130-nm Albumin–Bound Paclitaxel Enhances Tumor Radiocurability and Therapeutic Gain

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Abstract Purpose: 130-nm albumin–bound paclitaxel (nab-paclitaxel) is a novel solvent-free albumin-bound paclitaxel, designed to avoid solvent-related toxicity. Nab-paclitaxel has been successfully introduced into the clinic but its radiation-enhancing potential has not yet been evaluated. We conducted a preclinical evaluation of the radiation-modulating effects of nab-paclitaxel in tumor and normal tissues.

Experimental Design: Mice bearing syngeneic ovarian or mammary carcinomas were treated with nab-paclitaxel, radiation, or combination of both. Nab-paclitaxel was administered at 90 mg/kg, 1.5 times the maximum tolerated dose for solvent-based paclitaxel. End points were antitumor efficacy (growth delay, radiocurability, and cellular effects) and normal tissue toxicity (gut and skin).

Results: Nab-paclitaxel showed single-agent antitumor efficacy against both tumor types and acted as a radiosensitizer. Combined with radiation, nab-paclitaxel produced supra-additive effects when given before radiation. Nab-paclitaxel significantly increased radiocurability by reducing the dose yielding 50% tumor cure (TCD50) from 54.3 to 35.2 Gy. Tumor histology following nab-paclitaxel treatment was characterized by pronounced necrotic and apoptotic cell death and mitotic arrest. Nab-paclitaxel did not increase normal tissue radioresponse.

Conclusions: Nab-paclitaxel exhibited strong antitumor efficacy against both tumors as a single agent and it improved radiotherapy in a supra-additive manner. These improved effects were achieved without increased normal tissue toxicity to either rapidly or slowly proliferating normal tissues although the drug dose was 1.5 times higher than the maximum tolerated dose of solvent-based paclitaxel. These preclinical findings show that combining nab-paclitaxel with radiotherapy would improve the outcome of taxane-based chemoradiotherapy. This novel taxane is thus a good candidate for testing in clinical chemoradiotherapy trials.

Besides being very efficient cytotoxic drugs on their own, taxanes are also potent enhancers of tumor radiation response. In the clinical setting, taxanes are being used as radiation-enhancing drugs in a variety of disease sites including non–small-cell lung cancer, head and neck, esophageal, gastric, cervical, urothelial, and nasopharyngeal carcinoma (1–7).

Taxanes are complex diterpenoids characterized by an extremely hydrophobic structure with sparse aqueous solubility (8). To overcome solubility problems, chemical solvents such as ethanol, Tween 80, and castor oil (Cremophor EL) are used in currently approved solvent-based taxane formulations. These solvents are known to cause biological and pharmacologic side effects, including dose-limiting toxicity, acute hypersensitivity reactions, and altered pharmacokinetics (resulting in a nonlinear pharmacokinetic profile). As a result, intensive research is being aimed at developing alternative formulations (8–12).

One such alternative is nab-paclitaxel (Abraxane), a novel, solvent-free 130-nm nanoparticle albumin–bound formulation, designed without the dose-limiting solvent Cremophor EL of the standard solvent-based paclitaxel clinical formulation. A dose-finding study showed a higher maximum tolerated dose for nab-paclitaxel than solvent-based paclitaxel and linear pharmacokinetics (13, 14). Nab-paclitaxel was well tolerated without steroid or H1/H2 blocker premedication when given at doses higher than standard solvent-based paclitaxel and produced significant tumor response in patients with non–small-cell lung cancer (15), metastatic breast cancer (16), or head and neck and anal canal cancers (17). In a phase III study of patients with metastatic breast carcinoma, nab-paclitaxel had a favorable safety profile and higher efficacy than standard solvent-based paclitaxel (18). Preclinical xenograft studies showed that cellular transport of nab-paclitaxel differs from...
that of standard solvent-based paclitaxel. Albumin was found to be transported via a gp60-mediated mechanism into endothelial cells and via a secreted protein acidic and rich in cysteine (SPARC)-mediated mechanism into SPARC-overexpressing tumor cells, resulting in selective nab-paclitaxel accumulation in tumors (11, 12) with a 33% higher intratumoral concentration of paclitaxel following administration of ABI-007 compared with an equal dose of Cremophor-based paclitaxel in the MX-1 xenograft model (12). Further studies in other tumor models in vivo are needed to confirm and extend these observations.

