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Plasma pharmacokinetics and tissue distribution of paclitaxel in CD_2F_1 mice

Julie L. Eiseman[1, 2], Natalie D. Eddington[3], James Leslie[3], Claudia MacAuley[1], Dorothy L. Sentz[1], Michael Zuhowski[1], Jacqueline M. Kujawa[3], David Young[3], Merrill J. Egorin[1, 4]

[1] Division of Developmental Therapeutics, University of Maryland Cancer Center, Baltimore, Maryland, USA
[2] Department of Pathology, University of Maryland School of Medicine, Baltimore, Maryland, USA
[3] Pharmacokinetics-Biopharmaceutics Laboratory, University of Maryland Pharmacy School, Baltimore, Maryland, USA
[4] Department of Medicine, University of Maryland School of Medicine, Baltimore, Maryland, USA

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Abstract. We defined the pharmacokinetics of paclitaxel after i.v., i.p., p.o., and s.c. administration of 22.5 mg/kg to CD_2F_1 mice. Additional mice were studied after i.v. bolus dosing at 11.25 mg/kg or 3-h continuous i.v. infusions delivered at 43.24 μg kg^{-1} min^{-1}. Plasma was sampled between 5 min and 40 h after dosing. Brains, hearts, lungs, livers, kidneys, skeletal muscles, and, where applicable, testicles were sampled after i.v. dosing at 22.5 mg/kg. Liquid-liquid extraction followed by isocratic high-performance liquid chromatography (HPLC) with UV detection was used to determine paclitaxel concentrations in plasma and tissues. After i.v. administration to male mice, paclitaxel clearance (CL_{tb}) was 3.25 ml min^{-1} kg^{-1} and the terminal half-life ($t_{1/2}$) was 69 min. After i.v. administration to female mice, paclitaxel CL_{tb} was 4.54 ml min^{-1} kg^{-1} and the terminal $t_{1/2}$ was 43 min. The bioavailability of paclitaxel was ~10%, 0, and 0 after i.p., p.o., and s.c. administration, respectively. Paclitaxel bioavailability after i.p. administration was the same when the drug was delivered in a small volume to mimic the delivery method used to evaluate in vivo antitumor efficacy or when it was delivered in a large volume to simulate clinical protocols using i.p. regional therapy. Paclitaxel was not detected in the plasma of mice after i.p. delivery of the drug as a suspension in Klucel:Tween 80. Pharmacokinetic parameters were similar after i.v. delivery of paclitaxel at 22.5 and 11.25 mg/kg; however, the CL_{tb} calculated in these studies was much lower than that associated with 3-h continuous i.v. infusions. After i.v. administration, paclitaxel was distributed extensively to all tissues but the brain and testicle. These data are useful in interpreting preclinical efficacy studies of paclitaxel and predicting human pharmacokinetics through scaling techniques.

Key words: Paclitaxel – Taxol – Pharmacokinetics – Mice

Introduction

Paclitaxel is a novel antineoplastic agent that is derived from the bark of *Taxus brevifolia* and acts by stabilizing microtubules [17, 20, 28, 32, 36, 38]. Paclitaxel has shown considerable efficacy in clinical trials against ovarian, breast, lung, and head and neck carcinomas [6–8, 10, 22, 24, 25, 31, 35]. Pharmacokinetics studies in humans have indicated that during the 24 h after i.v. administration, only about 5% of paclitaxel is eliminated in the urine, suggesting that the drug may be sequestered in tissues, extensively metabolized, or excreted via the bile [27, 39]. In spite of the clinical development of paclitaxel, little is known about the pharmacokinetics of this agent in rodents, particularly mice, which are used for the preclinical toxicology and efficacy studies on which clinical development of the drug was predicated. To date, only a few studies of paclitaxel pharmacokinetics have been performed in rodents, and all of these have been done in rats [15, 23, 37]. Understanding the pharmacokinetics of paclitaxel is likely to be necessary for optimal application of the drug in the clinical setting and for adequate interpretation of preclinical toxicology and efficacy studies. We therefore undertook studies in CD_2F_1 mice to elucidate the pharmacokinetics of paclitaxel after i.v., p.o., i.p., and s.c. administration. In addition, the tissue distribution of paclitaxel was determined after i.v. bolus dosing.

