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Review Paper

Taxol: a review of its preclinical *in vivo* antitumor activity

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Taxol has been demonstrated in numerous laboratories worldwide to have broad-spectrum antitumor activity against many tumor models. The susceptible tumors include murine leukemias and solid tumors, and human solid tumor xenografts. The initial findings of taxol's ineffectiveness against most distal site tumor models was probably a consequence of the insolubility of taxol in nearly all the vehicles used in those early studies. On the occasions when an ethanol-based vehicle was used to dissolve taxol, substantial distal site antitumor activity was observed. Although no definitive schedule dependency data have evolved, once-a-day or every-other-day i.v. injections for several treatments have proved to be reproducibly effective in stringent s.c. tumor models. Attempts to discern a therapeutically synergistic cytotoxic drug combination was made on two occasions without success. In the manner evaluated, taxol plus either adriamycin, cisplatin, cyclophosphamide or etoposide (VP-16) were not meaningfully more efficacious than the more effective drug in each of those combination settings.

Key words: Antitumor, *in vivo*, preclinical, taxol.

Introduction

During a natural products screening program conducted by the National Cancer Institute (NCI) in the late 1960s, crude alcoholic extracts of bark from the Pacific yew tree (*Taxus brevifolia*) were found to be active against several murine tumors.[1,2] The active principle contained in those extracts was isolated and identified as taxol by Wani *et al.*[2]

Taxol (Figure 1) was selected for preclinical development on the basis of subsequent antitumor studies, particularly those in the i.p. B16 melanoma and the subrenal capsule (s.r.c.) MX-1 human breast xenograft models.[3–5] Yet many of the more stringent preclinical models, e.g. those involving i.v. or s.c. implantation of tumors, were not affected by taxol therapy. Conceivably, the failure to demonstrate taxol's activity versus distal site or disseminated tumors was probably due to the failure to solubilize the drug and administer it at effective yet tolerated dose levels. The use of ethanol and a suitable suspending agent has, however, recently allowed us and others[6,7] to successfully administer taxol and achieve meaningful antitumor activity against the more stringent tumor models.

The preclinical antitumor data reviewed herein include historical NCI results but also emphasizes other recent results with solubilized taxol, both alone and as part of combination chemotherapies.

Materials and methods

Mice, tumors and activity criteria

The names of each tumor model used by the NCI, Bristol-Myers Squibb (BMS) and other laboratories, and the host strain of mice used to perform the *in vivo* assays with those tumors, are described in Table 1. Also provided are the criteria for activity relevant to each of the tumor models presented. Details regarding the antitumor assay methodologies can be found in the publications referenced for the NCI screening procedures,[8,9] BMS[10–13] or other laboratories.[14–16] The only known significant deviation from a published procedure or interpretation is that no result of therapy is presented if it was known to be accompanied by >5 g of body weight loss or $>20\%$ lethality.

Drug preparation

Many different vehicles have been used to suspend or dissolve taxol for administration to tumor-bearing mice. The vehicles have included the following:

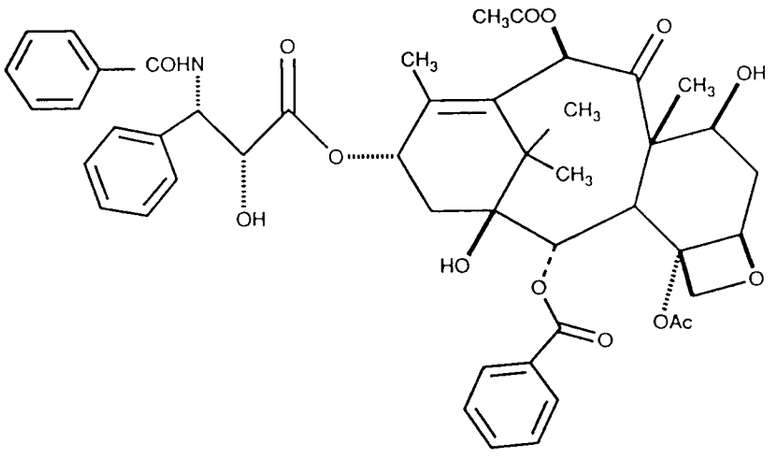

Figure 1. Structure of taxol.

Table 1. A description of tumor models, host strain of mouse, and activity criteria

		Criteria for activity[b]		
Tumor, site	Mouse strain[a]	MST %T/C	MTW %T/C	T-C (days)
P388[c], i.p.; i.v.; s.c.; i.c.	CDF_1 or BDF_1	≥125		
L1210, i.p.	CDF_1 or BDF_1	≥125		
P1534, i.p.	CDF_1	≥125		
ADJ/PC6, i.p.	BALB/c	≥125		
B16, i.p.	BDF_1	≥125		
B16, s.c.	BDF_1	≥140		≥4.5
$CD8F_1$, s.c.	$CD8F_1$		≤20	
C26, i.p.	CDF_1	≥125		
C38, s.c.	BDF_1		≤42	
Epend, i.c.	$B6C3F_1$	≥125		
LL, s.c.	BDF_1	≥125	≤42	
LL, i.v.	BDF_1	≥140		
M109, i.p.; s.c.	CDF_1	≥125		≥4.0
M109, s.r.c.	CDF_1		≤20	
M5076, i.p.; s.c.	BDF_1	≥125		>13.0
MAM 11/A, s.c.	CDF_1	≥125		
MAM 16/C, s.c.	$B6C3F_1$	≥125		
A2780, s.r.c.; s.c.	athymic		≤20	≥9.0
A431, s.r.c.	athymic		≤20	
CX-1, s.r.c.; s.c.	athymic		≤20	
CX-2, s.c.	athymic		≤20	
CX-5, s.r.c.; s.c.	athymic		≤20	
H2981, s.r.c.	athymic		≤20	
HCT-116, s.r.c.	athymic		≤20	
L2981, s.r.c.	athymic		≤20	
LOX, i.p.	athymic	≥125		
LX-1, s.r.c.; s.c.	athymic		≤20	
MX-1, s.r.c.; s.c.	athymic		≤20	

