Effect of Docetaxel on the Therapeutic Ratio of Fractionated Radiotherapy *in Vivo*[1]

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ABSTRACT

The aim of this investigation was to determine whether docetaxel increases the therapeutic ratio of fractionated radiotherapy *in vivo*. Two tumor types were chosen based on their sensitivity to docetaxel as a single agent: (*a*) docetaxel-sensitive MCa-4 mammary adenocarcinoma, which responds to docetaxel by G_2-M-phase cell cycle arrest, apoptosis, and subsequent reoxygenation of surviving tumor cells; and (*b*) docetaxel-resistant SCC-VII squamous cell carcinoma, which responds to docetaxel treatment only by G_2-M-phase arrest. Response of the normal jejunal mucosa in mice was compared to the response of both tumor types to confirm therapeutic gain. We conducted micromorphometric analysis of tumor cell mitosis, assayed apoptosis by its histological appearance in tissue sections, and determined tumor response by tumor growth delay. Normal tissue response of the jejunum was assayed by micromorphometric analysis of mitotic and apoptotic indices, and clonal crypt stem cell survival was measured using the microcolony assay. Two clinically relevant treatment schedules were tested for both antitumor efficacy and normal tissue toxicity: (*a*) a single bolus of docetaxel (33 mg/kg i.v.) 24 h before five daily fractions of radiation; and (*b*) daily administration of docetaxel (8 mg/kg i.v.) with radiation delivered at the peak of mitotic arrest (9 h for MCa-4 and 6 h for SCC-VII tumors). The best therapeutic gain for docetaxel-sensitive MCa-4 was achieved with a single bolus of drug 24 h before the start of fractionated radiotherapy (therapeutic gain = 2.04). This schedule takes advantage of reoxygenation of hypoxic tumor cells during the interval between drug treatment and radiation delivery. The best therapeutic gain for docetaxel-resistant SCC-VII was achieved with intermittent multiple doses of docetaxel given during the course of fractionated radiotherapy. This schedule maximized the exposure of cells to radiation while they were arrested by docetaxel in the radiosensitive G_2-M phases of the cell cycle (enhancement factor = 2.0). Final therapeutic gain was reduced to 1.59 because of increased normal tissue toxicity in mice treated with multiple intermittent doses of docetaxel in combination with fractionated radiotherapy. Thus, docetaxel greatly enhanced tumor response to fractionated radiotherapy, but the magnitude of therapeutic efficacy depended on drug-radiation scheduling. The greatest therapeutic gain in the treatment of docetaxel-sensitive tumors was achieved by a single large dose of docetaxel administered 1 day before the initiation of fractionated radiotherapy and in the treatment of docetaxel-resistant tumors by daily concomitant docetaxel-radiation treatments.

INTRODUCTION

Taxanes, primarily paclitaxel, have recently undergone extensive investigation for their radiosensitizing potential. The biological rationale for testing taxanes was based on the cell cycle effects of these drugs, specifically on their ability to arrest cells in G_2 and M phase (1, 2), the most radiosensitive phases of the cell cycle (3, 4). A recent review of preclinical studies performed mostly *in vitro* using a variety of different tumor cell lines showed that the taxanes were not universally radiosensitizing (5). Although taxanes had a supra-additive effect in the majority of cell lines when combined with radiation, their effect was additive in a high percentage of cell lines and subadditive in several cell lines.

In most studies demonstrating radiosensitization, the cells were incubated with the drug before irradiation, and the enhanced radiosensitivity occurred when radiation was delivered at the time of accumulation of cells in G_2 and M phase (6–8). A contributing mechanism observed specifically for docetaxel was that the drug, on its own, was toxic for radioresistant S-phase cells (9, 10). The magnitude of enhancement varied greatly, ranging from as low as 1.1 to as high as 3.21 (11). The degree of enhancement was primarily dependent on the cell line and its proliferative status, drug concentration, and the duration of drug exposure. In general, the presence of paclitaxel in moderate concentrations in culture medium (5–100 nM) for extended periods of time (≥24 h) resulted in greater radioenhancement (6–8).

An additive effect was noted in situations of short incubation periods (9, 12), when paclitaxel was added after irradiation (11) or when paclitaxel induced G_2-M-phase block that, on its own, resulted in cell death (13). The reduction in radiosensitivity, although infrequent, occurred when the irradiated cells were kept in culture in the presence of paclitaxel for several hours after irradiation (14), or when paclitaxel was added to cells after irradiation and kept in the medium for 24 h more (15). Radia-

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tion-induced G_1 and G_2 cell cycle arrest allowed repair of sublethal damage but blocked the cell cycle effects of paclitaxel (14, 15).

In contrast to extensive *in vitro* investigations, only a limited number of studies have addressed the radiosensitizing action of taxanes *in vivo* (16–23). These studies showed that paclitaxel can enhance the radioresponse of both paclitaxel-sensitive (16–20) and -resistant (19) tumors when the drug was given as a single treatment. They supported *in vitro* observations that G_2-M-phase arrest is a major mechanism of tumor radiopotentiation but showed that this mechanism dominates only in paclitaxel-resistant tumors. In these tumors, the arrested cells are not destined to die from drug treatment only (19, 24); consequently, they respond to radiation by enhanced sensitivity. Tumors sensitive to paclitaxel treatment alone also exhibited an enhanced radioresponse (16–20) even stronger than that of paclitaxel-resistant tumors, but the major underlying mechanism was reoxygenation of radioresistant hypoxic cells (17, 19, 22). In this type of tumor, taxanes induce mitotic arrest, but the majority of these cells die by apoptosis or necrosis. This massive cell loss results in increased tumor oxygenation, as demonstrated to occur in tumors treated with paclitaxel (24, 25) or docetaxel (20). Potentiation of radioresponse developed earlier in the paclitaxel-resistant (peak, about 10 h after paclitaxel) than in the paclitaxel-sensitive tumors (peak, between 24 and 72 h after paclitaxel; Refs. 16–20). These studies used combinations of taxanes and large single doses of radiation.

