Interaction of Ionizing Radiation with Paclitaxel (Taxol) and Docetaxel (Taxotere) in HeLa and SQ2OB Cells

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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 SQ2OB 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 IC₅₀ determined for drug alone. This antagonistic effect was also observed with SQ2OB cells. It disappeared for drug concentrations exceeding 0.9 (SQ2OB), 1.6 (HeLa; paclitaxel), and 3.4 (HeLa; docetaxel) IC₅₀ 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-G₁ 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 SQ2OB cells, the cytotoxicity of either drug or radiation alone was primarily dependent on the state of growth, with quiescent (G₀) 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. SQ2OB cells showed monophasic radiation recovery with both drugs over the same time range.

INTRODUCTION

Paclitaxel (TAX) 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 binds specifically to microtubules (9–11), alters their 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 antineoplastic potential of taxoids (21). However, they usually require exposure to large amounts of drugs relative to their IC₅₀ (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 G₂-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 radioreponse 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 alternation 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 S. A., were stored as 10 mm sterile solutions in absolute ethanol at −20°C. Drugs were adjusted
extemporarily at the appropriate concentrations by successive dilutions in pure DMSO was low enough (<0.5%) as not to alter cell growth.

DMSO and in growth medium through vortexing. The final concentration of products for cell culture were from Life Technologies, Inc. 2'-deoxyuridine, and propidium iodide came from Sigma Chemical Co. All (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 × 10^4 cells/cm^2, unless otherwise stated, and grown as monolayers in DMEM with CO_2), plus 0.4 mg/liter hydrocortisone for SQ2OB cells only. The number of doubling times (mid-log phase) were 21 ± 1 h (HeLa) or 23 ± 1 h (SQ2OB).

Colonies were fixed with methanol, stained, and scored visually. For colony formation assays, 600–1200 cells from subculture 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.

Exposure to TAX or TXT was carried out in dim light to prevent photo-degradation 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 mm intervals, after which cells were returned to normal growth medium.

**Cytolfluorometric 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 5-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 cytolfluorometric 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; Abellbeck/Synergy Software, Inc.).

**RESULTS**

**Cytotoxic Response to Drugs and Radiation Alone.** The viability of proliferating HeLa or SQ2OB 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 1 In vitro studies (clonogenic assays) on the outcome of combined radiation-drug treatment with taxoids

<table>
<thead>
<tr>
<th>Authors (Ref.)</th>
<th>Drug</th>
<th>Cell line⁶</th>
<th>Concentration range (nm)</th>
<th>Exposure (h)⁷</th>
<th>G₂-M block</th>
<th>Flow cytometry</th>
<th>Additivity status</th>
<th>Comments¹</th>
<th>Comments¹</th>
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<tr>
<td>Tischler et al. (23, 24)</td>
<td>TAX</td>
<td>G18</td>
<td>10</td>
<td>24</td>
<td>Yes</td>
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<td>Supra</td>
<td>[1]</td>
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<td>Choy et al. (25)</td>
<td>TXT</td>
<td>HL-60</td>
<td>30</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Supra</td>
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<td>Liebmann et al. (40)</td>
<td>TAX</td>
<td>MCF-7, PC-Sh, A549</td>
<td>1–10</td>
<td>24</td>
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<td>No</td>
<td>Add</td>
<td>[3]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TAX</td>
<td>MCF-7, PC-Sh</td>
<td>100–1000</td>
<td>24</td>
<td>Yes</td>
<td>ND</td>
<td>Supra</td>
<td>[3]</td>
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<tr>
<td></td>
<td></td>
<td>A549</td>
<td>100–1000</td>
<td>24</td>
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<tr>
<td></td>
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<td>SiHa</td>
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<tr>
<td>Liebmann et al. (29, 30)</td>
<td>TAX</td>
<td>MCF-7, PC-Sh, V79</td>
<td>1000</td>
<td>24</td>
<td>Yes</td>
<td>Yes</td>
<td>Supra</td>
<td>[8]</td>
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<tr>
<td>Minnik and Hall (27)</td>
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<td>SiHa</td>
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<td>Lokeshwar et al. (47)</td>
<td>TAX</td>
<td>PC-3, TSU-Prl</td>
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<td>Blackinton et al. (48)</td>
<td>TAX</td>
<td>LBC</td>
<td>4–200</td>
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<td>Chi et al. (49)</td>
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<td>HeLa</td>
<td>2</td>
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<td>Rave-Frank et al. (50)</td>
<td>TAX</td>
<td>MCF-7, CaSki, OC-JI</td>
<td>7</td>
<td>24</td>
<td>Yes</td>
<td>Yes</td>
<td>Add</td>
<td>[12]</td>
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</table>

