Complete Regression of Well-established Tumors Using a Novel Water-soluble Poly(L-Glutamic Acid)-Paclitaxel Conjugate

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ABSTRACT

Despite an intensive search, few water-soluble paclitaxel derivatives have been shown to have a therapeutic index superior to paclitaxel itself. We now report a water-soluble poly(L-glutamic acid)-paclitaxel conjugate (PG-TXL) that produces striking antitumor effects with diminished toxicity. A single i.v. injection of PG-TXL at its maximum tolerated dose (defined as that dose that produces a maximum 12-15% body weight loss within 2 weeks after a single i.v. injection) equivalent to 60 mg of paclitaxel/kg and at even a lower dose equivalent to 40 mg of paclitaxel/kg resulted in the disappearance of an established implanted 13762F mammary adenocarcinoma (mean size, 2000 mm3) in rats. (An equivalent dose of PG-TXL is the amount of conjugate that contains the stated amount of paclitaxel.) Similarly, mice bearing syngeneic OCA-1 ovarian carcinoma (mean size, 500 mm3) were tumor-free within 2 weeks after a single i.v. injection of the conjugate at a dose equivalent to 160 mg of paclitaxel/kg. The conjugate has little if any intrinsic tubulin polymerization activity in vitro and is >20 times less potent in supporting the growth of a paclitaxel-dependent CHO mutant cell line. PG-TXL has a prolonged half-life in plasma and greater uptake in tumor as compared with paclitaxel. Furthermore, the aqueous solubility of paclitaxel in either plasma or the tumor tissue within 144 h after drug injection. Histological studies of tumor tissues obtained from mice treated with PG-TXL show fewer apoptotic cells but more extensive tumor necrosis as compared with paclitaxel treatment. These data suggest that in addition to its role as a carrier for selective delivery of paclitaxel to the tumor, PG-TXL exerts distinct pharmacological actions of its own that may contribute to its remarkable antitumor efficacy.

INTRODUCTION

Paclitaxel (Taxol®) has shown significant antineoplastic activity against various human cancers, including breast and ovarian tumors (1, 2). However, a major difficulty in the clinical use of paclitaxel has been its poor water solubility. Previous attempts to prepare water-soluble prodrugs of paclitaxel involved placing low-molecular-weight solubilizing moieties at the C2′ or C7 position. These prodrugs are mainly ester derivatives (including succinate, sulfonic acid, and amino acid) and phosphate derivatives (3-7). Although some of these prodrugs possess adequate aqueous solubility, few have antitumor activity comparable to that of the parent drug (3, 4, 7). Several of these derivatives are not suitable for i.v. injection because of their instability in aqueous solution at neutral pH. Recently, Nicolau et al. (8) reported the synthesis and in vitro biological evaluation of a novel series of prodrugs termed "protaxols." These compounds possess greater aqueous solubility and are converted to paclitaxel as the active drug through an intramolecular hydrolysis mechanism. However, no in vivo data on the antitumor activity of protaxols are yet available.

Conjugation of chemotherapeutic agents to macromolecular carriers is typically performed not only to improve solubility characteristics of a compound but also to produce desirable pharmacokinetics and to enhance antitumor activity (9-12). Conjugation of paclitaxel to PEG, for example, has been reported to result in highly water-soluble prodrugs that are almost completely converted to free paclitaxel upon exposure to aqueous environments. Unfortunately, although the association with PEG improves the water solubility of paclitaxel, it has not greatly improved the therapeutic index of the PEG-paclitaxel complex compared with free paclitaxel itself (13, 14). With a goal of preparing water-soluble paclitaxel derivatives that possess adequate stability and improved antitumor efficacy, we selected a synthetic polyamino acid, PG (15-17), as a potential carrier for paclitaxel based on the following rationale: (a) PG contains a large number of side chain carboxyl functional groups for drug attachment; (b) PG can be readily degraded by lysosomal enzymes to its nontoxic basic component, L-glutamic acid; and (c) sodium glutamate has been reported to prevent manifestations of neuropathy induced by paclitaxel, thus enabling higher doses of paclitaxel to be tolerated (18). This report describes the synthesis, characterization, and in vivo antitumor activity of PG-TXL. Attempts are also made to compare the pharmacological properties of PG-TXL with those of paclitaxel. Our findings suggest that in addition to its role as a carrier for selective delivery of paclitaxel to the tumor, PG-TXL also exerts its own pharmacological actions different from those of paclitaxel.

