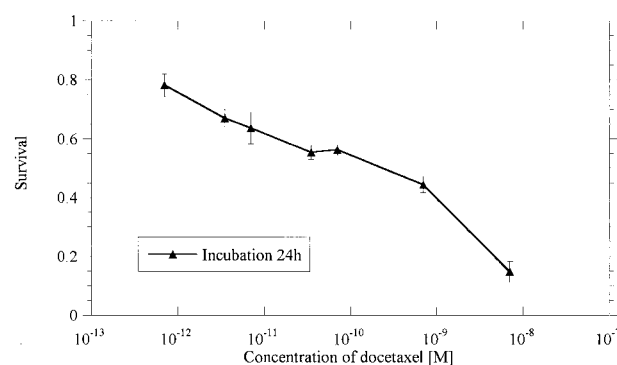


FIGURE 2 – Surviving CaSki cells plotted as a function of docetaxel (DOC) concentration for an incubation time of 24 hr. The curve represents the cytotoxic activity of DOC for a 24 hr incubation. Data represent mean values \pm SD. Error bars are standard deviations from three independent experiments.

TABLE 1 – INDUCTION OF APOPTOSIS OR NONAPOPTOTIC CELL DEATH BY DOCETAXEL (DOC) AND/OR RADIATION IN ZMK-1 AND CaSki CELLS¹

Cell type and treatment	Vital cells (%)	Mitotic figures (%)	Apoptosis (%)	Necrotic/lysed cells (%)
24 hours after treatment				
ZMK, C	91.02	0.73	3.45	4.81
ZMK + 4 Gy	92.00	1.05	3.62	3.33
ZMK + DOC	78.63	0.66	13.37	7.34
ZMK + DOC + 4 Gy	52.29	0.29	15.29	32.13
48 hours after treatment				
ZMK, C	93.61	1.35	1.26	3.78
ZMK + 4 Gy	74.62	0.89	8.58	15.90
ZMK + DOC	64.53	0.37	9.78	25.30
ZMK + DOC + 4 Gy	38.44	0.19	13.95	47.41
24 hours after treatment				
CaSki, C	92.58	3.13	1.07	3.22
CaSki + 4 Gy	89.67	3.68	1.99	4.67
CaSki + DOC	86.22	1.97	1.78	10.03
CaSki + DOC + 4 Gy	80.61	3.14	1.67	14.57
48 hours after treatment				
CaSki, C, 48 hr	95.08	2.27	0.57	2.08
CaSki + 4 Gy	82.61	0.44	3.30	13.65
CaSki + DOC	84.69	2.21	1.94	11.16
CaSki + DOC + 4 Gy	72.55	1.15	4.89	21.40

¹Percentages of vital cells, mitotic figures, apoptotic cells and necrotic/lysed cells were scored from fixed cells stained with acridine orange 24 or 48 hr after treatment.

by using the dose-response data for the individual agents to create an envelope that consisted of a mode I and a mode II line.

Once the envelope had been constructed, the experimental points for the chosen level of survival (*e.g.*, 0.37) were plotted in relation to the envelope and corresponded to the doses of the individual agents given in combination that resulted in the level of effect. If the experimental point was above the envelope, the interaction was termed *sub-additive*; if it was below the envelope, the interaction was termed *supra-additive*. If the plotted point was within the envelope, the interaction was termed *additive*, and the mechanism of the interaction was uncertain.

Flow cytometry

Both cell types, ZMK-1 and CaSki cells, were exposed to DOC for 0, 3, 9, 12, 24, 36 or 48 hr. After this, the cells were trypsinized,