There is even less information available on the combination of taxanes with fractionated irradiation *in vivo*. Response to fractionated radiation of a human hypopharyngeal tumor xenograft in nude mice was enhanced by paclitaxel, as assessed by tumor growth delay (21). Radiation, at a dose of 2 Gy/fraction, was given twice daily for 10 days, and paclitaxel was administered to mice either as a single bolus or daily for 10 days immediately before the first daily fraction. Another study (23), using the rat Dunning tumor, showed only an additive effect when paclitaxel, given daily for 5 days, was combined with radiation (1.5 Gy daily for 5 days).

Our present study investigated whether docetaxel can enhance tumor response to fractionated irradiation in docetaxel-sensitive and -resistant tumors. To model clinical situations, two approaches were explored: (*a*) one approach tested the effect of a single large bolus of docetaxel given before the initiation of daily fractionated radiation; and (*b*) the other tested the effect of daily administration of smaller amounts of docetaxel given before each fraction of irradiation. We used a murine mammary carcinoma designated MCa-4, which is responsive to docetaxel by tumor growth delay, mitotic arrest, and apoptosis (20), and a murine squamous cell carcinoma designated SCC-VII, which exhibits only mitotic arrest when treated with docetaxel (26). As reported previously (20), docetaxel was highly effective in enhancing the radioresponse of MCa-4 tumor to single-dose radiation. We also investigated whether and to what degree these two treatment approaches affected normal tissue response to radiation and whether they provided therapeutic gain. As in our earlier studies on the combination of taxanes with single-dose irradiation (18, 20, 27, 28), we used jejunal mucosa as an example of an acutely responding radiation dose-limiting tissue.

MATERIALS AND METHODS

Mice and Tumors. C3Hf/Kam mice, bred and maintained in our specific-pathogen-free mouse colony, were 3–4 months old at the beginning of the experiments and housed five per cage. Two transplantable syngeneic carcinomas, MCa-4 and SCC-VII, were used in their fourth and seventh isotransplant generations, respectively. These tumors arose spontaneously and have since been stored in liquid nitrogen. Solitary tumors were produced in the muscles of the right leg by the inoculation of 5×10^5 cells. Tumor cell suspensions were prepared by mechanical disruption and enzymatic digestion of nonnecrotic tumor tissue (28).

Docetaxel. Docetaxel was obtained from Rhone-Poulenc Rorer (Vitry Sur Seine Cedex, France) as a pure crystalline powder and stored at 4°C. A stock solution of 50 mg/ml was prepared in absolute ethanol and stored at −20°C for the duration of the experiments. Treatment solutions were prepared by mixing 1 volume of the ethanolic stock solution, 1 volume of polysorbate 80 (Sigma Chemical Co., St. Louis, MO), and 18 volumes of 5% glucose in water. Docetaxel was given to mice i.v. either as a single dose of 33 mg/kg or daily for 5 consecutive days at a dose of 8 mg/kg. Treatment solutions were kept on ice and injected within 10 min of formulation.

Histological Analysis of Mitotic Arrest and Apoptosis. To determine the effect of docetaxel on two known cellular hallmarks of exposure to docetaxel, mitotic arrest and apoptosis, mice were treated with docetaxel (33 mg/kg) when tumors (MCa-4 or SCC-VII) were 8 mm in diameter and sacrificed 0, 1, 3, 6, 9, 12, 16, 24, 48, or 72 h later. Tumors were removed and placed immediately in neutral buffered formalin. After fixation, 4-μm histological sections were prepared and stained with H&E. The micromorphometric method was used for scoring the percentage of cells in mitosis or apoptosis (20, 25, 27, 29). Briefly, five nonnecrotic fields were randomly selected from each tissue section and examined at ×400 magnification. One hundred nuclei per field were counted and scored as interphase, mitotic, or apoptotic. The mitotic and apoptotic indices were presented as a percentage based on counting 1500–2500 nuclei from three to five mice per group.

Mice without tumors were used to assess the effect of docetaxel on mitosis and apoptosis in jejunum. The dose of docetaxel and the time intervals for scoring mitosis and apoptosis after docetaxel administration were the same as described above for tumors. Mice were killed, and a 2-cm segment of jejunum was excised and processed for histology as described above. One hundred epithelial cells in complete longitudinal crypt sections from five random areas of jejunal transverse sections were scored as interphase, mitotic, or apoptotic. Mean apoptotic and mitotic indices per treatment group were based on 1500–2500 crypt cells from three to five mice per group.

Tumor Growth Delay. Tumors were locally irradiated when they grew to 8 mm in mean diameter. Radiation was delivered at a dose rate of 6.25 Gy/min to the tumor-bearing legs using a dual-source ^{137}Cs γ-ray unit. During irradiation, air-breathing mice were immobilized in a jig, with the tumor centered in the 3-cm-diameter circular irradiation field. Total radiation doses of 15 or 25 Gy were given as a single dose or daily for 5 days with doses per fraction of 3 or 5 Gy. No

anesthetic was given. To obtain tumor growth curves, three mutually orthogonal tumor diameters were measured at 2-day intervals with a vernier caliper, and the mean values were calculated. Regression and regrowth of tumors were followed until tumor diameter reached approximately 14 mm. Tumor growth delay was expressed as the time in days for tumors treated with radiation to grow from 8 to 12 mm in diameter minus the time in days for untreated tumors to reach the same size. This is termed the AGD.[3] The effect of the combined docetaxel plus radiation treatment was expressed as the NGD. It is defined as the time for tumors treated with both docetaxel and radiation to grow from 8 to 12 mm in diameter minus the time in days for tumors treated with docetaxel alone to reach the same size. Groups consisted of six to eight mice each.