MATERIALS AND METHODS

Synthesis of PG-TXL. To a solution of 75 mg of PG (M, 36,200; Sigma Chemical Co., St. Louis, MO) in 1.5 ml of dry N,N-dimethylformamide were added 20 mg of paclitaxel (Hande Tech, Houston, TX), 15 mg of dicyclohexylcarbodiimide, and a trace amount of dimethylaminopyridine. The reaction was allowed to proceed at room temperature overnight. TLC [silica plate; eluent, CHCl3:methanol (10:1)] showed complete conversion of paclitaxel (Rf = 0.55) to the polymer conjugate (Rf = 0). To stop the reaction, the mixture was poured into chloroform. The resulting precipitate was converted to the sodium salt of PG-TXL by dissolving the crude product in NaHCO3. The mixture was poured into chloroform. The resulting precipitate was converted to the sodium salt of PG-TXL by dissolving the crude product in NaHCO3. The aqueous solution of PG-TXL was dialyzed against distilled water, filtered, and lyophilized to obtain 88.6 mg of white powder. Paclitaxel content was 21% (w/w, UV method). Yield (conversion to polymer-bound paclitaxel, UV) was 93%. Values for 1H NMR (General Electric model GN 500 spectrometer, 500 MHz, in D2O) were: for aromatic components of paclitaxel, δ = 7.75-7.36 ppm; for aliphatic components of paclitaxel, δ = 6.38 ppm (C4-H), 5.97 ppm (C6-H), 5.63 and 4.78 ppm (C7-H), 5.55-5.36 ppm (C2-H and C4-H, m), 5.10 ppm (C7-H), 4.39 ppm (C2-H), 4.10 ppm (C6-H), 1.97 ppm (OCOCH3), and 1.18-1.20 ppm (C-CH3). Other resonances of paclitaxel were obscured by those of PG. PG resonances at 4.27 ppm (H-O), 2.21 ppm (H-N), and 2.04 ppm (H-B) were in accordance with the spectrum of pure PG.

Characterization of PG-TXL. UV spectra were obtained on a Beckman DU-640 spectrophotometer (Fullerton, CA). The content of paclitaxel conjugated to PG was estimated by UV measurements based on a standard curve generated with known concentrations of paclitaxel in methanol (A = 226 nm).
TUMOR REGRESSION USING A POLYMER-TAXOL CONJUGATE

Fig. 1. Structures of PG-TXL and paclitaxel.

Paclitaxel + \( \text{NHCHCO}_4^- \) \( \text{CH}_2\text{COOH} \)

\( \text{(1) DCC/DMF} \)

\( \text{(2) NaHCO}_3 \)

\( \text{CH}_2\text{COO}^- \text{Na}^+ \)

\( \text{COO}^- \text{C}_7\text{Paditaxel} \)

\( \text{O-Ca'-Paditaxel} \)

The relative molecular weight of PG-TXL was characterized by gel permeation chromatography. A TSK-gel column suitable for analysis of water-soluble polymer was used, and the system was eluted with 0.2 mm PBS (pH 6.8) at 1.0 ml/min. Solubility of PG-TXL was estimated by dissolving various amounts of polymer conjugate in 100 μl of water.

**In Vitro Studies.** The tubulin assembly reaction was performed at 32°C in PEM buffer (80 mm PIPES buffer, 1 mM EGTA, and 1 mM MgCl\(_2\) (pH 6.9)) at a tubulin (bovine brain; Cytoskeleton Inc., Boulder, CO) concentration of 1 mg/ml (10 μM) in the presence or absence of drugs (1.0–50 μM equivalent paclitaxel) and 1.0 mM GTP. Tubulin polymerization was followed by measuring the absorbance of the solution at 340 nm for a period of time.

