Skin Ulceration Potential of Paclitaxel in a Mouse Skin Model In Vivo

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Supported by a research grant from Bristol-Myers Oncology Division, Bristol-Myers Squibb Company, Princeton, New Jersey, U.S.A. and by Grant No. 17094 from the National Institutes of Health, National Cancer Institute, Bethesda, MD.

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Received March 18, 1996; accepted March 27, 1996.

BACKGROUND. The antimitotic agent paclitaxel is highly active in the therapy of several tumor types, including ovarian and breast cancer. The commercial formulation (Taxol®) is supplied in a vehicle containing alcohol and the surfactant Cremophor EL® (polyethoxylated castor oil). Whereas Phase I studies did not describe extravasation necrosis, more recent case reports have suggested that paclitaxel can cause soft tissue necrosis if inadvertently extravasated. The efficacy of various antidotal maneuvers, if any, was not known.

METHODS. Dehaired, BALB/c mice were given intradermal (ID) injections of paclitaxel 0.3 mg, 0.6 mg, or 1.2 mg, or Cremophor EL®, 0.1 mL, into the dorsal skin. The sites were observed thrice weekly for evidence of ulceration. Perpendicular widths of skin ulcers were measured by caliper and multiplied to yield a lesion area in cm^2. The lesion area multiplied by time in days was integrated by computer to yield cumulative ulceration areas in ($cm^2 \cdot$ days). Potential pharmacologic adjuvants were injected ID after paclitaxel. These included saline (0.05 mL), albumin (0.05 mL), hyaluronidase (15 Units), and hydrocortisone (2.5 mg). Topical adjuvants included dimethylsulfoxide solution, (0.1 mL), cooling to 8–10 °C or heating to 43–44 °C for 30 minutes after ID paclitaxel.

RESULTS. Dose-dependent skin ulcers that lasted 12–17 days were created with the 3 ID paclitaxel doses. The two higher paclitaxel dose levels, 0.6 mg and 1.2 mg, were selected for antidote studies. Hyaluronidase and saline were effective ID antidotes for lesions induced by the 0.6-mg paclitaxel dose, but not for the higher paclitaxel dose of 1.2 mg ($P < 0.05$ by analysis of variance). None of the topical adjuvants or other ID adjuvants significantly reduced paclitaxel-induced skin ulcers in the mice.

CONCLUSIONS. Paclitaxel has experimental vesicant potential in the ID mouse skin model. Clinical extravasations of paclitaxel may be treated by subcutaneous injections of hyaluronidase diluted in saline. ***Cancer* 1996; 78:152–6.**

KEYWORDS: extravasation, antidotes, paclitaxel infiltration, skin toxicity.

Paclitaxel (Taxol®; Bristol-Myers Squibb Oncology, Princeton, NJ) is a microtubule antagonist with a unique mechanism of action.[2] Unlike the vinca alkaloids, which bind to tubulin dimers,[3] paclitaxel preferentially binds to a single high-affinity binding site (K_d = 0.87 μM) on the β-tubulin subunit.[4] These sites are distinct from those for GTP, colchicine, and vinblastine. As a result of paclitaxel binding, microtubule assembly is stabilized with the formation of parallel bundles of microtubules.[4] This blocks tumor cell division in mitosis. Clinically, paclitaxel is active in a variety of nonhematologic tumors, including advanced ovarian carcinoma,[5] metastatic breast cancer,[6] nonsmall cell lung cancer,[7] and malignant melanoma.[8] The usual dose-limiting toxicity of paclitaxel is neutropenia,[5] but peripheral

neuropathy also occurs after cumulative doses are administered or if the length of the paclitaxel infusion is increased to 24 hours.[9,10] Other toxicities include total body alopecia, moderate nausea and vomiting, and rare hypersensitivity reactions that are thought to be caused at least in part by the surfactant Cremophor EL® (polyethoxylated castor oil) in the commercial Taxol® formulation.[11]

More recently, soft tissue injury has been described after the inadvertent extravasation of paclitaxel solutions.[1,12] In these instances, patients developed painful erythematous lesions over the site of the extravasation. Biopsies of the subcutaneous tissues in two patients showed subcutaneous necrosis with cytologic abnormalities noted in the adjacent endothelial cells. In one report, 4 patients developed superficial skin ulcers and 1 required local surgery 1 year after the extravasation to close an open ulcer on the forearm.[1] Both warm and cold compresses were anecdotally used to treat paclitaxel extravasation sites in this clinical series.[1] Other extravasations of paclitaxel in Phase I/II studies were similarly painful but not ulcerogenic.[5,13] This calls into question whether paclitaxel is a vesicant if inadvertently extravasated into soft tissues. To address this question, paclitaxel was evaluated in a murine skin model of extravasation using dehaired BALB/c mice given intradermal injections of paclitaxel. In addition, the effect of potential local antidotes was also evaluated.