Jejunal Radiation Damage. The microcolony assay introduced by Withers and Elkind (30) was used to determine the survival of crypt epithelial cells in the jejunum of mice exposed to radiation. Groups of six mice were whole body-irradiated with five doses of 250 KVP X-rays ranging from 3.90–6.15 Gy given at a dose rate of 1.62 Gy/min. The mice were given docetaxel using the same treatment schedules described previously for tumors and killed 66 h after whole body irradiation. The jejunum was prepared for histological examination, and the number of regenerating crypts in the jejunal cross-section was counted microscopically at ×100 magnification. To construct radiation survival curves, the number of regenerating crypts was converted to the number of surviving cells by applying a Poisson correction for crypts regenerating from more than one stem cell. Lines were fitted to data by least-squares regression analysis.

RESULTS

Radioresponse of Docetaxel-sensitive MCa-4. We reported previously that docetaxel strongly inhibited the growth of MCa-4 carcinoma (26) and enhanced the response of this tumor to single-dose ionizing irradiation (20). Histologically, docetaxel-treated tumors displayed significant mitotic arrest and cell death by apoptosis or necrosis (Ref. 26; Fig. 1*A*). The arrest of cells in mitosis was rapid and already visible 3 h after treatment, and it increased rapidly with time, achieving its peak of more than 20% at 9 h after treatment. After peaking, the percentage of arrested mitoses declined, returning to the background level by 72 h after treatment. The induced apoptosis began to increase 6 h after docetaxel administration and reached a peak of about 10% at 24 h. The percentage then declined to the control value at 72 h after docetaxel administration. The majority of mitotically arrested cells died by apoptosis or necrosis.

To investigate whether docetaxel enhances the response of MCa-4 to fractionated irradiation, two combination treatment schedules were used. In one schedule, a single dose of 33 mg/kg docetaxel was given i.v., followed by local tumor irradiation daily for 5 consecutive days at 3 Gy/fraction starting 24 h after docetaxel administration. In the other schedule, both docetaxel and local tumor irradiation were given daily for 5 consecutive days. The dose of docetaxel was 8 mg/kg/injection; the first injection was given 24 h before the first fraction of irradiation, and the remaining doses of docetaxel were given 9 h before the subsequent radiation doses. The radiation dose was 3 Gy/fraction (total dose, 15 Gy). Thus, in both schedules, the first radiation dose was given when a high percentage of mitotically arrested cells disappeared due to apoptosis or cell lysis (the 24 h interval point), with subsequent radiation doses delivered at the time of peak mitotic arrest (9 h). The control groups consisted of tumor-bearing mice that received docetaxel only, local tumor irradiation only, or no treatment. Also, for comparison, the effect of a single dose of docetaxel (33 mg/kg) combined with a 15-Gy single dose of local tumor irradiation (docetaxel was given 24 h before or 3 h after irradiation) was determined. Tumor growth delay, *i.e.*, the time in days it took the tumor to

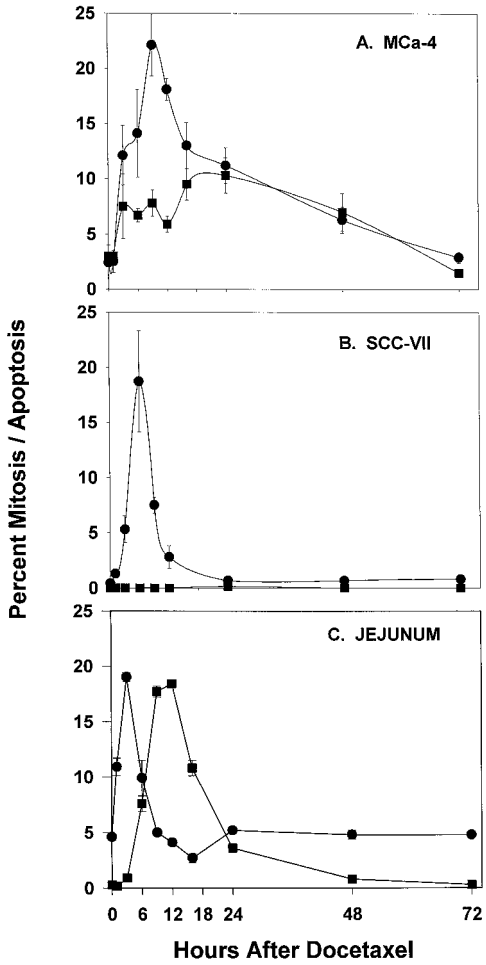

Fig. 1 Mitotic arrest and apoptosis in MCa-4 tumor (*A*), in SCC-VII tumor (*B*), and in jejunal mucosa (*C*) treated with docetaxel. Mice bearing 8-mm leg tumors were injected i.v. with 33 mg/kg docetaxel. Groups of three to five mice were sacrificed 1, 3, 6, 9, 12, 16, 24, 48, and 72 h later. Tumor or jejunum was surgically removed and fixed in neutral buffered formalin before routine histological processing. H&E-stained tissue sections were scored microscopically at ×400. A total of 500 cells/mouse were scored as interphase, mitotic, or apoptotic for MCa-4, SCC-VII, and jejunum. ●, mitotic index; ■ apoptotic index. *Bars*, SE. *A* and *C* are reprinted from Ref. 20 with permission.

[3] The abbreviations used are: AGD, absolute growth delay; EF, enhancement factor; NGD, normalized growth delay.

