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Interaction of Ionizing Radiation with Paclitaxel (Taxol) and Docetaxel (Taxotere) in HeLa and SQ20B Cells[1]

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ABSTRACT

Altered γ-ray response by brief (1 h), concomitant exposure to paclitaxel (Taxol) or docetaxel (Taxotere) was investigated in growing HeLa and SQ20B human tumor cells *in vitro*. For both cell lines, both taxoids were able to reduce or enhance radiation cell killing, depending on the drug concentration. Large reduction of radiosensitivity (up to 3.3-fold reduction relative to radiation alone) was observed in HeLa cells over a wide range of drug concentrations, extending to 1.5- (paclitaxel) or 3.3-fold (docetaxel) the IC_{50}s determined for drug alone. This antagonistic effect was also observed with SQ20B cells. It disappeared for drug concentrations exceeding 0.9 (SQ20B), 1.6 (HeLa; paclitaxel), and 3.4 (HeLa; docetaxel) IC_{50} equivalents, above which a drug dose-dependent, supra-additive radiation-drug interaction was observed.

Reduction of radiation susceptibility in the low-drug dose range also held for mid-G_1 synchronized HeLa cells, *i.e.*, in the cell cycle compartment characterized as the most resistant one to docetaxel (C. Hennequin *et al.*, Br. J. Cancer, *71:* 1194–1198, 1995). In the case of SQ20B cells, the cytotoxicity of either drug or radiation alone was primarily dependent on the state of growth, with quiescent (G_0) cells showing increased radiosensitivity and reduced drug toxicity compared to the growing fraction.

The effect of taxoids (1-h contact) was finally investigated in sequential treatment as a function of the time elapsed between radiation and exposure to drugs. In HeLa cells, the postirradiation time-dependence of the response to combined treatment was biphasic. The radioprotecting potential of either taxoid disappeared in ~1.5 h following radiation. At longer postirradiation delays, radiation-induced redistribution in the cell cycle appeared to be the major determinant of HeLa cell survival, in relation to the differential cell cycle phase specificity of each drug. Pronounced paclitaxel recovery *versus* increased sensitivity to docetaxel occurred over 8 h after irradiation. SQ20B cells showed monophasic radiation recovery with both drugs over the same time range.

INTRODUCTION

Paclitaxel (TAX[3]) and docetaxel (TXT) are the prototypes of a new class of microtubule-targeting diterpenoids referred to as taxoids. Paclitaxel (TAX) was first isolated from the bark of the Pacific yew *Taxus brevifolia* (1). TXT, a hemisynthetic analogue of TAX, has been prepared using English yew *T. baccata* needle extracts (2, 3). TAX and TXT kill proliferating mammalian cells *in vitro* (4–6). Both drugs have proven to be efficient in the treatment of solid tumors in humans from the very beginning of clinical studies (7, 8).

TAX binds specifically to microtubules (9–11), alters their dynamics (12, 13), promotes the reorganization of the microtubular network into bundles or asters, and stabilizes microtubules against disruption by various agents (14–17). TXT produces similar effects, yet it appears more potent than TAX on a molar basis (3, 18–20). These processes are currently considered to play a leading role in the antineoplasic potential of taxoids (21). However, they usually require exposure to large amounts of drugs relative to their IC_{50}s (17). Moreover, we have shown recently through the use of synchronized cells that TAX and TXT demonstrate different cell cycle-phase specificities, thus eliciting differential targets for cell killing (22).

Continuous exposure of human cells to cytotoxic amounts of TAX or TXT brings about a sustained block at the metaphase-anaphase boundary in many cell lines. Based on this observation and on the widely held opinion that cells in mitosis are considerably more sensitive to radiation than interphasic cells, a combination of prolonged taxoid exposure with ionizing radiation has been proposed to produce increased radiation response. Pioneering studies of radiation interaction with TAX (23, 24) or TXT (25) seemed consistent with this view. However, further studies showed that enhanced radiation cell killing by taxoids does not work in all cell lines (Table 1). Antagonistic interaction was even reported in some instances, and it has been recognized that prometaphase arrest upon prolonged contact with drugs, if it occurs, may not be a sufficient condition for increased radiation sensitivity (26–30). In fact, large differences in the efficiency of the mitotic block occur among cell lines (31, 32); in some instances, drug-treated cells escape the mitotic block without cytokinesis and give rise to multinucleated, nonviable cells (33). Moreover, characterization of the intrinsic radiation sensitivity of the various compartments of the cell cycle has only been completed in a very limited number of cell lines, including human HeLa cells and Chinese hamster V79 fibroblasts (34–37). The radiation sensitivity of HeLa and V79 cells does culminate in G_2-M, but whether the same holds true for all cell lines is open to question. Liebmann *et al.* (30), for instance, have shown recently that two human adenocarcinoma cell lines, although readily blocked at mitosis upon incubation with TAX, do not exhibit increased radiation sensitivity compared to drug-free replicates. Finally, recent *in vivo* studies (Table 2) suggest that other mechanisms than prometaphase arrest may act to provide synergistic interaction between taxoids and radiation. In particular, the manipulation of the oxygen supply has shown that reoxygenation plays a major role in enhancing tumor radioresponse *in vivo* when combined with TAX (38, 39).

From what is said above, whether and by which mechanisms taxoids may interact with radiation response seemed to be worth a careful reinvestigation. We chose to address the question using pulse (1-h) exposure to TAX or TXT concomitantly with γ-ray irradiation in two tumor cell lines *in vitro*. With these experimental conditions, there is no time for drugs to induce significant cell cycle redistribution at the time of irradiation. In spite of this, the results show that profound alteration of radiation survival may occur in the presence of TAX or TXT, ending in reduced or enhanced radiation response, depending on the drug concentration, the cell line, the state of growth, and the time scheduling of drug and radiation.

MATERIALS AND METHODS

Reagents. TAX (NSC 125973) and TXT (RP 56976; NSC 628503), both obtained as pure crystalline powders from Rhône-Poulenc Rorer, were stored as 10 mM sterile solutions in absolute ethanol at −20°C. Drugs were adjusted

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[3] The abbreviations used are: TAX, Taxol (paclitaxel); TXT, Taxotere (docetaxel).