Whereas nab-paclitaxel has already been successfully introduced into the clinic as a single agent, its radiation-enhancing potential compared with solvent-based paclitaxel has not yet been evaluated. Given the known radiation-enhancing ability of standard solvent-based paclitaxel (19–21), we hypothesized nab-paclitaxel would also be a strong radiation-enhancing agent with the potential to significantly improve therapeutic gain. To test this hypothesis, we conducted a preclinical evaluation of the radiation modulating effects of nab-paclitaxel using two murine tumors and normal skin and gut.

Materials and Methods

Mice and tumor models

Three- to four-month-old C3H/KamLaw mice, bred in our specific-pathogen-free facility, were used for these experiments. Mice were housed four or five per cage, exposed to 12- h light-dark cycles, and given free access to sterilized pelleted food (Prolab Animal Diet, Purina, Indianapolis, IN) and sterilized water. Mice were maintained in an American Association for Laboratory Animal Care–accredited facility and in accordance with current regulations of the U.S. Departments of Agriculture and Health and Human Services. The experimental protocol was approved by and in accordance with institutional guidelines established by the Institutional Animal Care and Use Committee.

Studies were done using two transplantable and nonimmunogenic syngeneic murine tumors: the ovarian adenocarcinoma OCa-I and the murine mammary carcinoma MCA-4. Both tumors arose spontaneously in C3H mice and are currently in their 7th and 3rd isotransplant generations, respectively. Single-cell suspensions were prepared by mechanical disruption and enzymatic digestion of parent tumors, as previously described (22). Solitary tumors were then established in the right hind legs of female mice by i.m. injection of 5 × 10³ viable tumor cells. Tumors were then measured at 2- to 3-day intervals with Vernier calipers in three orthogonal dimensions, and mean tumor diameter was taken as the mean of these three measurements.

Nab-paclitaxel

Nab-paclitaxel (Abraxane; refs. 11, 12, 23–25) was supplied by American BioScience, Inc. (Santa Monica, CA). The compound was dissolved in 0.9% NaCl solution to a concentration of 9 mg/mL and administered i.v. in a concentration of 90 mg/kg in a volume of 0.01 mL/g of body weight. In all experiments, nab-paclitaxel was given once as a single tail vein injection without premedication.

Effect of nab-paclitaxel on tumor radioresponse

To determine optimal sequence and schedule for combining nab-paclitaxel and radiation therapy, we assessed the antitumor efficacy of different treatment schedules using a tumor growth delay assay. OCa-I tumors were established in mice, and when tumors grew to a mean diameter of 7 mm, the mice were treated with a single radiation dose of 10 Gy, a single injection of nab-paclitaxel (90 mg/kg) i.v., or a combination of both. Radiation was delivered to the tumor-bearing leg using a 137Cs animal irradiator consisting of parallel-opposed sources at a dose rate of 5.2 Gy/min. During irradiation, the mice were immobilized in a jig, with their tumor-bearing leg centered in a 3-cm-diameter circular treatment field. Nab-paclitaxel was given 9 h or 1, 2, 3, 4, or 5 days before irradiation, or 1 day after irradiation. Tumor regression and regrowth were followed at 2- to 3-day intervals until tumor size reached – 14 mm in diameter. Treatment end point was absolute tumor growth delay, defined as the difference in days to grow from 7 to 12 mm in diameter between treated and untreated tumors. For groups treated with the combination of nab-paclitaxel and radiation, normalized growth delay was determined as the time for tumors treated with the combined regimen to grow from 7 to 12 mm minus time for tumors treated with nab-paclitaxel alone to reach that same size. For groups treated with the combination of nab-paclitaxel and radiation, an enhancement factor was calculated as the ratio of the difference in days to grow from 7 to 12 mm between the tumors treated with the combination and those treated with nab-paclitaxel alone to the tumor growth delay of tumors treated with radiation alone.