[a] Typical mouse strain used for taxol evaluation. Abbreviations used and their explanation: BDF_1, $(C57BL/6 \times DBA/2)F_1$; CDF_1, $(BALB/c \times DBA2)F_1$; $B6C3F_1$, $(C57BL/6 \times C3H)F_1$; $CD8F_1$, $(BALB/c \times DBA/8)F_1$.

[b] MST %T/C refers to maximum increases in median lifespan, relative to control values. MTW %T/C refers to minimum values of median tumor weight of treated versus (divided by) control groups of mice (i.e. the inverse of percentage tumor inhibition). T-C (days) refers to delays in primary tumor growth and the various criteria described reflect delays equivalent to about 1 log cell kill for the tumor models listed.

[c] Including drug-resistant sublines.

hydroxypropylcellulose (Klucel or HPC); 50% polyethyleneglycol-400 in distilled water (50% PEG), saline plus Tween 80 (in varying final concentrations); carboxymethylcellulose (CMC); dimethylsulfoxide (DMSO) in water or saline, and ethanol plus cremophor (emulphor) in varying final concentrations in distilled water or saline. Often, taxol will merely be described as having been administered either as a suspension or a solution in an ethanol-based vehicle. When specific vehicles were important determinants of activity, they have been identified.

When taxol was prepared for use in BMS experiments in ethanol-based vehicles, it was initially dissolved in ethanol with an equal volume of either Tween 80 or cremophor, facilitated by sonification for up to 30 min and kept at 4°C for as many as 10 days. As required for each experiment's daily taxol injections, an amount of the aforementioned stock solutions of taxol would be removed from refrigeration, brought to room temperature and diluted with saline (as needed) within 20–30 min of each group's injections.

Other drugs were included in certain BMS experiments. Cisplatin, adriamycin and cytoxan were dissolved in saline, etoposide (VP-16) was suspended in CMC and water.

Drug evaluation

Detailed descriptions of the assay and evaluation methods used for most of the antitumor testing contained herein have been reported.[6–16] Therapeutic results are presented in terms of maximum effect obtained and the dose yielding the effect is the optimal dose. When more than one therapeutic endpoint was determined, it is possible to derive more than one optimal dose. In the absence of activity, the maximum tolerated dose may be described.

Increases in lifespan were reflected by the relative median survival time (MST) of treated (T) versus control (C) groups (i.e. %T/C values). Tumor inhibition was determined by either calculating relative median times for T and C mice to grow tumors to 1 g in size (i.e. T − C values), or the ratio of median or log transformed mean tumor weights (MTW) of T and C groups of mice (i.e. MTW %T/C) at varying time points post-therapy. Tumor weights were interchangeable with tumor size on the basis of assuming 1 mm^3 = 1 mg. Statistical evaluations of BMS data were performed using Gehan's generalized Wilcoxon text.[17]

With specific regard to the BMS assays involving tumor implantation within the s.r.c., the approach used was a slight modification of the method of Bogden *et al.*[18] in that tumor size was estimated *in situ* by applying the formula, size = (length) × (width), and assuming a thickness of 1 mm. The changes in log transformed mean tumor sizes among taxol-treated and control mice were used to determine the MTW %T/C and percent regression values.

Results

Data from the NCI

A summary of the *in vivo* antitumor test results conducted by numerous laboratories under NCI auspices is presented in Table 2.

Against i.p. implanted murine leukemias, i.p. taxol treatment resulted in modest (L1210), moderate (P388) or good (P1534) activity, depending upon the tumor model involved. Other routes of taxol administration (e.g. s.c., i.v. and p.o.) were essentially ineffective versus i.p. P388 leukemia, as were any taxol therapies attempted versus s.c., i.c. or i.v. implanted P388.

The i.p. P388 leukemia model was used to explore many different vehicles and injection schedules. Most of the experiments performed which involved i.p. taxol therapy yielded moderately active results, with T/C values in the 150% range. Among different schedules evaluated concomitantly, only minor variation in efficacy was found between once daily injections for 5 or 9 days and intermittent injections (every fourth day for three injections); however, a single bolus injection failed to achieve an active result, whereas many injections per day ($q3h \times 8$), for one or more days, was associated with the greatest extensions in lifespan in this model (data not shown). While these data may appear to support the conclusion for schedule dependency (i.e. the advantage of a fractionated dose schedule), it should be noted that an obvious maximum tolerated dose level was not reached using many of the treatment schedules, thereby precluding a firm basis for drawing any such conclusion. Additionally, based on non-concomitant comparisons made during i.p. P388 testing, there were no substantial consistent therapeutic differences associated with the use of suspending media as opposed to vehicles capable of taxol's dissolution (e.g. ethanol plus cremophor).