Table 1 *In vitro* studies (clonogenic assays) on the outcome of combined radiation-drug treatment with taxoids

Authors (Ref.)	Drug	Cell line[a]	Concentration range (nM)	Exposure (h)[b]	G_2-M block	Flow cytometry	Additivity status	Comments[c]
Tischler *et al.* (23, 24)	TAX	G18	10	24	Yes	Yes	Supra	[1]
Choy *et al.* (25)	TXT	HL-60	30	ND	ND	ND	Supra	[2]
Liebmann *et al.* (40)	TAX	MCF-7, PC-Sh, A549	1–10	24	Yes	ND	Add	[3]
		MCF-7, PC-Sh	100–1000	24	Yes	ND	Supra	
		A549	100–1000	24	Yes	ND	Add	
Chang *et al.* (41)	TAX	A549	10	24	Yes	Yes	Supra	[4]
			10	0.5–3	No		Anta	
Choy *et al.* (42)	TAX	HL-60	30	1	No	Yes	Supra	[5]
Steren *et al.* (43, 44)	TAX	OVCAR-3, SK-OV-3	0.5–50	1.5	ND	ND	Supra	
		BG-1	5	1.5	No	Yes	Supra	
Hei and Hall (26)	TAX	C_3H 10T1/2	100	24	No	ND	Add	[6]
Gupta *et al.* (45)	TAX	U-251 MG	5	24	Yes	Yes	Supra	
Geard and Jones (28)	TAX	SiHa	10	24	Yes	ND	Add	[7]
Liebmann *et al.* (29, 30)	TAX	MCF-7, PC-Sh, V79	1000	24	Yes	Yes	Supra	[8]
		A549, OVG-1	1000	24	Yes	Yes	Add	
Minarik and Hall (27)	TAX	SiHa	10	24	Yes	Yes	Anta	[9]
Stromberg *et al.* (46)	TAX	MCF-7, DUT-145, HT-29	2–25	12–24	No	Yes	Add	
Lokeshwar *et al.* (47)	TAX	PC-3, TSU-Pr1	10	24	Yes	Yes	Supra	[10]
Blackinton *et al.* (48)	TAX	LBC	4–200	ND	ND	ND	Add	
Chi *et al.* (49)	TAX	HeLa	ND	2	Yes	Yes	Supra	[11]
Rave-Fränk *et al.* (50)	TAX	MCF-7, CaSki, OC-II	7	24	Yes	Yes	Add	[12]

[a] Human (tumor) cell lines: G18, grade III astrocytoma; HL-60, promyelocytic leukemia; MCF-7, breast adenocarcinoma; PC-Sh, pancreas adenocarcinoma; A549, lung carcinoma; BG-1, OVCAR-3, SK-OV-3, and OVG-1, ovary (adeno)carcinoma; OC-II, primary ovary carcinoma; U-251 MG, glioma; SiHa, cervix squamous carcinoma; HeLa, cervix epitheloid carcinoma; CaSki, cervical epidermoid carcinoma; DUT-145, PC-3, and TSU-Pr1, prostate (adeno)carcinoma; HT-29, colon adenocarcinoma; LBC, lymphoblastoid B cells. Rodent (normal) cell lines: C_3H 10T1/2 mouse embryo fibroblasts; V79, Chinese hamster fibroblasts.

[b] Length of drug exposure preceding irradiation. ND, not determined or not described. ERCKF, enhanced radiation cell killing factor (radiation dose, or radiation survival unreported). $ERCKF_{50}$ and $ERCKF_{10}$, ERCKF values for a γ-ray dose leaving 50 and 10% cell survival, respectively.

[c] [1], $ERCKF_{50} \approx 3.1$, $ERCKF_{10} \approx 30.3$; [2], $ERCKF_{50} \approx 1.53$, $ERCKF_{10} \approx 1.38$; [3], $ERCKF_{10}$ (MCF-7) ≈ 1.9; [4], ERCKF ≈ 1.13 (10 nM TAX; 24-h exposure); results at variance with those reported by Liebmann *et al.* (29, 30, 40) using the same cell line; [5], ERCKF ≈ 1.48; large accumulation of cells in G_2-M occurred 24 h after radiation/drug treatment and replating; [6], rodent cells were found to escape the mitotic block and undergo polyploidization upon prolonged incubation with taxoids; [7], synchronization by mitotic shake-off was used in this experiment to demonstrate M-phase specificity of the cytotoxicity of TAX; [8], $ERCKF_{10} \approx 1.8$ (MCF-7), 1.6 (OVG-1) or 1.5 (PC-Sh); the ERCKF values at low-radiation dose, determined in this study from the ratio of the α parameter of the radiation survival curves fitted to the linear-quadratic model, were 3.81 (MCF-7) or 3.64 (V79); [9], ERCKF ≈ 0.73 (determined in the same way as in [8]; [10], ERCKF (determined from the mean inactivation doses D_0) ≈ 1.52 (PC-3) or 1.15 (TSU-Pr-1); [11], short contact with TAX and radiation exposure at 24-h intervals; [12], some radiation sensitization may occur at low survival.

extemporarily at the appropriate concentrations by successive dilutions in pure DMSO and in growth medium through vortexing. The final concentration of DMSO was low enough (≤0.5%) as not to alter cell growth.

Rat monoclonal antibody directed against 5-bromo-2′-deoxyuridine was purchased from CeraLab. FITC-conjugated goat antirat IgG (heavy chain specific) was from Southern Biotechnology Associates. Thymidine, 5-bromo-2′-deoxyuridine, and propidium iodide came from Sigma Chemical Co. All products for cell culture were from Life Technologies, Inc..

Cell Cultures. HeLa (human cervix carcinoma) and SQ20B (head and neck squamous carcinoma) cells were kindly provided by Dr. J. Coppey (Institut Curie) and Dr. J. B. Little (Harvard School of Public Health), respectively. Cells were routinely subcultured every 4 days at a density of 6×10^3 cells/cm^2, unless otherwise stated, and grown as monolayers in DMEM with 4.5 g/liter glucose, 0.1 g/liter pyruvate, 10^5 IU/liter penicillin, 0.1 g/liter streptomycin, 0.86 g/liter Glutamax I, and 10% v/v fetal calf serum (37°C; 7% CO_2), plus 0.4 mg/liter hydrocortisone for SQ20B cells only. The number of passages from the same primary subculture was kept below 7–8. The mean doubling times (mid-log phase) were 21 ± 1 h (HeLa) or 23 ± 1 h (SQ20B). Control plating efficiencies were in the range of 80% for both cell lines. Synchronization of HeLa cell cultures was performed using a double thymidine block in the same way as described by Tsao *et al.* (57).

Treatments. For colony formation assays, 600–1200 cells from subcultures were plated in triplicate or more in 25-cm^2 flasks and incubated at 37°C for 4 h prior to treatment. Following radiation and/or drug exposure, the flasks were rinsed, and cells returned to normal growth medium for 11 or 12 days. Colonies were fixed with methanol, stained, and scored visually.

γ-Ray irradiation of cells with or without concomitant drug exposure was performed at room temperature (21–24°C) in medium saturated with 93% air-7% CO_2, using an IBL-637 (^{137}Cs) irradiator (CIS-BioInternational). The dose rate was 1.0 Gy/min.

Exposure to TAX or TXT was carried out in dim light to prevent photodegradation of the drugs. Contact with either taxoid lasted 1 h, unless otherwise stated. Drug was carefully removed through two washes with HBSS (37°C) at 3–4 min intervals, after which cells were returned to normal growth medium.

Cytofluorimetric Analysis and Data Handling. Cell cycle progression in subcultures and in treated cells was monitored by dual parameter flow cytometry using a FACStarPLUS cytofluorometer (Becton Dickinson). Cells were incubated with 5-bromo-2′-deoxyuridine (10 μM; 15 min) for pulse-labeling of S-phase cells and then harvested by trypsinization, pelleted, washed once with cold PBS, and fixed in 70% ice-cold ethanol. Treatment of fixed cells for cytofluorimetric analysis and bivariate data acquisition and processing were done according to Demarcq *et al.* (58). In experiments using synchronized (HeLa) cells, corrections for cellular multiplicity were performed in the same way as reported previously (59).

