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Review paper

Preclinical pharmacokinetics of paclitaxel and docetaxel

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The taxanes paclitaxel and docetaxel represent a novel class of antineoplastic agents. A major problem of both drugs is their low aqueous solubility and the design of suitable formulations has been a difficult step in the process of therapeutic development. The formulations currently used are mixtures of Cremophor EL:ethanol for paclitaxel (Taxol®) and Tween 80:ethanol for docetaxel (Taxotere®), but many new approaches have been tested or are under investigation. Paclitaxel and docetaxel have a similar mechanism of action, which is based on promotion of tubulin assembly and inhibition of microtubule disassembly. Pharmacokinetic studies revealed a marked non-linearity of paclitaxel in mice, which appeared to result exclusively from Cremophor EL, the major component present in the pharmaceutical formulation. An almost linear pharmacokinetic behavior was observed in the case of docetaxel. The reported plasma protein binding of both compounds ranged from 76 to 97% in different animal species. Paclitaxel and docetaxel widely distribute into most tissues of mice and rats, including tumor tissue, but only low concentrations were detected in the central nervous system. Despite the great similarity in the chemical structures of paclitaxel and docetaxel, their metabolic profile is very distinct. Furthermore, whereas paclitaxel metabolism is largely species dependent, docetaxel metabolism is similar across species in both isolated hepatic microsomal fractions and *in vivo* models. For both taxanes, hepatobiliary excretion is the major pathway of elimination and a major fraction of the dose is excreted in feces as parent drug or hydroxylated metabolites. [© 1998 Rapid Science Ltd.]

Key words: Docetaxel, paclitaxel, pharmacokinetics.

Introduction

During screening programs of natural products in the 1960s accomplished by the National Cancer Institute (NCI), the crude extract of the bark of the Western yew tree, *Taxus brevifolia*, was found to have activity against several murine tumors. In 1971 Wani *et al.* identified paclitaxel (Taxol; NSC 125973) as the active ingredient of the bark.[1] However, further development of paclitaxel was suspended for more than a decade because of the lack of superior activity in existing experimental models and because of problems in drug formulation. After the discovery that paclitaxel works as a mitotic-spindle poison with a unique mechanism of action,[2] i.e. enhancement of the formation and stabilization of microtubule bundles,[3,4] the clinical development of paclitaxel was started.[5] To address the difficulty in obtaining the Western yew bark and to seek alternative methods of drug supply, the NCI and the Bristol-Myers Squibb Company signed a cooperative research and development agreement, providing the pharmaceutical industry with exclusivity to the data for submission of a new drug application. Once the initial problems of allergic reactions and the persistent supply shortage were solved, development progressed rapidly and phase II trials indicated significant clinical activity in ovarian, breast and non-small cell lung cancers.[6–8]

In 1981, Rhône-Poulenc Rorer and the Institute de Chemie des Substances Naturelles concluded a co-operative research agreement about natural products extracted from yews and thujas, which led to the development of docetaxel (Taxotere; NSC 628503) in 1986.[9,10] Docetaxel is a semisynthetic paclitaxel derivative, prepared from 10-deacetyl baccatin-III, a non-cytotoxic precursor isolated from the needles of the European yew tree, *Taxus baccata*.

Paclitaxel and docetaxel have now been licensed in a number of countries for treatment of platinum-

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resistant ovarian and anthracycline-resistant breast cancers, and both compounds are currently undergoing extensive clinical phase II/III evaluation. A substantial number of reviews have dealt with the clinical aspects of the development of these drugs.[5,11-16] The present review, however, is focussed on preclinical pharmacokinetic aspects of paclitaxel and docetaxel development, with the emphasis on those issues that are considered to be of clinical importance.

Pharmaceutical properties

Physiochemistry

Paclitaxel (chemical name: 5β,20-epoxy-1,2β,4,7β,-10β,13α-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine) and docetaxel (chemical name: 4-acetoxy-2α-benzoyloxy - 5β,20 - epoxy - 1,7β,10β - trihydroxy - 9-oxotax-11-ene-13α-yl-(2*R*,3*S*)-3-*tert*-butoxycarbonylamino-2-hydroxy-3-phenylproprionate) are complex diterpenoid structures having a bulky fused taxane ring system with a rare four-membered oxetan ring at positions C_4 and C_5 and an ester side chain at C_{13} (Figure 1). Docetaxel differs from paclitaxel in two places: (i) a hydroxyl group instead of the acetyl group on the C_{10} position and (ii) a *tert*-butoxy moiety instead of a benzamide phenyl group on the C_3' position on the lateral C_{13} side chain.

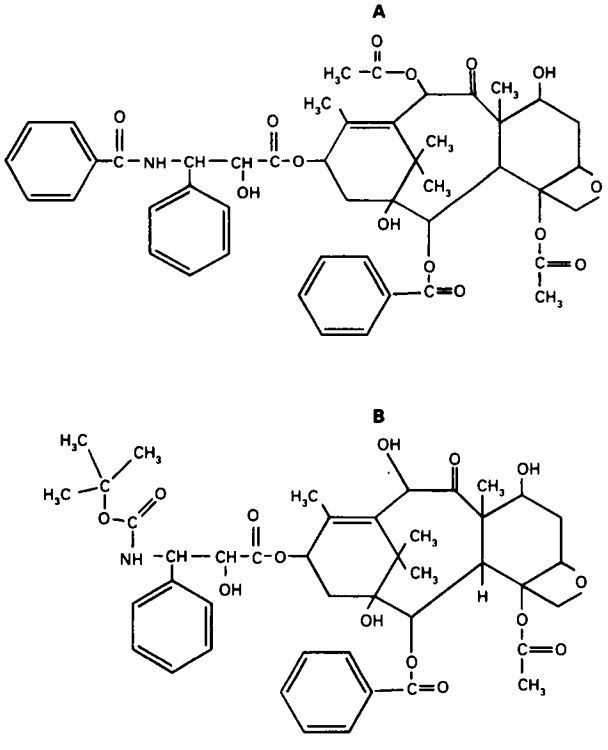


Figure 1. Chemical structures of paclitaxel (A) and docetaxel (B).