Mice implanted i.p. with P388 sublines resistant

Table 2. Condensed summary of taxol's preclinical antitumor activity: NCI data

Tumor, site	Range of optimal effects[a]		Predominant score[b]
	MST %T/C (C/T)	MTW %T/C	
Murine leukemias			
L1210, i.p.	107–139	—	− → +
P1534, i.p.	150–300	—	+ → + + +
P388, i.p.	101–190	—	+
P388, s.c.; i.c.; i.v.	101–107	—	−
P388/AMSA, i.p.	130	—	+
P388/ellipticine, i.p.	129–147	—	+
P388/vincristine, i.p.	98	—	−
Murine solid tumors			
ADJ/PC6, i.p.	124–133	—	+
B16, i.p.	106–428 (0–9/10)	—	+ + → + + + +
B16, s.c.	96–126	30–109	−
$CD8F_1$ Mam, s.c.	—	57–61	−
C26, i.p.	142–161	—	+
C38, s.c.	—	69–76	−
Ependymoblastoma, i.c.	91–106	—	−
Lewis lung, s.c.	104–113	39–55	−
Lewis lung, i.v.	93–98	—	−
M5076, s.c.	109–130	58–78	−
MAM 11/A, s.c.	95	—	−
MAM 16/C, s.c.	100	—	−
Human tumor xenografts			
CX-1, s.r.c.	—	(−38)–42	+ → + + +
CX-1, s.c.	—	54–118	−
CX-2, s.c.	—	68	−
CX-5, s.r.c.	—	21–89	−
CX-5, s.c.	—	57–159	−
LX-1, s.r.c.	—	28–63	−
LX-1, s.c.	—	82	−
LOX, i.p.	158–173 (0–3/10)	—	+ + → + + + +
MX-1, s.r.c.	—	−100[c]	+ + + +
		21–36[d]	−
MX-1, s.c.	—	87–142[c]	−

[a] MST %T/C (C/T) refers to maximum increase in median lifespan relative to control values, with cures/total shown in parentheses. MTW %T/C refers to minimum values of median tumor weight of treated versus (divided by) control groups of mice (i.e. the inverse of percentage tumor inhibition), with negative values reflecting percentage tumor regression.
[b] − = no activity; + = mild or modest activity; + + = moderate activity; + + + = substantial activity; + + + + = potentially curative activity.
[c] Taxol treatment, i.p.
[d] Taxol treatment, s.c.

to either 4′-(9-acrindylamino)-methanesulfon-*m*-anisidide (*m*-AMSA), ellipticine or vincristine were subjected to i.p. treatment with taxol. The P388 subline resistant to vincristine was cross-resistant to taxol as reflected by a maximum T/C of 98% (concomitant testing versus the parental line of P388 was associated with a T/C of 171%). The other two drug-resistant P388 sublines retained limited sensitivity to taxol (maximum T/C values obtained were 129–147%).

The therapeutic effects of taxol versus murine solid tumors are also included in Table 2.

Taxol had borderline activity against the i.p. implanted ADJ/PC6 plasmacytoma. Maximum T/C values ranged between 124 and 133% following multiple injection treatment.

Extensive testing of taxol was conducted by the NCI using the i.p. implanted B16 melanoma model. Multiple injection i.p. therapy with taxol administered in several different vehicles resulted in reproducible increases in lifespan, with T/C values typically in the 150–250% range. The greater taxol-induced extensions of lifespan, as well as frequent cures, were obtained when the drug was given as

a suspension in Klucel. Yet despite the activity of taxol versus i.p. implanted B16, no activity was observed against s.c. implanted B16 (all of which testing involved i.p. therapy with suspended taxol).

With regard to other murine solid tumor models, the $CD8F_1$, MAM 11/A and MAM 16/C breast tumors, and colon 38 carcinoma implanted s.c. were insensitive to taxol therapy, but all the testing conducted involved i.p. drug administered in Klucel. Colon 26 implanted i.p. was modestly sensitive to i.p. taxol therapy as reflected by maximum T/C values of 142–161%, but i.c. implanted ependymoblastoma was unaffected by i.p. taxol treatment. Mice bearing s.c. or i.v. implanted Lewis lung carcinoma did not have their lifespan increased following i.p. taxol therapy, although a borderline active inhibition of tumor growth was observed in one s.c. Lewis lung experiment. Against s.c. M5076, i.p. taxol treatment was essentially inactive save for one minimally positive result, a T/C of 130% obtained when taxol was administered in 50% PEG.

A summary of the NCI evaluation of taxol against human tumor xenografts is also included in Table 2. In all of the studies performed, taxol was administered either i.p. or s.c. as a suspension in Klucel.

In the CX-1 human colon carcinoma s.r.c. model, taxol given s.c. was active in three of the four experiments performed, including one study in which the maximum effect was partial tumor regression. Despite success in the s.r.c. model, s.c. implanted CX-1 tumors were not meaningfully affected by i.p. taxol therapy. Two other human colon tumor xenograft models, CX-2 and CX-5, were also subjected to treatment with taxol. No activity was observed in either s.r.c. or s.c. implant experiments involving these tumors. Additionally, taxol failed to produce an active result at acceptable levels of toxicity versus the LX-1 human lung carcinoma implanted either s.c. or in the s.r.c.