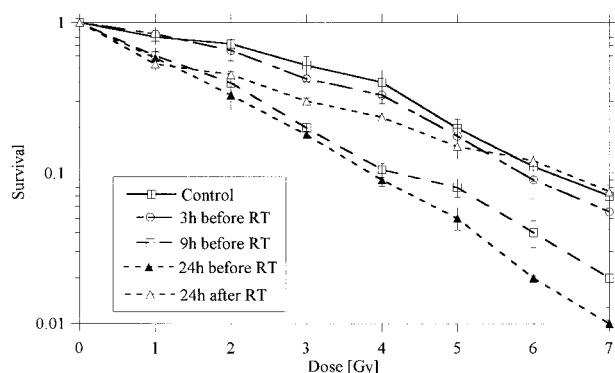


FIGURE 3 – Cytotoxic effect of radiation and DOC in ZMK-1 cells. Colony-forming assay with treatment of cells with DOC and escalating radiation doses. The cells were incubated immediately before radiation for durations of 3, 9 and 24 hr after radiation for a duration of 24 hr. The drug concentrations were 0.7, 0.7 and 0.35 nM, for incubation times of 3, 9 and 24 hr, respectively. The curves show the inhibitory effect of radiation alone (solid line), radiation with 3 hr of DOC incubation (open circles), radiation with 9 hr of DOC incubation (open squares) and 24 hr of incubation with DOC before (solid triangles) or after radiation (open triangles). Data represent mean values \pm SD. Error bars (shown when greater than the symbol) are standard deviations from three independent experiments.

washed twice and resuspended in PBS. For cell cycle analysis, 10^5 cells were fixed with 70% ice-cold ethanol and stained with 4,6-diamidino-2-phenylindole (DAPI). DNA measurements were performed on a PARTEC PAS III flow cytometer (Partec GmbH, Münster, Germany). Cell cycle histograms were analyzed by a standard flow cytometric software (MCYCLE), and the proportions of cells in G1, S and G2/M were determined for each time point. Each measurement was repeated two times, and mean values and standard deviations were calculated.

Determination of apoptosis

The presence of apoptosis, originally described by Kerr *et al.*,¹¹ was determined morphologically (fragmented nuclei, pyknotic appearance, cytoplasmic blebbing) in both cell lines. Cells ($50,000$ cells per cm^2) were plated on chamberslides and allowed to attach. Then the medium was replaced by DOC-containing medium or by fresh medium for specific controls, followed by a 24 hr incubation. Treatment was stopped by a second medium exchange. In experiments in which cells were also irradiated, they received 4 Gy immediately before the second medium exchange. After treatment, the cells grew for another 24 or 48 hr; then the medium was removed, the chambers were dismantled and the slides were fixed in alcohol and stained with 0.01% acridin-orange solution (Merck, Darmstadt, Germany). These times have been established previously as suitable for the detection of apoptosis in adherent cells. For each experimental point, 10×100 cells out of two individual chambers were scored for the appearance of apoptosis. Additionally, the method allows for scoring of mitotic figures and necrotic/lysed cells.

RESULTS

DOC cytotoxicity

Treatment with DOC alone reduced the colony-forming ability of CaSki and ZMK-1 cells in a time- and concentration-dependent manner. When cell survival is plotted as a function of DOC concentration, this effect is visible in very steep survival curves. In order to obtain comparable results for the two cell lines, we attempted to determine an equitoxic combination of incubation time and drug concentration for both cell lines. For ZMK-1 cells, the surviving fraction as a function of drug concentration is shown in Figure 1. A 3, 9 or 24 hr exposure to 0.7, 0.7 or 0.35 nM DOC

TABLE II – D_0 VALUES FOR THE TWO DIFFERENT CELL LINES AND FOR THE DIFFERENT INCUBATION TIMES

Incubation time with docetaxel	D_0 (Gy)	
	ZMK-1	CaSki
Control (irradiation alone)	2.56	3.41
Hours before irradiation		
3	2.38	—
9	1.82	—
24	1.51	2.45
Hours after irradiation		
24	2.25	3.03

resulted in a reduction of ZMK-1 cell survival to approximately 50% of the untreated control. The exact survival data, taken from the fitted survival curves are as follows: 0.50 ± 0.09 (3 hr, 0.7 nM); 0.43 ± 0.07 (9 hr, 0.7 nM); 0.45 ± 0.05 (24 hr, 0.35 nM).

For CaSki cells, the surviving fraction as a function of drug concentration is shown in Figure 2. A 24 hr exposure to 0.07 nM DOC resulted in a reduction of CaSki cell survival to approximately 50% of the untreated control. Both cell lines were very sensitive to small variations in DOC concentrations. The results presented are the mean of three independent experiments for each incubation time (Figs. 1, 2).

These concentrations, which are effective *in vitro*, are relevant for a clinical application. The plasma DOC concentration obtained in patients treated with this drug ranges from 0.1 to 1 $\mu\text{mol/l}$, which is higher than the concentrations applied *in vitro*.