To assess whether nab-paclitaxel affects tumor radioresistance, a tumor cure assay (TCRD) was done in OCa-I tumors. TCRD was defined as the dose of radiation yielding tumor cure in 50% of animals. Mice bearing 7-mm tumors were treated with single doses of radiation, ranging from 15 to 70 Gy. The time interval of 72 h between dose and radiation was determined to be optimal by the tumor growth delay study. Control mice received only local tumor irradiation to 7-mm tumors. After treatment, the irradiated site was checked in 3- to 8-day intervals for regression and recurrence of tumor. Tumor regression was defined as a tumor regrowing to the initial tumor size of 7-mm diameter. Mice were killed by CO2 inhalation when tumors reached a size of 14 mm. Tumor cure in surviving mice was assessed 140 days after conclusion of treatment. For each group, the percentage of mice cured was determined and plotted against administered dose. Radiation dose-response curves and the doses required to yield 50% tumor control (TCRD values) were calculated using the logit method of analysis (27).

To determine whether nab-paclitaxel enhances the efficacy of fractionated radiation, which is a more relevant treatment scheme for clinical translation, we did a tumor growth delay assay using fractionated local tumor radiation. Analogous to the previous experiments, treatment was initiated in mice bearing 7-mm OCa-I tumors. Groups of 8 to 10 mice were treated with fractionated radiation only, a single injection of nab-paclitaxel (90 mg/kg i.v.) only, or a combination of nab-paclitaxel and radiation, in which nab-paclitaxel was given 24 h before the first dose of fractionated radiation. Controls received no treatment. Radiation was given as local tumor radiation of 2-Gy fractions of daily treatment over 5 consecutive days, resulting in 10 Gy total dose. Tumor regression and regrowth were followed at 2- to 3-day intervals until tumor size reached – 14 mm. Treatment end points were absolute and normalized tumor growth delay and the resulting enhancement factors.

Effect of nab-paclitaxel on normal tissue radioresponse

Jejunum microcolony assay. The microcolony assay introduced by Withers and Elkind (28) was used to determine the survival of crypt epithelial cells in the jejunum of mice exposed to radiation. Mice were exposed to whole-body irradiation with 300-kV X-rays at a dose rate of 1.84 Gy/min. Whole-body irradiation was given to groups of eight mice either as single doses ranging from 10.0 to 13.5 Gy or as daily-fractionated doses of 4.7 to 6.3 Gy given for 5 consecutive days, resulting in total doses of 23.5 to 31.5 Gy. Controls were treated with graded doses of whole-body irradiation only, whereas mice treated with

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a combined treatment regimen were injected once with nab-paclitaxel (90 mg/kg i.v.) before whole-body irradiation. The most efficient treatment schedules used in the tumor experiments were used for the normal tissue study: nab-paclitaxel was given 72 h before single-dose radiation or 24 h before the first fraction of fractionated radiation. For single-dose radiation, we additionally assessed the effect of a 24-h interval between nab-paclitaxel administration and radiation. Mice were sacrificed and jejunal tissues harvested at times optimal for quantification of surviving jejunal crypts. Mice treated with single-dose radiation were sacrificed 86 h after completion of treatment, and mice treated with fractionated radiation were sacrificed 66 h after completion of treatment. Jejunum sections were paraffin embedded and stained with H&E. The number of regenerating crypts per 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 points by least-squares regression analysis.

**Skin desquamation assay.** The effect of nab-paclitaxel on acute radiation injury to the skin was determined in mice used in the TCD50 assay. Only mice that had no recurrent tumors represent at the time of assessment were analyzed. The degree of dry and moist skin desquamation was scored daily on the ventral surface of the irradiated leg using a scale of 0 to 3.5, as previously published (29). A score of 0 denotes normal skin; 1, occurrence of erythema; 2, appearance of a confluent area of moist desquamation; and 3.5, a breakdown of entire skin within the treatment field with severe exudation. Skin reactions were scored starting 16 days after the first radiation dose until day 35. Peak radiation-induced acute skin reaction during the scoring period was assessed for each mouse, and the mean determined for each dose group.

**Leg contracture.** To determine the effect of nab-paclitaxel on chronic radiation-induced damage to normal tissues of the leg, an assay quantifying leg contracture was applied using mice in the TCD50 experiment (30). Radiation-induced leg contracture in mice free from tumor recurrence was determined 120 days after radiation. Mice were positioned in a jig, without anesthesia, and the difference in leg extensibility between the unirradiated left and the irradiated right hind leg was quantified in millimeter.