In the human amelanotic melanoma i.p. xenograft model, LOX, taxol given i.p. was active in both experiments performed, including several cured mice in one instance.

The human tumor xenograft displaying the greatest sensitivity to taxol was the MX-1 breast carcinoma, but only when implanted in the s.r.c. and taxol was given i.p. Under those circumstances, 100% tumor regression was observed in two experiments. However, taxol administered s.c. versus s.r.c. MX-1 just failed to produce active levels of tumor inhibition at acceptable levels of toxicity. Taxol treatment of mice bearing s.c. implanted MX-1, even though administered i.p., was without effect.

Data from BMS

A condensed summary of the effects of taxol in the many experimental tumor models used to evaluate the drug is presented in Table 3.

Against the P388 leukemia implanted i.p., maximum T/C values obtained following i.p. taxol treatment, all vehicles considered, ranged from 118 to 194%, but most maximum effects fell within 140–160%. There was no consistent advantage to administering taxol i.p. as either a suspension or solution, or using consecutive daily injections versus an intermittent treatment schedule. Data addressing these issues are included in Table 4. In one i.p. P388 experiment (no. 8309) in which three different vehicles were compared using an identical qd1 → 5 treatment regimen, taxol in 10% DMSO/saline yielded a maximum T/C of 144% compared with a maximum T/C of 194% when 10% Tween 80/saline was the vehicle (taxol was administered as a suspension in both instances); taxol administered as a solution in 5% ethanol–5% cremophor/saline provided an intermediary result, 178% T/C. A comparison of both schedule and vehicle took place in experiment no. 8365. Taxol administered as a suspension in 10% Tween 80/saline achieved maximum T/C values of 130 and 140% on intermittent (q4d × 2) or consecutive daily (qd × 5) injection regimens, respectively. In comparison, taxol given on the same schedules but in 12.5% ethanol–12.5% cremophor/saline produced slightly greater maximum T/C values of 160% in both instances. Intermittent versus consecutive daily injection regimens were compared again in experiment no. 8373, and very similar maximum T/C values were obtained, 155 and 159%, respectively, following taxol's administration as a solution. Cumulative tolerated dose levels of taxol were similar on both intermittent and consecutive daily injection schedules when compared for use with a given vehicle.

Against i.v. implanted P388, i.p. dosing of taxol was ineffective and only on one occasion, using a consecutive daily regimen of taxol in an ethanol-based solution, was minimal activity (T/C of 129%) obtained following i.v. therapy.

Taxol was also evaluated against two sublines of P388, one resistant to adriamycin (P388/ADR) and another resistant to mitomycin C (P388/MMC). No activity was observed against either of these drug-resistant leukemias despite a concomitantly obtained active result against the parental line.

Taxol was evaluated in several murine solid tumor models (Table 3). Administration of taxol

Table 3. Condensed summary of taxol's preclinical antitumor activity: BMS data

Tumor, site	Range of optimal effects[a]			Predominant score[b]
	MST %T/C	T-C (days)	MTW %T/C	
Murine leukemias				
P388, i.p.	118–194	—	—	+ → + +
P388, i.v.	100–129	—	—	− → ±
P388/MMC, i.p.[c]	108–117	—	—	−
P388/ADR, i.p.[c]	100–105	—	—	−
Murine solid tumors				
B16, i.p.	> 295 (5/8)	—	—	+ + + +
B16, s.c.	100	0–0.3	—	−
M109, i.p.	190– > 594 (0–3/6)	—	—	+ + → + + + +
M109, i.p. (staged)[c]	144–276	—	—	+ + → + + +
M109, s.c.[c]	124–173	4.3–14.8	—	+ +
M109, s.r.c.[c]	—	—	(−21)–3	+ +
M5076, i.p.[c]	123	—	—	−
M5076, s.c.[c]	122	1.8	—	−
Human tumor xenografts[c]				
A431, s.r.c.	—	—	(−54)	+ + +
A2780, s.r.c.	—	—	(−63)	+ + +
A2780, s.c.	—	14.5	—	+ +
HCT-116, s.r.c.	—	—	(−1)	+ +
LX-1, s.r.c.	—	—	4	+ +
H2981, s.r.c.	—	—	(−72)	+ + +
L2987, s.r.c.	—	—	5	+ +

[a] See footnote 'a' in Table 2. For 'T-C (days)', see footnote 'b' in Table 1.
[b] − = no activity; + = mild activity; + + = moderate acitivity; + + + = substantial activity; + + + + = potentially curative activity.
[c] Data obtained exclusively with enthanol-based vehicles.