Paclitaxel is a white to off-white crystalline powder. Reported estimates of the aqueous solubility of paclitaxel vary considerably from 0.7 μg/ml at equilibrium[17,18] to 30 mg/ml when measured by reversed-phase HPLC immediately after preparation of a saturated paclitaxel solution.[10,19] After hydration of dry paclitaxel in saline buffer, sonication, centrifugation to remove precipitates and quantitation by HPLC, the initial solubility was observed to be 5 mg/ml.[13,20] Over the subsequent 24-36 h solubility decreased to a nadir of approximately 0.4 μg/ml, which is in agreement with the lower estimate reported.[17] Like paclitaxel, docetaxel is a white powder and also virtually insoluble in water.[10] Because both compounds lack acidic or basic moieties that are charged in a pharmaceutically useful pH range, manipulation of pH does not enhance the aqueous solubility. Furthermore, other common approaches to improve solubility, e.g. addition of charged complexing agents, are also not feasible.

Solutions of paclitaxel can be prepared at millimolar concentrations in a variety of alcohols, like methanol, ethanol (around 39 mg/ml), isopropanol (around 12 mg/ml), *tert*-butanol or octanol.[21] Paclitaxel is also soluble in polyoxyethyleneglycerol triricinoleate 35 (Cremophor EL; BASF, Ludwigshafen, Germany), polyethyleneglycols (PEGs) 300 and 400, chloroform, acetone, methylene chloride or acetonitrile (around 20 mg/ml), and freely soluble in dimethylacetamide (DMA) and dimethylsulfoxide (DMSO).[15] Docetaxel is sparingly soluble in acetone, soluble in chloroform, and freely soluble in dimethylformamide, 96% (v/v) of aqueous ethanol and methanol.[15] Reversible precipitation of both drugs has been observed at low temperatures, e.g. paclitaxel in ethanol at 5 mg/ml at −20°C (AS and OvT, unpublished data).

Drug formulation

Current taxane formulations. A large variety of excipients, including hydroxypropylcellulose (Klucel), polyethyleneglycol 400, polysorbate 80 (Tween 80), carboxymethylcellulose, DMSO, DMA and Cremophor EL, have been investigated to enable administration of the drugs (reviewed elsewhere).[22-24]

Currently, paclitaxel is commercially available from Mead Johnson Oncology Products (Bristol-Myers Squibb) as vials containing 30 mg of drug dissolved in 5 ml of Cremophor EL:dehydrated ethanol USP

(1:1, v/v) (Taxol®). Prior to use, the drug must be diluted 5- to 20-fold in saline or 5% (v/v) dextrose to a final concentration of 0.3–1.2 mg/ml. The diluted solutions are stable for upto 27 h at 25°C and day light conditions, although NCI guidelines advise administration within 3 h after dilution.[18] Ongoing shelf-life studies suggest that the undiluted formulation is stable in unopened vials for at least 5 years at 4°C.[21]

Docetaxel (Taxotere®) is developed and marketed by Rhône-Poulenc Rorer. Initially, a formulation which contained 75 mg of docetaxel in 2.5 ml of dehydrated ethanol and 2.5 ml of Tween 80 was used during clinical phase I studies. The currently used drug formulation consists of a solution of 80 mg of docetaxel in 2 ml of Tween 80. This formulation requires the preparation of a premix of 10 mg/ml of docetaxel with ethanol–saline and final dilution with saline or 5% (w/v) dextrose prior to administration. Although Cremophor EL and Tween 80 are essential components of the parenteral drug formulations, these compounds have been associated with serious or fatal hypersensitivity in both animals and patients.[25–28] Unique adverse effects have been observed for Taxotere in the clinic, which include fluid retention and peculiar nail disorders.[29–33] It is, however, unclear if this is related to the drug itself or to substances in the formulation. The potential role of the Cremophor EL and Tween 80 vehicles in mediating the observed toxicity (and antitumor activity) is currently under further investigation. Several studies showed that the Cremophor EL and Tween 80 solvents are able to modulate P-glycoprotein-mediated multidrug resistance (MDR) *in vitro*, which led to the suggestion that these compounds may enhance the antitumor effects of the taxane drugs in those cases where tumors display the MDR phenotype.[34–42] The extremely low volume of distribution of Cremophor EL,[43] the undetectable levels in (mouse) tissues,[44] and recent results in *mdr1a* P-glycoprotein knockout mice[45] and *in vivo* tumor-bearing models[46] suggest, however, that this compound is not a very effective MDR modulator *in vivo* at all.

Alternative taxane formulations. A number of other drug formulations have been and are being evaluated (reviewed elsewhere[24]). Important properties of these alternatives will be that they allow paclitaxel or docetaxel to be delivered at dose levels upto 300–400 mg/m^2 and the drug should be stable for several hours in order to be handled in the clinical setting. Furthermore, there must be a real improvement in vehicle-related side effects compared to the currently used formulations. Despite the tremendous efforts invested so far, none of the alternatives have fulfilled the requirements to justify clinical testing.

(a) *Co-solvents and emulsions.* PEGs and polyvinylpyrrolidone (PVP) are water-soluble polymers, and have frequently been used to modify the solubility behavior of drugs. PEGs are available in a range of molecular weights (300–20 000 Da), and both melting point and viscosity decrease with molecular weight. PEG of 400 Da (PEG 400) and PVP are liquids at 20°C, and both have been investigated as a co-solvent for paclitaxel.[24] Under most conditions, neither polymer would be suitable for the preparation of parenteral formulations, given the low solubility of paclitaxel in dilute solutions of PEG or PVP, and the risks of precipitation upon dilution. In one study, a chemically modified PEG, PEG-hydroxystearate, was mixed with DMSO (1:1, v/v) and the mixture diluted 10-fold in saline before bolus injection to urethane-anesthetized rats at a dose level of 10 mg/kg.[47] The stability of this paclitaxel formulation was not reported.

Another reported co-solvent system for paclitaxel formulation consists of ethanol, Tween 80 and the surfactant Pluronic L64 in the ratios of 3:1:6 (v/v/v).[48] No therapeutic data were given for the formulation, but limited *in vitro* toxicity data indicated that the formulation did not cause hemolysis and the investigators surmise from existing data that a dose of 30 mg of paclitaxel could be given to humans without vehicle toxicity.

To avoid the potential problem of precipitation upon dilution that may occur with co-solvent systems, the suitability of a series of oil-in-water emulsions have been investigated.[21,49] Intralipid, an emulsion containing soybean oil stabilized with lecithin, was unsuited due to the low solubility of paclitaxel in soybean oil (below 0.3 mg/ml).[21] The reported solubility of paclitaxel in triacetin, a water-soluble oil, was 75 mg/ml[49] and a prototype emulsion based on triacetin was characterized extensively. It contained paclitaxel at 10 mg/ml and consisted of triacetin:soy lecithin:Pluronic F68:ethyl acetate:water (50:1.5:1.5:2.0:45, v/v/v/v/v), in which the chemical stability of paclitaxel exceeded 6 months. The LD_{50} (lethal dose in 50% of the mice) was found to be about 0.03 ml of emulsion administered to mice of 25 g, corresponding to a maximum paclitaxel dose level of 24 mg/kg.