**Table 1. Effect of nab-paclitaxel on radioresponse of OCa-I tumors measured by tumor growth delay: timing of administration**

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Time in days required to grow from 7 to 12 mm</th>
<th>Absolute growth delay</th>
<th>Normalized growth delay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no treatment)</td>
<td>15.8 ± 1.0</td>
<td>20.9 ± 3.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Nab-paclitaxel only</td>
<td>36.7 ± 3.5</td>
<td>13.6 ± 2.1</td>
<td>1.4</td>
</tr>
<tr>
<td>Single-dose radiation only (10 Gy)</td>
<td>29.4 ± 2.1</td>
<td>38.7 ± 2.7</td>
<td>2.4</td>
</tr>
<tr>
<td>Nab-paclitaxel + 10 Gy, 9 h later</td>
<td>54.5 ± 2.7</td>
<td>40.5 ± 3.9</td>
<td>2.4</td>
</tr>
<tr>
<td>Nab-paclitaxel + 10 Gy, 1 d later</td>
<td>56.3 ± 3.9</td>
<td>53.8 ± 2.6</td>
<td>1.9</td>
</tr>
<tr>
<td>Nab-paclitaxel + 10 Gy, 2 d later</td>
<td>69.6 ± 2.6</td>
<td>53.1 ± 3.0</td>
<td>1.6</td>
</tr>
<tr>
<td>Nab-paclitaxel + 10 Gy, 3 d later</td>
<td>68.9 ± 3.0</td>
<td>47.3 ± 3.0</td>
<td>0.4</td>
</tr>
<tr>
<td>Nab-paclitaxel + 10 Gy, 4 d later</td>
<td>63.1 ± 3.0</td>
<td>42.6 ± 3.6</td>
<td>1.9</td>
</tr>
<tr>
<td>Nab-paclitaxel + 10 Gy, 5 d later</td>
<td>58.4 ± 3.6</td>
<td>26.0 ± 5.0</td>
<td>1.9</td>
</tr>
<tr>
<td>Radiation 10 Gy + nab-paclitaxel, 1 d later</td>
<td>41.8 ± 5.0</td>
<td>5.1 ± 0.5</td>
<td>1.9</td>
</tr>
</tbody>
</table>

*Groups of 8 to 9 mice bearing 7-mm tumors in the right hind leg were given a single dose of nab-paclitaxel (90 mg/kg i.v.), a single dose of local tumor irradiation, or a combination of both agents at varying time intervals.

†Enhancement factors obtained by dividing normalized growth delay in mice treated with nab-paclitaxel plus radiation by absolute tumor growth delay in mice treated with radiation alone.

‡Absolute tumor growth delay caused by nab-paclitaxel, radiation, or the combination of agents is defined as the time in days tumors required to grow from 7 mm to 12 mm minus the time in days untreated tumors required to grow from 7 mm to 12 mm.

§Normalized tumor growth delay is defined as the time in days for tumors to reach 12 mm in mice treated with the combination of nab-paclitaxel and radiation minus the time in days to reach 12 mm in mice treated with drug alone.

**Tumor histology**

To assess the effect of nab-paclitaxel on the time course of mitotic arrest, apoptosis, and necrosis, OCa-I tumors were collected at sequential time intervals following drug administration. Mice bearing 7-mm OCa-I tumors were treated with a single tail vein injection of nab-paclitaxel (90 mg/kg). Groups of four mice were then sacrificed and tumors harvested at 4, 9, 24, 48, 72, and 96 h after nab-paclitaxel injection. Tumors were fixed in formalin for 24 to 48 h, paraffin embedded, and cut into 4-μm-thick sections. The histologic sections were stained with H&E for micromorphometric analysis. As previously described, five randomly chosen fields per tumor section were scored at ×400 magnification, and 100 cells per viewing field were counted and classified based on morphologic features as either viable tumor or necrosis. The total area of viable tumor and the total area of necrosis were determined, and the percentage of necrosis was calculated as the fraction of necrotic area to total segmented tumor area.