Materials and methods

Reagents. Paclitaxel and cephalomannine, the internal standard for high-performance liquid chromatography (HPLC) assays, were obtained from the Developmental Therapeutics Program, National Cancer Institute (Bethesda, Md., USA). Klucel and diluent 12, a 1:1 mixture of cremophor:ethanol, were also obtained from the Developmental Therapeutics Program.

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Correspondence to: Dr. Julie L. Eiseman, Division of Developmental Therapeutics, University of Maryland Cancer Center, BRB, Room 9-029, 655 West Baltimore Street, Baltimore, MD 21201, USA

Paclitaxel was dissolved at 6 mg/ml in diluent 12 and then diluted further with sterile 0.154 *M* NaCl such that i. v. bolus doses of 30, 22.5, or 11.25 mg/kg paclitaxel could be delivered in a volume of 15 ml/kg. In studies evaluating i. p. administration, paclitaxel was delivered in one of three ways. To mimic preclinical efficacy and toxicology studies, a dose of 22.5 mg/kg was prepared as described above for i. v. studies. For studies designed to mimic clinical i. p. protocols, paclitaxel was dissolved at 6 mg/ml in diluent 12 and then diluted 1 : 39 with sterile 0.154 *M* NaCl such that the dose of 22.5 mg/kg was delivered i. p. at 150 ml/kg. In a third study aimed at duplicating the conditions of initial murine efficacy trials, paclitaxel was prepared as a suspension in Klucel : 0.05% Tween 80 and given i. p. at 30 mg/kg in a volume of 10 ml/kg. For studies evaluating s. c. administration, paclitaxel was formulated in the manner described for i. v. dosing and was given at 22.5 mg/kg. In studies evaluating oral administration, a paclitaxel dose of 22.5 mg/kg was used, and the drug was formulated in the manner described for i. v. dosing except that sterile, pyrogen-free water replaced the 0.154 *M* NaCl. For studies using a 3-h continuous i. v. infusion schedule of drug administration, paclitaxel was dissolved at 6 mg/ml in diluent 12 and then diluted such that a dose of 43.24 μg kg^{-1} min^{-1} could be delivered at a rate of 10 μl/min.

Mice. Virus-free, adult CD_2F_1 mice (5–6 weeks of age) were obtained from the Animal Program administered by the Animal Genetics and Production Branch of the National Cancer Institute. Mice were allowed to acclimate to the University of Maryland Animal Facility for at least 1 week before studies were initiated. To minimize exogenous infection, mice were maintained in conventional cages in a separate room and handled in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH number 85-23, 1985). Ventilation and air flow in the Animal Facility were set to 12 changes/h. Room temperatures were regulated at 72° ± 2° F, and the rooms were on automatic 12-h light/dark cycles. Mice received Purina 5001 Chow and water ad libitum except on the evening prior to dosing, when all food was removed and withheld until 4 h after dosing. Sentinel mice (CD-1 mice housed in cages containing one-fifth bedding removed from study-mice cages at cage change) were maintained in the animal room and assayed at monthly intervals for specific murine pathogens by the mouse antibody production (MAP) test (Litton Bionetics, Charleston, S. C., USA). These mice remained free of specific pathogens throughout the study period, indicating that study mice were free of specific pathogens.

Both male and female mice were given i. v. doses of 22.5 mg/kg; however in all other studies, only female mice were used. Three mice per dose group and sex were euthanized at each time point of sampling. Because of the toxicity of diluent 12, i. v. bolus doses of paclitaxel were given over 90 s through a tail vein. Paclitaxel was delivered via other parenteral routes as a rapid bolus. The oral dose of paclitaxel was given by 22-gauge gavage needle. For continuous i. v. infusion studies, mice were sedated with i. p. pentobarbital at a dose of 60 mg/kg, and a 27-gauge butterfly needle was inserted into a tail vein. The drug (0.0864 mg/ml) was delivered at a rate of 10 μl/min with a Sage model 352 pump (Orion Research, Inc., Boston, Mass., USA; calibration, 0.583 ± 0.08 g/h).