Table 1 Effect of docetaxel (DOC) on radioresponse of MCa-4 cells measured by tumor growth delay

Treatment[a]	Tumor growth delay			
	Time in days required for tumor to grow from 8 to 12 mm[b]	AGD[c]	NGD[d]	EFs[e]
No treatment	5.4 ± 0.4			
DOC (single)	14.2 ± 0.9	8.8 ± 0.9		
15 Gy XRT (single)[f]	12.1 ± 1.4	6.7 ± 1.4		
DOC (single) + XRT (single)	24.7 ± 1.2	19.3 ± 1.2	10.5 ± 1.2	1.57
XRT (single) + DOC (single)	19.6 ± 1.5	14.2 ± 1.5	5.4 ± 1.5	0.81
DOC (multiple)	22.9 ± 1.5	17.5 ± 1.5		
3 Gy XRT × 5 (fractionated)	10.5 ± 0.5	5.1 ± 0.5		
DOC (single) + XRT (fractionated)	24.5 ± 1.2	19.1 ± 1.2	10.3 ± 1.2	2.02
DOC (multiple) + XRT (fractionated)	29.2 ± 1.2	23.8 ± 1.2	6.3 ± 1.2	1.2

[a] Mice bearing 8-mm tumors in the right legs were given i.v. docetaxel as a single dose of 33 mg/kg or as five daily doses of 8 mg/kg and/or local tumor irradiation.
[b] Mean ± SE.
[c] AGD, days for tumors in treated groups (DOC or radiation) to grow from 8 to 12 mm minus the time for tumors in the untreated control group to reach the same size.
[d] NGD, time for tumors treated with both DOC and radiation to grow from 8 to 12 mm minus the time for tumors treated with DOC alone to reach the same size.
[e] EF, ratio of NGD for tumors treated with DOC plus radiation to AGD for tumors treated with radiation alone.
[f] XRT, radiation.

grow from 8 to 12 mm, was used as the treatment end point (Table 1).

Both single-bolus docetaxel and single-dose radiation were effective as single treatments, but when docetaxel was given 24 h before local tumor irradiation, the tumor growth delay was longer than the additive effects of individual treatments, indicating that docetaxel enhanced tumor radioresponse. The EF was 1.57, which was similar to that reported by us in a previous study (20). Docetaxel given after irradiation did not augment tumor radioresponse. The effect was even somewhat reduced (EF = 0.81). When a single injection of docetaxel was combined with fractionated irradiation, the enhancement of tumor radioresponse was increased from 1.57 (single-dose irradiation) to 2.02.

For the MCa-4 tumor, the antitumor efficacy of docetaxel alone was greater (17.5- *versus* 8.8-day AGD) when, instead of a large single bolus (33 mg/kg), smaller doses (8 mg/kg) were given daily for 5 days (Table 1). The combination of daily docetaxel administrations with fractionated irradiation resulted in the largest tumor growth delay (23.8-day AGD), but this effect could be attributed more to the antitumor efficacy of docetaxel than to its enhancing effect on tumor radioresponse. Although tumor radioenhancement was achieved (EF = 1.2), it was much smaller than that achieved by single bolus administration of docetaxel (EF = 2.02). Fig. 2 shows the effect of docetaxel and fractionated irradiation on the growth of MCa-4 tumors.

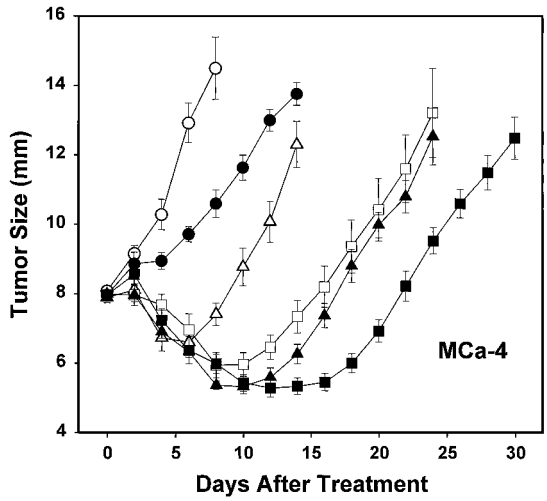

Fig. 2 Effect of docetaxel and fractionated radiation on the growth of MCa-4 tumors in mice. Mice bearing 8-mm tumors in the right hind leg were untreated (○), treated with docetaxel as a single injection of 33 mg/kg (△) or as five daily injections of 8 mg/kg (□), treated with fractionated radiation of 3 Gy daily for 5 days (●), or treated with a combination of fractionated radiation and either a single injection of docetaxel given 24 h before the start of radiation (▲) or five doses of docetaxel (■), with the first injection given 24 h before the first radiation, and the remaining injections applied 9 h before subsequent radiation fractions. Each data point represents the mean size of six or seven tumors; *bars*, SE.

Radioresponse of Docetaxel-resistant SCC-VII. SCC-VII is resistant to a single i.v. bolus (33 mg/kg) of docetaxel (26). At the cellular level, docetaxel induced mitotic arrest but caused no increase in apoptosis (Fig. 1*B*). As in the case with the MCa-4 tumor, SCC-VII was 8 mm in diameter when the mice were given a single i.v. bolus of 33 mg/kg docetaxel. The arrest of cells in mitosis was rapid: mitotic figures were already visible 3 h after treatment. The percentage of arrested cells increased rapidly with time, achieving its peak of 18.7% at 6 h after docetaxel administration. After peaking, the percentage of arrested mitoses declined rapidly, returning to the background level 24 h after the administration of docetaxel.

