Metabolism of paclitaxel in mice

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Previous mass balance studies in humans and mice have shown that the fecal and urinary recovery of paclitaxel and known metabolites (3′*p*-hydroxypaclitaxel, 6α-hydroxypaclitaxel and 3′*p*,6α-dihydroxypaclitaxel) was not complete. Obviously this discrepancy is caused by the existence of other yet unknown metabolites. *Mdr1a/1b*$^{-/-}$ mice excrete very low quantities of unchanged paclitaxel. We have therefore used these mice receiving i.v. [^{3}H]paclitaxel to further study the metabolic fate of paclitaxel. The major part of the radiolabel, being 70%, was excreted in the feces. A lipophilic sample, containing about 70% of the radioactivity present in the feces sample, was obtained by diethyl ether extraction. The aqueous residue containing about 30% of the radioactivity was further extracted using methanol. The high-performance liquid chromatography (HPLC) chromatograms of the lipophilic and aqueous sample revealed two and five putative new metabolites of paclitaxel, respectively. The HPLC fractions containing substantial amounts of radioactivity were subjected to tandem mass spectrometry. Two novel monohydroxylated paclitaxel structures were identified, which are probably 2*m*-hydroxypaclitaxel and 19-hydroxypaclitaxel, structures previously identified in rats. Including these metabolites, about 60% of the mass balance of paclitaxel could be quantified. *Anti-Cancer Drugs* 14:203–209 © 2003 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2003, **14**:203–209

Keywords: high-performance liquid chromatography, mass spectrometry, metabolism, mice, paclitaxel

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Sponsorship: Supported by grant NKI 98-1799 from the Dutch Cancer Society.

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Received 20 December 2002 **Accepted** 14 January 2003

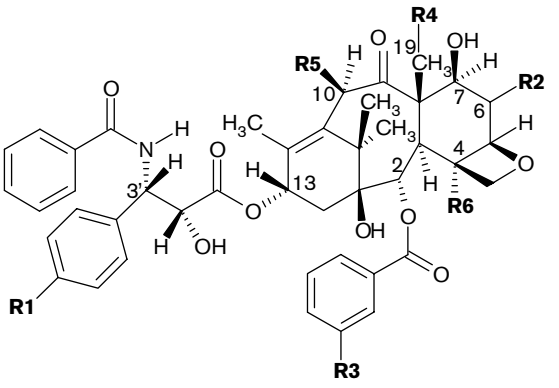
Introduction

Since its introduction in the clinic in the 1980s, paclitaxel (Fig. 1) has become an important anticancer agent, used in the treatment of many solid tumors (reviewed in [1,2]). Pharmacokinetic studies in humans, rats and mice have shown that after i.v. administration paclitaxel is mainly excreted via the hepato-biliairy route into the feces. Renal excretion is much less important, with only about 10% of the dose being recovered in the urine [3]. In humans, metabolism is the most important route of detoxification, since only a minor fraction of the administered dose is excreted in the feces and urine as unchanged drug [4,5]. The first studies concerning paclitaxel metabolism were performed by Monserrat *et al.* using high-performance liquid chromatography (HPLC), mass spectrometry (MS) and nuclear magnetic resonance (NMR) [3,6]. Whereas nine metabolites were found in bile samples of rats and five in human bile, none of these were glucuronated or sulfated products. The structures of the metabolites in rats that could be identified were all monohydroxylated, except for baccatin III, which lacks the side chain at C-13 of the taxane ring (Fig. 1). Some of the rat metabolites were as active as paclitaxel in stabilizing microtubules in cell-free test systems; however, they were all less active *in vitro* against L1210 leukemia cells [3]. In human bile the structures of three metabolites were identified as 3′*p*-hydroxypaclitaxel, 6α-hydroxypaclitaxel and 3′*p*,6α-dihydroxypaclitaxel (Fig. 1). Strikingly, only 3′*p*-hydroxypaclitaxel was found in rat bile, indicating marked species differences in paclitaxel metabolism [6]. In later studies, several other metabolites were identified in bile samples, hepatocytes and/or liver microsomes of the rat (structures in Fig. 1) [7–10]. However, except for baccatin III [11,12], these were absent in the bile of humans, where only minor amounts of deacetylated metabolic products were found [11,12]. Two cytochrome P-450 isoenzymes were identified to participate in the metabolism of paclitaxel in humans. Isoenzyme 3A4 catalyses the formation of 3′*p*-hydroxypaclitaxel [13–17], whereas isoenzyme 2C8 is responsible for the formation of 6α-hydroxypaclitaxel [13,15,17]. The formation of 3′*p*,6α-dihydroxypaclitaxel was assumed to be the result of a stepwise process by both isoenzymes [13,14,17,18].

The metabolism of paclitaxel in mice most likely occurs by comparable mechanisms to those observed in humans, since 3′*p*-hydroxypaclitaxel, 6α-hydroxypaclitaxel and 3′*p*,6α-dihydroxypaclitaxel have also been detected in the bile, feces, plasma and urine of these animals [19].