Sampling. In studies in which paclitaxel was injected i. v. at a dose of 22.5 mg/kg, blood, brains, hearts, lungs, livers, kidneys, skeletal muscles, and, in males, testicles were collected at 5, 10, 15, 30, 45, 60, 120, 240, 480, 960, 1440, and 2400 min after dosing. In all subsequent studies, only blood was sampled, but in some experiments, additional time points were included such that blood was also sampled at 75, 90, 120, 150, 180, and 210 min after dosing. Blood was collected by cardiac puncture into heparinized syringes and stored on ice until centrifuged at 1500 *g* for 10 min to obtain plasma. Tissues were rapidly dissected, placed on ice until weighed, and then snap-frozen in liquid nitrogen. Plasma, tissues, and dosing solutions were stored frozen at –70° C until analysis.

Analysis of paclitaxel. Plasma and tissue concentrations of paclitaxel were determined by HPLC using a modification of the method of Jamis-Dow et al. [12]. Briefly, 100-μl samples of plasma were mixed with 200 μl acetonitrile that contained 25 μg/ml cephalomannine as the internal standard and were then centrifuged at 5000 *g* for 4 min. To avoid base-catalyzed degradation of paclitaxel, 200 μl of the resulting supernatant was mixed with an equal volume of 0.02 *M* sodium acetate (pH 4) [19]. Tissue samples were thawed and immediately homogenized in 2 parts (w/v) of 0.154 *M* NaCl, and the resulting homogenate was then rapidly extracted as described above for plasma. The acetonitrile : sodium acetate solutions (100 μl) were analyzed with a Hewlett Packard 5 μm Hypersil ODS (100×4.6 mm) column and an isocratic mobile phase of acetonitrile : distilled, deionized water (40 : 60, v : v) delivered at a flow rate of 1.8 ml/min. Column effluent was monitored at 227 nm, and the detector signal was processed so as to integrate the area under each peak eluted.

The paclitaxel concentration in each sample was calculated by determining the ratio of paclitaxel peak area to that of the corresponding internal standard peak and comparing that ratio with a concomitantly performed standard curve prepared in the appropriate matrix. There was no endogenous material in mouse plasma or tissues or in cremophor that interfered with the determination of paclitaxel or internal standard. The limit of quantitation was 0.5 μg/ml, and the assay was linear over the plasma drug-concentration range of 0.5–170 μg/ml. The limit of quantitation in tissues was 0.8 μg/g and the assay was linear to 170 μg/g. Recoveries of drug from spiked samples of plasma and tissues were greater than 95% at low (0.8 μg/g or 0.8 μg/ml) and at higher concentrations (3.3 μg/ml or 3.3 μg/g). The coefficient of variability for the analysis was ≤15% with regard to both intraday analysis of any concentration on the standard curve and interday comparison of standard curves.

Pharmacokinetic analysis. The area under the curve from zero to infinity (AUC 0 – infinity) and the terminal half-life ($t_{1/2}$) were estimated by noncompartmental analysis with the LaGrange function [41] as implemented by the LAGRAN computer program [26]. Total body clearance (CL_{tb}) was calculated from the equation

$$CL_{tb} = \frac{Dose}{AUC},$$

and the volume of distribution (V_d) was calculated from the formula $Vd = CL_{tb}/k_{el}$. In addition, the profiles of plasma paclitaxel concentration versus time following i. v. administration were fit to a two-compartment, open linear model with the program ADAPT II [4], which uses a Nelder Mead simplex as the algorithm. A two-compartmental, open linear model was chosen because it was the simplest model that best approximated the actual data and resulted in the lowest sum of squares.