The effect of PG-TXL on the growth of paclitaxel-dependent CHO mutant Tax-18 cells was carried out in 24-well tissue culture dishes. Approximately 100 cells were added to wells containing growth medium (α-MEM containing 5% fetal bovine serum, 50 units/ml penicillin, and 50 μg/ml streptomycin) and equivalent concentrations of paclitaxel varying from 0 to 10 μM. After 6 days of incubation at 37°C, the medium was removed, and the cells were stained with 0.1% methylene blue solution.

**In Vivo Antitumor Activity.** All animal work was carried out at the animal facility of the University of Texas M. D. Anderson Cancer Center in accordance with institutional guidelines. C3H/Kam mice were bred and maintained in a pathogen-free facility in the Department of Experimental Radiation Oncology. Solitary tumors were produced in female C3H/Kam mice (25–30 g) by injecting \( 5 \times 10^5 \) viable murine ovarian carcinoma cells (OCA-1) into the muscle of the right thigh. In a parallel study, female Fischer 344 rats (125–150 g) received injections of \( 1.0 \times 10^5 \) viable 13762F tumor cells in 0.1 ml PBS. Treatments were initiated when the tumors in the mice had grown to 500 mm\(^3\) or when the tumors in the rats had grown to 2200 mm\(^3\). A single dose of PG-TXL in saline or paclitaxel in a Cremophor EL vehicle was given in doses varying from 40 to 160 mg of equivalent paclitaxel/kg body weight. In control experiments, saline, the Cremophor vehicle (50:50 Cremophor:ethanol diluted with saline, 1:4), PG solution in saline, and paclitaxel plus PG were used. Tumor growth was determined daily by measuring three orthogonal tumor diameters. Tumor volume was calculated according to the formula \( V = \frac{A \times B \times C}{2} \).

**Pharmacokinetic and Biodistribution.** Normal female C3Hf/kam mice (25–30 g) received injections of a dose of 20 mg of equivalent paclitaxel/kg of

In each case, the drug was injected i.v. in a single dose. Data are presented as means of tumor volumes; bars, SD. In a, mice bearing OCA-1 tumor received injections of: □, PG control (800 mg/kg; \( n = 9 \)); ▼, paclitaxel (80 mg/kg; \( n = 7 \)); ●, paclitaxel (80 mg/kg) plus PG (800 mg/kg; \( n = 5 \)); ○, PG-TXL (80 mg equivalent paclitaxel/kg; \( n = 6 \)); ●, PG-TXL (160 mg equivalent paclitaxel/kg; \( n = 26 \)). In b, rats bearing 13762F tumor received injections of: □, PG control (220 mg/kg; \( n = 7 \)); ▼, paclitaxel (20 mg/kg; \( n = 5 \)); ●, paclitaxel (40 mg/kg; \( n = 7 \)); ○, PG-TXL (20 mg equivalent paclitaxel/kg; \( n = 5 \)); ●, PG-TXL (40 mg of equivalent paclitaxel/kg; \( n = 9 \)); ○, PG-TXL (40 mg of equivalent paclitaxel/kg; \( n = 9 \)); PG-TXL was dissolved in saline (10 mg of equivalent paclitaxel/ml), and paclitaxel was dissolved in Cremophor EL vehicle (6 mg/ml). Other controls (saline and Cremophor EL vehicle) produced essentially the same results as the PG control and are not presented in these figures for the sake of clarity.