Results have been expressed as means ± SD. Least-squares regression analyses were performed using a Gauss-Levenberg-Marquardt algorithm (Kaleidagraph 3.0; Abelbeck/Synergy Software, Inc.).

RESULTS

Cytotoxic Response to Drugs and Radiation Alone. The viability of proliferating HeLa or SQ20B cells exposed for 1 h to TAX or TXT was assessed by clonogenic assays as described in "Materials and Methods." With both drugs and both cell lines, the reproductive ability decreased following an exponential dose-effect relationship. For HeLa cells, the mean IC_{50}, *i.e.*, the amount of drug that reduces the proliferation of treated cells to 50% of that in controls, was in the range of 25 nM for TXT and 225 nM for TAX, as reported (22). These

Table 2 *In vivo* studies on the outcome of combined treatment with paclitaxel and radiation

Authors (Ref.)	Cell line	Drug schedule	Growth delay (days)		Radiation alone	Combined treatment outcome[a]	Comments[b]
			Drug alone	Radiat. alone			
Joschko *et al.* (51)	FaDu	Single bolus (125 mg/kg)	13.6	53		Complete regression	[1]
		10-day boluses (20 mg/kg/day)	23.0	53		Complete regression	
Milas *et al.* (52)	MCA-4	Single bolus (60 mg/kg)	7.2	5.7		Growth delay 8.4[c]–9.7[d]–14.2[e] days	[2]
Lokeshwar *et al.* (47)	DU R23327G	5-day boluses (0.4 mg/kg/day)	3.6	12		ND	[3]
		5-day boluses (4 mg/kg/day)	9.1	12		Growth delay, 21 days	
Milas *et al.* (38)	MCA-4	Single bolus (60 mg/kg)	7.1	8.5[h] *vs.* 67.0[i]		Growth delay 14.8[f] *vs.* 7.2[g] days	[4]
Hampel *et al.* (53)	PEC-MB	Single bolus (10 mg/kg)	ND	ND		ERCKF ~ 1.9	[5]
Milas *et al.* (39)	MCA-4	Single bolus (60 mg/kg)	6.8[h]	8.5[h] *vs.* 6.2[i]		Growth delay (days) 19.0[h] *vs.* 12.5[ij] 22.5[h] *vs.* 13.5[ik] 32.1[h] *vs.* 13.9[il]	[6]
Mason *et al.* (54)	MCA-4 and Intestinal Crypts	Single bolus (40 mg/kg)			TCD_{50} = 69.5 Gy	TCD_{50} = 60.0 Gy[j] 52.5 Gy[k], 45.7 Gy[l]	[7]
Sigdestad *et al.* (55)	Intestinal Crypts	Single bolus (40 mg/kg)				Infraadditive[m] Additive[n]	[8]
Mason *et al.* (56)	Intestinal Crypts	Single or 4-day boluses (total, 40 mg/kg)				ERCKF ~ 1.07[o] ERCKF ~ 0.89–0.92[p]	[9]
Milas *et al.* (39)	MCA-4	Single bolus (40 mg/kg)			TCD_{50} = 70.2[h] Gy *vs.* 76.2[i] Gy	TCD_{50}[i] (Gy) 60.6[h] *vs.* 71.6[ij] 54.9[h] *vs.* 71.9[ik] 50.0[h] *vs.* 73.8[il]	[10]

[a] Footnote explanations are within the "Comments" footnote b.

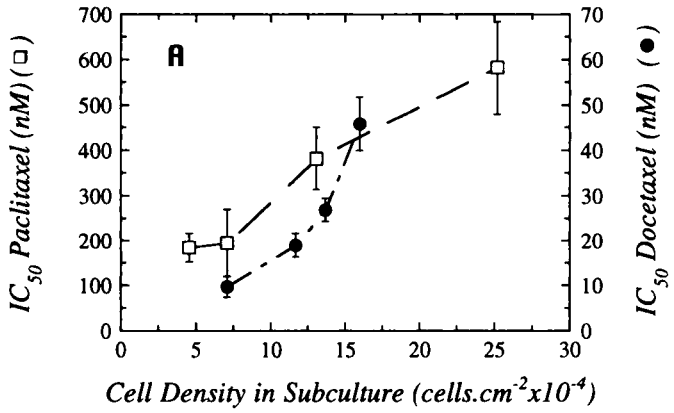
[b] [1], human hypopharynx squamous carcinoma cells grafted in female BALBc *(nu/nu)* mice. Fractionated irradiation (2 Gy/day for 10 days). No tumor recurrence after 80 days (combined treatment). Vehicle (solvent) is unreported; [2], nonimmunogenic, syngeneic murine mammary carcinoma cells grafted in female C3Hf/Kam mice. Single-dose irradiation (15 Gy). Drug injection with Chremophor EL at 1 (c), 9 (d), or 24 h (e) prior to irradiation; [3], Dunning androgen-sensitive human prostatic carcinoma cells grafted in male CopxF344F1 rats. Fractionated irradiation (1.5 Gy/day, 5 days). Drug was injected with Chremophor EL; [4], same cells as in [2] but grafted in male C3Hf/Kam mice. Single-dose irradiation (21 Gy). Drug was injected with Chremophor EL 24 h prior to irradiation. Tissue oxygenation at the time of irradiation was normoxyc (f) or hypoxic (g); [5], human squamous cell carcinoma cells xenotransplanted on NMRI nude mice. Single-dose irradiation (18 MeV electrons; dose unreported). Drug was injected (vehicle unreported) 12, 24, or 36 h prior to radiation. Synergistic effect increased with the delay between drug and radiation and was maximum at 48 h. ERCKF, enhanced radiation cell killing factor; [6], same animals, grafted cell line, vehicle, and radiation single-dose as in [4]. The results refer to normoxyc (h) or hypoxic conditions (i). Irradiation was given 9 (j), 24 (k), or 48 h (l) after paclitaxel; [7], same animals and grafted cell line as in [2]. Single-dose irradiation (varying doses). Drug was injected with Chremophor EL 9 (j), 24 (k) or 48 h (l) prior to radiation. The maximum differential therapeutic index was reached at 48 h, with ~5% protection of normal tissue (intestinal crypts) and 1.5-fold increased tumor radioresponse; [8], normal tissue (jejunum) response. Random-bred HA/ICR male mice. Single-dose irradiation (12.5 Gy). Drug was injected with Chremophor EL at varying times (48 h before to 48 h after) relative to radiation, and tissue survival was determined at 12-h intervals by microcolony assays. The interaction was found to be additive when drug was introduced 2–10 h prior to radiation (m), and additive or slightly supraadditive when drug was introduced later (n); [9], same animals, grafted cell line, and solvent as in [2]. Single-dose irradiation (10–15 Gy; 1.6 Gy/min). Drug was injected at different times (4–24 h) prior to radiation, and tissue dynamics and survival were determined for up to 4 days following drug exposure. TAX-induced mitosis and apoptosis were peaking at 2–4 h and 10–12 h following drug injection, respectively. Radiation-drug interaction was slightly supraadditive at 4 h after injection (o) but afforded significant radioprotection after 24 h (p). ERCKF, enhanced radiation cell killing factor; [10], same experiment as in [6]. Irradiation was given under normoxyc (h) or hypoxic conditions (i) at 9 (j), 24 (k), or 48 h (l) after TAX exposure.

values did not change appreciably with the age of the subcultures as long as these were in the exponential phase of growth. In contrast, the resistance of SQ20B cells to TAX and TXT grew considerably with cell density in the subcultures (Fig. 1*A*), matching the accumulation of cells in G_0-G_1 (Fig. 1*B*). This may be explained by the propensity of SQ20B cells to grow as tight, well-delimited microcolonies in which inner cells form a confluent-arrested, drug-resistant monolayer and require over 6–8 h to resume cell cycle progression after dislocation by trypsin and plating.