Determination of paclitaxel binding in mouse plasma. The extent of paclitaxel binding to proteins in mouse plasma was determined by equilibrium analysis. SprectraPor 7 dialysis menbranes with a molecular-weight cutoff of 22,000 Da (Spectrum Medical Industries, Inc., Los Angeles, Calif., USA) were placed between the two halves of five-cavity dialysis-chamber apparati (BEL-ART Products, Pequannock, N. J., USA), and 1 ml phosphate-buffered saline (PBS; 0.144 *M* NaCl, 0.1 *M* KPO_4; pH 7.46) was placed into each half of each chamber. The assembled chambers were incubated overnight at 4° C. [^{3}H]-Paclitaxel (radiochemical purity, 98% as determined by reverse-phase thin-layer chromatography; specific activity, 2.2 Ci/mmol; Amersham Life Science, Arlington Heights, Ill., USA) was added to either normal mouse plasma or PBS to produce a final concentration of 1 μCi/ml (0.454 μ*M*). The PBS that had been incubated overnight was aspirated from each dialysis chamber and replaced with 900 μl [^{3}H]-paclitaxel in either PBS or mouse plasma on one side of the dialysis membrane and with fresh PBS on the other. The dialysis apparati were subsequently incubated at 37° C, and after 0, 4, 8, and 24 h of incubation, 100-μl aliquots were removed from each half of each dialysis chamber and mixed with Ready Safe liquid scintillation cocktail (Beckman Instruments, Inc., Fullerton, Calif., USA), and the radioactivity was counted with a Beckman model LS 6000IC liquid scintillation counter. The pipet tips were rinsed twice with scintillation cocktail to avoid loss of paclitaxel to the tips.

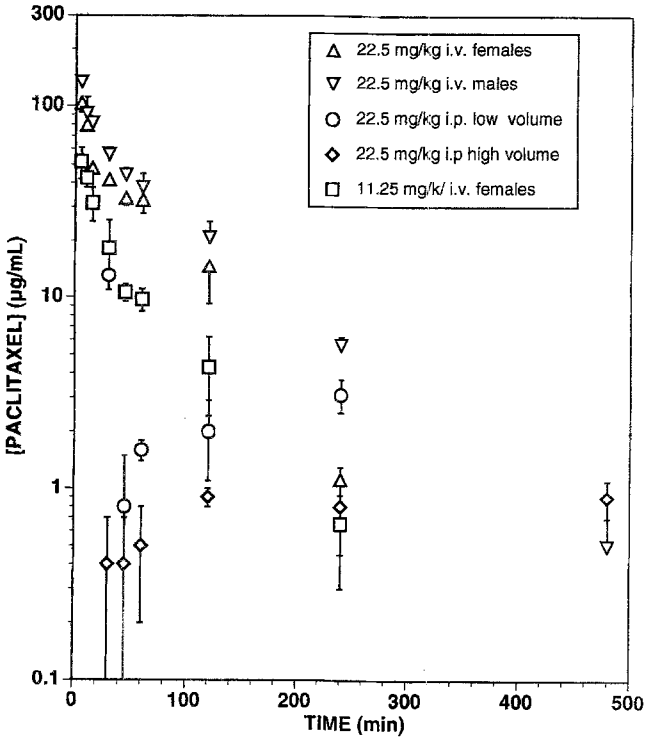

Fig. 1. Concentrations of paclitaxel detected in the plasma of mice after administration by a variety of routes of either 22.5 or 11.25 mg/kg paclitaxel

The percentage of bound drug was calculated by the following equation:

$$\text{Percentage bound} = 100 \times \left[1 - \frac{\text{Radioactivity PBS side}}{\text{Radioactivity plasma side}}\right].$$

The total of counts on both sides of the dialysis membrane were consistent with the amount of radioactivity initially added to the plasma.

Results

Plasma pharmacokinetics

Because no information was available with regard to the toxicity of paclitaxel after i.v. administration to mice, preliminary efforts were directed at defining a tolerable i.v. dose. Paclitaxel was dissolved at its maximal solubility of 6 mg/ml in diluent 12 and further diluted with 0.154 *M* NaCl such that doses of 30, 22.5, 15, and 7.5 mg/kg could be delivered in a maximal volume of 20 ml/kg to groups of male and female mice (10 mice per sex per group). Administration of vehicle (i.v.) associated with the 30-mg/kg dose resulted in prostration, lethargy, and loss of righting reflex for the 1st h after dosing; however, no other drug-related toxicity was observed during the 2-week observation period, and no unusual drug-related toxicity was observed in the animals upon necropsy.