MATERIALS AND METHODS

Adult female BALB/c mice (weight: 25–30 grams) were obtained from Jackson Laboratories, Bar Harbor, ME. The mice were housed four to a cage and given access to food and water as desired. After 2 weeks of acclimatization, a 2 cm^2 area on the dorsal surface was dehaired using the topical depillatory agent Neet® lotion (Whitehall Laboratories, New York, NY). Twenty-four hours later, the mice received intradermal injections of paclitaxel in the commercial Taxol® formulation. This contains per mL 6 mg paclitaxel and 527 mg polyethoxylated castor oil, in 49.7% (volume to volume ratio) dehydrated alcohol. The highest intradermal (ID) dose tested was 1.2 mg of paclitaxel or 0.2 mL of the undiluted concentrate representing a dose of approximately 170 mg/m^2 per mouse body surface area (converted using the method of Freireich et al.).[14] Two lower ID doses of paclitaxel solution were also evaluated; 0.6 mg (0.1 mL) and 0.3 mg (0.05 mL). After injection, the mice were observed every other day for the formation of a visible ulcer at the injection site. Perpendicular ulcer widths were measured using a micrometer and the diameters were multiplied to yield an ulcer area in cm^2. All lesions were observed until complete healing had occurred. To quantitate the cumulative amount of skin toxicity for each dose level, the lesion area (cm^2) multiplied by time (days) was integrated by computer for each mouse.[15] This value represents the cumulative area under the ulcer multiplied by time curve (AUC in $cm^2 \cdot$ days) for ulcerative toxicity within a given treatment group.

A number of local adjuvants were evaluated for their potential to reduce paclitaxel ulcerative toxicity. Intradermally injected agents were administered immediately adjacent to and after the ID paclitaxel injection. These included 0.1 mL of 25% human albumin U.S.P. (Alpha Therapeutic Corporation, Los Angeles, CA) to locally bind the paclitaxel, 0.05 mL of 0.9% sodium chloride or 15 Units of hyaluronidase in 0.05 mL saline (Wydase®; Wyeth Laboratories, Philadelphia, PA) to dilute the drug, and the antiinflammatory corticosteroid, hydrocortisone (Solu-Cortef®; Upjohn Company, Kalamazoo, MI). Topical adjuvants included 0.1 mL of 99% dimethylsulfoxide (DMSO, HPLC Grade; Burdick Jackson Company, Muskegon, WI). This was applied to the site once after ID paclitaxel. Topical heating to 43–44 °C, or cooling to 8–10 °C, were applied to the dorsal skin site for 30 minutes after an ID injection of paclitaxel. The latter procedure followed a protocol described in detail previously.[16]

Statistical tests for significant differences among treatment groups involved an initial analysis of variance (ANOVA). This was followed by a multiple range test using the Student Neuman Keuls procedure for any ANOVA results indicating a significant subset. Statistical significance was assessed at the $P < 0.05$ level for both tests. Statistical analyses were performed on the following treatment groups: 1) the 3 different doses of paclitaxel with no adjuvants; 2) all treatments at the 1.2-mg dose level; and 3) all treatments at the 0.6-mg dose level. Each treatment was evaluated in mice initially and repeated once for a total of eight mice per treatment.

RESULTS

The three doses of undiluted paclitaxel solution produced skin ulcers in proportion to the injected dose (Table 1). In all cases, the skin ulcers peaked in size by Day 3 and had completely resolved after 17 days for the 1.2-mg and 0.6-mg doses, and by Day 13 for the 0.3-mg dose. Variability in cumulative ulcer multiplied by time areas was much higher with the highest paclitaxel dose of 1.2 mg. The ID injection of 0.1 mL of Taxol® diluent (Cremophor® in alcohol) also produced measurable toxicity in this system (Table 1). This toxicity was less than that produced by the 0.3-mg dose of paclitaxel solution, which includes 0.05 mL of the diluent solution along with the paclitaxel. Thus,

TABLE 1
Dose-Response for Paclitaxel or Cremophor®-Induced Skin Ulceration in Mice

