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Enhancement of Radiation Effects by Combined Docetaxel and Carboplatin Treatment In Vitro

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SUMMARY This study was designed to evaluate the combination of docetaxel (Taxotere) and carboplatin for radiopotentiation in vitro. H460 human lung carcinoma cells were treated with docetaxel (or paclitaxel) for 1 h and rinsed. After 24 h, the cells were treated with carboplatin for 1 h, irradiated, and colony forming ability was assesed. Using various doses of docetaxel with 100 μM carboplatin, the dose enhancement ratio (D.E.R.) for drugs only was 1.26. When 25 nM docetaxel was used with various doses of radiation, the radiation D.E.R. was 1.41. With all three agents combined, and after normalization for combined drug effects, the radiation D.E.R. was 1.55. Similar values were obtained using paclitaxel with these agents. Significant redistribution of cells into the radiosensitive G_2/M phase was observed using a dose of paclitaxel (750 nM), which also caused radiation enhancement. However, an equally cytotoxic dose of docetaxel (25 nM) did not result in any cell cycle redistribution; this phenomenon was only observed at higher doses. This study shows that the combination of docetaxel and carboplatin enhance the effects of radiation in vitro more effectively than either drug seperately. In addition, our data show that the mechanism of radiopotentiation by docetaxel probably does not involve a G_2/M block in H460 cells. *Radiat. Oncol. Invest. 7:343–352, 1999.*

Key words: docetaxel, paclitaxel, carboplatin, lung cancer, radiotherapy

INTRODUCTION

Paclitaxel (taxol) and docetaxel (taxotere) belong to a class of microtubule-inhibiting compounds called taxanes which are currently used as agents to treat various malignancies. Combined paclitaxel and carboplatin with radiotherapy has been evaluated in clinical trials against nonsmall-cell lung cancer [1–6], and recently against head and neck cancer [7,8]. However, clinical trials using the combination of docetaxel, carboplatin, and radiotherapy have not yet been reported. Therefore, in vitro mechanistic studies may provide useful information in determining the optimum timing and sequencing of these agents in the clinic. Very little is known about the combined effects of docetaxel and platinum agents (such as carboplatin or cisplatin) in vitro, but there have been several studies using paclitaxel and cisplatin. These preclinical studies have shown that cisplatin combined with paclitaxel can be either antagonistic or supra-additive, depending upon the sequencing and the cell line used [9–14]; a single study has shown a similar sequencing dependence with carboplatin and paclitaxel in vitro [15]. In general, paclitaxel treatment followed by a platinum compound has been more effective in cell killing than platinum followed by paclitaxel; the timing between these two drugs is also a significant factor.

The mechanisms of cytotoxicity for taxanes and carboplatin are distinct from each other, as well

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as their proposed mechanisms of radiosensitization. The taxane agent, paclitaxel, is a potent promoter of microtubule polymerization, enhancing the rate and yield of microtubular assembly while preventing microtubular inhibition [16,17]. The result of this promotion is that cells are blocked in the G_2/M phase of the cell cycle, which is the most radiosensitive phase [18,19]. Paclitaxel has been shown to have radiosensitizing properties both in vitro [20,21,22] and in vivo [23,24], and this radiosensitization is attributed to the mitotic block induced by the drug. Docetaxel is also a microtubule inhibitor and has been shown to potentiate the effects of ionizing radiation in vitro [22], but little is known about its mechanism of radiosensitization. In contrast, the cytotoxicity of platinum drugs such as cisplatin and carboplatin results from the formation of bifunctional adducts with DNA (interstrand and intrastrand crosslinks), although it is not known which adduct is responsible for the cytotoxicity [25,26]. Both cisplatin and its less toxic analogue, carboplatin, are radiopotentiating agents in vivo and in vitro [27-30]. The mechanism of radiopotentiation by carboplatin is less understood than that of taxanes, but it is generally agreed that the platinum DNA adducts are responsible for this effect. Possible proposed mechanisms are direct interaction of platinum adducts and radiation-induced single-strand breaks to form double-stranded breaks [29], and/or inhibition of repair of radiation-induced DNA damage [28]. Since the mechanisms of cytotoxicity and radiopotentiation are different for these two drug classes, we hypothesized that the interaction between taxanes, carboplatin, and radiation would be at least additive, if not supra-additive, if the proper sequencing and timing strategy was used.

In this study, we have evaluated the combination of docetaxel, carboplatin, and radiation on the killing of human nonsmall-cell lung cancer cells. We have shown that treatment with docetaxel followed by carboplatin and radiation 24 h later results in radiation enhancement, when drug effects are normalized out. The effects observed using docetaxel are similar to the results with paclitaxel. We have also shown that the mechanism of radiation enhancment for docetaxel alone does not involve a G_2/M arrest, although this arrest may be involved with paclitaxel.