Because radiosensitivity of mitotically arrested cells forms the biological basis for combining docetaxel with radiation in

Table 2 Effect of docetaxel (DOC) on radioresponse of SCC-VII cells measured by tumor growth delay

Treatment[a]	Tumor growth delay			
	Time in days required for tumor to grow from 8 to 12 mm[b]	AGD[c]	NGD[d]	EFs[e]
No treatment	3.8 ± 0.3			
DOC (single)	3.8 ± 0.2	0		
25 Gy XRT (single)[f]	7.1 ± 0.3	3.3 ± 0.3		
DOC (single) + XRT (single)	7.9 ± 0.7	4.1 ± 0.7	4.1 ± 0.7	1.24
DOC (multiple)	5.9 ± 1.0	2.1 ± 1.0		
5 Gy XRT × 5 (fractionated)	5.7 ± 0.3	1.9 ± 0.3		
DOC (single) + XRT (fractionated)	6.8 ± 0.2	3.0 ± 0.2	3.0 ± 0.2	1.58
DOC (multiple) + XRT (fractionated)	9.7 ± 0.5	5.9 ± 0.5	3.8 ± 0.5	2.0

[a] Mice bearing 8-mm tumors in the right legs were given i.v. docetaxel as a single dose of 33 mg/kg or as five daily doses of 8 mg/kg and/or local tumor irradiation.
[b] Mean ± SE.
[c] AGD, days for tumors in treated groups (DOC or radiation) to grow from 8 to 12 mm minus the time for tumors in the untreated control group to reach the same size.
[d] NGD, time for tumors treated with both DOC and radiation to grow from 8 to 12 mm minus the time for tumors treated with DOC alone to reach the same size.
[e] EF, ratio of NGD for tumors treated with DOC plus radiation to AGD for tumors treated with radiation alone.
[f] XRT, radiation.

this tumor, we tested two treatment approaches. One approach consisted of giving a large i.v. single bolus (33 mg/kg) of docetaxel 6 h before the initiation of daily fractionated radiotherapy at a dose of 5 Gy/fraction for 5 days (total dose, 25 Gy). Thus, the first radiation dose was delivered at the peak of mitotic arrest. The other approach consisted of administering 8 mg/kg docetaxel daily for 5 days 6 h before each radiation fraction of 5 Gy. Thus, in this schedule, each subsequent irradiation was delivered at the peak of mitotic arrest. In theory, this approach should result in higher enhancement of tumor radioresponse than the first approach. As in the experiment with MCa-4, the control groups consisted of tumor-bearing mice that received docetaxel only, local tumor irradiation only, or no treatment. Also, for comparison, the effect of a single dose of docetaxel (33 mg/kg) combined with a 25-Gy single dose of local tumor irradiation was determined (docetaxel was given 6 h before irradiation). Tumor growth delay, as defined previously for MCa-4, was used as the treatment end point (Table 2).

Single-bolus docetaxel was ineffective against this tumor on its own, but it enhanced the effect of single-dose radiation by a factor of 1.24. When combined with fractionated irradiation, it enhanced the tumor radioresponse even more, increased it by a factor of 1.58. When docetaxel was administered for 5 consecutive days, it produced some antitumor effect; the growth of SCC-VII was delayed by 2.1 days. However, when daily docetaxel administration was combined with fractionated irradiation, the enhancement of tumor radioresponse was the greatest; the EF was 2.0.

Radioresponse of Jejunal Mucosa. To provide therapeutic benefit in combination with radiotherapy, any radiopotentiating agent must increase tumor radioresponse more than the radioresponse of normal tissues that limit radiotherapy. We tested whether docetaxel, given in the schedules described above, modulates radiation-inflicted injury to the jejunal mucosa. We reported previously (Ref. 20; Fig. 1*C*) that docetaxel induces both mitotic arrest and apoptosis in the jejunum. The induction of mitotic arrest was more rapid than that in both MCa-4 and SCC-VII tumors, with the peak of mitotic arrest occurring 3 h after treatment, when 19% of crypt cells were mitotic. The decline in the percentage of mitotic cells was also rapid, returning to background levels 9 h after treatment. Docetaxel was also effective in inducing apoptosis in jejunal crypt cells, which started to increase 6 h after docetaxel administration and reached its peak between 9 and 12 h after treatment, when about 18% of cells were apoptotic. After this, the percentage of apoptotic cells declined rapidly, reaching a level only slightly above the background at 24 h after docetaxel administration.

Table 3 Radiation EFs for jejunum pretreated with docetaxel (DOC)

Treatment	Dose (Gy) @ 20 surviving cells/ circumference	EF[a]
FXRT[b] only	28.62	
DOC (multiple) 6 h before FXRT	22.78	1.26
DOC (multiple) 24 & 9 h before FXRT	26.10	1.10
DOC (single) 6 h before FXRT	28.02	1.02
DOC (single) 24 h before FXRT	28.78	0.99

[a] EF, radiation EF.
[b] FXRT, fractionated radiotherapy.

The effect of docetaxel on radioresponse of jejunal mucosa was quantified using drug and radiation schedules similar to those designed for the two tumor types (Table 3; Fig. 3). The radiation dose modification factors were determined at an isosurvival level of 20 surviving cells/circumference of jejunum. The control consisted of mice treated with five daily doses of radiation only. A single i.v. dose of 33 mg/kg docetaxel was administered either 6 or 24 h before the first of five radiation exposures, simulating the tumor treatment schedules for the SCC-VII and MCa-4 tumors, respectively. Docetaxel given by this schedule did not influence the response of jejunal mucosa to radiation: the EF for docetaxel given 6 h before the first radia-