ID Dose mg (mL)	Mean ulceration (SD) Peak (cm^2)	Duration (median days)	Cumulative area ($cm^2 \cdot$ days)
Cremophor			
0.01 mL (0.10)	0.33 ± 0.17[a]	12	1.67 ± 0.76[a]
Paclitaxel			
0.30 mg (0.05)	0.32 ± 0.21[a]	15	2.61 ± 1.19[a,b]
0.6 mg (0.10)	0.53 ± 0.37	15	4.85 ± 2.11[b]
1.2 mg (0.20)	1.16 ± 1.15	17	13.05 ± 7.68

SD: standard deviation; ID: intradermal.
[a] Not significantly different from 0.6 mg dose.
[b] Statistically significant from 1.2 mg dose subset after analysis of variance and Student Neuman Keuls tests ($P < 0.05$).

TABLE 2
Effect of Local Adjuvants on Paclitaxel-Induced Skin Ulceration in the Mouse

Local adjuvant			Mean skin ulceration (SD) Paclitaxel 0.6 mg		Paclitaxel 1.2 mg	
Agent	Dose	Route	Peak (cm^2)	AUC ($cm^2 \cdot$ days)	Peak (cm^2)	AUC ($cm^2 \cdot$ days)
Control	(From Table 1)		0.53 ± 0.37	4.85 ± 2.11	1.16 ± 1.15	13.05 ± 7.68
Saline	0.05 mL	ID	0.085[a]	0.46[a]	0.57 ± 0.31	5.45 ± 2.39
Albumin	0.05 mL	ID	ND	ND	0.62 ± 0.50	7.635 ± 2.65
DMSO	0.1 mL	TOP	0.37 ± 0.21	3.43 ± 1.77	0.54 ± 0.47	4.78 ± 3.94
HYAL	15 U	ID	0.003[a] ± 0.001	0.12[a] ± 0.15	0.85 ± 0.41	7.07 ± 1.69
HYDCORT	2.5 mg	ID	0.29 ± 0.24	2.07 ± 2.14	1.27 ± 1.11	10.81 ± 2.89
TEMP (°C × 30 minutes)						
Cold	8–10 °C	TOP	0.16 ± 0.22	1.17 ± 1.01	0.54 ± 0.60	9.69 ± 3.97
Heat	43–44 °C	TOP	0.34 ± 0.29	3.27 ± 1.72	1.11 ± 0.91	17.75 ± 5.85

SD: standard deviation; AUC: area under the curve; ID: intradermal; TOP: topically applied to paclitaxel injection site; ND: not done; DMSO: dimethyl sulfoxide; HYAL: hyaluronidase; HYDCORT: hydrocortisone; TEMP: temperature.

although the diluent does not contribute the majority of ulcerative toxicity to the commercial Taxol® formulation, it is nonetheless an active vesicant agent when undiluted.

The results of the local adjuvant studies were disappointing in that none of the seven agents consistently reduced paclitaxel-induced skin ulcers for both ID paclitaxel doses (Table 2). The only agents that demonstrated efficacy were active only against the lower ID paclitaxel dose of 0.6 mg. These included ID injections of either 0.05 mL of saline or 15 Units of hyaluronidase diluted in 0.05 mL of saline. The hyaluronidase treatment provided nearly complete protection from ulceration after the 0.6-mg paclitaxel ID injection. In this experiment, only 1 mouse developed very slight ulceration from 0.6 mg of paclitaxel followed by 15 Units of hyaluronidase. Hyaluronidase decreased the peak ulcer size and cumulative area by approximately 50% against the higher 1.2-mg paclitaxel dose (Table 2). However, these changes did not reach statistical significance. The ID injection of 0.05 mL saline without hyaluronidase also reduced ulceration significantly for the lower (0.6 mg) paclitaxel dose but the degree of protection was substantially less than with hyaluronidase. The results presented in Table 2 also indicate that neither topical heating nor cooling of the skin over the paclitaxel injection site was effective as an antidotal maneuver.

DISCUSSION

This study shows that paclitaxel produces dose-dependent skin ulcers in mice given ID injections of concen-

trated Taxol® solution. Similar results have previously been reported in the same model using the vinca alkaloids vinblastine, vindesine, vincristine,[17] and vinorelbine,[18] which do not utilize the Cremophor®-based solvent system.[17] In general, a 1.2-mg paclitaxel dose produced an ulcer AUC comparable to that previously produced by a 0.1-mg ID injection of vincristine in the same BALB/c mouse model.[17] However, unlike the vinca alkaloids, hyaluronidase and saline were less effective as local antidotes, and topical heating was completely ineffective. Although hyaluronidase reduced ulceration by about half against the higher dose of paclitaxel, the lack of statistically significant antidotal efficacy may relate to the relatively large amount of variability in the cumulative ulcer areas produced by this dose of paclitaxel in the mice (Table 1).