MATERIALS AND METHODS

Cell Culture

NCI-H460 human large cell lung carcinoma cells were obtained from the American Type Culture Collection (ATCC). H460 cells were cultured at 37°C, 5% CO_2 in RPMI 1640 medium (Gibco BRL, Gaithersburg, MD) + 10% fetal bovine serum (Gibco) + 50 U/ml penicillin (Gibco) + 50 μg/ml streptomycin (Gibco). Cells were passaged twice weekly using 0.05% trypsin-EDTA (Gibco), and kept in log phase prior to experiments. Cells were frozen in liquid nitrogen using growth medium with 10% dimethylsulfoxide (DMSO), and newly thawed cells were used for experiments after approximately 10 passages.

Drug Exposures

Docetaxel (Taxotere) powder was obtained from Rhone-Poulenc Rorer (France), and stock solutions were made at 1 mM in ethanol and stored at −20°C until dilution immediately before experiments. Paclitaxel (taxol) powder was obtained from Sigma Chemical Co. (St. Louis, Mo.). Stock solutions of paclitaxel were made at 5 mM in DMSO (Sigma), and diluted appropriately for experiments. The final ethanol or DMSO concentration in the drug medium was 0.2% or less. Cis-Diammine[1,1-cyclobutane-dicarboxylato] platinum (carboplatin) was obtained from Sigma, and fresh stock solutions were made before each experiment in water at 5 mM. Drugs were added directly to flasks containing 5 ml growth medium using innoculation volumes not exceeding 100 μl. Cells were incubated at 37°C for 1 h with docetaxel or paclitaxel, rinsed with phosphate-buffered saline (PBS), and then 5 ml of fresh medium was added to flasks. After various times (usually 24 h), cells were treated with 100 μM carboplatin for 1 h, and then immediately irradiated. Flasks were kept at room temperature for 10 min (irradiation period), then returned to 37°C for 20 min. The drug medium was then immediately aspirated and cells were rinsed with phosphate-buffered saline, then 5 ml of drug-free medium was replaced and cells were returned to 37°C. Untreated controls and cells treated with radiation only were also sham-treated by rinsing with PBS.

Radiation Treatments

Irradiation of cells was performed using a ^{137}Cs irradiator (JL Shepherd Mark I, Glendale, CA) at a dose rate of 2.38 Gy/min. Six 25 mm^2flasks were irradiated simultaneously on a rotating stand, with 5 ml medium per flask, and thermoluminescent dosimetry for this specific system was performed immediately prior to this study. Cells were kept at room temperature for 10 min during the irradiation period. Flasks with drug only or untreated controls

were also kept at room temperature for 10 min, in order to simulate conditions of irradiation.

Survival Analysis

Log-phase cells were trypsinized from 75 mm^2 flasks and counted using a particle data counter (Coulter Electronics, Hialeah, FL). Cells were then diluted serially to appropriate concentrations, and plated out in triplicate per data point into 25 mm^2 flasks in 5 ml total growth medium. The number of cells plated depended upon the toxicity of the treatment (minimum 100 cells for controls), in order to improve statistics by avoiding counts of less than 20 colonies. Cells were allowed to attach for 24 h at 37°C and then treated with docetaxel or paclitaxel. For the single-cell colony formation protocol, 100,000 cells were plated into flasks (one per data point) and allowed to attach for 24 h. Cells were then treated with drugs, irradiated, and immediately trypsinized and plated in triplicate at appropriate numbers for colony formation. After drug/radiation treatment using either protocol, cells were returned to 37°C for 8 days. Cells were then fixed for 15 min with 3:1 methanol:acetic acid and stained for 15 min with 0.5% crystal violet (Sigma) in methanol. Colonies were counted by eye, with a cut-off of 50 viable cells. Surviving fraction was calculated as mean colonies/(cells innoculated × plating efficiency), where plating efficiency is defined as mean colonies/cells innoculated for untreated controls.