Table 4. The effects of treatment schedule and vehicle on taxol's antitumor activity

Tumor, site	Experiment no.	Treatment			Maximum	
		schedule, route	vehicle[a]	optimal dose (mg/kg/injection)	MST %T/C	T-C (days)
P388, i.p.	8309	qd1 → 5, i.p.	10% T80/saline	12	194	—
		qd1 → 5, i.p.	10% DMSO/saline	24	144	—
		qd1 → 5, i.p.	5% E&C/saline	24	178	—
	8365	d.2&6, i.p.	10% T80/saline	64	130	—
		qd2 → 6, i.p.	10% T80/saline	25.6	140	—
		d.2&6, i.p.	12.5% E&C/saline	45	160	—
		qd2 → 6, i.p.	12.5% E&C/saline	18	160	—
	8373	d.1&5, i.p.	12.5% E&C/saline	40	155	—
		qd1 → 5, i.p.	12.5% E&C/saline	24	159	—
M109, s.c.	211	d. 1, 5&9, i.v.	10% T80&E/saline	40/80[b]	124	6.0
		d.1, 5&9, i.v.	12.5% E&C/saline	40/80[b]	126	4.3
		qd1 → 5, i.v.	10% T80&E/saline	25	145	12.5
		qd1 → 5, i.v.	12.5% E&C/saline	50	145	10.8
	215	qd5 → 9, i.v.	12.5% E&C/saline	48	155	14.8
		qd5 → 9, i.v.	5% E&C/saline	48	152	13.8

[a] T80 = Tween 80; E = ethanol; C = cremophor.
[b] Lower dose yielded the maximum lifespan increase whereas higher dose caused the greatest delay in tumor growth.

i.p. as a suspension was very effective against i.p. implanted B16 melanoma as reflected by a maximum T/C of >295% with the majority of mice having been cured. In the same experiment, such treatment was completely unsuccessful against s.c. implanted B16. This result was confirmed in a subsequent s.c. B16 experiment. Although taxol was not found to be active against the s.c. B16 tumor model, it was never administered i.v. as a solution.

Most of the murine solid tumor data for taxol was accumulated using M109 lung carcinoma, either i.p. or s.c. implant models. Initially, taxol was evaluated i.p. as a suspension in Tween 80/saline against unstaged i.p. implanted M109 and good to excellent activity was observed, including several instances of cured mice. In subsequent i.p. M109 studies, i.p. therapy was withheld until day 5 post-implant (i.e. 'staged' M109 experiments) and taxol was always administered in vehicles containing ethanol and cremophor. Using this staged M109 model, taxol failed to produce cures but nevertheless caused substantial increases in lifespan (T/C values of 144–276%).

In mice bearing s.c. M109, i.v. treatment with taxol suspended in Tween 80 and saline was not effective, nor was s.c. treatment with taxol suspended in CMC or 70% DMSO/saline (data not shown in Table 3). A completely different outcome was obtained when taxol was initially solubilized with ethanol plus either Tween 80 or cremophor, and subsequently diluted with saline. A comparison between these two vehicles was made in a s.c. M109 experiment involving two different treatment regimens (see experiment M109 no. 211, Table 4). When administered in 10% ethanol–10% Tween 80/saline or 12.5% ethanol–12.5% cremophor/saline on a q4d × 3 treatment schedule, taxol achieved similar maximum effects using either vehicle, T/C values of 124–126%, and T-C values of 4.3–6.0 days. There was no obvious indication of having reached a maximum tolerated dose on this schedule, although historical data using these vehicles would suggest that any greater dose escalation would not be tolerated. In the same experiment, consecutive daily injections for 5 days was associated with a maximum T/C of 145% on both schedules and maximum T-C values of 10.8–12.5 days. The successful application of the consecutive daily injection schedule was observed in subsequent studies and i.v. taxol has reproducibly achieved moderate (10–15 day) delays in s.c. M109 tumor growth and active levels of extensions in lifespan (Table 3).

Taxol's activity in the s.c. M109 tumor model also includes positive effects against staged s.c. M109. When a concomitant comparison was made between taxol's activity against unstaged versus staged s.c. M109, optimal therapy resulted in similar maximum effects as reflected by T/C values of 148–155% and T-C values of 13.3–13.5 days. In yet another staged s.c. M109 experiment, a comparison was made between vehicles, 12.5% cremophor–12.5% ethanol/saline versus 5% cremophor–5% ethanol/saline (see experiment M109 no. 215, Table 4). Very similar maximum effects were observed regardless of the vehicle; the maximum T/C values were 152–155%, and maximum T-C values were 13.8–14.8 days, reflecting effective treatments (Figure 2A).

Taxol in ethanol plus cremophor was also administered to mice bearing M109 implanted in the s.r.c. Both i.p. and i.v. therapies delivered qd1 → 5 resulted in partial regressions or essentially complete inhibition of tumor growth.

The use of an ethanol–cremophor vehicle did not, however, provide a means for taxol to demonstrate its effectiveness against i.p. or s.c. M5076 tumors. In the i.p. tumor model, maximum tolerated doses of taxol failed to achieve increases in lifespan consistent with activity. Against s.c. M5076, i.v. taxol therapy similarly failed to achieve an active result.

Each of the remaining assessments of taxol's antitumor activity involved human tumors implanted s.c. or in the s.r.c. (Table 3). In all of these experiments, taxol was administered i.v., q2d × 5, in ethanol–cremophor/saline vehicles. Three tumor lines showed particular susceptibility to taxol, A2780 ovarian, A431 vulva and H2981 lung carcinomas. Mice bearing these tumors in the s.r.c. had them regress by >50% following treatment with taxol. The remaining three tumors, HCT-116 colon, L2987 lung and LX-1 lung carcinomas, implanted in the s.r.c., had their growth nearly completely inhibited by taxol, as reflected by MTW %T/C values of −1 to 5%.