Fig. 2. Antitumor effect of PG-TXL and paclitaxel on mice bearing murine ovarian tumor OCA-1 and rats bearing rat breast tumor 13762F. In each case, the drug was injected i.v. in a single dose. Data are presented as means of tumor volumes; bars, SD. In a, mice bearing OCA-1 tumor received injections of: □, PG control (800 mg/kg; \( n = 9 \)); ▼, paclitaxel (80 mg/kg; \( n = 7 \)); ●, paclitaxel (80 mg/kg) plus PG (800 mg/kg; \( n = 5 \)); ○, PG-TXL (80 mg equivalent paclitaxel/kg; \( n = 6 \)); ●, PG-TXL (160 mg equivalent paclitaxel/kg; \( n = 26 \)). In b, rats bearing 13762F tumor received injections of: □, PG control (220 mg/kg; \( n = 7 \)); ▼, paclitaxel (20 mg/kg; \( n = 5 \)); ●, paclitaxel (40 mg/kg; \( n = 7 \)); ○, PG-TXL (20 mg equivalent paclitaxel/kg; \( n = 5 \)); ●, PG-TXL (40 mg of equivalent paclitaxel/kg; \( n = 9 \)); ○, PG-TXL (40 mg of equivalent paclitaxel/kg; \( n = 9 \)); PG-TXL was dissolved in saline (10 mg of equivalent paclitaxel/ml), and paclitaxel was dissolved in Cremophor EL vehicle (6 mg/ml). Other controls (saline and Cremophor EL vehicle) produced essentially the same results as the PG control and are not presented in these figures for the sake of clarity.

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Fig. 3. Growth of CHO mutant Tax-18 cells in various concentrations of paclitaxel and PG-TXL. After 6 days of growth, cell culture medium was removed, and the cells were stained with methylene blue. Numbers above the plate wells refer to drug concentration (μM).

Fig. 4. Plasma clearance of radioactivity after an i.v. injection of PG-[3H]TXL and [3H]paclitaxel in C3H/HeJ mice. Data are presented as mean values of four to five mice/time point. Total radioactivity in plasma was determined, and radioactivity was identified as "Paclitaxel" after an extraction/HPLC procedure.

[3H]paclitaxel or PG-[3H]TXL into the tail vein. Each mouse received 6 μCi of radiolabeled drug. [3H]Paclitaxel was dissolved in Cremophor vehicle, whereas PG-[3H]TXL was dissolved in saline. The volume injected into each mouse was between 0.2 and 0.3 ml. At 0, 5, 15, and 30 min, and 1, 2, 4, 8, 16, 24, and 48 h after injection, animals were killed, and blood samples were collected (four to five mice/time point). Total radioactivity in the plasma was measured by liquid scintillation counting (Beckman Model LS 6500) using 10-μl aliquots of plasma. Up to 200 μl of plasma were extracted with three volumes of ethyl acetate according to Longnecker et al. (19). The extraction efficiency for paclitaxel was 80%. The samples were centrifuged for 5 min at 2500 rpm, and the supernatant was separated and brought to dryness. The dried extract was reconstituted with 195 μl of HPLC mobile phase and mixed with 5 μl of cold paclitaxel (0.2 mg/ml), and 100 μl were injected onto the HPLC for determination of free paclitaxel radioactivity. Pharmacokinetic parameters were analyzed by a noncompartmental model using the WinNonlin software package.

In a separate experiment, mice bearing OCA-1 (500 mm³) received injections of a dose of 20 mg of equivalent paclitaxel/kg of [3H]paclitaxel or PG-[3H]TXL into the tail vein. Animals were killed at 2, 5, 9, 24, 48, and 144 h after injection. Tumors were removed, weighed, and homogenized with three volumes of PBS (w/v). An aliquot of tissue homogenate was mixed with tissue solubilizer, followed by the addition of scintillation solvent, and counted for total radioactivity. The counting efficiency was verified by the method of standard addition. Alternatively, aliquots of tissue homogenates were extracted with ethyl acetate and analyzed for free paclitaxel by HPLC. The uptake of total drugs in OCA-1 tumor was expressed as a percentage of the administered dose/g of tissue; the association of radioactivity within OCA-1 tumor as free paclitaxel was expressed as dpm/g tissue.

The HPLC system consisted of a 150 × 3.9-mm Nova-Pak column (Waters, Milford, MA), a flow scintillation analyzer (Packard model 500TR, Downers Grove, IL), and a Packard radiomatic software for data analysis. The eluting solvent (methanol:water, 2:1) was run at 1.0 ml/min.