Surviving fraction values for radiation plus a single drug were normalized by dividing by the surviving fraction for that drug only. When taxanes (docetaxel or paclitaxel) plus carboplatin were used with radiation, normalization was performed using the surviving fraction representing the combined effect of both drugs. Surviving fractions for taxanes plus carboplatin were normalized by dividing by the surviving fraction of carboplatin only. The radiation dose enhancement ratio (D.E.R.) was calculated as the dose (Gy) for radiation alone divided by the dose (Gy) for radiation plus drug(s) (normalized for drug toxicity), at a surviving fraction of 0.1. For taxanes plus carboplatin, the taxane D.E.R. was calculated as the dose (nM) for taxane alone divided by the dose (nM) for taxane plus carboplatin, at a surviving fraction of 0.25. Error bars were calculated as S.E.M. (standard error of the mean) by pooling the results of three independent experiments, each of which were plated in triplicate. Multiplicity correction was performed according to previously published protocol [31,32]. Briefly, 1000 cells were plated in T25 flasks, and allowed to attach for 24 h. Cells were treated with taxanes for 1 h, rinsed, and incubated for 24 h. The flasks were then stained for 30 sec using Wright stain (Sigma). After analyzing 30 microcolonies per flask, the average number of cells per microcolony was determined. Curves were then corrected using the equation $S = 1\text{-}\ (1 - f)^{1/N}$, where f = measured surviving fraction, S = single cell survival, and N = average cells/microcolony, or cellular multiplicity. Although multiplicity corrections are not shown on the graphs, the multiplicity correction was incorporated into the calculation of DER values.

Cell Cycle Analysis by Flow Cytometry

3.3×10^5 H460 cells were plated into 25 cm^2 flasks for each data point. After 48 h at 37°C, cells were treated with taxanes for 1 h, rinsed with PBS (phosphate-buffered saline), then fresh medium was added. At 24 h after drug treatment, the cells were fixed for flow cytometry as follows: cells were trypsinized, counted, and 2×10^6 cells were centrifuged down from each flask. Cells were then resuspended in 2 ml of ice-cold PBS by vortexing, and then 2 ml of ice-cold absolute ethanol was added dropwise while vortexing. Samples were brought to 70% ethanol and stored at 4°C for 20 min to 2 weeks before staining. Fixed cells in 70% ethanol were centrifuged for 5 min at 1000 rpm, and resuspended in 50 μg/ml propidium iodide (Sigma) with 40 KU/ml DNase-free RNase (Stratagene, La Jolla, Calif.). Cells were incubated with propidium iodide for 20 min-2hr before measurement. Cells were then run on a Beckton Dickinson FACScan flow cytometer. Excitation was monitored at 488 nm and emission at 530-595 nm, and 2-parameter DNA histograms (number of cells vs. DNA content) were collected. The percentage of cells in G_2/M phase were then determined by computer analysis of the histograms. Three independent experiments were performed, and error bars represent the standard error of the mean (S.E.M.).

RESULTS

Figure 1 presents drug cytotoxicity survival curves for docetaxel alone and docetaxel plus carboplatin in H460 human lung carcinoma cells. A one-hour exposure to docetaxel was used, then the cells were rinsed and fresh medium was added. Carboplatin (100 μM) was then added 24 h later, and cells were rinsed after 90 min. Surviving fraction values are based upon a colony formation assay. The mean IC_{50} (inhibitory concentration at 50% survival) value for docetaxel was 22.5 nM; for paclitaxel, the IC_{50} value was 625 nM. The mean surviving frac-

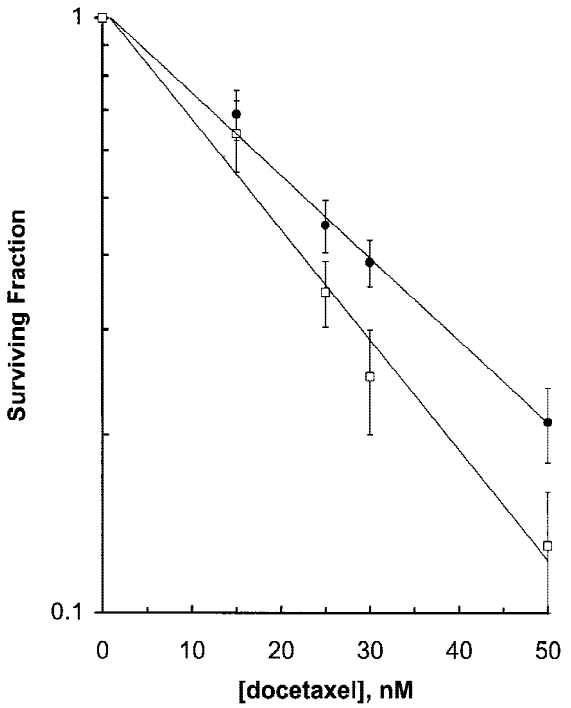

Fig. 1. Combined effect of docetaxel and carboplatin on survival of H460 cells. Cells were incubated with various concentrations of docetaxel for 1 h at 37°C, rinsed, incubated for 24 h, and exposed to 100 μM carboplatin for 90 min. (●), docetaxel only; (□), docetaxel plus carboplatin (normalized for the effects of carboplatin alone; mean S.F. = 0.72.)