(b) *Liposomes.* Liposomes are microparticulate carriers that consist of one or more lipid bilayer membranes enclosing an internal aqueous phase. The most common constituents are synthetic or naturally occurring phospholipids and cholesterol. Although several reports address the activity of paclitaxel encapsulated in liposomes,[50–58] relatively little information has been presented on the physical properties

of these formulations as well as the considerations in choosing the specific liposome constituents or properties selected. Recently, however, a family of active paclitaxel-liposome formulations has been prepared and a number of formulation parameters have been examined in detail.[59] Liposomes containing the (overall) neutral Zwitterionic phospholipid phosphatidylcholine (PC) were found to have the highest incorporation of paclitaxel and physical stability of the liposomes was found to be dependent on the mole fraction of paclitaxel incorporated.[59]

Several methods, including the thin-film hydration method,[60] were used to make paclitaxel liposomes. Paclitaxel and phospholipids can also be suspended in organic solvent followed by lyophilization to a fluffy powder. It is hoped that the long shelf-life anticipated for such a formulation and the prior human use of popular liposomes[61] will increase the feasibility of producing a paclitaxel-liposome formulation suitable for human parenteral testing.

One study has reported a greater activity of paclitaxel-liposome formulations after i.p. administration to mice with s.c. implanted tumors, compared to the clinical paclitaxel formulation.[51] This difference may arise from a higher systemic paclitaxel bioavailability of the liposomal formulation, since human pharmacokinetic studies have indicated that paclitaxel administered in the clinical formulation is retained and even concentrated within the peritoneal cavity.[62-64] The bioavailability of the liposome formulation, however, has not been tested by measurement of paclitaxel plasma concentrations. After i.v. administration to mice bearing the paclitaxel-resistant Colon-26 tumor, paclitaxel liposomes retarded tumor growth at all dose levels tested (10–45 mg/kg), in contrast to free paclitaxel where no delay in tumor progression was discerned.[53,54] Considering the paclitaxel concentration in the liposome preparations and the maximum volume that can be injected safely into a mouse, paclitaxel-liposome formulations showed lower acute toxicity in mice than the Cremophor EL formulation after a single i.v. dose (LD_{50}: 50–100 versus 28 mg/kg, respectively).[58]

Paclitaxel has also been encapsulated in nanocapsules of 250 nm diameter, composed of an oily benzyl benzoate core, the surfactant Pluronic F68 and a polymeric wall of polylactic acid,[50] human serum albumin[65] or PVP.[66] No information was given on the physical or chemical stability of the formulations. The *in vitro* activity against L1210 and P388 leukemias was found to be lower than that of free paclitaxel formulated in 0.2% DMSO. Recent studies have also been directed towards developing new paclitaxel delivery systems based on microspheres composed of poly(ε-caprolactone),[67] triolein and dipalmitoyl phosphatidylcholine,[68] a blend of ethylenevinyl acetate copolymer and poly(*d,l*-lactic acid),[69,70] bile salt/phospholipid mixed micelles[71] or lipid vesicles composed of 1-palmitoyl-2-oleyl-*SN*-glycero-3-phosphocholine.[72]

(c) *Cyclodextrins.* Alpha (α), beta (β) and gamma (γ) cyclodextrins are naturally occurring cyclic oligosaccharides containing six, seven and eight covalently linked glucopyranose rings, respectively. Cyclodextrines have been used extensively to increase the aqueous solubility of various antitumor drugs, through inclusion yielding water soluble complexes. Recently, it was shown that paclitaxel forms inclusion complexes with α-cyclodextrin[73] and hydroxy-propyl-β-cyclodextrin.[20,74] Taking into account the bulky structure of paclitaxel, it is not very likely that it can enter the relatively small cavity of α-cyclodextrin. The investigators assume that various substructures of paclitaxel can insert more or less deeply in the cavity, resulting in the decrease of hydrophobicity observed. β-Cyclodextrins in general allowed paclitaxel aqueous concentrations in the millimolar range, reaching values that, in theory, could be useful clinically as a formulation alternative.[20] Although it was hypothesized that larger internal cavities would be better to accommodate bulky paclitaxel hydrophobic constituents, the use of γ-cyclodextrins increased the solubility of paclitaxel only minimally.[20] The *in vitro* activity of the cyclodextrin-solubilized paclitaxel was comparable to that of free paclitaxel. The prospects of using cyclodextrin formulations is endangered by the fact that administration of paclitaxel doses near the maximum tolerated dose (MTD) of free drug in mice (around 30 mg/kg)[66] requires the injection of cyclodextrin quantities that are at the threshold of causing hemolysis.[20]

Taxane prodrugs

Because of the poor aqueous solubility of paclitaxel and docetaxel, major efforts have been put in the design and synthesis of more water soluble derivatives or prodrugs (reviewed elsewhere[75-77]). To date, only one of the derivatives, a hydroxypropyl-methacrylamide polymer prodrug with an amino acid spacer bound to paclitaxel at the C_{20} position (FCE28161; Pharmacia/Upjohn, Italy) has progressed to clinical evaluation.[78] Many compounds have shown only marginal improvements in solubility or are too unstable to allow administration in a clinical setting. The instability of prodrug forms of paclitaxel and docetaxel is a problem, since the product of degrada-

tion (generally the parent drug) is insoluble and precipitates in aqueous solutions. Other synthetic approaches have produced fairly stable prodrugs, but the rate of active drug liberation proceeds at a too slow and variable rate. Recently, the design of phosphate-ester prodrugs yielded compounds with improved solubility that are unmasked at a rate appropriate for therapy[79,80] (reviewed elsewhere[81]).

Pharmacokinetic properties

Analytical methods

Detailed pharmacokinetic studies of paclitaxel were not performed during preclinical drug development. Consequently, only limited information was available to investigators when paclitaxel entered early clinical trials. The lack of pharmacokinetic data of paclitaxel was mainly due to the absence of suitable bioanalytical methods. The only pharmacological information that was available before clinical trials began was from Hamel *et al.*, who developed a laborious biochemical assay that was based on the ability of paclitaxel to induce the formation of tubulin polymers that hydrolyze GTP at 0°C.[82,83] However, the lower limit of sensitivity of the biochemical assay was only about 100 ng/ml, which is suboptimal for accurate pharmacokinetic monitoring during preclinical and clinical trials.