Flow cytometry

The cell cycle distribution of ZMK-1 or CaSki cells was determined by flow cytometry. The proportion of cells in the S- and G2/M-phase of the cell cycle is shown in Figure 6. For ZMK-1 cells, we found moderate changes at both DOC concentrations. After 9 to 12 hr, an increased proportion of cells in S-phase was observed, and after 24 hr up to 52.3% of the cells were in the G2/M-phase. For CaSki cells no increased proportions of S-phase cells were detected; however, a pronounced accumulation of cells in the G2/M-phase, namely, 80.4%, after a 24 hr exposure to 0.07 nM DOC was observed.

DOC-induced apoptosis

The appearance of apoptosis, together with mitotic figures and necrotic/lysed cells, was determined for both cell lines. Table I shows that DOC alone, as well as radiation alone, induced apoptosis to a higher extent in ZMK-1 than in CaSki cells. The combination of both modalities did not further increase the proportion of apoptotic cells. In both cell lines DOC-induced apoptosis appeared before the radiation-induced apoptosis. A decrease in mitotic figures was also observed after DOC treatment in both cell lines. Besides normal, mitotic and apoptotic cells, a large number of cells with much weaker fluorescence, often swollen with indistinct cell borders and no sharp delineation between nucleus and cytoplasm, were scored as necrotic or lysed cells. For both cell lines, their contribution to cell death was higher than that of apoptosis.

Combination of DOC and radiation

For clonogenic testing of cell survival after combined treatment with DOC and radiation, concentrations and incubation periods for DOC were chosen close to the 50% survival level of the DOC-alone treatment.

Figure 3 shows the results of experiments in which ZMK-1 cells were treated with combinations of DOC and irradiation. For the incubation times of 9 or 24 hr, enhanced radiosensitivity of the ZMK-1 cells was observed. The sensitizer enhancement ratios (determined from normalized curves) at the 37% survival level were 2.01 and 2.4 for the 9 or 24 hr pretreatment, respectively. The incubation of ZMK-1 cells with DOC after irradiation resulted in a sensitizer enhancement ratio of 1.54. The corresponding D_0

values for the survival curves are given in Table II. Analysis of the colony-forming assay data by the isobologram approach (Fig. 4a–c) revealed additivity for DOC and radiation for the 3 hr incubation and supra-additivity for the 9 and 24 hr incubations.

Figure 5 gives the results of experiments in which CaSki cells were treated with DOC for 24 hr before or 24 hr after irradiation. Even at these long incubation times, the combined effects were much smaller than for the ZMK-1 cells. The sensitizer enhancement ratios (determined from normalized curves) at the 37% survival level were 1.44 and 1.17, respectively. The corresponding D_0 values for the survival curves are given in Table II. Analysis of the colony-forming assay data by the isobologram approach (Fig. 4d) revealed additivity for DOC and radiation for the pre-irradiation as well as the post-irradiation incubation experiments.

DISCUSSION

The assumption of a cell cycle-specific radiosensitization by the taxane drugs is very attractive and has been investigated extensively with respect to PAC. However, DOC, the second taxane that is clinically available at present, has been rarely studied for its ability to enhance tumor radioresponsiveness. Phase I/II trials showed that weekly DOC doses of 15 to 20 mg/m² combined with conventional radiotherapy resulted in high response rates, which were, however, accompanied by high levels of side effects.³

Using colony formation as an endpoint, we determined the cytotoxic and radiosensitizing effects of DOC in two squamous cell carcinoma cell lines, ZMK-1 and CaSki. The results of the present study will be compared with the effects of PAC, which have been previously determined in the same cell lines by using the same experimental methods.⁸

In accordance with previously published studies,^{12,13} we found that DOC was more cytotoxic than PAC for both cell lines and for each

incubation time (3, 9 and 24 hr). As shown in Table III, the DOC concentration that reduced the cellular survival to approximately 50% of the untreated controls was up to 100-fold lower than the corresponding PAC concentration. Interestingly, for ZMK-1 cells, these differences in cytotoxicity diminished with increasing incubation times. This result and the activity differences of DOC and PAC could be explained by their pharmacokinetics. Uptake and efflux studies¹⁴ revealed that a 3-fold higher intracellular concentration of DOC was