The radiation sensitivity of SQ20B cells was also primarily dependent on the growth fraction in subcultures (Fig. 2*A*). The α parameter determined from fitting the radiation survival curves to the linear-quadratic model (Fig. 2*A*) demonstrated a linear relationship with the cell density; the effect on the quadratic component, β, was comparatively minor (Fig. 2*B*). In other words, with SQ20B cells, the quiescent fraction was the most radiosensitive, whereas it was the most resistant to drugs. HeLa cells did not show significant variations of the α (0.350 ± 0.028 Gy^{-1}) and β (0.0061 ± 0.0009 Gy^{-2}) parameters with the cell density in subcultures. It is also worth mentioning that with the HeLa cell line used in this study, the value of the β component was comparatively low, as frequently found with high-passage aneuploid tumor cells.

Concomitant Treatment with Radiation and Taxoids in HeLa Cells. Asynchronous, growing HeLa cells were exposed to TAX or TXT for exactly 30 min, irradiated, and immediately returned to the incubator. Drug was subsequently washed off, and cells were supplied with fresh growth medium in such a way that the total length of contact with drug was 1 h.

The effect of combined treatment was first explored as a function of the radiation dose using fixed drug concentrations in the range of the IC_{50}s. Typical results are shown in Fig. 3*A*. Drug survivors clearly showed reduced radiation sensitivity compared to drug-free controls. In many instances, the survival curve for combined treatment was found to intercept the one drawn from survival to radiation alone. Therefore, taxoids are able to produce a large decrease of the radiation susceptibility among the drug surviving fraction over the whole range of radiation doses investigated. Interestingly, this effect persisted in HeLa cell cultures synchronized in G_1 (Fig. 3*B*), corresponding to peak resistance to TXT (22).



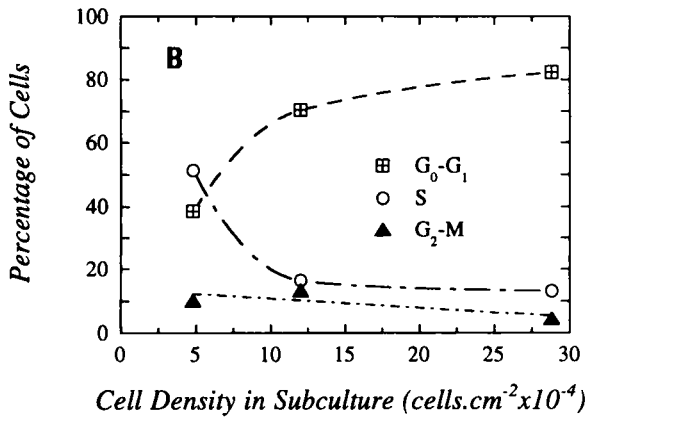


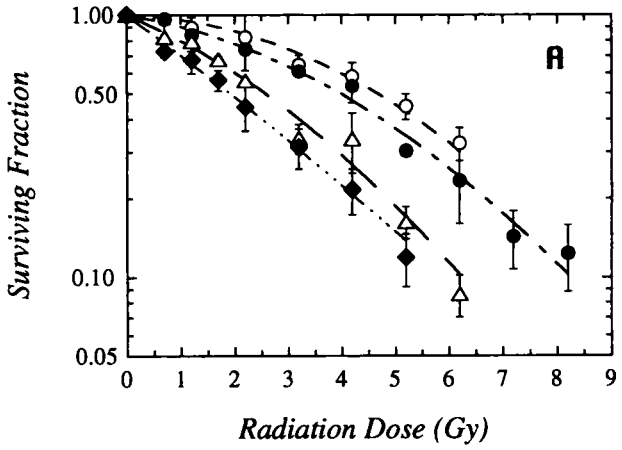
Fig. 1. *A*, variation of the cytotoxicity of paclitaxel (□) and docetaxel (●) against SQ20B cells with the cell density in subcultures. Cells were plated (in triplicate) 4 h prior to drug exposure for colony formation assays. The length of contact with drug was 1 h. IC_{50} is defined as the drug concentration that reduces the clonogenic ability of cells to 50% of that of controls. The IC_{50}s for HeLa cells were 25.2 ± 1.8 nM (docetaxel) and 225 ± 22 nM (paclitaxel). *Bars*, SD. *B*, flow cytometric determination (duplicate measurements) of the growth fraction in SQ20B cultures as a function of the cell density. Quiescent (G_0) cells represent ~70% of the bulk cell population at the maximum density reached in the experiment. Deviation was less than the size of the symbols representing the means.

Experiments were also carried out by varying the drug concentration at a fixed dose of radiation to establish the drug concentration dependence of combined treatment survival. Antagonistic interaction ceased in the presence of 1.5 (TAX) to 3.3 (TXT) IC_{50} equivalents, and enhanced radiation response was observed at larger drug concentrations, leaving residual survival values to combined treatment of 5% or less (Fig. 4).

Concomitant Treatment with Radiation and Taxoids in SQ20B Cells. The outcome of combined radiation-drug treatment with SQ20B cells was found to depend on the radiation dose and on the growth state in subcultures in addition to the drug concentration which, for HeLa cells, was the only major determinant. Fig. 5 shows radiation survival following concomitant treatment, with radiation and TAX or TXT of SQ20B cells plated from subcultures of differing cell density. With quiescent or poorly cycling subcultures, a low concentration of TAX or TXT virtually annihilated the lethal effect of radiation below 2 Gy (Fig. 5*B*). At high radiation doses, this antagonistic effect was only minor and was no longer dependent on the γ-ray dose. Reduction of the radiation response was still more pronounced with cells from actively growing subcultures (Fig. 5*A*).

Fig. 6 shows the drug concentration dependence of the additivity status of drugs and 5 Gy radiation. The drug dose range within which TAX or TXT reduces the radiation toxicity was much narrower than for HeLa cells, and supraadditive interaction consistently took place at comparatively lower concentrations.