**Histological Analysis of Apoptosis.** OCA-1 tumor-bearing mice were prepared as described previously. When tumor volume reached 500 mm³, animals received injections of either paclitaxel (80 mg/kg) or PG-TXL (160 mg of equivalent paclitaxel/kg). At different times ranging from 0 to 144 h after treatment, tumors were histologically analyzed to quantify mitotic and apoptotic activity according to Milas et al. (20). In brief, the mice were killed by cervical dislocation, and the tumors were immediately excised and placed in neutral-buffered formalin. The tissues were then processed and stained with H&E. Both mitosis and apoptosis were scored in coded slides by microscopic examination (X400). Five fields of nonnecrotic areas were randomly selected...
in each histological specimen; in each field, the number of apoptotic nuclei and cells in mitosis were recorded as numbers/100 nuclei and were expressed as a percentage. The values were based on scoring 1500 nuclei obtained from three mice/time point.

RESULTS

Synthesis and Characterization. A dicyclohexylcarbodiimide-mediated coupling reaction between the carboxyl groups of PG and the hydroxyl groups of paclitaxel yielded PG-TXL in almost complete conversion (Fig. 1). Evidence of covalent conjugation after linkage came from UV absorption spectra and gel permeation chromatographic analyses. The UV spectrum of PG-TXL obtained in water had slightly shifted (λmax 230 nm) as compared with that of paclitaxel in methanol (λmax 228 nm). Gel permeation chromatography of the product yielded a macromolecular peak with a negligible amount of low-molecular-weight contaminants (<0.1%, data not shown). Additionally, high frequency NMR (500 MHz, in D2O) indicated that the conjugate contained both PG and paclitaxel moieties. The specific site of esterification for paclitaxel, however, is at present unclear because of the interference of PG peaks and inadequate NMR resolution. Although a peak at 5.63 ppm could be tentatively assigned to the C-2’ proton of the C-2’ ester, the C-2’ proton of unsubstituted paclitaxel at 4.78 ppm was also present, suggesting that the resulting conjugate may contain paclitaxel substitutions at both the C-2’ and C-7 positions. A 100-mg/ml solution of the conjugate produces a clear, viscous, yet flowable liquid. This simple, one-step procedure consistently produces a PG-TXL conjugate containing 20% paclitaxel by weight, i.e., approximately seven paclitaxel molecules are bound to each polymer chain.

PG-TXL Exhibits Remarkable Antitumor Activity in Vivo. To evaluate the antitumor efficacy of systemically administered PG-TXL, female C3Hf/kam mice were inoculated i.m. with syngeneic ovarian carcinoma OCA-1 (20). Approximately 3 weeks after inoculation, when the tumors were well-established (mean volume, 500 mm\(^2\) - 1-cm diameter), mice received injections of a single dose of PG-TXL in saline (10 mg of equivalent paclitaxel/ml) or paclitaxel in a Cremophor EL vehicle (6 mg/ml) through the tail vein. Paclitaxel in Cremophor EL administered at its MTD\(^4\) of 80 mg/kg initially delayed tumor growth, but tumors regrew as rapidly as those in control mice after 10 days (Fig. 2a). The combination of paclitaxel and PG as separate compounds gave essentially the same tumor growth profile as

\(^{4}\) MTD is defined as that dose that produces a maximum 12-15% body weight loss within 2 weeks after a single i.v. injection.

Fig. 5. a, time-dependent OCA-1 tumor content of radioactivity after injection of either PG-\(^{1}\)H\(_2\)TXL or \(^{1}\)Hpaclitaxel into mice. Conversion of PG-\(^{1}\)H\(_2\)TXL to \(^{1}\)Hpaclitaxel within OCA-1 tumor. Data are presented as means from three mice/time point; bars, SD. The percentage of injected dose/g tissue was calculated based on total radioactivity associated with the tumor, which was determined by counting prepared tissue homogenate aliquots. Extraction with ethyl acetate and the subsequent analysis by HPLC provided the "Paclitaxel" radioactivity.