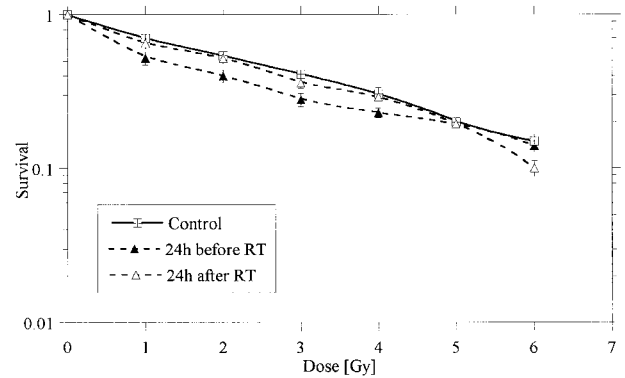


FIGURE 5 – Cytotoxic effect of radiation and DOC in CaSki cells. Colony-forming assay with treatment of cells with DOC and escalating radiation doses. The cells were incubated with DOC before, during and after radiation for a duration of 24 hr. The drug concentration was 0.07 nM. The curves show the inhibitory effect of radiation alone and radiation with DOC before (solid triangles) or after (open triangles) incubation with DOC. Data represent mean values \pm SD. Error bars (shown when greater than the symbol) are standard deviations from three independent experiments.

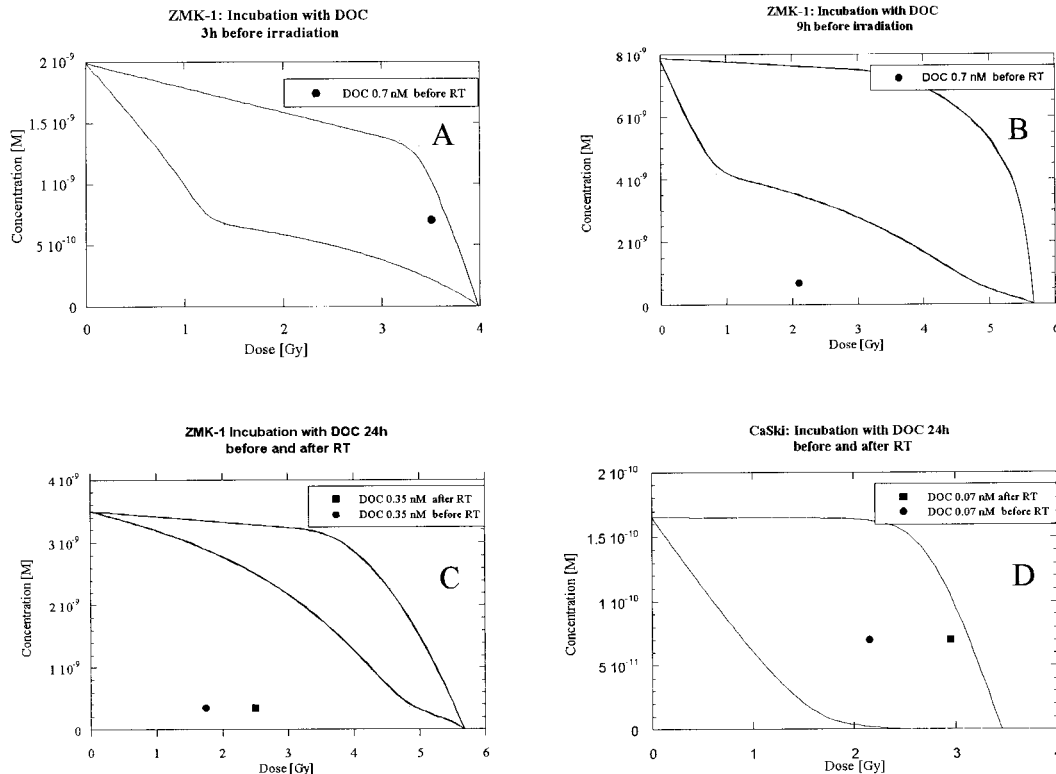


FIGURE 4 – Isoeffect analysis of colony-forming assay data. Isoeffect plots of DOC and radiation were calculated from single-agent colony-forming assays with ZMK-1 and CaSki cells. Solid squares or circles represent the results from combination treatment experiments with DOC applied before (a–d) or after irradiation (c,d).