On the basis of the above-mentioned observations, 22.5 mg/kg was selected as the highest dose for use in studies investigating the pharmacokinetics of paclitaxel after i.v. administration. After delivery of paclitaxel to mice by 90-s i.v. infusion, plasma paclitaxel concentrations declined in a manner well described by a two-compartment, open linear model (Fig. 1, Table 1). In mice injected with 22.5 mg/kg, peak plasma paclitaxel concentrations ranged between 88 and 139 µg/ml (Fig. 1, Table 1). Administration of paclitaxel via the i.v. route at 11.25 mg/kg resulted in peak plasma paclitaxel concentrations varying between 46 and 63 µg/ml, i.e., 50% of those produced by the 22.5-mg/kg dose (Fig. 1, Table 1). In these i.v. studies, plasma concentrations of paclitaxel declined with a $t_{1/2\lambda_1}$ value of approximately 5–7 min and a $t_{1/2\lambda_2}$ value of approximately 45–65 min (Table 1). The CL_{tb} of paclitaxel ranged between 3 and 6 ml min^{-1} kg^{-1} and was similar in female mice injected with doses of 11.25 and 22.5 mg/kg. In no study employing i.v. administration did plasma paclitaxel concentrations exceed the lower limit of quantitation of the HPLC assay after 240 min. Other parameters describing the decline in plasma concentrations of paclitaxel observed after i.v. administration of doses of 11.25 and 22.5 mg/kg were similar (Table 1).

Administration of paclitaxel via the i.p. route at a dose of 22.5 mg/kg resulted in much lower plasma concentrations of paclitaxel than those measured after 90-s i.v. infusions of the same dose (Fig. 1). Formulation of paclitaxel as a solution in diluent 12 and 0.154 *M* NaCl resulted in an apparent bioavailability of approximately 10% (Table 2). There was no obvious difference associated with administration of paclitaxel in either a small volume, comparable with that used in preclinical efficacy and toxicology studies, or a large volume, scaled to reproduce the volumes used in clinical trials of i.p. regional chemotherapy. In both cases, plasma concentrations of paclitaxel did not exceed the lower limit of quantitation of the HPLC assay until 30–45 min after i.p. administration. Thereafter, plasma concentrations of paclitaxel increased slowly until 240 min after administration and then fell below the lower limit of quantitation of the HPLC assay by 6 h. When paclitaxel was formulated as a suspension in a mixture of Klucel and Tween 80 and given i.p. at a dose of 30 mg/kg, plasma concentrations of paclitaxel never exceeded the 0.5-µg/ml lower limit of quantitation of the HPLC assay. Similarly, formulation of paclitaxel in diluent 12 and distilled, deionized water and administration at 22.5 mg/kg by oral gavage failed to produce plasma drug concentrations above the 0.5-µg/ml lower limit of quantitation of the HPLC assay. Finally, s.c. administration, at 22.5 mg/kg, of a solution of paclitaxel formulated in diluent 12 and 0.154 *M* NaCl also failed to produce plasma paclitaxel concentrations that exceeded the 0.5-µg/ml limit of quantitation of the HPLC assay.

On the basis of the CL_{tb} calculated for paclitaxel after 90-s i.v. infusion, a dosing rate of 43.24 µg kg^{-1} min^{-1} delivered with an infusion rate of 0.01 ml/min was selected for a 180-min continuous i.v. infusion designed to produce plasma paclitaxel concentrations of approximately 10 µg/ml. The rate of drug delivery was calculated by rearranging the relationship

$$\text{Steady} - \text{state concentration} = \frac{\text{Rate of infusion}}{CL_{tb}}$$

into the form

Table 1. Pharmacokinetic parameters describing the decline in plasma paclitaxel concentrations observed in mice after the administration of paclitaxel via 90-s i.v. infusions and other routes