Several reversed-phase HPLC methods using liquid-liquid extraction (LLE) for sample clean-up were developed during early phase I investigations, permitting the characterization of the pharmacokinetic behavior of paclitaxel in both brief and prolonged schedules of administration.[62,84-88] In general, the procedures for paclitaxel that utilize LLE are not very sensitive due to the presence of endogenous interferences. More sensitive and specific reversed-phase HPLC methods capable of detecting paclitaxel or docetaxel concentrations in human plasma as low as 10 ng/ml have been developed recently by the utilization of solid-phase extraction (SPE)[90-96] or combined plasma protein precipitation and solvent extraction.[97,98]

Several HPLC assays have also been described for the determination of paclitaxel in mouse plasma with lower limits of quantitation of approximately 125-500 ng/ml.[99-101] To enable a more sensitive and selective determination of paclitaxel and hydroxylated metabolites in various biological matrices of mice (i.e. plasma, tissues, urine and feces), LLE has been utilized with diethyl ether for the primary clean-up step.[102] After evaporation of the organic solvent, the residue was dissolved in blank human plasma. This enabled processing of the samples by SPE and HPLC with UV detection at 227 nm as if they were human plasma samples, according to a slightly modified procedure reported previously.[90] The lower limit of quantitation was 10-125 ng/ml (or ng/g) for all compounds in the various biological matrices. The same method (but with UV detection at 231 nm) has recently been modified for the determination of docetaxel and its major metabolites in mouse plasma with similar validation characteristics (AS and OvT, unpublished data). Preclinical pharmacokinetic studies with docetaxel reported by the Rhône-Poulenc Rorer investigators have determined the drug in plasma by HPLC with UV detection at 225 nm after SPE similar to the method described by Vergniol *et al.*[93]

Methods using HPLC-electrospray mass specrometry (MS),[103-105] capillary electrophoresis[106] and enzyme-linked immunosorbent assay techniques[107,108] have been developed recently and are more sensitive (0.1-0.4 ng/ml) than HPLC with UV detection. As such, they may have potential for clinical monitoring in studies utilizing prolonged infusion schedules which result in plasma concentrations that approach or are below the sensitivity limits for HPLC-UV.[109,110] However, although most authors emphatically claim superiority of their methods over existing assays, there is no convincing evidence to date that such methods offer reproducibility and/or facility of operation better than or equivalent to HPLC-UV techniques.

Plasma pharmacokinetics

The first pharmacokinetic study with paclitaxel was done in a single rabbit receiving 8.5 mg/kg by i.v. bolus injection (Table 1). By using the tubulin assay, the plasma concentrations could be monitored until 4 h after drug administration, and the distribution and elimination half-lives were 2.7 and 45 min, respectively.[82] A later study using a more sensitive HPLC assay showed that the elimination half-life was much longer.[111] The plasma pharmacokinetics of paclitaxel in mice has now been addressed in several studies, and taking into account that these data are from different strains and laboratories, the results are fairly comparable. In general, these mice received paclitaxel formulated in Cremophor EL:ethanol at relatively high dose levels (10-30 mg/kg).[111,112] Therefore, these studies did not recognize the non-linear plasma pharmacokinetics, which was most apparent between dose levels of 2 and 10 mg/kg, where the clearance of 2.37 l/h/kg was markedly reduced to 0.33 l/h/kg, respectively.[113,113] It is important to realize that the

Table 1. Plasma pharmacokinetic parameters of paclitaxel formulated in Cremophor EL:ethanol in animals

Species (strain)	Route	Dose (mg/kg)	Assay method	C_{max} (μg/ml)	$t_{1/2}$ (h)	AUC (μg/ml·h)	CL (l/h/kg)	F (%)	Reference
Rabbit	i.v.	8.5	tubulin assay	85	0.70	NA	–	–	82
	i.v. (1 h)	8.6	HPLC	6.4	9.5	NA	–	–	111
Mouse									
ICR									
CD_2F_1	i.v.	30	HPLC	100	2.2	118	0.25	–	111
CD_2F_1	i.v.	11.3	HPLC	51	0.65	33.2	0.34	–	112
FVB	i.v.	22.5	HPLC	103	0.72	82.6	0.27	–	112
FVB	i.v.	2	HPLC	1.1	2.0	0.85	2.37	–	43
FVB	i.v.	10	HPLC	34	2.2	30	0.33	–	43
FVB	i.v.	20	HPLC	120	2.2	134	0.15	–	43
FVB	i.v.	2[a]	HPLC	1.1	1.6	0.78	2.57	–	43
FVB	i.v.	10[a]	HPLC	5.1	1.5	3.82	2.62	–	43
CD_2F_1	i.v.	10[b]	HPLC	5.9	1.4	3.76	2.66	–	43
Swiss	i.p.	22.5	HPLC	2.0	NA	7.65	2.94	NA	112
Swiss	i.p.	18	HPLC	13.0	3.0	113	0.16	NA	126
$B_6D_2F_1$	i.p.	36	HPLC	25.7	3.7	142	0.25	NA	126
ICR	i.p.	20	HPLC	14.1	2.9	42.5	0.47	NA	127
CD_2F_1	p.o.	30	HPLC	0.3	NA	0.53	–	0.45	111
FVB	p.o.	22.5	HPLC	<0.5	NA	~0	–	~0	112
CD_2F_1	p.o.	10	HPLC	0.14	NA	0.50	–	11.2	44
	s.c.	22.5	HPLC	<0.5	NA	~0	–	~0	112
Rat									
SD	i.v. (5 min)	8–9	radio-assay	NA	1.1	0.40	20	–	130

[a] Paclitaxel formulated in 100% (v/v) dimethylacetamide instead of Cremophor EL:ethanol.
[b] Paclitaxel formulated in Tween 80:ethanol instead of Cremaphor EL:ethanol.
Abbreviations: C_{max}, maximum plasma concentration; $t_{1/2}$, terminal elimination half-life; AUC, area under the plasma concentration–time curve; CL, total body clearance; F, bioavailability; NA, not available.

plasma concentrations achieved with these dose levels are close to those in patients, where a non-linear pharmacokinetic behavior has also been observed.[114-117]

The hypothesis that the substantial amount of concurrently administered Cremophor EL is responsible for this non-linear pharmacokinetic behavior was confirmed by using various Cremophor EL-free formulations.[43] If paclitaxel formulated in Tween 80:ethanol (1:1, v/v) or dimethylacetamide (DMA) was administered at a dose level of 10 mg/kg, the plasma AUC was almost 20-fold lower as compared to the same dose in Cremophor EL:ethanol, whereas the levels in tissues were essentially similar (see below). Furthermore, since the clearance of paclitaxel given in Tween 80:ethanol or DMA at 2 or 10 mg/kg was about 2.6 l/h/kg, it was concluded that the non-linearity resulted from Cremophor EL exclusively. It appears that in the presence of Cremophor EL, paclitaxel prefers to stay within the plasma compartment and although the exact nature of this process is unclear, it may be due to the formation of micelles from Cremophor EL, or by the lipoprotein products generated by Cremophor EL.[118-120] Recent pharmacokinetic investigations in mice with doxorubicin,[121,122] epirubicin,[123] etoposide,[124] cyclosporin A[125] and the photosensitizer C8KC[118] indicate that this feature may not be unique for paclitaxel.