Postirradiation Recovery of HeLa and SQ20B Cells. The effect of taxoids was finally investigated in sequential exposure to radiation and drugs by varying the radiation-to-drug delay. Biphasic time-dependent profiles were obtained with HeLa cells, showing a rapid loss of cell survival, followed either by pronounced recovery, indicative of decreasing TAX toxicity, or by a time-related increase of TXT cytotoxicity (Fig. 7*A*). The first phase of the whole process evokes a rapid loss with time of the radioprotecting ability of either drug; the half-life of this phase was estimated at ~15 min from curve fitting, but uncertainties about the rate of drug intake and efflux definitely precluded a precise determination. On the other hand, cell cycle redistribution by radiation was determined to correlate it with the postirradiation time-dependence of drug cytotoxicity. Acute irradiation of HeLa cells produced accumulation of cells in S phase and at the S-G_2 junction preceding arrest in G_2, but exit from G_1 was not hindered, thus resulting in depletion of the G_1 compartment with time



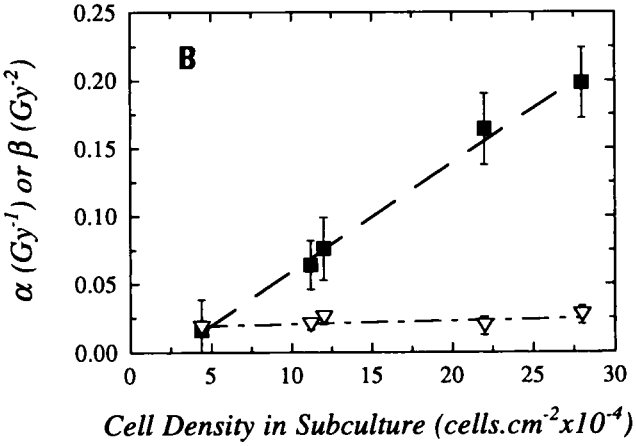


Fig. 2. *A*, γ-ray dose-dependence of asynchronous growing HeLa (◆) and SQ20B (△, ●, and ○) cell survival. Cell densities in SQ20B subcultures were 0.46 × 10^5 (○), 1.2 × 10^5 (●), and 2.8 × 10^5 (△) cells/cm^2. Survival curves were fitted to the linear-quadratic equation:

$$\ln \frac{S}{S_0} = -\alpha.D - \beta.D^2,$$

where D is the radiation dose and S_0 the plating efficiency (unirradiated cells). *Bars*, *SD*. *B*, variation of the α (■) and β (▽) parameters of radiation survival of SQ20B cells with the age of subcultures. α and β for HeLa cells were 0.350 ± 0.028 Gy^{-1} and 0.0061 ± 0.0009 Gy^{-2}, respectively. *Bars*, SD.

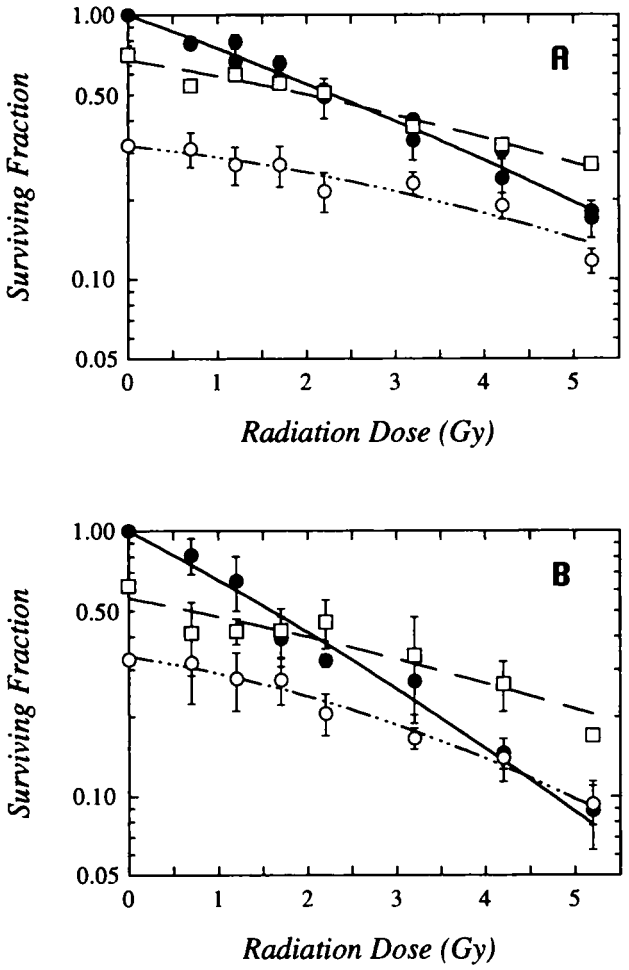


Fig. 3. *A*, radiation survival of asynchronous growing HeLa cells without (●) or with (□, ○) concomitant treatment with 30 nM docetaxel (○) or 200 nM paclitaxel (□). Drugs were introduced 30-min prior to irradiation and were present for 60 min. Fitting survival curves to a linear-quadratic equation as in Fig. 2 yielded α (Gy^{-1}) and β (Gy^{-2}) values of 0.282 ± 0.043 and 0.0087 ± 0.0035 for radiation alone (●); 0.088 ± 0.034 and 0.0145 ± 0.0070 with docetaxel (○); and 0.132 ± 0.032 and 0.0099 ± 0.0038 with paclitaxel (□). *Bars*, SD. *B*, radiation survival of HeLa cells synchronized in mid-G_1, *i.e.*, 14 h after release from double thymidine block (59). ●, radiation alone (α = 0.410 ± 0.094 Gy^{-1}, β = 0.0154 ± 0.0030 Gy^{-2}); ○, concomitant treatment with 20 nM docetaxel (α = 0.121 ± 0.043 Gy^{-1}; β = 0.0252 ± 0.0063 Gy^{-2}); □, concomitant exposure to 200 nM paclitaxel (α = 0.154 ± 0.058 Gy^{-1}; β = 0.0074 ± 0.0025 Gy^{-2}). Data were corrected for the effect of cell multiplicity. *Bars*, SD.

(Fig. 8*A*). With HeLa cells, therefore, acute irradiation brings about accumulation in compartments of the cell cycle which, from studies performed using synchronized HeLa cells (22), appear to be considerably more sensitive to TXT and more resistant to TAX, than mid-log asynchronous cell populations.

Altered cell cycle progression by radiation was comparatively less pronounced in SQ20B cells. With early-log cultures, a 5-Gy exposure resulted in some G_1 depletion (−11%) and accumulation in G_2 (+14%) after 9 h postirradiation incubation, but the bulk S-phase content was affected only poorly (Fig. 8*B*). With mid-log and late-log subcultures, very few cells experienced cell cycle redistribution by radiation. Accordingly, there were no significant changes in the sensitivity of mid- and late-log cells to drugs with the postirradiation time. With early-log cells, irradiation did produce altered drug response with time, but the narrow domain of drug concentration for switching from antagonistic to supraadditive interaction (Fig. 6) made the results somewhat puzzling in the low-dose range. Fig. 7*B* shows the effect of postirradiation drug exposure in early-log SQ20B cells using a high enough drug concentration to allow for additive or slightly supraadditive interaction. This effect was similar with both drugs and consisted of loss of drug toxicity progressing slowly within the time range considered. The initial, rapid decrease of drug-induced radioresistance typical of HeLa cells response was not observed.