Two-compartment, open linear model:													
Sex	Dose (mg/kg)	Route	Peak [paclitaxel] (μg/ml)[a]	V_1 (ml/kg)	k_{10} (min^{-1})	k_{12} (min^{-1})	k_{21} (min^{-1})	CL_{tb} (ml min^{-1} kg^{-1})	Vdss (ml/kg)	λ_1 (min^{-1})	$t_{1/2\lambda_1}$ (min)	$t_{1/2\lambda_2}$ (min^{-1})	$t_{1/2\lambda_2}$ (min)
Male	22.5	i.v.	132 ± 5	123	0.025	0.072	0.065	3.09	258	0.152	4.6	0.011	64
Female	22.5	i.v.	103.0 ± 12.5	175	0.029	0.043	0.067	5.12	288	0.124	5.6	0.016	44
Female	11.25	i.v.	51 ± 9	160	0.036	0.036	0.036	5.7	319	0.094	7.3	0.014	50

Noncompartmental estimation:							
Sex	Dose (mg/kg)	Route	AUC (μg ml^{-1} min)	k_{el} (min^{-1})	$t_{1/2}$ (min)	V_D (ml/kg)	CL (ml min^{-1} kg)
Male	22.5	i.v.	6931	0.01	69	325	3.25
Female	22.5	i.v.	4956	0.016	43	284	4.54
Female	11.25	i.v.	1990	0.018	39	320	5.77
Female	22.5	i.p.[b]	459	–	–	–	–
Female	22.5	i.p.[c]	480	–	–	–	–
Female	22.5	p.o.	0	–	–	–	–
Female	22.5	s.c.	0	–	–	–	–

[a] Mean value ± SD
[b] Low volume
[c] High volume

Table 2. Plasma and tissue concentrations of paclitaxel determined in female mice injected with 22.5 mg/kg i.v. (*Sk. Musc.* Skeletal muscle, *ND* not detectable)[a]

Time (min)	Plasma (μg/ml)	Brain (μg/g)	Heart (μg/g)	Lungs (μg/g)	Liver (μg/g)	Kidney (μg/g)	Sk. Musc. (μg/g)
5	103.0 ± 12.5	2.1 ± 0.8	38 ± 4	61 ± 13	155 ± 19	127 ± 4.6	6.2 ± 1.7
10	77.9 ± 1.4	1.8 ± 0.3	45 ± 3	44 ± 1.4	119 ± 26	128 ± 31	9 ± 2
15	47.9 ± 10.0	1.4 ± 1.6	55 ± 11	41 ± 6	125 ± 12	104 ± 16	8.1 ± 1.8
30	41.6 ± 0.7	0.3 ± 0.2	39 ± 9	35 ± 0.9	97 ± 7	74 ± 3.6	10.7 ± 0.4
45	33.4 ± 2.8	0.1 ± 0.2	27 ± 5	29 ± 0	102 ± 18	66 ± 5.1	10 ± 0.4
60	32.6 ± 4.9	0.3 ± 0.3	44 ± 10	32 ± 1	91 ± 9	59 ± 8.2	10.8 ± 2.1
120	14.7 ± 5.5	0.1 ± 0.2	22 ± 5	17 ± 2.3	68 ± 23	45 ± 21	10.2 ± 1
240	1.1 ± 0.2	ND	10 ± 1.7	5.4 ± 0.2	32 ± 6.2	13 ± 1.5	2 ± 0.2
480	ND	ND	0.5 ± 0.7	ND	4.9 ± 1.9	1.6 ± 0.8	ND
960	ND	ND	ND	ND	ND	ND	ND
1440	ND	ND	ND	ND	ND	ND	ND
2400	ND	ND	ND	ND	ND	ND	ND

[a] Data represent mean values ± SD

Rate of infusion = (steady-state concentration) (CL_{tb}).

We used 10 μg/ml as the desired steady-state concentration, 4.5 ml min^{-1} kg^{-1} as the CL_{tb}, and 0.02 kg as the representative weight of a mouse. The rate of fluid delivery was selected so as not to produce excessive fluid overload in the treated mice. In two separate studies, this method and rate of drug delivery produced plasma paclitaxel concentrations that only slightly, if at all, exceeded the 0.5-μg/ml lower limit of quantitation of the HPLC assay. In each of these studies, the concentration of paclitaxel in the dosing solution was measured as being equal to that desired, and additional studies showed that no drug was lost through interaction with the drug delivery system.