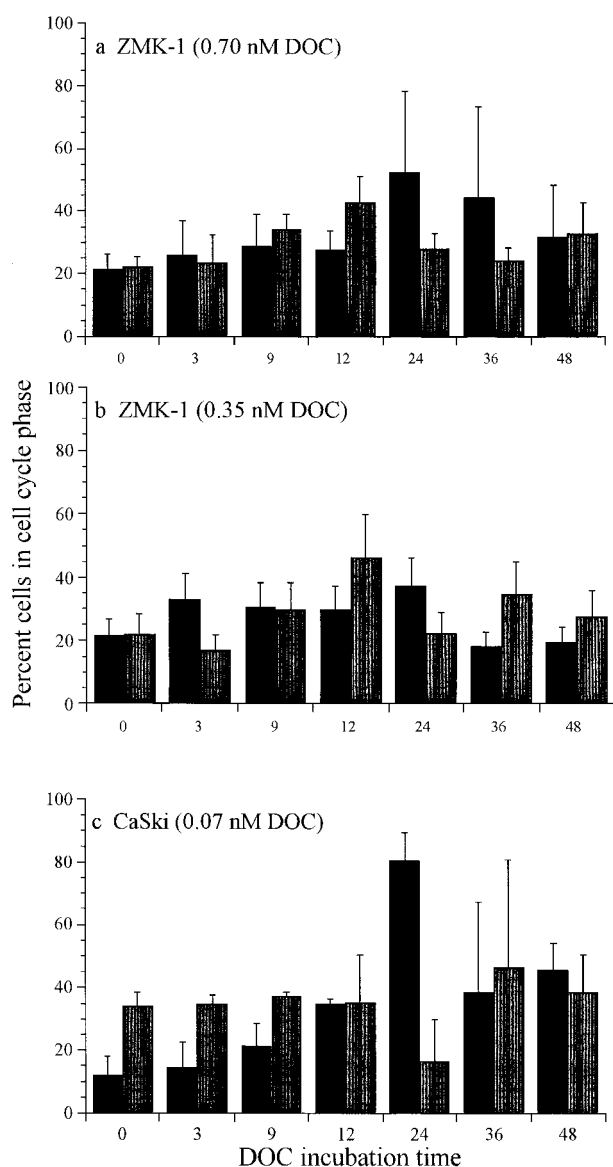


FIGURE 6—DOC-induced changes in cell cycle distribution for ZMK-1 cells (a, b) or CaSki cells (c). Black bars represent cells in G2/M-phase, and gray bars represent cells in S-phase. Error bars are standard deviations from two independent experiments.

TABLE III—COMPARISON OF THE CONCENTRATIONS OF PAC AND DOC THAT REDUCED CELL SURVIVAL TO 50% OF THE UNTREATED CONTROL FOR ZMK-1 AND CaSki CELLS

Hours of incubation	Concentration ($\times 10^{-10}$ M)		Factor
	PAC ¹ (Pradier et al, 1999)	DOC	
ZMK-1			
3	700	7	100
9	70	7	10
24	7	3.5	2
CaSki	70	0.7	100
24			

¹According to Pradier et al.⁸

obtained, compared with PAC, for the same initial extracellular concentration. Efflux studies revealed that the half-time of efflux of DOC was at least three times slower than that of PAC.

Hanauske et al.⁵ and Alberts et al.¹⁵ compared the antiproliferative action of DOC with that of PAC in a variety of freshly explanted human tumor cells at clinically relevant concentrations using an *in vitro* soft agar colony formation assay. Cytotoxicity of DOC was tested against tumor-forming units from breast, lung, ovarian, colorectal cancer and melanoma explants at concentrations that are achievable in human plasma (3.7 μ g/ml, following a dose of 100 mg/m², administered as a 1 to 2 hr infusion).¹⁶ In a direct comparison of 78 tumor specimens, 29 were more sensitive to DOC than to PAC, whereas only 13 were more sensitive to PAC. These data also indicate that cross-resistance between these two agents was incomplete with freshly explanted human tumors.

In the study by Ringel et al.,¹² DOC was 2.5 times more effective than PAC in the two murine cell lines J774.2 and P388. Another noteworthy finding in this study was that DOC was more than 5 times more active than the parent drug in a PAC-resistant variant of the J774.2 cell line. In this study it was postulated that at least part of the increased *in vitro* potency of DOC over PAC might have been attributable to the greater water solubility of DOC compared with PAC (47 vs. 35 μ M). The higher *in vitro* activity of DOC compared with PAC (1.3- to 12-fold) was also confirmed by studies of Riou et al.¹⁷ and Kelland et al.¹³ using human tumor cell lines. Braakhuis et al.⁴ compared the *in vitro* anti-proliferative activity of DOC and PAC against human tumors and normal bone marrow cells. DOC and PAC were much more potent than cisplatin. DOC generally was 2- to 4-fold more cytotoxic than PAC.