The A2780 ovarian carcinoma was also implanted s.c. in one experiment (Figure 2B). Taxol treatment was initiated on day 7 post-implant and given i.v. q2d × 5; optimal treatment with 24 mg/kg/injection caused a 14.5 day T-C reflective of moderate activity in this model.

Several experiments were performed in which taxol was evaluated in combination chemotherapy settings versus the unstaged s.c. M109 tumor model. In each instance, taxol was administered i.v. in a cremophor–ethanol/saline vehicle, qd1 → 5, and

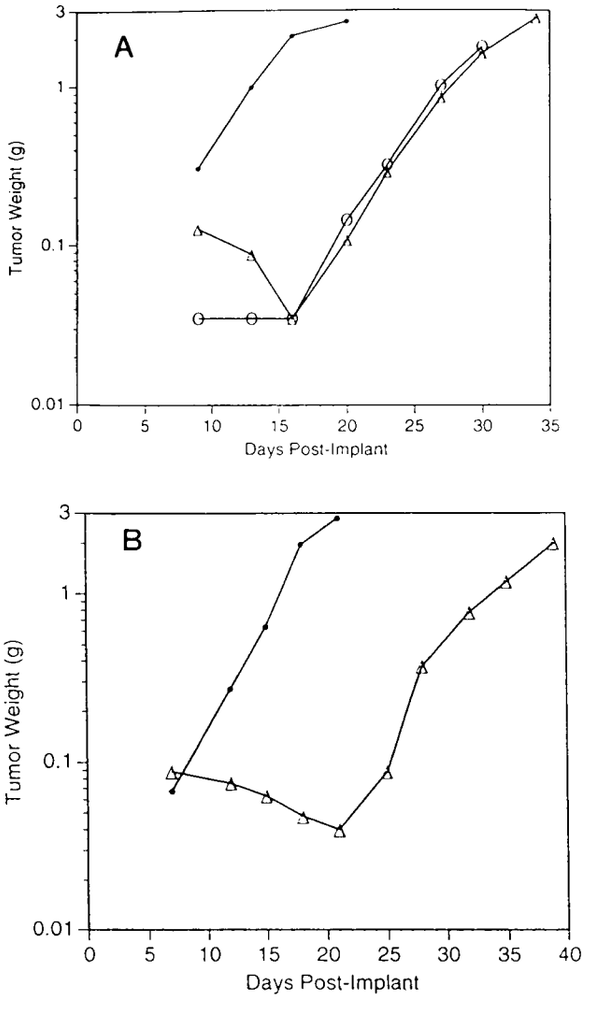

Figure 2. (A) Effect of taxol versus staged s.c. murine M109 lung carcinoma. Taxol, 48 mg/kg/injection, i.v. qd5 → 9, administered in either 12.5% (△) or 5% (○) each of ethanol and cremophor in saline compared to tumor-bearing control mice (●). (B) Effect of taxol versus staged s.c. human A2780 ovarian carcinoma. Taxol, 24 mg/kg/injection, i.v. d7, 9, 11, 13 and 15, administered in 5% ethanol–5% cremophor/saline (△) compared to tumor-bearing control mice (●).

the other drugs were administered i.p. or i.v. on days 1 and 5; on the days when both taxol and another drug were administered, taxol was given 1 h prior to the other drug.

In the initial experiment, taxol, cisplatin and VP-16 were each evaluated singularly as well as in two-drug, taxol-based combinations. The maximum effects for taxol alone were a 150% T/C and a 13.5 day T-C value, which was superior to the best solo effects of either i.p. cisplatin or i.p. VP-16. The best effect obtained using taxol plus cisplatin was a T/C of 155% accompanied by a 17.8 day T-C; this delay in tumor growth, but not the increased lifespan, was significantly ($p < 0.05$) greater than the delay caused by taxol alone (Figure 3B). The therapeutic synergy suggested by this result occurred at only one dose combination and was a maximum tolerated regimen. In practical terms, the enhanced delay in primary tumor growth was, even if experimentally reproducible, of limited likely utility. The best effects obtained using taxol plus VP-16, a T/C of 158% accompanied by a T-C of 14.5 days, was not significantly superior to the optimum effects of taxol alone (Figure 3A).

In the second combination chemotherapy experiment, taxol, adriamycin and cyclophosphamide were each evaluated singularly as well as in two-drug, taxol-based combinations. Taxol alone achieved a maximum T/C of 173% and a 10.8 day T-C, i.v. adriamycin achieved a maximum T/C of 170% and also a 10.8 day T-C, and i.p. cyclophosphamide caused effects inferior to these two drugs. Optimal combination chemotherapy involving taxol plus adriamycin resulted in a maximum T/C of 175% and a maximum T-C of 13.5 days, and for taxol plus cyclophosphamide, a maximum T/C of 161% and an 11.5 day T-C was observed. These combination chemotherapy results were not superior to the best effects of the individual drugs (Figure 3C and D).

Published descriptions of taxol's preclinical antitumor activity

An early reference to taxol's *in vivo* activity was provided by Wani *et al.*[2] who noted the NCI's results in several murine tumor models, including L1210, P388 and P1534 leukemias, sarcoma 180, Lewis lung carcinoma, and the Walker 256 carcinoma. Other than reports describing NCI data,[3,5,19,20] Bartoli *et al.*[14] confirmed taxol's activity against i.p. P388 leukemia; taxol solubilized in 5% cremophor–5% DMSO–90% saline and administered qd1 → 4 produced a T/C of 166%. Similarly, Lavelle *et al.*[15] reported that taxol was active versus i.p. P388 (T/C of 125–179%) and i.p. B16 (T/C of 125–149%) but not i.p. L1210 following qd1 → 4 i.p. injections. The latter group also found taxol to be inactive following i.v. administration against i.m. Lewis lung and s.c. colon 26, but no mention was made of the vehicle used.