Eiseman *et al.* were the first to report on the pharmacokinetics of paclitaxel (22.5 mg/kg) administered i.p. to mice.[112] The systemic availability of the drug formulated in Cremophor EL:ethanol was only about 10% and there was no difference with the drug given in a small or large volume. Although this poor systemic availability is consistent with the pharmacokinetic data from clinical trials[62-64] and would explain why paclitaxel was inactive in murine tumor models when given i.p.,[22] more recent reports by Innocenti *et al.*[126] and Adler *et al.*[127] showed that the plasma AUC values after i.p. administration were very similar to those when given by the i.v. route. These high plasma levels after i.p. administration have also been verified by us (unpublished data). From the description of the methods in the reports of Eiseman *et al.* and others, however, we cannot explain what may have caused the rather large discrepancy.

It is obvious that the availability of a suitable oral formulation of paclitaxel would be a welcome addition. However, since initial studies reported that the oral bioavailability was less than 1%, it appeared that administration by this route is not feasible.[111,112] Recent work in wild-type and *mdr1a* P-glycoprotein knockout mice has shed new light on this important issue.[44] In the first place, the bioavailability had been underestimated in the previous reports, due to the fact that the plasma concentrations had been compared to the AUC achieved after i.v. administration of paclitaxel formulated in Cremophor EL:ethanol. Calculated relative to the AUC after i.v. administration in Tween 80:ethanol, the bioavailability was about 11%. Secondly, by using *mdr1a* P-glycoprotein knockout mice it was shown that when P-glycoprotein was absent in the intestinal lumen, the absolute plasma AUC increased around 5-fold with a concomitant increase in oral bioavailability upto 35%.[44] Based upon this observation, it was hypothesized that concomitant administration of a blocker of P-glycoprotein activity may increase the absorption of orally administered drugs from the intestinal lumen. Preliminary experiments indeed confirmed that the oral bioavailability of paclitaxel can be substantially increased when given in combination with P-glycoprotein blockers such as cyclosporin A[128] or the structurally related compound SDZ PSC 833.[129]

The pharmacokinetics of i.v. administered [^{14}C]paclitaxel at 8-9 mg/kg was characterized by terminal half-lives of 1.1 and 2.1 h and total body clearance values of 3.9 and 12.6 l/h/kg in female and male Sprague-Dawley rats, respectively.[130] Low plasma concentrations of paclitaxel were found in three out of five i.p. dosed rats, although all of the radiolabel was excreted in urine (below 10%) and feces (above 98%) in 6 days, indicating that the overall absorption is complete.[130]

Data on the plasma pharmacokinetic parameters of docetaxel in animals published to date are very limited (Table 2). The plasma concentration-time curve of docetaxel in mice receiving i.v. bolus doses of 13-62 mg/kg was biphasic with a distribution and an apparent elimination half-life of 7 min and 1 h, respectively, with a total body clearance of 1.6 l/h/kg, and a plasma AUC of 17 (μgh)/ml.[131] Similar plasma pharmacokinetic data with docetaxel administered i.v. to female FVB mice at dose levels of 2.5, 10 and 33 mg/kg have been obtained recently (unpublished data). Within the plasma concentration range that is clinically relevant, the pharmacokinetic behavior in mice was linear, whereas the pharmacokinetics was apparently slightly non-linear between the 10 and 33 mg/kg dose levels. Furthermore, the finding that toxicity is related with a threshold drug level will be important for further pharmacokinetic/dynamic studies in patients.

In the rat, docetaxel half-lives were comparable to those in the mouse, with 0.8-13 min for the first phase and 0.8-1.7 h for the second phase.[132] Between dose levels of 2.5 and 10 mg/kg, the AUC increased linearly from 0.6 to 2.5 (μgh)/ml, whereas at

Table 2. Plasma pharmacokinetic parameters of docetaxel formulated in Tween 80:ethanol in animals

Species (Strain)	Route	Dose (mg/kg)	Assay method	C_{max} (μg/ml)	$t_{1/2}$ (h)	AUC (μg/ml.h)	CL (l/h/kg)	Reference
Mouse								
$B_6D_2F_1$	i.v.	37	HPLC	54	1.1	24	1.5	131
FVB	i.v.	2.5	HPLC	4.9	1.2	1.5	1.7	–[a]
FVB	i.v.	10	HPLC	9.8	1.7	4.8	2.1	–[a]
FVB	i.v.	33.3	HPLC	66	1.6	31	1.1	–[a]
Rat								
SD	i.v.	5	HPLC	4.1	0.8	0.91	5.5	132
Dog								
beagle	i.v. (10 min)	1.5	HPLC	3.5	6.6	1.7	0.88	132

[a]AS and OvT (unpublished data).
Abbreviations: see Table 1.

a dose level of 20 mg/kg the AUC increased more than proportional to 9.4 (μgh)/ml.[132] These findings are consistent with results obtained in the isolated perfused rat liver, where a non-linear increase in the AUC was observed upon escalation of the concentration of docetaxel in the perfusate from 5 to 50 μM, which was probably caused by cholestasis and/or saturation of metabolic enzymes.[133] This conclusion is further supported by the low K_m of docetaxel metabolism (about 6 μM) in rat liver microsomes.[134]

Plasma protein binding

An important factor in the pharmacology of drugs is its binding to plasma proteins. By using an equilibrium dialysis assay and [^{3}H]paclitaxel formulated in Cremophor EL:ethanol, protein binding in mouse plasma of 90-92% has been found.[112] Plasma protein binding in human samples, as determined by both equilibrium dialysis and/or ultrafiltration, has also been demonstrated to be extensive and independent of the paclitaxel concentration within the therapeutic range, ranging from 88% to greater than 97%.[86,94,135] Human serum albumin and α_1-acid glycoprotein contributed equally to the overall binding, with a minor contribution from lipoproteins.[135] As paclitaxel therapy involves numerous other drugs, displacement of paclitaxel by these drugs may occur. However, none of the drugs commonly co-administered with paclitaxel, including dexamethasone, diphenhydramine, ranitidine, doxorubicin, 5-fluorouracil and cisplatin, altered the binding of paclitaxel significantly.[135] When examining the influence of the protein binding on the paclitaxel distribution into red blood cells as a model for drug-receptor interactions and intracellular distribution, there was a dramatic inhibition.[135] Thus, when *in vivo* concentrations of paclitaxel are compared to *in vitro* concentrations in protein-free media, the unbound fraction *in vivo* should be considered.