Paclitaxel binding to plasma proteins

Paclitaxel was highly bound to proteins in mouse plasma. A period ranging between 8 and 24 h was required for equilibrium to be established across the SpectraPor membrane. At this time, 90%–92% of the [^{3}H]-paclitaxel added to mouse plasma was protein-bound.

Tissue concentrations of paclitaxel following i.v. administration

After 90-s i.v. infusion at a dose of 22.5 mg/kg, paclitaxel was widely distributed to most tissues (Tables 2, 3). The highest concentrations of paclitaxel were measured in the liver, kidney, and lung. In these organs, paclitaxel concentrations were maximal at 5 min after drug administration and declined progressively with time thereafter (Tables 2, 3). In contrast, at 5 min after paclitaxel injection, drug concentrations measured in the heart were 2–6 times lower than those found in the liver, kidney, and lung but increased progressively over the next 10 min before decreasing with time. As a result, peak cardiac paclitaxel concentrations were not observed until 15 min after drug

Table 3. Plasma and tissue concentrations of paclitaxel determined in male mice injected with 22.5 mg/kg i.v. (*Sk. Musc.* Skeletal muscle, *ND* not detectable)[a]

Time (min)	Plasma (μg/ml)	Brain (μg/g)	Heart (μg/g)	Lungs (μg/g)	Liver (μg/g)	Kidney (μg/g)	Sk. Musc. (μg/g)	Testes (μg/g)
5	132.2± 4.8	1.7±0.3	33 ± 5	394 ± 61	208 ±45	79 ±12	8 ±1.5	0.3±0.6
10	90.5±20.1	1.4±0.2	39 ±10	325 ± 50	158 ±53	74 ± 8	9.7±0.5	0.7±0.6
15	81.3± 4.2	1.0±0.1	38 ± 8	269 ±193	175 ±28	63 ±10	12.5±1.1	0.7±0.6
30	56.0± 4.7	1.4±0.8	26 ± 8	64 ± 9	159 ± 9	48 ± 8	14.5±3.2	1 ±0.2
45	49.3± 4.0	0.4±0.7	27 ± 5	64 ± 13	148 ±11	40 ± 4	14.6±6	1 ±0.1
60	37.9± 7.0	ND	14 ± 0.8	45 ± 29	164 ±34	36 ± 7	12.1±4.9	0.8±0.7
120	20.8± 4.5	ND	12.6± 5	27 ± 4	85 ±30	18 ± 2	11.4±5.8	0.9±1.0
240	5.6± 0.6	ND	7.2± 1.2	20 ± 8	32 ±28	9.8± 5.6	7.9±2.1	ND
480	0.5± 0.4	ND	1 ± 1.4	5.8± 3.1	5.7± 3.7	2 ± 1.2	0.9±1.4	ND
960	ND	ND	ND	8.4± 3.6	3.7± 0.9	ND	2.9±4.2	ND
1440	ND	ND	ND	0.8± 1.1	1.9± 0.4	ND	ND	ND
2400	ND	ND	ND	ND	ND	ND	ND	ND

[a] Data represent mean values ± SD

administration (Tables 2, 3). At 5 min after drug delivery, paclitaxel concentrations detected in skeletal muscle were ≥ 1 log lower than the concentrations observed in the lung, liver, and kidney and 4–5 times lower than those found in the heart. However, in both male and female mice, paclitaxel concentrations in skeletal muscle increased during the first 30 min after drug administration and remained relatively constant at 10–15 μg/g tissue (wet wt.) until 2 h, after which they declined with time. Very low concentrations of paclitaxel were observed in the brain and testes, consistent with the existence of both a blood-brain barrier and a blood-testes barrier. In many tissues, the ratio of the tissue paclitaxel concentration to the concomitant plasma concentration of drug remained approximately 1. In both male and female mice, the ratio for skeletal muscle remained <1. Conversely, the liver was the only organ in both male and female mice in which the tissue:plasma ratio of paclitaxel concentrations consistently showed tissue accumulation of drug. As implied by the very low concentrations of paclitaxel measured in the brain and testes, the ratio of tissue-to-plasma concentration of paclitaxel in these organs was extremely low (Tables 2, 3).