**Results**

**Effect of nab-paclitaxel on OCa-I tumor radioresponse.** We first tested whether nab-paclitaxel can enhance radiation-induced tumor growth delay and whether the effect depends on the time interval between nab-paclitaxel administration and radiation delivery. All treatments delayed the time to grow from 7 to 12 mm in diameter (Table 1). Nab-paclitaxel at this dose...
was more effective than 10 Gy only. When the two agents were combined, the effect was greater than the sum of the effects of individual treatments when nab-paclitaxel administration preceded tumor irradiation. To quantify the extent of the supra-additive effect, the tumor growth delay data were expressed as absolute and normalized growth delays, and radiation enhancement factors were calculated. They ranged from 1.3 to 2.4, indicating that the magnitude of the supra-additive effect depended on the time interval between nab-paclitaxel administration and irradiation. The largest enhancement of tumor radioresponse was achieved when nab-paclitaxel was given 2 or 3 days before tumor irradiation. The effect of nab-paclitaxel given 1 day after irradiation was, however, less than the sum of the effects of individual treatments (enhancement factor = 0.4).

Additional experiments were done to test whether nab-paclitaxel improved the efficacy of fractionated radiotherapy, a more clinically relevant treatment schedule. Radiation was delivered daily for 5 consecutive days at a daily dose of 2 Gy (to a total dose of 10 Gy), a common fractional dose of radiation used in clinical radiotherapy. When nab-paclitaxel was combined with radiation, the drug was given 1 day before initiation of radiotherapy. This schedule was selected based on results obtained with single dose irradiation (Table 1) in which we observed that nab-paclitaxel induced a strong radio-enhancement if given within several days before radiation. Thus, it was logical to hypothesize that in the fractionated irradiation schedule, nab-paclitaxel would affect each radiation fraction given within 5 days after nab-paclitaxel administration. Both nab-paclitaxel and radiation as individual treatments strongly delayed tumor growth, but the combined treatment was the most effective, resulting in supra-additive tumor growth delay (Fig. 1B). The tumor growth delay was 22.2 ± 1.7 days after nab-paclitaxel, 14.0 ± 1.2 days after radiation, and 43.5 ± 3.8 days after nab-paclitaxel plus radiation. The enhancement factor was 1.5. Clearly, nab-paclitaxel was thus also effective in enhancing tumor growth delay induced by fractionated radiotherapy.

**Effect of nab-paclitaxel on MCa-4 tumor radioresponse.** To determine whether nab-paclitaxel also enhances the efficacy of radiation in a different tumor type, a tumor growth delay study was done with mammary adenocarcinoma MCa-4. Nab-paclitaxel was given 72 h before radiation. This time interval was based on the optimal schedule found for OCa-1 tumors.
The tumor growth curves presented in Fig. 3 show that all treatments resulted in tumor growth delay. Radiation (20 Gy single dose) was more effective than a single dose of nab-paclitaxel (90 mg/kg), and the longest growth delay resulted from combining nab-paclitaxel and radiation. The combined treatment was supra-additive and resulted in an enhancement factor of 1.4.

**Effect of nab-paclitaxel on normal tissue radioresponse.** To increase the therapeutic gain of radiotherapy, nab-paclitaxel must produce a bigger effect on tumor than on normal tissue radioresponse. We tested the effect of nab-paclitaxel on radioresponse of jejunal mucosa and skin, two highly proliferating early responding tissues that manifest acute radiation damage within days or weeks from the start of radiotherapy. We also quantified the response of slowly proliferating s.c. tissue leading to development of leg contractures observed months after radiotherapy (late responding tissue). Results of jejunal crypt survival, skin response, and leg contracture assays all showed that nab-paclitaxel did not enhance normal tissue damage. Figure 4 shows a dose-dependent increase in jejunal crypt cell killing with increase in radiation dose after both single and fractionated irradiation. Fractionated radiation was much less effective due to interfraction cell repair and repopulation. Importantly, there was no significant difference in the radiation-dose response curves between radiation only groups and those that received both nab-paclitaxel and radiation. Thus, nab-paclitaxel caused no significant modification of radiation injury to jejunal crypt cells inflicted by either single dose or fractionated radiation.

The effect of nab-paclitaxel on radiation-induced skin desquamation and radiation-induced leg contractures was assessed in the mice used in the TCD50 single dose radiation experiment (Fig. 2). Figure 5A and B shows that the degree of skin desquamation and leg contractures, respectively, increased with increase in radiation dose. As was the case with the jejunal mucosa, treatment of mice with nab-paclitaxel had no significant modifying effect on either acute radiation-induced skin desquamation or late radiation-induced leg contractures.