Bissery *et al.*[16] demonstrated taxol's activity in a distal site solid tumor model, s.c. B16. They found

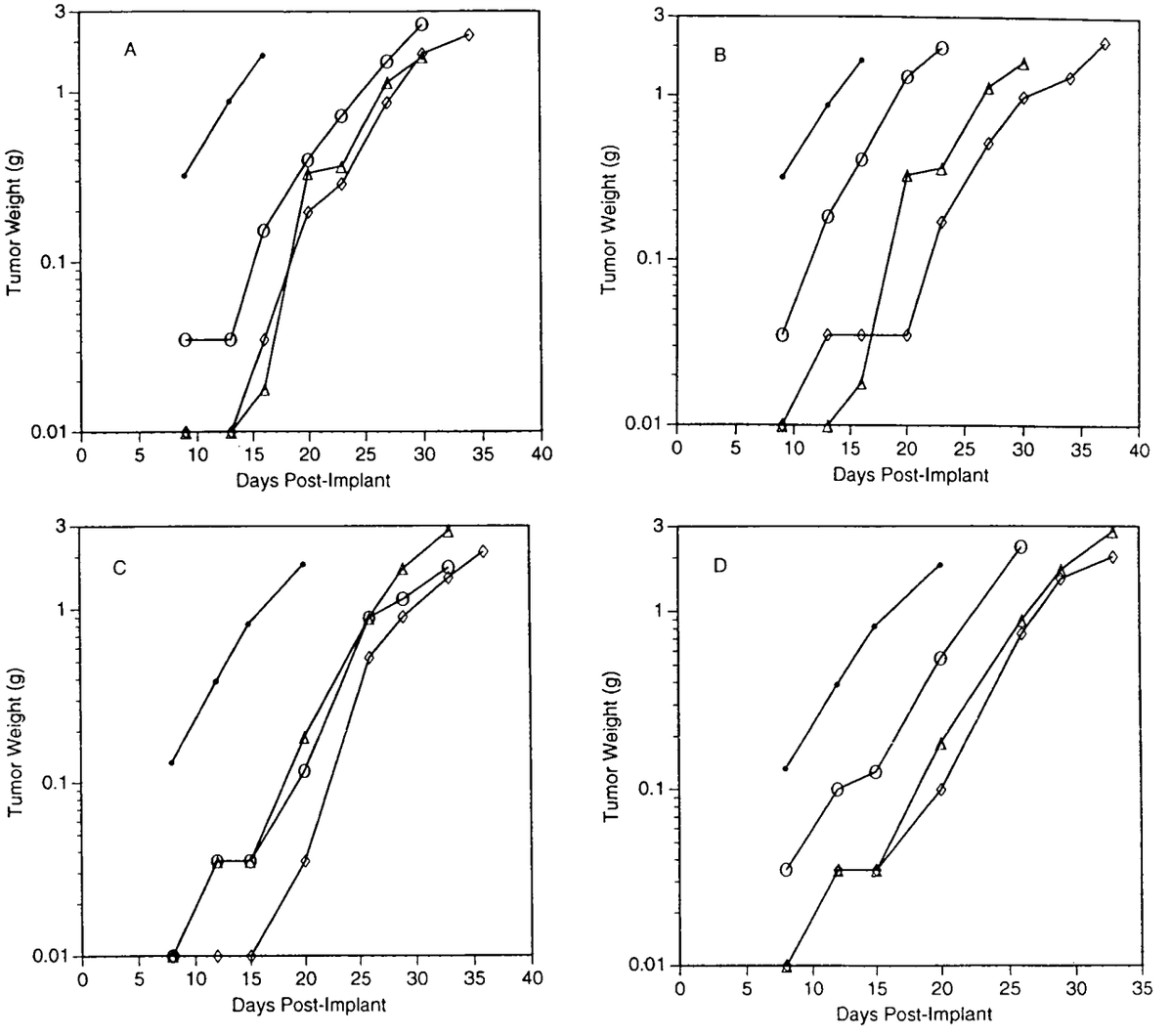

Figure 3. Taxol-based combination chemotherapy of s.c. M109 lung carcinoma. Taxol was always given i.v. qd1 → 5, and each of the other drugs was given i.v. or i.p. day 1 and 5. (A) Taxol, 48 mg/kg/injection (△); VP-16, i.p. 120 mg/kg/injection (○); taxol, 24 mg/kg/injection, plus VP-16, i.p. 60 mg/kg/injection (◇); controls (●). (B) Taxol, 48 mg/kg/injection (△); cisplatin, i.p. 8 mg/kg/injection (○); taxol, 36 mg/kg/injection, plus cisplatin, i.p. 6 mg/kg/injection (◇); controls (●). (C) Taxol, 36 mg/kg/injection (△); adriamycin, i.v. 10 mg/kg/injection (○); taxol, 36 mg/kg/injection, plus adriamycin, i.v. 5 mg/kg/injection (◇); controls (●). (D) Taxol, 36 mg/kg/injection (△); cyclophosphamide, i.p. 160 mg/kg/injection (○); taxol, 16 mg/kg/injection, plus cyclophosphamide, i.p. 80 mg/kg/injection (◇); controls (●).

that taxol dissolved in ethanol, Tween 80 and 5% dextrose in water, and administered i.v., q4d × 4, against an early-staged s.c. B16 model, produced a 4.8 day delay in primary tumor growth which translated into >1 $\log_{10}$ cell kill.