The cytotoxicity of PAC in human leukemia cell lines has been shown to be directly related to the PAC-induced formation of irreversible microtubule bundles.¹⁸ In sensitive cell lines, these arrays of disorganized microtubules were formed during all phases of the cell cycle. However, in PAC-resistant cells, microtubule bundling appeared to be reversible, and cells remained unaffected as they passed through the G0/G1- and S-phases. Thus sensitive cells were critically affected during interphase, whereas resistant cells accumulated in the G2/M-phase and formed multiple abnormal spindle asters. The 2- to 4-fold difference in potency between PAC and DOC correlates closely with the respective ability of these agents to promote polymerization of tubulin.

Thus the higher potency of DOC observed *in vitro* may be explained by the combination of its greater water solubility, its higher affinity for microtubules, its higher achievable intracellular concentration and the slower cellular efflux.

All these factors may have also contributed to the uniformly observed additivity or even supra-additivity for the DOC and radiation interaction in both cell lines (Figs. 3–5). CaSki cells showed an accumulation of cells in the G2/M-phase after a 24 hr incubation with DOC (Fig. 6c). When radiation was delivered at that time, enhanced radiosensitivity of the cells was observed. This supports the original rationale, that G2/M arrest is a major mechanism for taxane-induced radioenhancement. This assumption is supported by the observation that DOC incubation after irradiation caused only additive effects (Fig. 5).

In ZMK-1 cells, the relationship between radiosensitization and G2/M accumulation is less clear, as the experiments showed a high variability (Fig. 6), and the DOC-induced G2/M accumulation at 24 hr was much weaker. However, it was preceded by an accumulation of S-phase cells at 9 and 12 hr, which may have contributed to the enhanced radiosensitivity, observed after the 9 hr pretreatment, as DOC has specifically been described as toxic for radioresistant S-phase cells.¹⁹

Another noteworthy observation on ZMK-1 cells is their susceptibility to DOC-induced apoptosis (Table I), which could also explain the observed radiosensitization. Creane et al.²⁰ reported on greater radiosensitizing effects for colon carcinoma cells with high levels of DOC- and radiation-induced apoptosis, compared with cells with lower levels. In a murine tumor model, PAC-induced levels of apoptosis correlated highly with increased tumor growth delay; however, for DOC, neither the induced mitotic arrest nor the induced apoptosis correlated with its anti-tumor effect.^{21,22} A

possible mechanism for the correlation of apoptosis and radiosensitization was suggested by Haldar *et al.*,²³: stabilization of the microtubule system might signal the cells to undergo more radiation-induced apoptosis than usual, possibly because, as was observed, stabilization of microtubules by taxanes results in phosphorylation and inactivation of bcl-2, leading to increases in BAX levels and a consequent increase in apoptosis.

One striking observation in our experiments with PAC and irradiation was the existence of radiosensitizing as well as radioprotective effects, depending on the concentrations and timing schedule of PAC and irradiation that were applied.^{7,8} Similar observations have been reported by Leonard *et al.*,²⁴ Hennequin *et al.*,⁶ and Ingram and Redpath.²⁵

The absence of these effects with respect to DOC (Figs. 3, 5) could be due to the different modes of action between DOC and PAC, as mentioned above (greater water solubility, higher affinity for microtubules, higher achievable intracellular concentration and

slower cellular efflux), which may also result in differences in interaction with repair mechanisms after irradiation. The participation of repair processes in the combined effect of DOC and radiation is supported by the finding that ZMK-1 cells, which have a higher alpha component of radiation damage ($\alpha = 0.145 \text{ Gy}^{-1}$) are more sensitized than CaSki cells, in which the alpha component is $\alpha = 0.037 \text{ Gy}^{-1}$). Another interesting observation recently noted by Matsuura *et al.*²⁶ also supports the effectiveness of DOC treatment after irradiation. For a human ependyoblastoma in nude mice, they found that apoptosis was most common in tumors in which irradiation was followed by the DOC treatment.

In conclusion, we found that DOC is active against tumor cells at lower concentrations than PAC and that its ability to enhance radioresponsiveness is less influenced by the concentration and the time schedules applied. A further evaluation of DOC and concomitant radiotherapy should be performed *in vivo* to support further the basis for clinical studies.

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