DISCUSSION

TAX and TXT demonstrate large differences in the cell cycle phase specificity for cell killing. As a matter of fact, we showed that TAX toxicity peaks at mitosis, whereas TXT shows near-absolute lethality against S-phase cells (22). Unexpectedly also, we show here that brief exposure to TAX or TXT at concentrations in the range of their IC_{50}s may bring about marked reduction of the cell killing potential of radiation applied concomitantly (Figs. 3 and 5). With HeLa cells, an alteration of the α parameter of radiation survival (Fig. 2*A*) among the drug-surviving fractions appears to be the major outcome, and reduced radiation response takes place over the entire range of γ-ray doses investigated. In contrast, for SQ20B cells, the effect is strongly dependent on the radiation dose, showing optimal protection in the low-dose range. Moreover, in the case of SQ20B cells, the response to drug alone, to radiation alone, and to combined treatment is also affected by the state of growth (Figs. 2 and 5).

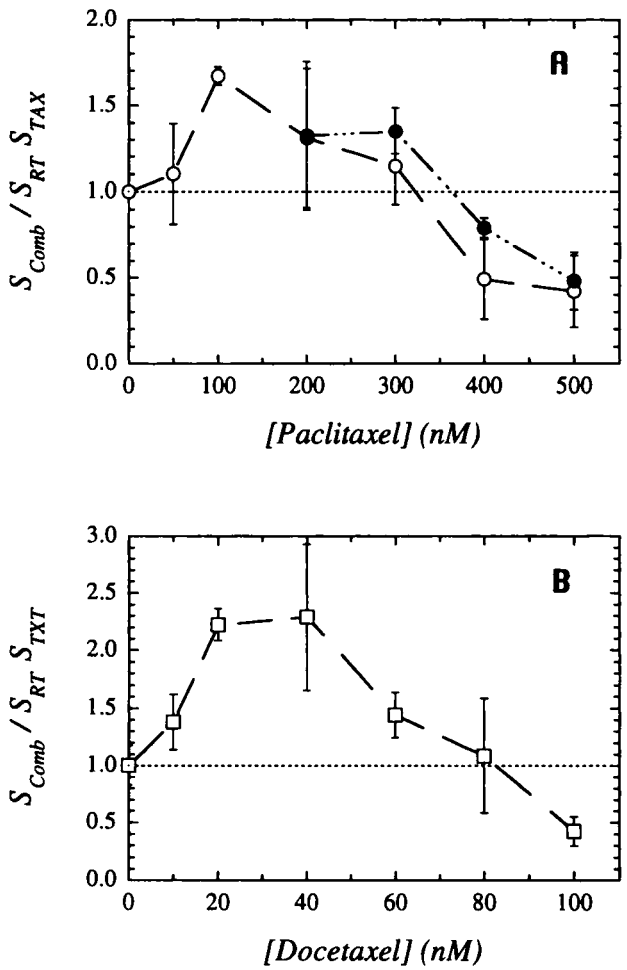


Fig. 4. Analysis of the outcome of combined treatment with 5 Gy (○, □) or 7 Gy (●) radiation and paclitaxel (*A*) or docetaxel (*B*) with survival as an end point. The additivity status was determined from the ratio of survival to combined treatment (S_{Comb}) relative to the product of survival to radiation (S_{RT}) and drug alone (S_{TAX}, S_{TXT}). $S_{Comb}/S_{RT} \cdot S_{Drug} > 1$ defines infraadditive interaction; $S_{Comb}/S_{RT} \cdot S_{Drug} < 1$ indicates supraadditive interaction. IC_{50}s for paclitaxel and docetaxel alone were 225 and 25 nM, respectively. *Bars*, SD.

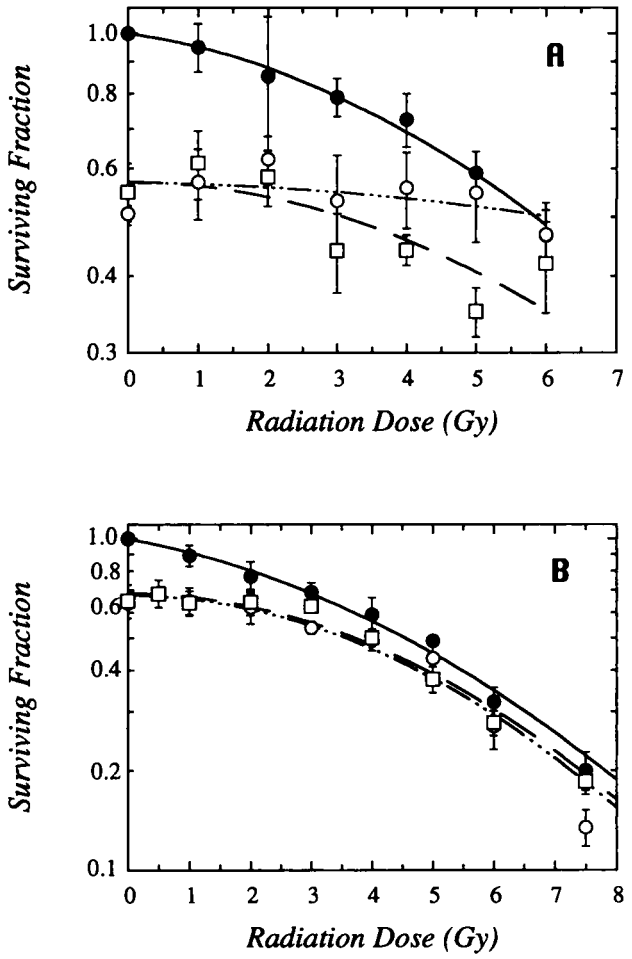


Fig. 5. Radiation survival of asynchronous growing SQ20B cells with or without concomitant exposure to docetaxel or paclitaxel. The time scheduling of drug and radiation was as in Fig. 3. Data were fitted to the linear-quadratic equation given in Fig. 2. *Bars*, SD. *A*, response of cells from low-density (0.46×10^5 cells/cm^2) subcultures to radiation alone (●; $\alpha = 0.0361 \pm 0.0097$ Gy^{-1}; $\beta = 0.0141 \pm 0.0018$ Gy^{-2}), to radiation plus 10 nM docetaxel (○; $\alpha \sim 0.0009$ Gy^{-1}; $\beta \sim 0.0034$ Gy^{-2}), or to radiation plus 150 nM paclitaxel (□; $\alpha \sim -0.0018$ Gy^{-1}; $\beta \sim 0.0130$ Gy^{-2}). *B*, replicate experiments using cells from subcultures grown at high density (2.8×10^5 cells/cm^2). ●, radiation alone ($\alpha = 0.077 \pm 0.012$ Gy^{-1}; $\beta = 0.0165 \pm 0.0021$ Gy^{-2}); ○, radiation with 20 nM docetaxel ($\alpha \sim 0.0035$ Gy^{-1}; $\beta \sim 0.0227$ Gy^{-2}); □, radiation with 200 nM paclitaxel ($\alpha \sim 0.0034$ Gy^{-1}; $\beta \sim 0.0220$ Gy^{-2}).

The interaction between radiation and drug becomes supraadditive in the high-drug dose range (Figs. 4 and 6). For the two cell lines studied, different drug doses, relative to the respective IC_{50}s, are required to observe this supraadditivity. For HeLa cells, it requires amounts of drug in excess of 2-fold the IC_{50}. SQ20B cells, on the other hand, are more sensitive and display supraadditive interaction with doses as low as 1 IC_{50} or even less. Therefore, pulse exposure to taxoids may act to induce infraadditive as well as supraadditive radiation-drug interaction in the low- and high-dose range of drug, respectively (Fig. 9). These results establish that a prometaphase block is not required for taxoids to act as radiation enhancers.