In the original Phase II study of Taxol® in advanced ovarian cancer, extravasations of a 24-hour infusion solution were described in two courses.[5] Both patients experienced local swelling, erythema, and mild pain that resolved without treatment after 5 to 10 days. No skin ulcers or other long term sequelae were noted in these two instances. Because a 24-hour infusion was used in these patients, the absolute amount of paclitaxel solution that extravasated was probably small. In an earlier Phase I trial, Donehower et al. observed local venous toxicity with 5 courses in 2 patients receiving paclitaxel infusions over 1 or 6 hours.[13] These reactions involved erythema and mild pain along the course of the vein used for infusion. The involved sites eventually resolved without treatment, leaving sclerotic and hyperpigmented tissues, but without any ulceration. Another Phase I study described Grade 2 cellulitis (Eastern Cooperative Oncology Group standards) in 3 patients experiencing an extravasation of >50 mL of paclitaxel solution.[19] In these patients, the drug was diluted into 1 liter of 5% dextrose in water for intravenous infusion over 6 hours to deliver total doses of 50, 200, and 230 mg/m^2, respectively.[19]

Paclitaxel also causes dose-limiting abdominal pain when administered diluted into 2 liters of normal saline; moderate to severe pain requiring narcotic analgesia occurred 8–12 hours after drug instillation in a majority of patients.[20] Finally, two reports from the M. D. Anderson Cancer Center in Houston clearly associate paclitaxel extravasation with subcutaneous necrosis of soft tissues.[1,12] In these reports, several patients developed Grade 2 or Grade 3 local toxicity (by National Cancer Institute or Gynecologic Oncology Group criteria) after inadvertent extravasations of paclitaxel solutions containing 125–200 mg/m^2 infused over 24 hours. The final drug concentration in these studies ranged from 1.2 mg/mL[12] to as low as 0.125 mg/mL. In each report, the solution was infused via a peripheral intravenous line. In the larger series, extravasation was suspected in 19 of 925 infusions (2%) given to 17 patients with gynecologic malignancies.[1] Infiltration was documented in 8 of 19 suspected events (42%) by the presence of painful, discolored, and swollen sites. Lesions appeared immediately in 6 of 19 suspected extravasations, or after 3–12 days in 11 patients.[1] Overall, there were 2 of 19 Grade 1 reactions (11%) noted by pain and erythema; 13 of 19 Grade 2 reactions (68%) noted by pain, erythema, and inflammation or phlebitis; and 4 of 19 Grade 3 reactions (21%) noted by frank ulceration. One of these patients died at 6 months with a lesion still present. Another patient required plastic surgery to close an open forearm ulcer 12 months after the extravasation occurred. Thus, paclitaxel lesions tended to heal slowly, over several weeks. Subcutaneous necrosis was documented histologically in two patients.

These clinical reports complement the animal data described in the current study. It is clear that concentrated paclitaxel solutions can cause local tissue damage if inadvertently delivered to subcutaneous tissues. Based on the mouse data, the contribution of the Cremophor® diluent to this local toxicity appears to be substantially less than that of the complete Taxol® formulation containing Cremophor® and paclitaxel. This is similar to prior mouse skin studies that showed relatively low toxicity for Cremophor® and the epipodophyllotoxins VM-26 and VP-16.[21]

Fortunately, paclitaxel-induced lesions in mice healed briskly over a 2-week period without any local therapy. This is similar to the pattern observed with vinca alkaloids[17,18] but is much more rapid than the slow recovery pattern observed with doxorubicin skin lesions in this same mouse model.[15] It is likely that as the clinical use of paclitaxel expands, the incidence and severity of reported local extravasation injuries with paclitaxel will increase. This is particularly pertinent to the current use of shorter infusion times and thus, more concentrated paclitaxel infusion solutions. Based on the mouse antidote studies, the local injection of hyaluronidase at the human dose of 150 Units diluted in 3 mL of saline may be beneficial for treating severe paclitaxel extravasations in the clinical setting. Clearly, topical heating or cooling were not effective antidotal maneuvers and therefore should be avoided based on the animal data.

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