⁶Human (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 OV-1, ovary (adenocarcinoma; OC-2, primary ovary carcinoma; U-251 MG, glioma; SiHa, cervix squamous carcinoma; HeLa, cervix epithelial carcinoma; CaSki, cervical epithelial carcinoma; DUT-145, prostate (adenocarcinoma; HT-29, colon adenocarcinoma; LBC, lymphoblastoid B cells. Rodent (normal) cell lines: C3H lOTl/2 mouse embryofibroblasts; V79, Chinese hamster fibroblasts.

⁷Length of drug exposure preceding irradiation. ND: not determined or not described. ERCKF₅₀ and ERCKF₁₀: ERCKF values for a γ-ray dose leaving 50 and 10% cell survival, respectively.

*1*, ERCKF₅₀ = 3.1, ERCKF₁₀ = 3.03; [2], ERCKF₅₀ = 1.53, ERCKF₁₀ = 1.38; [3], ERCKF₁₀ (MCF-7) = 1.9; [4], ERCKF ≈ 1.13 (10 nCi TAX; 24-h exposure); results at variance with those reported by Liebmann et al. (29, 30) using the same cell line; [5], ERCKF ≈ 1.48; large accumulation of cells in G₂-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], ERCKF₁₀ (MCF-7), 1.6 (OV-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 D₀) = 1.52 (PC-3) or 1.15 (TSU-Prl-1); [11], short contact with TAX and radiation exposure at 24-h intervals; [12], some radiation sensitization may occur at low survival.

**RESULTS**

**Cytotoxic Response to Drugs and Radiation Alone.** The viability of proliferating HeLa or SQ2OB 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 IC₅₀, 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
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 SQ2OB cells to TAX and TXT grew considerably with inner cells form a confluent-arrested, drug-resistant monolayer and SQ2OB cells to grow as tight, well-delimited microcolonies in which the quiescent fraction was the most radiosensitive, whereas it was the most resistant to drugs. HeLa cells did not show significant variations of the $\alpha$ (0.350 ± 0.028 Gy$^{-1}$) and $\beta$ (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 $\beta$ 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 mm, 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$_{50}$. Typical results are shown in Fig. 3A. Drug survivors clearly showed reduced radiation sensitivity compared to drug-free controls. 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 peak and usually between 2–4 h and 10–12 h after drug injection, respectively. Radiation-drug interaction was slightly supradadditive at 4 h after injection (o) but afforded significant radioprotection after 24 h (p). ERCKF, enhanced radiation cell killing factor; [10], same experiment as [6]. Irradiation was given under normoxic (h) or hypoxic conditions (i). Irradiation was given (9) 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 SQ2OB cells to TAX and TXT grew considerably with 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 SQ2OB cells was also primarily dependent on the growth fraction in subcultures (Fig. 2A). The $\alpha$ parameter determined from fitting the radiation survival curves to the linear-quadratic model (Fig. 2A) demonstrated a linear relationship with the cell density; the effect on the quadratic component, $\beta$, was comparatively minor (Fig. 2B). In other words, with SQ2OB 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 $\alpha$ (0.350 ± 0.028 Gy$^{-1}$) and $\beta$ (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 $\beta$ 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$_{50}$. Typical results are shown in Fig. 3A. 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 resistance of SQ2OB cells to TXT (22).