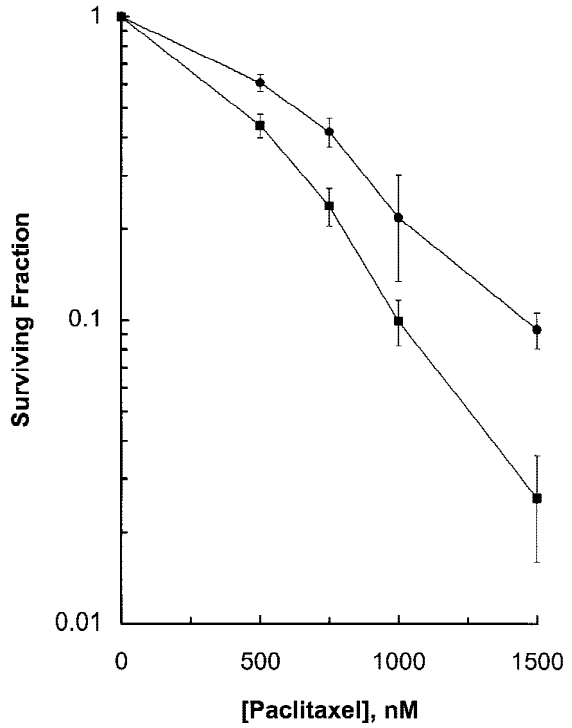

Fig. 2. Combined effect of paclitaxel and carboplatin on survival of H460 cells. Protocol is the same as in Figure 1, except with paclitaxel. (●), paclitaxel only; (■), paclitaxel plus carboplatin.

tion for carboplatin alone was 0.72. Figure 2 shows a similar set of experiments, using paclitaxel instead of docetaxel. The addition of 100 μM carboplatin resulted in drug dose enhancement ratios (D.E.R.) of 1.26 and 1.40 for docetaxel and paclitaxel, respectively. Thus, carboplatin potentiated the cell killing effects of either docetaxel or paclitaxel. The D.E.R. values for the various agent combinations used are also listed in Table 1.

The results of combined radiation, docetaxel, and carboplatin treatments are shown in Figure 3. The same drug treatment protocol was used as in Figure 1. Radiation treatment was delivered 24 h after treatment with docetaxel. When cells were incubated with carboplatin, radiation was delivered at 60 min after drug addition, and cells were rinsed 30 min later. Radiation plus carboplatin resulted in a radiation D.E.R. of 1.29; the D.E.R. for radiation plus docetaxel was 1.41. The mean surviving fraction for 25 nM docetaxel alone was 0.45 ± 0.04; for paclitaxel alone, the value was 0.42 ± 0.04. When radiation, docetaxel, and carboplatin were combined, the D.E.R. value was 1.55. This three-agent survival curve was normalized for the combined effects of both docetaxel and carboplatin; thus, the increase in D.E.R. does not include the interaction between these two drugs shown in Figure 1. Therefore, this drug combination is a more effective radiopotentiator than either drug separately. A similar set of experiments was performed using paclitaxel and carboplatin plus radiation (Figure 4). The resulting DER values from Figure 4 are also listed in Table 1. Although the interactions for paclitaxel appear larger on the graphs than for those observed

Table 1. Dose Enhancement Ratios Corresponding to Figures 1–4

Treatment	Dose enhancement ratio (DER)
Drugs only (taxane dose varied)	
Paclitaxel + carboplatin	1.40
Docetaxel + carboplatin	1.26
Taxanes + radiation (radiation dose varied)	
Paclitaxel + radiation	1.33
Docetaxel + radiation	1.41
Carboplatin + radiation	1.29
3-Agent response (radiation dose varied)	
Paclitaxel + carboplatin + radiation	1.50
Docetaxel + carboplatin + radiation	1.55

All carboplatin doses were 100 μM. All curves used to calculate DER values have been corrected for multiplicity. DER values were calculated at surviving fraction = 0.1.