When paclitaxel was incubated with fresh human blood at 37°C, the platelet/plasma concentration ratio was about 240, whereas the red blood cell/plasma concentration ratio was only 0.59.[136] In kinetic experiments using platelet-rich plasma, the platelet accumulation was found to be highly temperature and concentration dependent. Such a high degree of platelet accumulation has previously been shown to occur with other tubulin/microtubule-interactive drugs, such as the vinca alkaloids,[137-139] and presumably reflects paclitaxel binding to microtubules. The pharmaceutical vehicle Cremophor EL was recently shown to induce the formation of lipoprotein dissociation products for which paclitaxel has high binding affinity.[119]

In vivo plasma protein binding of radiolabeled docetaxel was, like for paclitaxel, high in both mice and rats (above 84%). Binding to mouse, rat and dog plasma proteins *in vitro* was also high (89-95, 70-76 and 83-89%, respectively).[132] In human plasma docetaxel is mainly bound to albumin and α_1-acid glycoprotein,[132] with more than 90% of plasma radioactivity bound to proteins.[140]

Tissue distribution

Most early studies dealing with the tissue distribution of paclitaxel have involved administration of radiolabeled drug. Lesser *et al.* determined drug levels in tissues of rats sampled at 2 h after the i.v. administration of [^{3}H]paclitaxel by autoradiographic measurements.[141] The tissue/plasma ratios were high in almost all tissues sampled, particularly in tissues involved in

organ barrier filtration, including the portal triad, renal medulla, choroid plexus and glomeruli. Gaver *et al.* demonstrated that after i.v. administration of [^{14}C]paclitaxel to Sprague-Dawley rats, the peak levels of radioactivity were measured at 1 h, and were greatest in liver followed by duodenum, jejunum, kidneys, adrenals, salivary gland, pancreas, lungs, spleen and plasma.[142] The concentrations in tissues were similar in both genders. Klecker *et al.* reported a very limited uptake of the radiolabel by rat testes, which is generally considered a pharmacological sanctuary.[143] In addition, although peripheral neurotoxicity is a frequently observed non-hematologic toxic side effect of paclitaxel, radioactivity was not detected in the peripheral nervous tissue, following the administration of [^{3}H]paclitaxel to rats.[141]

Using more selective analytical methods based on HPLC, tissue distribution of paclitaxel has been extensively studied in CD_2F_1 mice,[112] FVB mice[113] and CDF_1 mice.[144] Except for brain and testes, the distribution of paclitaxel to all the other tissues was substantial.[112] The maximum drug levels were achieved within 0.5 or 1 h, and declined to undetectable levels within 24–48 h.[113] A slow release of paclitaxel, albeit at low concentrations, was observed from ovary, uterus and thymus. The highest peak levels of paclitaxel were, as found in radioactivity measurements, observed in the liver, followed by kidneys, lungs, spleen, heart, gall bladder and small intestine. In contrast to the marked non-linear pharmacokinetics of paclitaxel in plasma, the AUC of the drug in tissues increased almost linear with the dose over the range of 2–10 mg/kg and 10–20 mg/kg.[113]

Maximum paclitaxel levels in tumor tissue, obtained from mice bearing a human ovarian carcinoma xenograft administered paclitaxel i.v. at 20 mg/kg, were attained within several hours, but were considerably lower than corresponding liver levels (AS and OvT, unpublished data). Despite this fact, exposure of the tumors was remarkably high, which was caused by extremely slow elimination of the drug from the tumor tissue. At 48 h after drug administration, tumor levels of paclitaxel were still above 2 μg/g. Administration of an alternative formulation of paclitaxel in a mixed vehicle containing Tween 80 and ethanol (instead of Cremophor EL and ethanol) had no significant effect on the tumor concentrations (AS and OvT, unpublished data). A significant drug retention in tumor tissue has also been observed in a study employing ICR mice bearing the S.180 or M109 tumors.[144] Paclitaxel levels could not be detected in brain tumor xenografts in rabbits following drug administration or in malignant central nervous tissue of Fisher rats with stereotactically implanted C6 glioma cells in the right frontal lobes.[145]

From studies with *mdr1a* P-glycoprotein knockout mice, it has been shown that, with the exception of the brain, the tissues are not effectively protected by this transport protein. At 1 h after paclitaxel administration, the brain accumulated about 3-fold more drug and, even more important, drug efflux from this tissue was markedly reduced as the drug levels remained essentially similar between 1 and 24 h after drug administration.[45] This effect in the brain warrants extreme caution when combining this potentially neurotoxic agent with potent P-glycoprotein blockers in clinical trials aimed to reverse P-glycoprotein-mediated MDR. Overall, the $AUC_{(0-24)}$ values in the other tissues were 1.5–2-fold higher, corresponding with the about 2-fold higher plasma AUC in these knockout mice versus wild-type controls when receiving paclitaxel formulated in Tween 80–ethanol.[44] This difference in plasma concentration was not seen when the drug was given in the clinical formulation, which is probably caused by the influence of the high amounts of Cremophor EL. This effect should be kept in mind, as plasma is usually the only material available from patients to monitor the effects of modulation of the pharmacokinetic behavior by P-glycoprotein blockers.

Distribution studies with [^{14}C]docetaxel have been carried out in mice and dogs.[132] By using autoradiographic determination, a similar tissue distribution pattern was found as seen with paclitaxel, with a rapid and substantial uptake in almost all tissues except for the brain. High levels also occurred in the stomach, in the hematopoietic tissues spleen and bone marrow, in muscle tissue, and in the pancreas. Radioactivity was further detected in fetal tissues and in milk. Levels in reproductive organs were higher in females than in males.[132] Also similar to what has been observed with paclitaxel was the distribution and retention of docetaxel in tumor tissue. In tumor-bearing mice given 37 mg/kg of drug, the drug AUC in the tumor was 5-fold higher than in plasma due to a substantially longer terminal elimination half-life in tumor tissue (i.e. 22 versus 1–4 h in plasma and other tissues).[131] The basis for this long retention in tumor tissue is unclear.