![Figure 3](https://example.com/fig3.png)

**Fig. 3.** Effect of nab-paclitaxel and single dose radiation on MCA-4 tumor growth. Mice bearing 7-mm tumors in the leg were untreated (○) or treated with 90 mg/kg nab-paclitaxel i.v. (⊗), a single dose of 20 Gy local tumor radiation (□), or a combination of both treatments, wherein nab-paclitaxel was given 72 h before radiation (■).

Thus, nab-paclitaxel greatly increased the therapeutic ratio of radiotherapy.

**Cellular effects of nab-paclitaxel.** Because mitotic arrest, apoptosis, and necrosis have previously been identified to be the major cellular effects underlying antitumor efficacy of taxanes as well as taxane-induced enhancement of tumor radioresponse (21), the following analysis was done to measure the effects of nab-paclitaxel. Nab-paclitaxel induced both mitotic arrest and apoptosis and increased necrosis in a time-dependent manner. The background level of mitotic index (MI) in untreated tumors was 1.1 ± 0.2%. MI increased to 13.0 ± 0.7% at 4 h, peaked at 9 h with a MI of 19.1 ± 2.7%, and then gradually declined but remained somewhat elevated even at 96 h (MI = 5.6 ± 0.9%) following nab-paclitaxel administration. The apoptotic index also increased with time but peaked later (24 h) than MI. The baseline apoptotic index of 2.3 ± 0.5% increased to a maximum of 9.9 ± 0.9% at 24 h and then gradually declined to the pretreatment level by 96 h (apoptotic index = 2.7 ± 0.8%). The extent and dynamics of these cellular changes are similar to those we previously reported for solvent-based paclitaxel (31, 32). The necrotic index in untreated tumors was 32.1 ± 1.9% and was only elevated between 48 and 96 h after nab-paclitaxel treatment. The maximum amount of necrosis was 73.9 ± 3.4% at 96 h.

**Discussion**

The data presented here show that nab-paclitaxel was a very effective antitumor agent and that it greatly improved the efficacy of radiotherapy for two murine carcinomas (OCa-I and MCA-4). Two important components contributed to the overall efficacy of the combined treatment, one being a strong...
enhancement of tumor radioresponse and the other a complete absence of influence on radiation-induced normal tissue toxicity. Thus, this agent, compared with solvent-based taxanes, improved the therapeutic gain. Our observation on the anti-tumor activity of nab-paclitaxel extends recent observations by Desai et al. (12) to additional tumor types. More important, our demonstration of the effects of the combined nab-paclitaxel and radiation is, to our knowledge, the first ever reported.

Nab-paclitaxel enhanced tumor response to radiation when assessed both by tumor growth delay and tumor cure and when radiation was delivered either as a single dose or as daily fractional doses similar to clinical radiotherapy regimens. The magnitude of the achieved radioenhancement depended on the sequence and timing of administration of the two agents. Enhancement was observed only when nab-paclitaxel preceded radiation delivery (9 h to 5 days, the time intervals tested in this study), with enhancement factors ranging from 1.3 to 2.4. The strongest enhancement was, however, observed when nab-paclitaxel was given 2 to 3 days before radiation, a time-dependent effect similar to that we reported earlier for solvent-based paclitaxel (21). This observation suggests that timing of nab-paclitaxel administration in relation to the start of radiotherapy is very important for achieving the optimal interaction between the two agents.