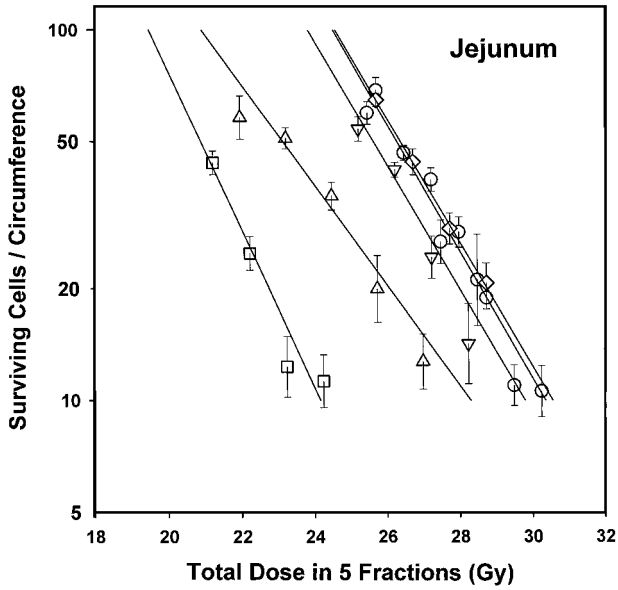

Fig. 3 Radiation dose-survival curves of mouse jejunal crypt cells after five daily fractions of radiation (○) or after injection of docetaxel before fractionated radiation. Docetaxel was given as a single injection of 33 mg/kg 6 (▽) or 24 h (◇) before the first fraction of radiation or as five daily injections of 8 mg/kg given 6 h before each radiation dose (□) or 24 h before the first radiation dose and 9 h before each subsequent radiation dose (△). Each data point represents the mean cell survival of four to six mice; *bars*, SE.

tion exposure was 1.02; and the EF for docetaxel given 24 h before the first radiation dose was 0.99. In contrast, potentiation of radiation injury was demonstrated when multiple doses of docetaxel were combined with fractionated irradiation. The MCa-4-type schedule (8 mg/kg docetaxel 24 h before the first radiation exposure and 9 h before the next four radiation exposures) resulted in an EF of 1.1. The SCC-VII-type schedule (8 mg/kg docetaxel 6 h before each of five radiation exposures) was substantially more effective in enhancing radiation damage to the jejunum. The resultant EF was 1.26. Thus, only the treatment schedules using multiple doses of docetaxel in combination with fractionated irradiation (particularly the 6-h interval schedule) resulted in increased normal tissue toxicity.

DISCUSSION

Our results show that docetaxel greatly enhances the response of murine tumors to fractionated irradiation and increases the therapeutic ratio of radiotherapy. The extents of radioenhancement and therapeutic gain were highly dependent on the administration schedules of the two agents. In turn, optimization of treatment schedules depended on intrinsic characteristics of tumor response to docetaxel as a single treatment, such as whether the tumor was docetaxel sensitive or docetaxel resistant. MCa-4 was used as a prototype of a docetaxel-sensitive tumor, and SCC-VII was used as a prototype of a docetaxel-resistant tumor.

Our previous studies showed that in taxane-sensitive tumors, where cellular response consisted of both mitotic arrest and apoptosis, reoxygenation was a dominant mechanism of radiosensitization, but mitotic arrest contributed as well (5, 19). Because of reoxygenation, the degree of radioenhancement was higher when single-dose radiation was delivered 1–3 days after taxane administration than when it was delivered at the time of the peak of mitotic arrest, which was approximately 9 h after paclitaxel or docetaxel administration (5, 16–19). To take advantage of the effects of tumor reoxygenation on MCa-4 radioresponse, the fractionated radiation in the present study was initiated 24 h after the administration of a single large dose of docetaxel. This schedule resulted in a strong enhancement of tumor response to daily radiation fractionation (EF = 2.02), a response stronger than that seen after single-dose irradiation given 24 h after docetaxel administration (EF = 1.57). This additional increase in radioenhancement after fractionated irradiation could be explained by an additional increase in reoxygenation of hypoxic tumor cells after each fraction of irradiation, radiosensitive mitotically arrested cells that remain present for a few days after docetaxel administration (Fig. 1*A*), or both.

When multiple daily small doses (8 mg/kg) of docetaxel instead of a single large bolus (33 mg/kg) were combined with fractionated irradiation, MCa-4 tumor radioenhancement was also achieved, but it was much smaller (EF = 1.2) than that achieved by the single bolus administration of docetaxel (EF = 2.02). However, this combination resulted in the longest tumor growth delay, although it could be attributed more to the antitumor efficacy of docetaxel than to the enhanced effect on tumor radioresponse. Smaller doses (8 mg/kg) of docetaxel given daily for 5 days were more effective against MCa-4 than a large single bolus (33 mg/kg) of docetaxel (Table 1); therefore, it is not clear why a smaller tumor radioenhancement was achieved by this intermittent daily docetaxel-radiation schedule. Because docetaxel given after radiation was somewhat inhibitory rather than radioenhancing (EF = 0.81; Table 1), as was also found to be the case in some *in vitro* studies (5, 14, 15), it may be possible that during fractionated irradiation, the radioenhancing efficacy of the first docetaxel dose given 24 h before the first dose of fractionated irradiation would be minimal. As already elaborated in the "Introduction," under these conditions, radiation would induce both G_1 and G_2 cell cycle arrest on its own, allowing repair of sublethal damage to occur and simultaneously preventing taxanes from exerting cell cycle effects (14, 15). However, this explanation might not be consistent with the observation that the multiple docetaxel-fractionated irradiation schedule in SCC-VII tumor was the most radioenhancing schedule (see Table 2). Another possibility is that in tumors highly sensitive to multiple administration of docetaxel, docetaxel would affect cell cycle redistribution so that at the time of delivery of daily radiation fractions, an increased proportion of tumor cells might be in the radioresistant S-phase of the cell cycle.