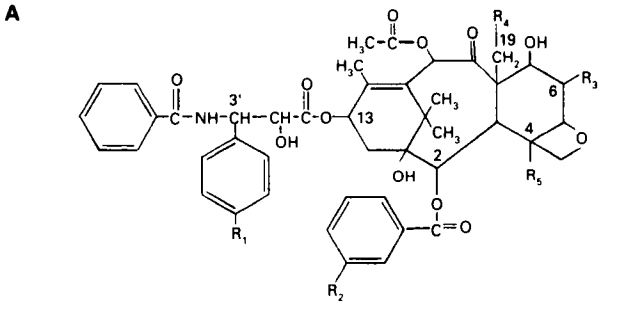
Metabolism

Several studies have shown that hepatic metabolism and biliary excretion is a major pathway of paclitaxel and docetaxel elimination in mice, rats and humans, with major fractions of the dose excreted in the

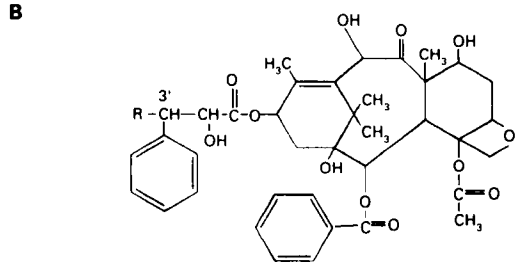
form of parent drug or hydroxylated metabolites. Detection and identification of paclitaxel metabolites in bile of rats was first reported by Monsarrat *et al.* using analytical HPLC, MS and nuclear magnetic resonance spectroscopy.[47,146,147] Neither glucuronated nor sulfated metabolites could be identified, although nine newly formed compounds were detected in rat bile. All rat biliary metabolites of paclitaxel identified to date are mono-hydroxylated derivatives, with the exception of baccatin-III, which lacks the side chain at position C_{13} of the taxane ring (Figure 2). The rat metabolites were found to be substantially less active *in vitro* against L1210 leukemia than paclitaxel, but some are as active as the parent drug in stabilizing microtubules against disassembly in cell-free tubulin preparations.[47] Two main metabolites of paclitaxel were separated by reversed-phase HPLC and shown to be mono-hydroxylated metabolites by tandem MS, i.e. 3′-*p*-hydroxypaclitaxel and 2-*m*-hydroxypaclitaxel, in freshly isolated rat hepatocytes[148] and rat liver microsomes.[149] Inhibition studies suggest that the oxidative attack leading to both metabolites may be mediated by cytochrome P450 3A enzymes.[148]

In *in vivo* metabolism studies with paclitaxel in male Sprague-Dawley rats, two metabolites were detected in liver, but not in plasma or other tissues.[143] Whereas cimetidine had no effect on metabolism, probenecid- and ketoconazole-pretreated rats displayed a 50 and 25% decrease in biliary secretion of both metabolites, respectively. In addition to the two metabolites described above, three new metabolites were formed in Sprague-Dawley rats treated with dexamethasone.[150] Mass spectra of two of these compounds were consistent with 4-deacetylpaclitaxel and 4-deacetyl-2-*m*-hydroxypaclitaxel.[150]

In a recent pharmacokinetic study performed in FVB mice, besides paclitaxel two other major compounds were detected in the cecum, small intestine, liver, gall bladder and feces, but not in plasma or other tissues.[113] Both the chromatographic retention times of these compounds and their UV spectra, as determined by on-line UV-photodiode array detection, corresponded to two mono-hydroxylated compounds



compound	R_1	R_2	R_3	R_4	R_5
6α,3′-*p*-di-OH-paclitaxel	OH	H	OH	H	Ac
3′-*p*-OH-paclitaxel	OH	H	H	H	Ac
6α-OH-paclitaxel	H	H	OH	H	Ac
2*m*-OH-paclitaxel	H	OH	H	H	Ac
19-OH-paclitaxel	H	H	H	OH	Ac
4-deacetyl-paclitaxel	H	H	H	H	H
2-*m*-OH-4-deacetyl-paclitaxel	H	OH	H	H	H
baccatin-III	13-OH	H	H	H	Ac
paclitaxel	H	H	H	H	Ac



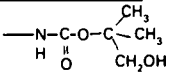
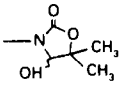
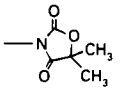
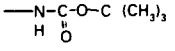
compound	R
3′-(1-OH-2-methyl)-docetaxel	
3′-[3-5,5-dimethyl-4-OH-2-oxo-1,3-oxazolidinyl)]-docetaxel (two diastereomers)	
3′-[3-5,5-dimethyl-2,4-dioxo-1,3-oxazolidinyl)]-docetaxel	
docetaxel	

Figure 2. Chemical structures of paclitaxel (A) and docetaxel (B) metabolites identified to date in mice, rats and humans.

with a hydroxyl group on the α position at C_6 of the taxane ring (6α-hydroxypaclitaxel) or on the *para* position of the phenyl group at C_3' in the C_{13} side chain (3′-*p*-hydroxypaclitaxel) (Figure 2). Interestingly, 6α-hydroxypaclitaxel was also shown to be the major metabolite in bile,[147,148] plasma[114,116] and feces[151] of Taxol-treated patients, human liver microsomes[152-154] and in liver slices,[149] but was not identified in rat samples. The human (and murine) metabolites were many-fold less active than paclitaxel in growth inhibition and clonogenic assays,[151,153,154] and less myelotoxic compared to paclitaxel in an *in vitro* progenitor toxicity assay.[151] Recently, 6α-hydroxypaclitaxel formation has been shown to be catalyzed by cytochrome P450 2C8 enzymes,[155,156] although involvement of other P450 enzymes, notably P450 3A, has also been evoked.[157] Formation of 3′-*p*-hydroxypaclitaxel appears to be dependent on cytochrome P450 3A4.[158,159] In addition, several *in vitro* studies have indicated that several classes of pharmacological agents, including immunosuppressives, benzodiazepines and epipodophyllotoxins, may alter the metabolism of paclitaxel.[149,160,161]