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

<table>
<thead>
<tr>
<th>Authors (Ref.)</th>
<th>Cell line</th>
<th>Drug schedule</th>
<th>Growth delay (days)</th>
<th>Combined treatment outcome$^a$</th>
<th>Comments$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joschko et al. (51)</td>
<td>FaDu</td>
<td>Single bolus (125 mg/kg)</td>
<td>13.6</td>
<td>Complete regression</td>
<td>[1]</td>
</tr>
<tr>
<td>Milas et al. (52)</td>
<td>MCA-4</td>
<td>Single bolus (60 mg/kg)</td>
<td>7.2</td>
<td>5.7</td>
<td>Growth delay</td>
</tr>
<tr>
<td>Lokeshwar et al. (47)</td>
<td>DU R23327G</td>
<td>5-day boluses (0.4 mg/kg/day)</td>
<td>3.6</td>
<td>ND</td>
<td>Growth delay</td>
</tr>
<tr>
<td>Milas et al. (38)</td>
<td>MCA-4</td>
<td>Single bolus (60 mg/kg)</td>
<td>7.1</td>
<td>5.7 vs. 67.0$^a$</td>
<td>Growth delay</td>
</tr>
<tr>
<td>Hample et al. (53)</td>
<td>PEC-MB</td>
<td>Single bolus (10 mg/kg)</td>
<td>ND</td>
<td>ND</td>
<td>ERCKF ~ 1.9</td>
</tr>
<tr>
<td>Milas et al. (39)</td>
<td>MCA-4</td>
<td>Single bolus (60 mg/kg)</td>
<td>6.8$^a$</td>
<td>5.7 vs. 6.2$^a$</td>
<td>Growth delay</td>
</tr>
<tr>
<td>Mason et al. (54)</td>
<td>MCA-4 and Intestinal Crypts</td>
<td>Single bolus (40 mg/kg)</td>
<td>TCD$_{50}$ = 69.5 Gy</td>
<td>TCD$_{50}$ = 60.0 Gy$^b$</td>
<td>ERCKF ~ 1.9</td>
</tr>
<tr>
<td>Sigestedt et al. (55)</td>
<td>Intestinal Crypts</td>
<td>Single bolus (40 mg/kg)</td>
<td>TCD$_{50}$ = 60.0 Gy$^b$</td>
<td>ERCKF ~ 0.89–0.92$^b$</td>
<td>[8]</td>
</tr>
<tr>
<td>Mason et al. (56)</td>
<td>Intestinal Crypts</td>
<td>Single or 4-day boluses (total, 40 mg/kg)</td>
<td>TCD$_{50}$ = 70.2$^b$ Gy vs. 76.2 Gy</td>
<td>TCD$_{50}$ = 70.2$^b$ Gy</td>
<td>ERCKF ~ 0.89–0.92$^b$</td>
</tr>
</tbody>
</table>

$^a$ 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 mouse 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], Dunnin androgen-sensitive human prostatic carcinoma cells grafted in male C3Hf/F441 rats. Fractionated irradiation (1.5 Gy/day, 5 days). Drug injection 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 normoxic (h) or hypoxic (g); [5], human squamous cell carcinoma cells xenotransplanted on NMRJ nude mice. Single-dose irradiation (18 MeV electrons; dose unreported). Drug was injected (vehicle unreported) 12, 24, or 48 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 normoxic (h) or hypoxic conditions (i). Irradiation was given (9) 24 h, (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 24 h prior to irradiation. The maximum differential therapeutic index was reached at 48 h, with ~5% protection of normal tissue (intestinal crypts) and 1.5-fold increased tumor radioreponse; [8], normal tissue (jejunal) response. Random-bred HA/RCR 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 micrometabolic assays. The analysis was found to be additive when drug was introduced 2–10 h prior to radiation (m), and additive or slightly supradadditive 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 peak at 2–4 h and 10–12 h after drug injection, respectively. Radiation-drug interaction was slightly supradadditive 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 normoxic (h) or hypoxic conditions (i) at 9 (j), 24 (k), or 48 h (l) after TAX exposure.
TAXOIDS AND IONIZING RADIATION

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 SQ2OB 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. 7A). 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-G2 junction preceding arrest in G2, but exit from G1 was not hindered, thus resulting in depletion of the G1 compartment with time.

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) IC50 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 SQ2OB Cells. The outcome of combined radiation-drug treatment with SQ2OB 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 SQ2OB 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. 5B). 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. 5A).

Fig. 6 shows the drug concentration dependence of the additivity status of drugs and 5 Gy radiation. The drug dose range within which

\[ \frac{S}{S_0} = -\alpha D - \beta D^2, \]

where \( D \) is the radiation dose and \( S_0 \) the plating efficiency (unirradiated cells). Bars, SD. B, variation of the \( \alpha \) (■) and \( \beta \) (□) parameters of radiation survival of SQ2OB cells with the age of subcultures. \( \alpha \) and \( \beta \) for HeLa cells were 0.350 ± 0.028 Gy\(^{-1}\) and 0.0061 ± 0.0009 Gy\(^{-2}\), respectively. Bars, SD.