Fig. 6. Kinetics of mitosis (a) and apoptosis (b) in OCA-1 tumors after a single i.v. dose of 160 mg of equivalent paclitaxel/kg PG-TXL and 80 mg/kg paclitaxel. The percentage of mitosis and apoptosis were scored from histological sections made from tumors at 0-144 h after treatment. Data are presented as means from three mice/time point; bars, SE.
Tumor regression using a polymer-taxol conjugate.

Fig. 7. Histological appearance of OCA-1 tumors. a, untreated control. Normal mitoses were present (arrowhead). b-d, after treatment with 160 mg of equivalent paclitaxel/kg PG-TXL. In b, at 24 h, PG-TXL-treated tumors show mitotically arrested cells with the characteristic coronal appearance of condensed chromatin (arrowhead); apoptotic bodies consist of small nuclear fragments surrounded by a narrow rim of cytoplasm (arrow). In c, by 96 h, edema and necrosis is extensive (N). In d, by 144 h, residual tumor cells exhibit greater pleomorphism but less mitotic activity than controls. ×400.

Paclitaxel alone. In contrast, PG-TXL at 80 mg of equivalent paclitaxel/kg caused significant tumor growth delay as compared with the same dose of paclitaxel. Furthermore, a striking antitumor response was observed in all mice who received injections of PG-TXL at its MTD of 160 mg/kg. In contrast, the MTD of free paclitaxel in Cremophor EL was 20 mg/kg. The growth of tumors was completely suppressed, and the tumors gradually shrank. After 2 weeks, tumors were undetectable in all 26 treated mice; at the end of the experiment (2 months), 25 remained histologically tumor-free. None of the animals treated with PG-TXL died during the experimental period, whereas 2 mice (of 13) treated with paclitaxel died of apparent hypersensitivity reactions.

In a parallel study, the antitumor activity of PG-TXL in Fischer rats with the well-established rat mammary adenocarcinoma 13762F was examined. Once tumors reached a mean volume of 2000 mm³, animals were treated using a similar protocol as described above. Again, complete tumor eradication at the MTD of PG-TXL (60 mg of equivalent paclitaxel/kg) was observed (data not shown). PG-TXL given at a lower dose of 40 mg of equivalent paclitaxel/kg also resulted in complete tumor regression (Fig. 2b). In contrast, the MTD of paclitaxel in Cremophor EL was 20 mg/kg. Paclitaxel at this dose caused a tumor growth delay of only 5 days, whereas the same equivalent paclitaxel dose of PG-TXL resulted in a tumor growth delay of 23 days (Fig. 2b).

PG-TXL does not promote microtubule assembly. To test whether intact PG-TXL has any intrinsic biological activity in promoting tubulin polymerization, paclitaxel and PG-TXL were compared for their relative ability to promote in vitro assembly of purified bovine brain tubulin. The addition of 1 μM paclitaxel to a solution of tubulin in an assembly buffer caused a clear increase in absorbance due to the increase in light scattering that resulted from the polymerization of tubulin into microtubules. In contrast, a 10 μM and even a 50 μM paclitaxel equivalent of PG-TXL had no effect on polymerization (data not shown).

We next investigated the ability of PG-TXL to rescue a paclitaxel-dependent mutant cell line. Tax 18, a CHO cell line selected for resistance to paclitaxel, is a well-characterized mutant that has been found to require the continuous presence of paclitaxel for cell division. In the absence of the drug, a functional mitotic spindle apparatus is unable to form (21). The mitosis phase of the cell cycle is prolonged, with subsequent failure to segregate chromosomes and to divide into daughter cells. Nonetheless, the cells continue to progress through the cell cycle and replicate their DNA, resulting in the formation of large polyploid cells that eventually die because of genomic instability (22). Low concentrations of paclitaxel are able to rescue the mutant phenotype by permitting microtubule assembly and the formation of sufficient mitotic spindle fibers. Thus, these cells provide a convenient bioassay for agents that promote microtubule assembly. Fig. 3 shows the results of a cloning efficiency assay comparing the relative abilities of PG-TXL and free paclitaxel to promote the growth of Tax-18. Little or no increase in cell number is seen in the absence of either drug, but concentrations of paclitaxel between 0.05 and 0.2 μM clearly support the growth of this cell line. Higher concentrations of paclitaxel are presumably toxic to the cells because of overstabilization of the microtubules, as is observed for normal cells. In contrast, freshly prepared PG-TXL shows little ability to rescue Tax-18 cell growth, even at the highest paclitaxel-equivalent concentration tested (1 μM). These data indicate that PG-TXL does not promote microtubule assembly.