The structures of docetaxel metabolites have been elucidated by the groups of Monsarrat *et al.* and Gaillard *et al.*[162-164] The main pathway of docetaxel metabolism consists of successive oxidations of the *tert*-butyl ester group on the C_{13} side chain, with spontaneous cyclization occurring for the putative aldehyde and acid derivatives (Figure 2). All four metabolites were significantly less potent *in vitro* against the A2780 human ovarian and CC531 colon carcinoma cell lines compared to docetaxel.[165] In addition, the alcohol and cyclized acid were found inactive *in vitro* against murine P388 leukemia cells and *in vivo* against B16 melanoma implanted in mice.[166]

In contrast to paclitaxel, no metabolites resulting from modification of the taxane ring have been detected for docetaxel, whereas hydroxylation on the C_3' phenyl group of the C_{13} side chain, a major pathway for paclitaxel metabolism in mice, rats and humans (see above), is only a very minor route for docetaxel (in rats).[162] *In vivo* metabolism studies in the mouse, rat, rabbit and dog did not indicate any major species or gender differences in the metabolic pathway. In these species, the four above-mentioned metabolites represented the large majority of fecal docetaxel derivatives, with the alcohol derivative as the most abundant one.[132,162] In mice, low levels of three metabolites were detectable in plasma after i.v. drug administration at high dose levels (unpublished data). The facts that docetaxel is the main circulating compound after i.v. administration and that the major metabolites are much less active than docetaxel indicates that docetaxel biotransformation represents an important detoxification pathway. *In vitro* studies using mouse, rat, dog and human liver microsomes showed a good correlation with the above *in vivo* data.[134]

The main enzymes involved in the biotransformation of docetaxel were recently identified as cytochrome P450 3A enzymes in human liver microsomes[167,168] and hepatocytes.[169] Studies in rat liver microsomes and in the isolated perfused rat liver also indicated a major role of cytochrome P450 3A in this species, but other enzymes may also be involved.[133,134] So far, no papers have addressed the question why, despite the involvement of the same cytochrome P450 isozymes, the metabolic pathways of paclitaxel and docetaxel are so differently. The formation of the alcohol derivative of docetaxel was strongly reduced by cytochrome P450 inhibitors like ketoconazole, midazolam, erythromycin, testosterone, orphenadrine, nifedipine and troleandomycin.[168,169] Furthermore, some vinca alkaloids and doxorubicin were shown to inhibit docetaxel metabolism in both hepatocytes and microsomes.[169]

Excretion

The excretion of paclitaxel in bile and urine of male Sprague-Dawley rats has been examined by several investigators.[47,143,147,148] As in humans,[62,84,86] no metabolites of paclitaxel were detected in rat urine, with only 10% of the injected dose recovered as intact drug over 24 h. In contrast, 11.5 and 29% of the injected dose was recovered in rat bile as unchanged drug and metabolites, respectively.[47] In another report, 24% of the dose was secreted into the bile of rats within 6 h, with 38% as parent paclitaxel.[143] In mice with biliary excretion monitored for 90 min after i.v. drug administration, approximately 5% of the dose was secreted as unchanged drug, and 2-3% each of 3′-*p*-hydroxypaclitaxel and 6α-hydroxypaclitaxel.[44] The finding that the hydroxylated metabolites were not detectable in plasma or any tissues, except liver and tissues of the gastrointestinal tract, indicates that the major part of these compounds is secreted directly into the bile and that reabsorption from the intestinal lumen is low.[113] Consistent with a high biliary secretion of paclitaxel and a low intestinal (re)absorption, a substantial fraction of i.v. administered paclitaxel (2 mg/kg) was recovered in 96 h feces either as unchanged drug (58%) or as hydroxylated metabolite (29%).[113] The rate of fecal excretion was independent of the dose, with most of the drug being recovered within 24 h. At higher dose levels (10 and 20 mg/kg),

fecal excretion of unchanged drug and of metabolites was significantly reduced, probably due to a saturation of transport proteins involved in the biliary excretion.[113] Like in rats, the excretion of unchanged paclitaxel in mouse urine was low (below < 1%), with trace levels of both hydroxylated derivatives.[113] The remaining fraction of the administered dose, i.e. 23% at 10 mg/kg, leaves the body via the urine as an unknown product,[44] possibly resulting from breakdown of the C_{13} side chain of the paclitaxel molecule as suggested recently by Walle *et al.*[170]

A substantial fraction of the i.v. paclitaxel dose was recovered in intestinal contents of mice with cannulated gall bladder, implicating that besides renal and hepatic drug excretion, the direct transport of taxanes through the gut wall can be an important elimination pathway.[44] Since the direct excretion through mucosal cells and total fecal excretion is drastically reduced in *mdr1a* P-glycoprotein knockout mice, P-glycoprotein was identified as an essential component of this paclitaxel secretion process.[44]

Radiolabeled docetaxel was also largely excreted by the fecal route in mice, rats and dogs.[132] Less than 10% of the dose was excreted unchanged in the feces within 7 days, whereas three to four metabolites accounted for approximately 75% of the dose. In patients given [^{14}C]docetaxel, the major part of the radioactivity was also recovered in the feces in the form of both the parent drug and the cyclized acid metabolite.[140,164,165,171]

Studies in rats with cannulated gall bladder and in the isolated perfused rat liver confirmed the predominance of biliary excretion of radiolabeled docetaxel biotransformation compounds.[133] In the rat, enterohepatic cycling of radioactivity was not important, with only 13% excreted in bile being reabsorbed.[162]

Conclusions and perspectives

Paclitaxel and docetaxel are hydrophobic antineoplastic agents demonstrating significant antitumor activity against a broad range of human malignancies, including refractory ovarian and breast cancers, non-small cell lung carcinoma, and melanoma. The enthusiasm evoked by the exciting clinical results of these relatively new agents has led to the publication of a substantial number of papers addressing their preclinical *in vivo* pharmacology. In a very short time period, these investigations have yielded valuable insight into their mechanism of action, the mechanisms of tumor resistance, toxicities, and considerations of dosage and schedule and route of drug administration. Many of these studies have been made possible by the development of selective analytical methodologies to specifically monitor the parent drugs and individual biotransformation products, with sufficient sensitivity to detect the compounds at levels achieved after therapeutic dosing. However, only through further investigations which may allow better definition of the biochemical pharmacology and pharmacokinetics of taxanes can the rational optimization of therapy involving these drugs be realized. This need is intensified in light of the current clinical use of such agents in combination with other antineoplastic drugs. The new dimension in chemotherapy provided by paclitaxel and docetaxel in the treatment of a variety of solid tumors assures growth in the area of taxane-related chemotherapeutic drugs. In general, with the continued application of animal pharmacokinetic studies, coupled with new approaches in taxane drug design, a more rational and selective chemotherapy should be possible and thus the treatment of cancer improved.

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