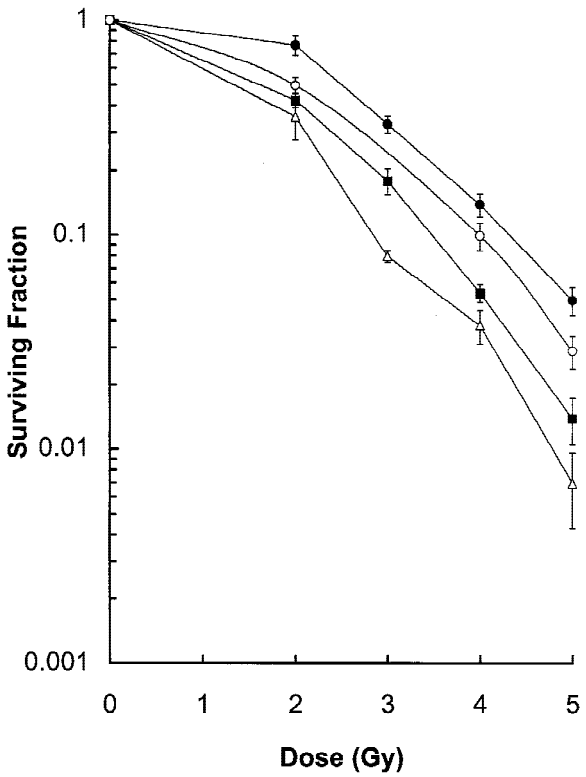

Fig. 3. Combined effects of docetaxel, carboplatin, and radiation on survival of H460 cells. Cells were incubated with 25 nM docetaxel for 1 h (mean S.F. = 0.45 ± 0.04 for docetaxel alone). Cells were rinsed, incubated for 24 h at 37°C, then exposed to 100 μM carboplatin for 90 min. Cells were irradiated at t = 60 min during carboplatin exposure. (●), radiation only; (○), radiation plus carboplatin; (■), radiation plus docetaxel; (Δ), radiation plus docetaxel plus carboplatin. All curves are normalized for killing by drug(s) alone. Curves are not corrected for multiplicity.

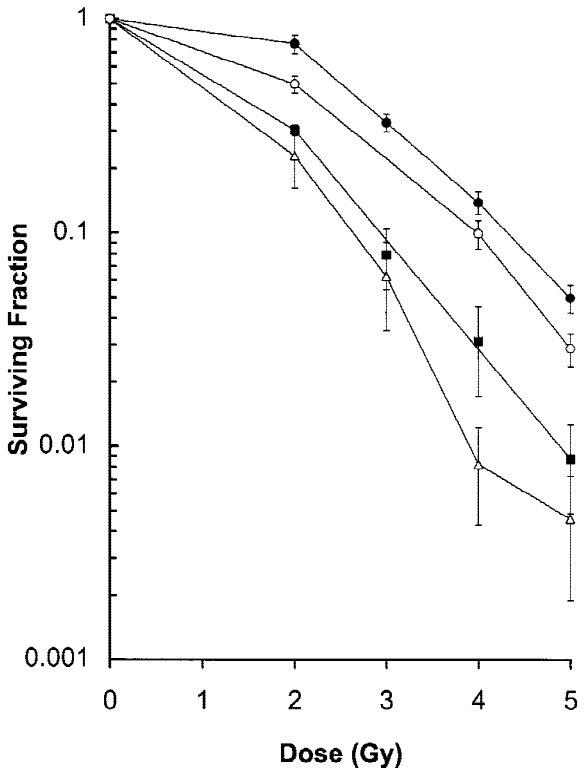

Fig. 4. Combined effects of paclitaxel, carboplatin, and radiation on survival of H460 cells. Cells were incubated with 750 nM paclitaxel for 1 h (mean S.F. = 0.42 ± 0.04 for paclitaxel alone). Protocol is the same as in Figure 3. (●), radiation only; (○), radiation plus carboplatin; (■), radiation plus paclitaxel; (Δ), radiation plus paclitaxel plus carboplatin.

using docetaxel, paclitaxel inhibited microcolony proliferation more effectively than docetaxel (data not shown). Therefore, the multiplicity-corrected DER values for docetaxel are slightly higher than those of paclitaxel. A single-cell plating protocol was also used with these agents to verify this point. When cells were treated with drugs and radiation, and then immediately trypsinized and plated as single cells, similar levels of enhancement were also observed, although the DER values were somewhat smaller in general. The DER values corresponding to the single-cell protocol are listed in Table 2.

The kinetics of these combined agent effects were evaluated using the same colony formation assay. Cells were incubated with docetaxel or paclitaxel for 1 h, and carboplatin and/or radiation treatments were given at various times after docetaxel treatment. Figure 5 displays the kinetics of interaction between radiation, docetaxel, and carboplatin. The interaction between docetaxel (25 nM), carboplatin (100 μM), and radiation (2 Gy) remained relatively constant from 0 to 24 h, while a modest peak occurred at 16 h with docetaxel (25 nM) and carboplatin without radiation. The surviving fraction with docetaxel plus radiation remained relatively constant with time between treatments. Thus, the radiosensitization by docetaxel and carboplatin using this particular dose and sequencing strategy does not appear to be dependent upon the

Table 2. Radiation Dose Enhancement Ratios for Various Agent Combinations Using the Single-Cell Plating Protocol

Agent combination	Dose enhancement ratio (DER)
Radiation + paclitaxel	1.2
Radiation + docetaxel	1.2
Radiation + paclitaxel + carboplatin	1.3
Radiation + docetaxel + carboplatin	1.4

Drug concentrations are the same as in Figures 3 and 4. DER values were calculated as described in Materials and Methods.