Discussion

Although paclitaxel has undergone extensive clinical testing and development [1, 5–10, 16, 18, 21, 22, 24, 25, 27, 29–31, 34, 35, 39, 40] and a number of clinical pharmacology studies have been presented [13, 14, 27, 29, 33, 39], the current studies provide important data not previously available.

The current data on the plasma pharmacokinetics of paclitaxel not only provide material with which to compare clinical pharmacokinetic data but can also be considered in the proposed framework of pharmacologically guided dose escalation of phase I trials [2, 3]. Moreover, the data allow intelligent assessment of early toxicology and in vivo activity testing performed in mice. In view of the failure to detect paclitaxel in plasma after administration of the drug as a suspension in Klucel:Tween 80, it is not surprising that paclitaxel, when given in this fashion, showed activity only against i.p. implanted tumors [11]. The poor bioavailability of paclitaxel after s.c. or p.o. administration implies that these are not viable means of delivering the drug to patients. The poor bioavailability of paclitaxel after its formulation in a vehicle similar to that used clinically and its delivery i.p. in large or small volumes is consistent with pharmacologic data generated in clinical trials of i.p. paclitaxel [9, 21]. Furthermore, the low systemic exposure of mice to paclitaxel after i.p. delivery is consistent with the frequent lack of activity of the drug when delivered this way in murine test systems.

Although there was no evidence of nonlinearity in the pharmacokinetic data generated with 11.25- and 22.5-mg/kg i.v. doses of paclitaxel, the failure of the 4.5-ml min^{-1} kg^{-1} CL_{tb}, calculated after i.v. delivery of those doses, to predict adequately the rate of paclitaxel infusion required to achieve a 10-μg/ml plasma steady-state concentration suggested possible nonlinearity of paclitaxel clearance. This was observed subsequently when clinical trials of paclitaxel began to use 3-h infusions instead of the 24-h continuous infusion schedule commonly used in the past [13, 14, 33]. Investigation of this question was somewhat restrained by several practical issues related to paclitaxel. The 22.5-mg/kg dose used in the current work was the practical upper limit for i.v. dosing as a result of (1) the extremely hydrophobic nature of paclitaxel, (2) the 6-mg/ml limit of solubility of paclitaxel in diluent 12, and (3) the amount of diluent 12 and the volume of fluid that could be delivered as a brief i.v. infusion to mice. The practicality of employing lower doses of paclitaxel was limited by the lower limit of quantitation of the HPLC assay used to measure paclitaxel. Administration of paclitaxel as a prolonged continuous infusion was rendered problematic because of the inability to achieve adequate drug concentrations in any vehicle compatible with devices such as Alzet osmotic pumps (Alza Corp., Palo Alto, Calif., USA).

The tissue distribution of paclitaxel defined in the current studies also augments and extends the previously available information with regard to the pharmacokinetics of this agent. The documentation of both a blood-brain barrier and a blood-testes barrier should prove helpful in view of current interest in evaluating paclitaxel versus intracranial neoplasms and the potential use of the drug against testicular tumors. Monsarrat et al. [23] have shown

that approximately 50% of a dose of paclitaxel delivered to rats was recovered in 24 h of bile collection. However, these investigators did not account for the remaining 50% of the dose. Klecker et al. [15] have extended these observations by studying the plasma pharmacokinetics, biliary excretion, and tissue distribution of radiolabeled paclitaxel after 6-h i.v. infusion in male Sprague-Dawley rats. Our data agree well with the tissue distribution of paclitaxel presented by Klecker and colleagues [15]. In both rats and mice, the highest tissue concentrations of paclitaxel were observed in the liver, kidney, and lung, with lower concentrations being measured in the heart and much lower concentrations being found in skeletal muscle. In both rodent species, there was evidence for a blood-brain barrier and a blood-testes barrier.

In conclusion, the studies presented herein further our understanding of the pharmacokinetics of paclitaxel and provide a basis for interpreting the results of preclinical efficacy trials. They also provide information that can be useful in interpretation of pharmacokinetic data generated with paclitaxel in the clinical setting.

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