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**Fig. 1.** A, variation of the cytotoxicity of paclitaxel (□) and docetaxel (●) against SQ2OB 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\(_{50}\) 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 SQ2OB cultures as a function of the cell density. Quiescent (G0) 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.

**Fig. 2.** A, rny-ray dose-dependence of asynchronous growing HeLa (●) and SQ2OB (Δ, ○, and ◆) cell survival. Cell densities in SQ2OB subcultures were 0.46 × 10⁶ (○), 1.2 × 10⁶ (●), and 2.8 × 10⁶ (Δ) cells/cm². 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 \( \alpha \) (■) and \( \beta \) (□) parameters of radiation survival of SQ2OB cells with the age of subcultures. \( \alpha \) and \( \beta \) for HeLa cells were 0.350 ± 0.028 Gy\(^{-1}\) and 0.0061 ± 0.0009 Gy\(^{-2}\), respectively. Bars, SD.
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 IC50s 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. 2A) 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 SQ2OB cells, the effect is strongly dependent on the radiation dose, showing optimal protection in the low-dose range. Moreover, in the case of SQ2OB 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).

With SQ2OB 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 SQ2OB cells. With early-log cultures, a 5-Gy exposure resulted in some G1 depletion (~11%) and accumulation in G2 (+14%) after 9 h postirradiation incubation, but the bulk S-phase content was affected only poorly (Fig. 8B). 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. 7B shows the effect of postirradiation drug exposure in early-log SQ2OB cells using a high enough drug concentration to allow for additive or 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**

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et al. (61), that the initiation of DNA replication in cells released from double thymidine block at the G1-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 G2 phases. Reduced radiation susceptibility by TAX or TXT also takes place in HeLa cells synchronized in mid-G1 phase (Fig. 3B), 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 supra-lethal 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-

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 IC50s, are required to observe this supraadditivity. For HeLa cells, it requires amounts of drug in excess of 2-fold the IC50. SQ20B cells, on the other hand, are more sensitive and display supraadditive interaction with doses as low as 1 IC50 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

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 × 10^6 cells/cm²) subcultures to radiation alone (□, α = 0.0361 ± 0.0097 Gy⁻¹; β = 0.0141 ± 0.0018 Gy⁻²), to radiation plus 10 nM docetaxel (○, α = 0.0090 Gy⁻¹; β = 0.0034 Gy⁻²), or to radiation plus 150 nM paclitaxel (□, α = 0.0018 Gy⁻¹; β = 0.0130 Gy⁻²). B, replicate experiments using cells from subcultures grown at high density (2.8 × 10^6 cells/cm²). ○, radiation alone (α = 0.077 ± 0.012 Gy⁻¹; β = 0.0165 ± 0.0021 Gy⁻²); □, radiation with 20 nM docetaxel (α = 0.0035 Gy⁻¹; β = 0.0227 Gy⁻²); □, radiation with 200 nM paclitaxel (α = 0.0034 Gy⁻¹; β = 0.0220 Gy⁻²).

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 (○, IC₅₀ = 212 nM), 1.9 (○, IC₅₀ = 314 nM), 0.72 (○, IC₅₀ = 9.7 nM), and 1.8 × 10⁶ cells/cm² (□, IC₅₀ = 45.3 nM). Bars, SD.
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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 SQ2OB 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 SQ2OB cells due probably to the fact that, at the concentrations used, TAX or TXT provide supraadditive interaction with radiation (Fig. 6). HeLa and SQ2OB 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-G2 junction, accompanied by depletion of the G1 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 G1 phase depletion and the accumulation of cells in S phase. With SQ2OB cells, no such postirradiation time-related differences in the cytotoxic response to TAX or TXT are observed. SQ2OB 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 SQ2OB cultures.

Peak plasma concentrations at an interval of 1–4 h after administration of 60–225 mg/m² 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 SQ2OB cells (Fig. 9). Although the large reoxygenation potential of taxoids determined from in vitro 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.


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

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