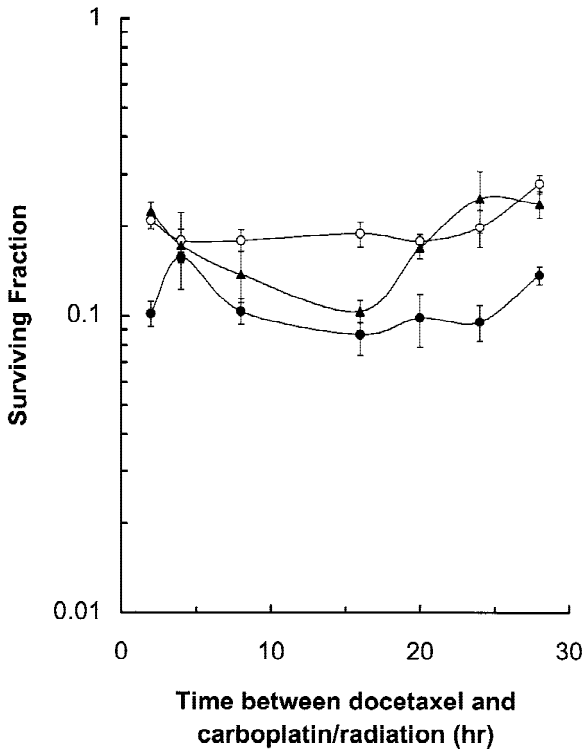

Fig. 5. Kinetics of interaction between docetaxel, carboplatin, and radiation in H460 cells. Cells were incubated with 25 nM docetaxel for 1 h, rinsed, incubated for various times at 37°C, and then treated with 100 μM carboplatin and radiation (2 Gy) as in previous experiments. Curves are *not* normalized for the effects of drug(s) alone. (●), docetaxel + carboplatin + radiation; (○), docetaxel + radiation; (◆), docetaxel + carboplatin.

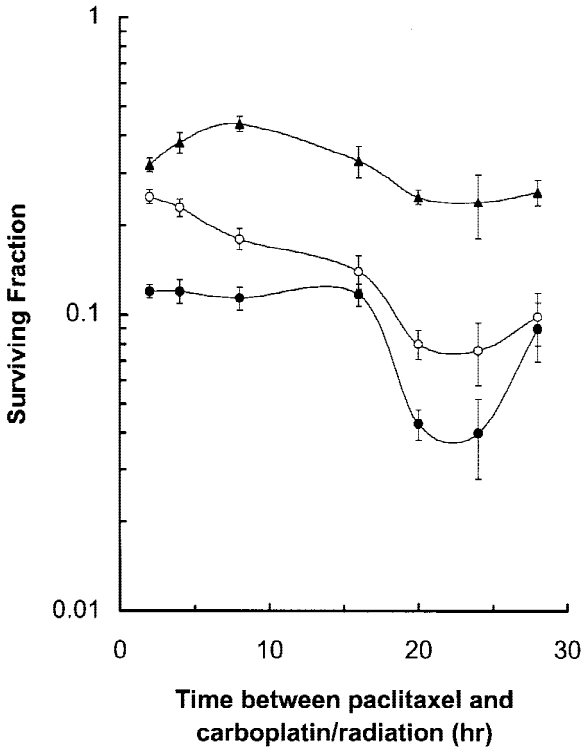

Fig. 6. Kinetics of interaction between paclitaxel, carboplatin, and radiation in H460 cells. Protocol is the same as in Figure 6, except 750 nM paclitaxel was used. (●), paclitaxel + carboplatin + radiation; (○), paclitaxel + radiation; (◆), paclitaxel + carboplatin.

time between treatments. In contrast, the response observed using paclitaxel (750 nM) plus radiation (or paclitaxel plus carboplatin plus radiation) varied over time, with a peak survival reached at 20–24 h after drug treatment (Figure 6).