To establish which of the above two schedules provides greater therapeutic benefit, we assessed how the two schedules differed in influencing radiation injury of the jejunal mucosa. The schedule with a single bolus followed 1 day later by fractionated radiation had no influence on intestinal mucosal damage (EF = 0.99), thus providing a high therapeutic gain factor of 2.04 (Table 4). The schedule combining multiple small doses of docetaxel with fractionated radiation was toxic, although not highly toxic. It enhanced the radiation damage of the

Table 4 Therapeutic gain factors after combining docetaxel (DOC) with fractionated irradiation

Treatment		EFs[a]		EFs jejunum		TG[b]	
DOC	XRT	MCA-4[c]	SCC-VII[d]	MCA-4[e]	SCC-VII[f]	MCA-4	SCC-VII
Single[g]	FXRT[h]	2.02	1.58	0.99	1.02	2.04	1.55
Multi[i]	FXRT	1.24	2.00	1.10	1.26	1.13	1.59

[a] EF, radiation EF.
[b] TG, therapeutic gain obtained by dividing EF for tumor by EF for jejunum.
[c] MCa-4, DOC-sensitive tumor.
[d] SCC-VII = DOC-resistant tumor.
[e] MCa-4 = type schedule with DOC 9 h before each radiation dose.
[f] SCC-VII = type schedule with DOC 6 h before each radiation dose.
[g] Single dose of 33 mg/kg DOC 24 h before irradiation.
[h] FXRT, fractionated irradiation once daily for 5 days.
[i] Multiple dose of 8 mg/kg DOC 6 or 9 h before irradiation for SCC-VII and MCa-4, respectively.

jejunum by a factor of 1.1, and, taking into account that tumor radioenhancement by this schedule was relatively small (a factor of 1.24), the resultant therapeutic gain of radiotherapy was small. However, considering that this combination schedule was highly effective against MCa-4, mainly because of the antitumor efficacy of docetaxel, it might be therapeutically beneficial for the treatment of taxane-sensitive tumors.

The two schedules, single large bolus of docetaxel *versus* multiple daily small doses of docetaxel, resulted in different therapeutic outcomes when combined with fractionated radiation for the treatment of docetaxel-resistant SCC-VII tumor and MCa-4 (20). Because docetaxel induced mitotic arrest in this tumor but caused no increase in apoptosis (Fig. 1*B*), our rationale was to give radiation at the peak of mitotic arrest, which was 6 h after docetaxel administration. Single bolus docetaxel enhanced the effect of single-dose radiation by a factor of 1.24 and the effect of fractionated irradiation by a factor of 1.58. This increase in radioenhancement after fractionated irradiation could not be attributed to mitotic arrest because, as shown in Fig. 1*B*, mitotically arrested cells returned to the background level 1 day after administration of a single bolus of docetaxel. Thus, at least the last three fractions of radiation were delivered at the time of no increased mitotic arrest. A likely possibility is that some degree of tumor reoxygenation occurred between radiation fractions. The enhancement of radioresponse of SCC-VII was the greatest when daily administration of small doses of docetaxel was combined with fractionated irradiation, providing an EF of 2.0. Docetaxel was administered 6 h before each radiation fraction; therefore, this strong enhancement of radioresponse could be largely attributed to the accumulation of radiosensitive mitotic cells. In the case of jejunal damage, the single bolus docetaxel schedule had little influence on mucosal radioresponse (EF = 1.02) and consequently resulted in a high therapeutic gain (gain factor, 1.55). However, the daily small dose docetaxel-fractionated radiation schedule was highly toxic for jejunal mucosa, resulting in a radiation EF of 1.26. Even with this high toxicity, however, this schedule provided the greatest therapeutic gain: a factor of 1.59. Therefore, we conclude that the latter schedule is the most effective in the treatment of docetaxel-resistant tumors, but increased mucosal damage must be taken into clinical consideration. These conclusions apply to clinical settings in which acutely responding normal epithelial tissues are at risk for treatment-related complications.

Overall, our results clearly show that docetaxel can greatly enhance tumor response to fractionated irradiation, but the degree of therapeutic efficacy depends on proper drug-radiation scheduling based on tumor biology (Table 4). The results suggest that the most effective treatment for docetaxel-sensitive tumors would be to apply a single large dose of docetaxel 1 day before initiation of fractionated radiotherapy. On the other hand, the most effective treatment for docetaxel-resistant tumors would consist of daily concomitant docetaxel-radiation treatments in which drug administration precedes radiation delivery by several hours. Combining taxanes with radiotherapy on a mechanistic basis calls for the identification of cellular correlates of taxane activity in individual patients. These correlates may include pretreatment levels of apoptosis (24, 26), assessment of oncogenes, such as p53, bax, and bcl-2, or changes in these parameters within 1–2 days after treatment with the drug.

REFERENCES

1. Schiff, P. B., Fant, J., and Horwitz, S. B. Promotion of microtubule assembly *in vitro* by Taxol. Nature (Lond.), *277:* 665–667, 1979.

2. Gueritte-Voegelein, F., Guenard, D., Lavelle, F., Le Goff, M-T., Mangatal, L., and Potier, P. Relationship between the structure of Taxol analogues and their antimitotic activity. J. Med. Chem., *34:* 992–998, 1991.

3. Terasima, T., and Tolmach, L. J. Variations in survival responses of HeLa cells to x-irradiation during the division cycle. Biophys. J., *3:* 11–33, 1963.

4. Sinclair, W. K., and Morton, R. A. X-ray sensitivity during the cell generation cycle of cultured Chinese hamster ovary cells. Radiat. Res., *29:* 450–474, 1966.

5. Milas, L., Milas, M. M., and Mason, K. A. Combination of taxanes with radiation: preclinical studies. Semin. Radiat. Oncol., *9:* 12–26, 1999.

6. Tishler, R. B., Geard, C. R., Hall, E. J., and Schiff, P. B. Taxol sensitizes human astrocytoma cells to radiation. Cancer Res., *52:* 3495–3497, 1992.

7. Liebmann, J., Cook, J. A., Fisher, J., Teague, D., and Mitchell, J. B. *In vitro* studies of Taxol as a radiation sensitizer in human tumor cells. J. Natl. Cancer Inst., *86:* 441–446, 1994.

8. Liebmann, J., Cook, J. A., Fisher, J., Teague, D., and Mitchell, J. B. Changes in radiation survival curve parameters in human tumor and rodent cells exposed to paclitaxel (Taxol). Int. J. Radiat. Oncol. Biol. Phys., *29:* 559–564, 1994.