Additional reports have also appeared describing taxol's activity in human tumor models. Riondel *et al.*[7] found taxol induced substantial regression or tumor growth delay when evaluated against several staged human tumor xenografts. They administered taxol (dissolved in ethanol, cremophor and water) s.c. at a fixed dose of 12.5 mg/kg/injection, for five consecutive days out of every 7 days, for a period of 3 weeks, to mice bearing well established (200 mm^3) tumors. Four of five mice implanted with a human ductal carcinoma had their tumors regress during treatment with taxol. Other groups of mice implanted with either endometrial, ovarian, brain,

lung or tongue tumors experienced significant delays in tumor growth compared to untreated parallel control tumor-bearing mice. In contrast to taxol's effectiveness in the aforementioned tumor models, Sternberg *et al.*[16] found taxol to be ineffective against two human pancreatic tumor lines, Capan-1 and P2, heterotransplanted into athymic mice. Considering that taxol was given only at a single dose level, 6 mg/kg, q4d × 7, by the i.p. route, and no toxicity was observed, the adequacy of taxol's evaluation must be questioned.

Discussion

Taxol has been demonstrated in numerous laboratories worldwide to have broad spectrum antitumor activity against many tumor models. The susceptible tumors include murine leukemias and solid tumors, and human solid tumor xenografts. Many of the studies conducted up until the late 1980s utilized taxol as a suspension and, consequently, taxol's activity was usually expressed in i.p. tumor models following i.p. therapy. The insensitivity of most distal site tumor models, both of murine and human origin, was probably a consequence of the insolubility of taxol in nearly all of the vehicles used in these early studies. On the occasions when an ethanol-based vehicle was used to dissolve taxol (Bissery *et al.*,[6] Riondel *et al.*[7] and BMS data included herein), substantial distal site antitumor activity was observed.

In relating data from the NCI, the assessment made herein may appear to be at odds with the published descriptions.[3,5] These published accounts ascribed good activity to s.c. taxol treatment of s.r.c. MX-1, but the conclusions reached in this review are that such therapy was just barely ineffective. The discrepancy is due to the use here of more stringent toxicity criteria which prevents the consideration of taxol regimens causing >5 g of body weight loss or $>20\%$ lethality. Nevertheless, subsequent application of taxol by the i.p. route versus s.r.c. MX-1 resulted in 100% tumor regression on two occasions, and so this model was definitely sensitive to taxol.

Despite a few attempts to address the question of schedule dependency, no definitive answer is yet forthcoming. The NCI i.p. P388 data would appear to support the preferred use of many closely spaced small doses of taxol rather than on large bolus injections, but it was noted that the one large bolus injection used was not very large at all, and no maximum tolerated dose was reached. Under such circumstances, one cannot draw definite conclusions. In the BMS experiments reviewed, daily versus intermittent regimens in the i.p. P388 model failed to discern an advantage for either treatment. A similar experiment evaluating i.v. taxol versus s.c. M109 gave an indication that daily dosing was more efficacious, but again a clearly toxic dose level was not obtained on the intermittent treatment schedule. We have utilized daily or every-other-day treatments in all our subsequent (to the aforementioned s.c. M109 studies) studies because it has provided reproducibly positive results in nearly all the tumor models evaluated.

Attempts to discern a therapeutically synergistic drug combination involving taxol was made on two occasions, but without success. The particular treatment regimens selected, using the various drugs evaluated, were made on the basis of historical data (unpublished date and Rose[10]) suggesting the likely activity in the s.c. M109 model of each of the drugs when used alone. The decision to administer taxol 1 h prior to another drug, on days when both taxol and another drug were given, was made on the basis of the data of Citardi *et al.*[21] and Rowinsky *et al.*[22] These investigators found that *in vitro* exposure of L1210 cells to taxol and cisplatin was most effective (greatest cytotoxicity) when taxol was given prior to cisplatin,[21] and that a more profound neutropenia occurred clinically in patients receiving cisplatin prior to taxol, relative to the reverse sequence, due perhaps to a 25% lower taxol clearance rate when cisplatin was given initially.[22] Although a statistically significant enhancement of tumor growth delay was found using taxol plus cisplatin, the effect was minimal in absolute terms and occurred at only one particular drug–dose combination which was considered a maximum tolerated regimen. In essence, none of the taxol-based combination chemotherapies tested yielded therapeutic advantages over the best antitumor effects associated with concomitantly evaluated solo drug treatment. It is possible, however, that drug treatment schedules other than those utilized may provide for improved therapeutic outcomes, and such additional experiments will be pursued.

In summary, taxol has a broad-based preclinical antitumor activity. Dissolution of taxol prior to its i.v. administration allowed for the demonstration of taxol's substantial activity against the more stringent distal site tumor models of both murine and human origin.

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