To address the possible difference in mechanisms, cell cycle analysis using propidium iodide staining and flow cytometry was performed on cells treated with docetaxel. When cells were treated with various doses of docetaxel for 1 h, incubated for 24 h, then fixed and stained for flow cytometry, a dose-dependent increase in the percentage of cells in G_2/M phase was observed (Figure 7a). However, at the docetaxel dose used for radiosensitization studies (25 nM), no accumulation of cells into G_2/M was observed at 24 h after treatment. No cell cycle redistribution was observed at other times between 0 and 30 h using 25 nM docetaxel (data not shown). Thus, the radiosensitization observed using 25 nM docetaxel does not appear to involve redistribution of cells into the radiosensitive G_2/M phase of the cell cycle. However, a characteristic increase in the percentage of G_2/M cells was observed using paclitaxel (Figure 7b). At a dose which significant G_2/M accumulation was observed with paclitaxel (750 nM), radiation enhancement was also achieved. The peak of radiosensitization for paclitaxel or paclitaxel plus carboplatin shown in Figure 6 also correlated with the approximate time at which cells reached their peak G_2/M (data not shown), which was approximately 18 h after drug treatment. There was no change in cell cycle distribution immediately after a 90 min treatment with 100 μM carboplatin (data not shown). Thus, cell cycle redistribution at the time of irradiation was due to treatment with paclitaxel only. Since cell cycle status at the time of irradiation can determine radiosensitivity, samples were fixed only at this time.

DISCUSSION

This study demonstrates that the combination of docetaxel and carboplatin potentiates radiation-induced cell killing more effectively than docetaxel or carboplatin alone. A 1 h exposure to docetaxel alone has also been shown to enhance the effects of radiation in both HeLa cells and SQ20B cells (head

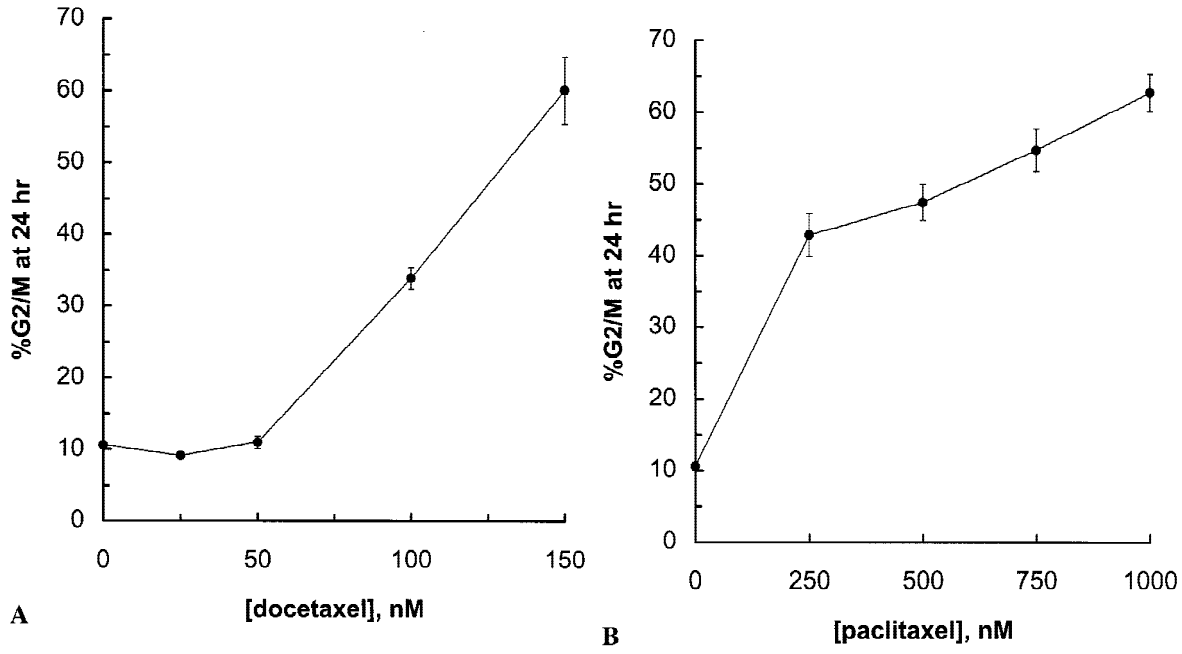

Fig. 7. Effect of various concentrations of docetaxel or paclitaxel on G_2/M block formation in H460 cells. Cells were incubated for 1 h with docetaxel (Figure 7a) or paclitaxel (Figure 7b), rinsed, and incubated at 37°C for 24 h. Cells were then fixed with 70% ethanol, stained with propidium iodide, then analyzed by flow cytometry.

and neck squamous carcinoma) [22]. A 1 h drug exposure was used in our study (as opposed to the 24 h treatment often used in other studies with paclitaxel) so that the kinetics of cell cycle-related changes in survival could be studied. These kinetic studies require that the killing by drug alone is constant for each irradiation time. We have found that 24 h exposures to paclitaxel or docetaxel require concentrations about 100-fold less than those necessary for a 1 h treatment to give the same surviving fraction (data not shown). Our D.E.R. value of 1.29 for radiation plus carboplatin is in agreement with previous studies on squamous cell carcinoma cells [33], RIF-1 tumor cells [28], and human lung cancer cells [30]. The combined effects of all three agents, using docetaxel or paclitaxel, have not been previously reported either in vivo or in vitro.