9. Hennequin, C., Giocanti, N., and Favaudon, V. Interaction of ionizing radiation with paclitaxel (Taxol) and docetaxel (Taxotere) in HeLa and SQ20B cells. Cancer Res., *56:* 1842–1850, 1996.

10. Hennequin, N., Giocanti, N., and Favaudon, V. S-phase specificity of cell killing by docetaxel (Taxotere) in sychronized HeLa cells. Br. J. Cancer, *71:* 1194–1198, 1995.

11. Zanelli, G. D., Quaia, M., Robieux, I., Bujor, L., Santarosa, M., Favaro, D., Spada, A., Caffau, C., Gobitti, C., and Trovo, M. G. Paclitaxel as radiosensitizer: a proposed schedule of administration based on *in vitro* data and pharmacokinetic calculations. Eur. J. Cancer, *33:* 486–492, 1997.

12. Gupta, N., Hu, L. J., and Deen, D. F. Cytotoxicity and cell-cycle effects of paclitaxel when used as a single agent and in combination with ionizing radiation. Int. J. Radiat. Oncol. Biol. Phys., *37:* 885–895, 1997.

13. Minarik, L., and Hall, E. J. Taxol in combination with acute and low dose rate irradiation. Radiother. Oncol., *32:* 124–128, 1994.

14. Ingram, M. L., and Redpath, J. L. Subadditive interaction of radiation and taxol *in vitro*. Int. J. Radiat. Oncol. Biol. Phys., *37:* 1139–1144, 1997.

15. Liebmann, J., Herscher, L., Fisher, J., Teague, D., and Cook, J. A. Antagonism of paclitaxel cytotoxicity by x-rays: implication for the sequence of combined modality therapy. Int. J. Oncol., *8:* 991–996, 1996.

16. Milas, L., Hunter, N. R., Mason, K. A., Kurdoglu, B., and Peters, L. J. Enhancement of tumor radioresponse of a murine mammary carcinoma by paclitaxel. Cancer Res., *54:* 3506–3510, 1994.

17. Milas, L., Hunter, N. R., Mason, K. A., Milross, C. G., Saito, Y., and Peters, L. J. Role of reoxygenation in induction of enhancement of tumor radioresponse by paclitaxel. Cancer Res., *55:* 3564–3568, 1995.

18. Milas, L., Saito, Y., Hunter, N., Milross, C. G., and Mason, K. A. Therapeutic potential of paclitaxel-radiation treatment of a murine ovarian carcinoma. Radiother. Oncol., *40:* 163–170, 1996.

19. Milross, C. G., Mason, K. A., Hunter, N. R., Terry, N. H. A., Patel, N., Harada, S., Jibu, T., Seong, J., and Milas, L. Enhanced radio response of paclitaxel-sensitive and -resistant tumors *in vivo*. Eur. J. Cancer, *33:* 1299–1308, 1997.

20. Mason, K. A., Hunter, N. R., Milas, M., Abbruzzese, J. L., and Milas, L. Docetaxel enhances tumor radioresponse *in vivo*. Clin. Cancer Res., *3:* 2431–2438, 1997.

21. Joschko, M. A., Webster, L. K., Groves, J., Ball, D. L., and Bishop, J. F. Taxol enhances radiation effect in a hypopharyngeal xenograft. *In:* Forty-Fifth Annual Meeting of Radiation Research Society Book of Abstracts, p. 206, Oakbrook, IL: Radiation Research Society, 1994.

22. Griffon-Ettienne, G., Boucher, Y., Taghian, A., Jain, R. K., and Suit, H. D. Effects of paclitaxel and docetaxel on oxygen partial pressure and interstitial fluid pressure in experimental tumors. *In:* Forty-Fifth Annual Meeting of Radiation Research Society Book of Abstracts, p. 154, Oakbrook, IL: Radiation Research Society, 1997.

23. Lokeshwar, B. L., Ferrell, S. M., and Block, N. L. Enhancement of radiation response of prostatic carcinoma by Taxol: therapeutic potential for late-stage malignancy. Anticancer Res., *15:* 93–98, 1995.

24. Milross, C. G., Mason, K. A., Hunter, N. R., Chung, W. K., Peters, L. J., and Milas, L. Relationship of mitotic arrest and apoptosis to antitumor effect of paclitaxel (Taxol). J. Natl. Cancer Inst., *88:* 1308–1314, 1996.

25. Milas, L., Hunter, N. R., Kurdoglu, B., Mason, K. A., Meyn, R. E., and Peters, L. J. Kinetics of mitotic arrest and apoptosis in murine mammary and ovarian tumors treated with Taxol. Cancer Chemother. Pharmacol., *35:* 297–303, 1995.

26. Schimming, R., Mason, K. A., Hunter, N., Weil, M., Kishi, K., and Milas, L. Lack of correlation between mitotic arrest or apoptosis and antitumor effect of docetaxel. Cancer Chemother. Pharmacol., *43:* 165–172, 1999.

27. Mason, K. A., Milas, L., and Peters, L. J. Effect of paclitaxel (Taxol) alone and in combination with radiation on the gastrointestinal mucosa. Int. J. Radiat. Oncol. Biol. Phys., *32:* 1381–1389, 1995.

28. Milas, L., Hunter, N., Mason, K. A., and Withers, H. R. Immunological resistance to pulmonary metastases in C3Hf/Bu mice bearing syngeneic fibrosarcoma of different sizes. Cancer Res., *34:* 61–71, 1974.

29. Stephens, L. C., Ang, K. K., Schultheiss, T. E., Milas, L., and Meyn, R. E. Apoptosis in irradiated murine tumor. Radiat. Res., *127:* 308–316, 1991.

30. Withers, H. R., and Elkind, M. M. Microcolony survival assay for cells of mouse intestinal mucosa exposed to radiation. Int. J. Radiat. Biol., *17:* 261–267, 1970.

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