PG-TXL is not simply a prodrug for the gradual release of paclitaxel. To assess the pharmacokinetic and release characteristics of paclitaxel in vivo, normal female C3Hf/Kam mice received i.v. injections of [3H]paclitaxel or PG-[3H]TXL. The clearance of both drugs from plasma is shown in Fig. 4. Whereas paclitaxel has an extremely short half-life in the plasma of mice (t1/2, 29 min), the apparent half-life of PG-TXL is prolonged (t1/2, 317 min). Surprisingly, the rate of conversion of PG-TXL to paclitaxel in plasma was slow, with <0.1% of the radioactivity from PG-[3H]TXL being recovered as [3H]paclitaxel within 48 h after drug injection (Fig. 4).

Quantitative assessment of the tumor uptake in C3Hf/Kam mice showed that relatively high levels of radioactivity appear in tumor tissue shortly after injection of radiolabeled PG-TXL as compared with the injection of radiolabeled paclitaxel (Fig. 5a). However, only small amounts of radioactivity within tumor tissue are due to the release of free paclitaxel (Fig. 5b). The percentage of radioactivity within tumor tissue due to paclitaxel does not appreciably increase.

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3 An equivalent dose of PG-TXL is the amount of conjugate that contains the stated amount of paclitaxel.

4 Tumor growth delay is defined as the time in days for tumors treated with the test drugs to grow from 2,000 mm² to 10,000 mm² minus the time in days for tumors treated with saline control to grow from 2,000 mm² to 10,000 mm².
with time, which suggests that PG-TXL is not simply a prodrug for the gradual release of paclitaxel.

**PG-TXL Induced Less Apoptosis Compared with Paclitaxel in OCA-1 Tumor.** Tumors from PG-TXL- and paclitaxel-treated mice were histologically analyzed for mitotic arrest and apoptosis. As shown in Fig. 6a, both PG-TXL and paclitaxel at their respective MTD doses were effective in arresting tumor cells in mitosis. The kinetics of mitotic arrest induced by PG-TXL was similar to that induced by paclitaxel (Fig. 6a).

In contrast, treatment with PG-TXL resulted in only a mild increase in apoptotic cells, whereas paclitaxel increased significantly the proportion of apoptotic cells in OCA-1 tumor from the control value of 2.4 ± 0.5% to the peak value of 13.9 ± 1.6% reached at 24 h after injection (Fig. 6b).

The morphological changes observed in the paclitaxel-treated mice were qualitatively similar to those described previously (20). The tumor cells showed marked nuclear fragmentation with formation of apoptotic bodies, which was especially marked on day one. Viable tumor cell clumps with normal mitoses were still present in these tumors by 144 h, which indicated that these tumors would eventually regress (data not shown). The histological features of OCA-1 tumors from PG-TXL-treated mice are presented in Fig. 7. At 24 h after PG-TXL treatment, an increase in mitotically arrested cells and a mild increase in apoptotic cells were observed (Fig. 7b). By 96 h, tumors from PG-TXL-treated mice developed extensive edema and necrosis, and only a small rim of viable tumor cells remained (Fig. 7c). By 144 h, the residual tumor clumps as compared with controls were comprised of cells that were larger and more pleomorphic and displayed less mitotic activity (Fig. 7d).

**DISCUSSION**

We have shown that the water-soluble form of paclitaxel prepared by conjugating paclitaxel to a large polymer, PG, produces impressive antitumor responses, which include complete cures of breast and ovarian cancers. Furthermore, this degree of antitumor activity can be achieved with single i.v. injections into animals. Such formulation of paclitaxel is comprised of cells that were larger and more pleomorphic and disgradual release of paclitaxel.

**REFERENCES**

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