Although combined cisplatin and paclitaxel treatment has been studied in vitro, the mechanisms of platinum drug-taxane interaction have not been established. Previous studies have demonstrated antagonism when the two drugs are given within a short interval of each other, especially if platinum treatment was followed by paclitaxel [9,10,11,12]. When paclitaxel treatment preceded platinum drug treatment, an additive or synergistic effect was observed which depended upon the time interval, drug doses, and cell lines used [13,14,15]. The most obvious mechanism for the effects observed in those studies between paclitaxel and carboplatin would be the accumulation of cells into the G_2/M phase by paclitaxel. However, previous studies have shown either a lack of cell cycle-related specificity in cell killing for cisplatin [34], or preferential killing of G_1 cells by cisplatin [35]. In addition, docetaxel does not exhibit a G_2 block in the dose range used for our studies. Other studies have shown that paclitaxel followed by cisplatin gives a time-dependent increase in the enhancement ratio [36,37]. One study showed that the efficacy of the drug combination was not attributed to increased apoptosis [36], while another demonstrated that it was not due to an increase in cisplatin-induced DNA adducts (interstrand and DNA-protein crosslinking) by paclitaxel [37]. Similarly, it was shown that either docetaxel or paclitaxel inhibit DNA-adduct formation and intracellular accumulation of cisplatin in human leukocytes [38]. Based upon our data and the results of other studies, we hypothesize that taxane-platinum drug interactions are due to cell-cycle-dependent phenomena (but not a G_2 block), based upon the kinetics of these interactions when taxoid treatment precedes platinum drug treatment.

We have found that when docetaxel is followed by carboplatin 24 h later, with radiation treatment during carboplatin incubation, the resulting radiopotentiation is greater than for either drug alone. The kinetics experiments show that optimum

cell killing for docetaxel plus carboplatin without radiation occurs at about 16 h between drug treatments. Although a similar peak might be expected for the three agents combined, there was no significant time-dependence observed. The combined effect of the three agents suggests that radiopotentiation by docetaxel occurs by a mechanism which is independent of the carboplatin mechanism, since the radiation D.E.R. values for each drug seperately are less than the radiation D.E.R. for both drugs together. The mechanism of radiopotentiation for carboplatin alone is probably either DNA repair inhibition by carboplatin [28] or direct interaction of carboplatin- and radiation-induced DNA lesions [29]. The radiopotentiation shown in this study for combined docetaxel/carboplatin treatment is in addition to the interaction between docetaxel and carboplatin, since the D.E.R.'s were calculated by normalizing for the combined effect of the two drugs.

The data presented here indicates that the mechanism of radiopotentiation by paclitaxel may be different than that of docetaxel, at least in H460 cells. Our experiments have demonstrated that doses of paclitaxel around IC_{50} result in similar radiation enhancement as docetaxel in H460 cells, and this enhancement was correlated with a G_2/M block. Others have previously found a G_2/M block using doses of paclitaxel which resulted in radiosensitization [20,21,24,39]. However, no G_2/M block was observed in our study using docetaxel at a radiation-enhancing dose. A previous report described a G_2/M block in human colon cancer cells using docetaxel at much higher doses (up to 1000 nM) [40], which is consistent with our results. A 1 h treatment of HeLa cells with nanomolar doses of docetaxel has been shown to affect centrosome organization and lead to catastrophic exit of mitosis; thus, lack of mitotic block was shown as the primary cause of cell lethality [41]. It has been previously postulated that the radiosensitizing mechanisms for these two drugs may be different [22]. This hypothesis was based upon a study which demonstrated that docetaxel was almost totally lethal to S-phase HeLa cells (surviving fraction less than 0.05), while an equally cytotoxic dose of paclitaxel (IC_{50}) yielded surviving fractions between 0.8 and 0.5 in S-phase cells [42]. Since S-phase cells are relatively radioresistant [18,19], but are essentially excluded from the analysis using docetaxel at doses around IC_{50}, the resulting population of irradiated cells are relatively radiosensitive according to this rationale.

In conclusion, the combination of docetaxel (or paclitaxel) and carboplatin enhances radiation-induced killing of human lung cancer cells more effectively than either drug separately. Since the taxanes also interact with carboplatin without radiation, the combination of these three agents results in significant tumor cell killing. Clinical trials using this regimen are currently being started for the treatment of nonsmall-cell lung cancer. The mechanisms of enhancement for docetaxel plus carboplatin plus radiation may be different than those for paclitaxel plus these agents, according to our data.

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