Based on earlier studies on taxane-radiation interactions (21), it is likely that the magnitude of the observed enhancement in tumor radioresponse is mechanistically linked to the cellular effects of nab-paclitaxel, primarily mitotic arrest and cell death by apoptosis and necrosis. Nab-paclitaxel induced mitotic arrest that lasted for 4 days after administration, although it peaked at 9 h. Earlier studies showed that solvent-based taxane-induced G2-M arrest is a major mechanism underlying radioenhancement produced by these agents (33) because G2-M cells are known to be more radiosensitive than cells in other phases of the cell cycle (34). Although this mechanism was likely involved in the presently observed enhancement of tumor radioresponse, it probably was not the dominant mechanism because radiation delivered at the peak of mitotic arrest was not the most effective combination schedule (enhancement factor = 1.3). The maximal enhancement occurred when radiation was delivered 2 to 3 days after nab-paclitaxel administration, a time when MI declined near to the background level. At this time after nab-paclitaxel administration, however, there was a significant loss of tumor cells by both apoptosis and necrosis, which, according to our previous studies with solvent-based paclitaxel (35), could have resulted in increased tumor oxygenation. Direct measurements of tumor oxygenation by the Eppendorf pO2 histogram showed that in the MCa-4 tumor treated with solvent-based paclitaxel, pO2 increased from the control median value of 6.8 to 10.5 mm Hg at 24 h to 31.2 mm Hg at 48 h (35). This change in oxygenation was associated with a reduction in the percentage of hypoxic cells from 32% in untreated tumors to 4% and 2% at 24 and 48 h after solvent-based paclitaxel administration, respectively (35). Reoxygenation by taxanes can be accomplished by a number of means including (a) lowering oxygen consumption due to cell loss by apoptosis or necrosis so that more oxygen becomes available to surviving cells; (b) reopening closed capillaries as a result of reduced tumor interstitial pressure; and (c) inducing active migration of tumor cells from previously hypoxic microregions closer to blood vessels. Grifon-Etienne et al. (36) reported that both solvent-based paclitaxel and docetaxel were highly effective in reducing tumor tissue interstitial fluid pressure in a number of murine tumors.

No improvement in tumor radioresponse was achieved when nab-paclitaxel was administered after radiotherapy (Table 1), an observation similar to that we previously reported for solvent-based paclitaxel (21). The reasons for this are unclear. One possibility, based on in vitro observations (37), is that G1 and G2 cell cycle arrest induced by radiation renders cells insensitive to additional cell cycle perturbations by taxanes when applied within several hours after radiation. Regardless of the mechanism, this information is important as it implies that sequencing of nab-paclitaxel administration is critical when this agent is combined with radiotherapy. Our earlier studies on solvent-based paclitaxel and docetaxel showed that both these taxanes enhanced radioresponse of jejunal mucosa, particularly that after fractionated irradiation (38). Therefore, in comparison, nab-paclitaxel has a clinical advantage because of its better tolerability in spite of administration of higher doses than those achievable with solvent-based paclitaxel or docetaxel.

Fig. 5. Effect of nab-paclitaxel on radiation-induced injury to normal tissues of the leg. Mice from the TCD150 study were evaluated for acute skin reaction (desquamation) at days 16 to 35 and for chronic leg contracture at 120 d after irradiation. Only the mice free of local recurrence at the time of observation were used. A, points, mean peak skin reaction scored for mice treated at each dose; bars, 95% confidence intervals. B, points, mean extent of leg contracture; bars, 95% confidence intervals. Linear regression line was determined using all data. Open symbols, mice treated with irradiation alone; closed symbols, mice treated with nab-paclitaxel at 72 h before irradiation.
In contrast to the strong enhancement of tumor radiosponse, there was a complete lack of modification of normal tissue radiosponse by nab-paclitaxel. This was observed for both highly proliferating epithelial cells of jejunum and skin, and slowly proliferating s.c. tissue damage manifested in leg contractions (Fig. 5). As shown by jejunal crypt assay, neither the damage inflicted by single dose nor that by fractionated radiation was influenced by nab-paclitaxel. The results indicate that nab-paclitaxel is highly effective in increasing therapeutic gain of radiotherapy. The therapeutic gain factors, obtained by dividing enhancement factors for tumor radiosresponse modification by those for normal tissue modification, are virtually equal to the enhancement factors for tumor radiosresponse. This lack of modification of normal tissue radiosponses may be attributable to selective accumulation of nab-paclitaxel in tumors (11, 12).

In conclusion, nab-paclitaxel exhibited strong antitumor effect against two murine tumors when administered as a single agent, and it improved the antitumor efficacy of tumor radiotherapy in a supra-additive manner. It enhanced tumor response in terms of increased tumor growth delay and tumor cure rate to both single and fractionated irradiation. These improved effects were achieved without any increase in normal tissue toxicity to either rapidly or slowly proliferating normal tissues although the drug dose used was 1.5 times higher than the maximum tolerated dose of standard solvent-based paclitaxel. These preclinical findings show that combining nab-paclitaxel with radiotherapy represents an improvement in taxane chemoradiotherapy, making this novel taxane a good candidate for testing in clinical chemoradiotherapy trials.

References

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