Contact with S phase-specific poisons usually bears pseudo-sensitization to radiation among the drug-surviving fractions, simply because the S-phase compartment is the most radioresistant one (59, 60). In this respect, reduced radiation sensitivity in asynchronous HeLa cells by concomitant exposure to TXT was unexpected. Moreover, TAX is less efficient in this process than TXT at equitoxic doses (Fig. 4). Alteration of DNA synthesis or interphase progression is unable to explain this effect. Actually, we found, in agreement with Kuriyama *et al.* (61), that the initiation of DNA replication in cells released from double thymidine block at the G_1-S junction is not impaired by taxoids; DNA, RNA, and protein syntheses are not hindered (62); moreover, our experimental results show that neither TAX nor TXT alters the rate of progression through S and G_2 phases. Reduced radiation susceptibility by TAX or TXT also takes place in HeLa cells synchronized in mid-G_1 phase (Fig. 3*B*), corresponding to a minimum drug sensitivity (22). This suggests that stabilization of the microtubule dynamics and radiation repair are inter-related, although TAX is not an inducer of immediate-early response genes, not even at supralethal doses (63).

At the present time, we have no straightforward explanation for the synergistic or antagonistic effects of taxoids applied concomitantly with, or at a short interval after radiation. We hypothesize that taxoids, by virtue of their ability to bind firmly to β-tubulin, may hinder microtubule-dependent transduction pathways involved in radiation-induced apoptotic cell death. In this regard, it is worth mentioning that TAX mimics the action of bacterial endotoxin in inducing rapid tumor necrosis factor-α induction and phosphorylation of the microtubule-associated protein-2-kinase in macrophages (64–66), correlated with nuclear factor-κB activation (67). In addition, combined treatments of B-lymphoblastoid cells with radiation and TAX or vincristine have been proposed recently as ways to alter drug-induced apoptosis in a dose-dependent fashion (68). That TAX may alter protein isoprenylation (69) suggests that taxoids may elicit many different transduc-

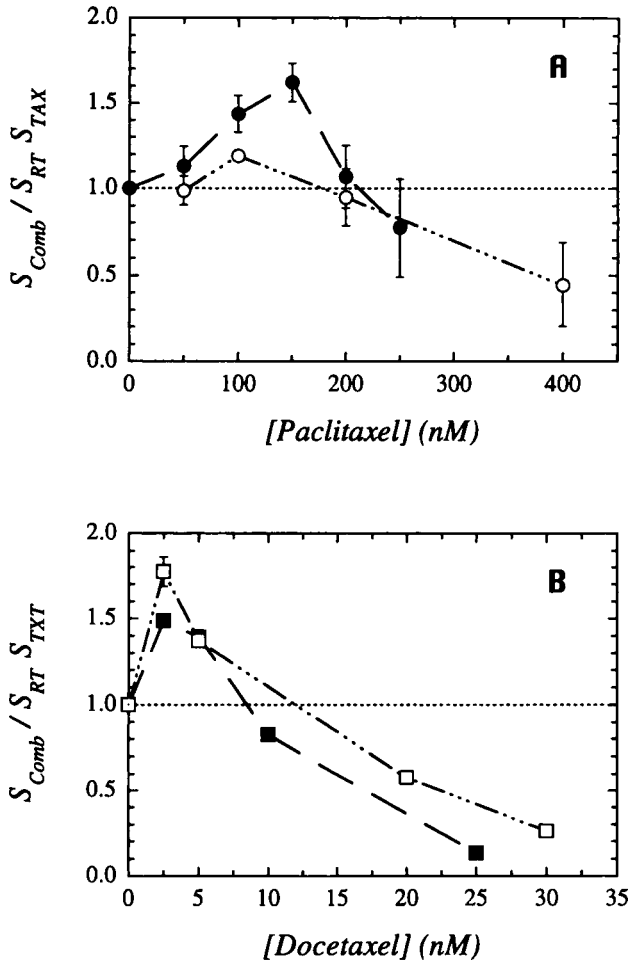


Fig. 6. Outcome of combined treatment with 5 Gy radiation and paclitaxel (*A*) or docetaxel (*B*). The additivity status was determined as in Fig. 4. Cell densities in subcultures were 0.68 (●; IC_{50} = 212 nM), 1.9 (○; IC_{50} = 514 nM), 0.72 (■; IC_{50} = 9.7 nM), and 1.8×10^5 cells/cm^2 (□; IC_{50} = 45.3 nM). *Bars*, SD.

tional targets. However, it should be kept in mind that TAX and TXT show a very different potential with regard to tumor necrosis factor-α induction in macrophages (70), and whether and to what extent similar processes may occur to alter radioresponse in epithelial cells is open to question.

Cell survival following exposure to cell cycle stage-specific drugs applied at an interval after radiation is under the control of cytokinetic cooperation, *i.e.*, of cell cycle phase redistribution by radiation (59, 60). However, analysis of postirradiation recovery (Fig. 7) shows differing pictures among HeLa and SQ20B cells. HeLa cells display a characteristic biphasic process, with a rapid phase corresponding to loss of the antagonistic ability (Fig. 7). This phase is missing in the case of SQ20B cells due probably to the fact that, at the concentrations used, TAX or TXT provide supraadditive interaction with radiation (Fig. 6). HeLa and SQ20B cell survival also show different profiles within the time domain, extending the time of drug exposure beyond 1 h after radiation. For HeLa cells, acute irradiation induces accumulation in S phase and at the S-G_2 junction, accompanied by depletion of the G_1 compartment (Fig. 8). As a result, the most responsive fraction to TAX (22) decays progressively over the time range considered. TAX survival accordingly grows as the time elapsed between radiation and drug exposure increases. In contrast, TXT survival drops within the same time intervals, reflecting the G_1 phase depletion and

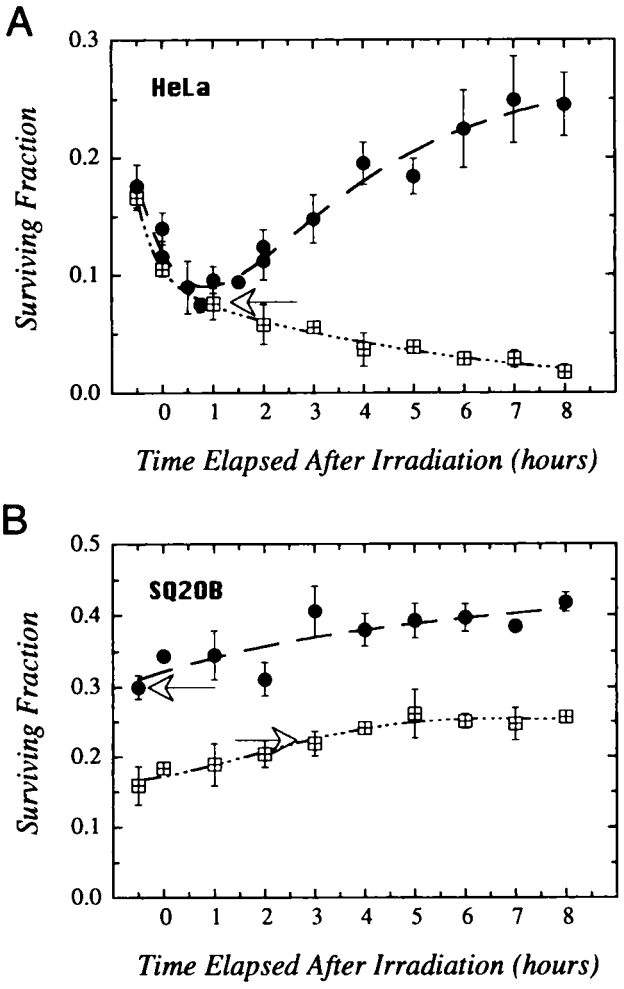


Fig. 7. Postirradiation evolution of the cytotoxicity of 20 nM docetaxel (⊞) or 200 nM paclitaxel (●) against asynchronous HeLa and SQ20B cells. The cell density in SQ20B subcultures was 2.0 × 10^5 cells/cm^2. The γ-ray dose given initially was 5 Gy. Drugs (60-min contact) were introduced at the times indicated. *Arrows*, combined treatment survival corresponding to additivity. *Bars*, SD.

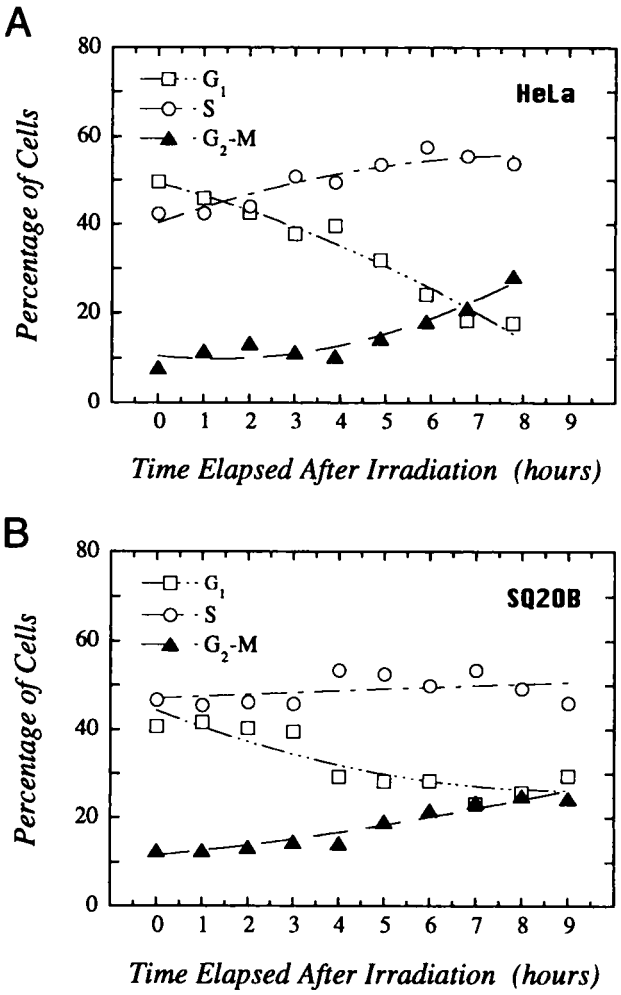


Fig. 8. Cell cycle phase redistribution of asynchronous HeLa and SQ20B cell cultures following 5 Gy irradiation. The cell density in SQ20B subcultures was 0.56 × 10^5 cells/cm^2.

the accumulation of cells in S phase. With SQ20B cells, no such postirradiation time-related differences in the cytotoxic response to TAX or TXT are observed. SQ20B cells show uniform, parallel radiation recovery with both drugs. This matches the weak cell cycle redistribution by radiation observed with these cells. Redistribution is still less pronounced with mid- to late-log SQ20B cultures.

Peak plasma concentrations at an interval of 1–4 h after administration of 60–225 mg/m^2 TAX in short (1–3 h) infusion may be as high as 2–5 μM (71–73). In contrast, residual plasma concentrations obtained after 12 to 24 h following drug infusion ranged between 50 and 100 nM (71–73). TXT shows similar pharmacokinetic and catabolic behavior (74). Our data suggest, on the one hand, that the full dosage of taxoids may provide suitable conditions for supraadditive interaction with radiation in concomitant exposure, and on the other hand, that induced radiation resistance by TAX or TXT may occur in the low-drug dose range. In addition, the outcome of combined treatment varies from one cell line to another, as shown here through the comparison of HeLa and SQ20B cells (Fig. 9). Although the large reoxygenation potential of taxoids determined from *in vivo* studies (38, 39, 52) is likely to result in an enhanced cytotoxic response to radiation, one should remain careful about the possibility of increased radiation resistance by taxoids in concomitant treatment.

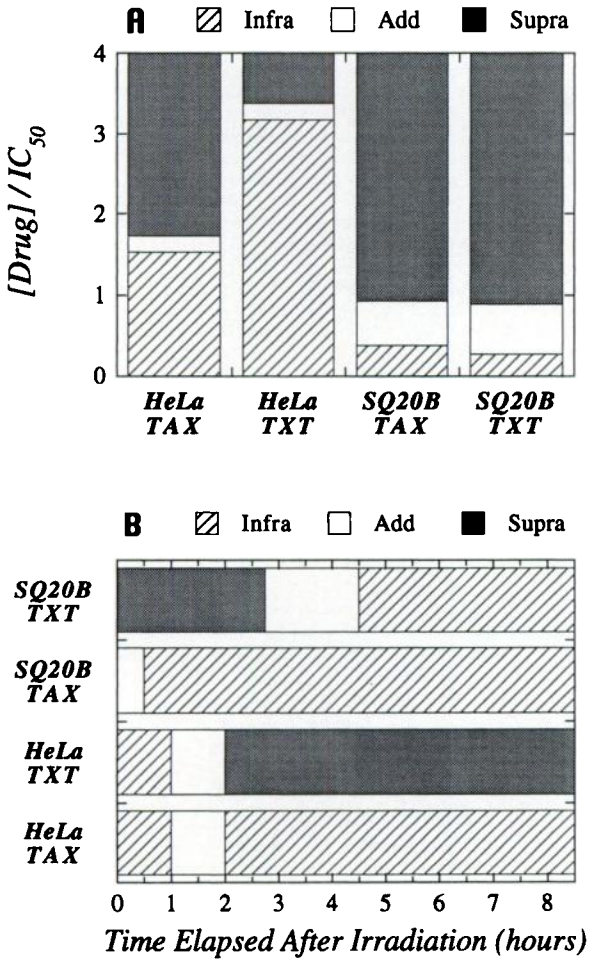


Fig. 9. Synopsis of the additivity status of radiation-taxoid interaction in HeLa and SQ20B cells as a function of the drug concentration relative to the IC_{50} in concomitant treatment (*A*) and of the postirradiation delay in sequential treatment (*B*). Radiation doses and drug concentrations are the same as given in the legends to Figs. 4, 6, and 7. *Infra,* infraadditive interaction; *Add,* additive interaction; *Supra,* supraadditive interaction.

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