0959-4973 © 2003 Lippincott Williams & Wilkins DOI: 10.1097/01.cad.0000060630.27490.b7

Fig. 1



Structure	R1	R2	R3	R4	R5	R6	Species
paclitaxel	H	H	H	H	$OCOCH_3$	$OCOCH_3$	–
7-epipaclitaxel	H	H	H	H	$OCOCH_3$	$OCOCH_3$	human
3'*p*-hydroxypaclitaxel	OH	H	H	H	$OCOCH_3$	$OCOCH_3$	human, rat, mouse
6α-hydroxypaclitaxel	H	OH	H	H	$OCOCH_3$	$OCOCH_3$	human, mouse
6α,3'*p*-dihydroxypaxlitaxel	OH	OH	H	H	$OCOCH_3$	$OCOCH_3$	human, mouse
10-deacetylpaclitaxel	H	H	H	H	OH	$OCOCH_3$	cell culture, human
baccatin III	13-OH	H	H	H	$OCOCH_3$	$OCOCH_3$	human, rat
10-deacetyl baccatin III	13-OH	H	H	H	OH	$OCOCH_3$	cell culture, human
2-*m*-hydroxypaclitaxel	H	H	OH	H	$OCOCH_3$	$OCOCH_3$	rat
19-hydroxypaclitaxel	H	H	H	OH	$OCOCH_3$	$OCOCH_3$	rat
4-deacetylpaclitaxel	H	H	H	H	$OCOCH_3$	H	rat
2*m*-hydroxy-4-deacetyl paclitaxel	H	H	OH	H	$OCOCH_3$	H	rat

Structure formulas of paclitaxel and its known metabolites.

However, it is also clear that the three hydroxylated metabolites are not the only paclitaxel metabolites formed in mice. Especially in mice that lack functional drug transporting P-glycoproteins (*Mdr1a/1b*$^{-/-}$ mice) we observed a considerable discrepancy between the amount of radioactivity in the feces (92% of the dose) and the amount of identified products (42% of the dose). Due to the absence of P-glycoprotein in these animals only minor amounts of unchanged paclitaxel are excreted in feces or urine. We have therefore used the feces from *Mdr1a/1b*$^{-/-}$ mice to search for novel metabolic products of paclitaxel. We here report the finding of putative metabolites in the feces of mice treated with [^{3}H]paclitaxel by using HPLC and MS.

Materials and methods

Drugs and chemicals

Paclitaxel pure compound originated from Yew Tree Pharmaceuticals (Haarlem, The Netherlands) and [^{3}H]paclitaxel (1.0 mCi/ml. in ethanol, specific activity 6.5 Ci/mmol) from Moravek Biochemicals (Brea, CA). 6α-Hydroxypaclitaxel, 3′*p*-hydroxypaclitaxel and 6α,3′*p*-dihydroxypaclitaxel reference compounds were isolated and purified from feces of patients as described before [20]. Lyophilized bovine serum albumin (fraction V) was obtained from Roche Diagnostics (Mannheim, Germany). Polyoxyethylenesorbitan monooleate (Tween 80) was purchased from Sigma (St Louis, MO) and saline from Braun (Emmer-Compascuum, The Netherlands). All other chemicals were purchased from Merck (Darmstadt, Germany) and were of analytical or Lichrosolv gradient grade. Water purified by the Milli-Q Plus system (Millipore, Milford, MA) was used in all aqueous solutions.

Excretion experiment

Several female FVB *mdr1a/1b*$^{-/-}$ mice [21], 13 weeks of age, were housed in a Ruco Type M/1 metabolic cage (Valkenswaard, The Netherlands). They were allowed to accustom to the cages for 2 days before receiving paclitaxel drug solution (Taxol; diluted 1 + 3 with saline), containing 1.5 mg/ml of paclitaxel, supplemented with a tracer amount of 1.5 μCi/ml of [^{3}H]paclitaxel was administered orally to the mouse, resulting in a dose level of 20 mg/kg (20 μCi/kg) of paclitaxel. Feces were collected in fractions of 0–8 and 8–24 h after drug

administration. Each sample was homogenized at 4°C in 10 volumes/g feces of 40 g/l bovine serum albumin in water using a Polytron PT1200 homogenizer (Kinematika, Littau, Switzerland) and were stored at −20°C until analysis. During the experiment the animals were allowed to take water and food *ad libitum* throughout the experiment, and were maintained and handled according to institutional guidelines based on Dutch law.

Analytical methods

Sample handling

Aliquots of 200 μl of each feces sample were extracted twice with 4 ml of diethyl ether. The supernatant diethyl ether layers were decanted and combined in a glass tube, evaporated at 37°C under nitrogen, and re-dissolved in 1 ml diethyl ether. The diethyl ether fractions of six aliquots of feces homogenate were combined, evaporated at 37°C under nitrogen, re-dissolved in 250 μl of a mixture of acetonitrile:methanol:water (4:1:5, v/v) and transferred into a polypropylene vial. After centrifugation at 4°C and 20 000 *g* for 5 min, 120–220 μl of the clear supernatant, containing lipophilic structures, was injected into HPLC system I. The residues that remained in the aqueous layer after diethyl ether extraction of the feces samples, thus containing the hydrophilic classes of metabolites, were extracted twice with 0.5 ml methanol. The methanol fractions were combined in a polypropylene vial and centrifuged at 4°C and 20 000 *g* for 5 min. The clear supernatant was transferred into a polypropylene vial, and dried under vacuum using a FDC206 freeze-drying chamber and a RVT 4104 refrigerated vapor trap (Savant, Farmingdale, NY) connected to a model Trivac D4B vacuum pump (Leybold, Köln, Germany). After reconstitution in 400 μl of methanol:water (1:3, v/v) and centrifugation at 4°C at 20 000 *g* for 5 min, 150–240 μl of the clear supernatant was injected into HPLC system II.

Chromatographic purification

The HPLC equipment consisted of a Model 300C or 480 solvent delivery system (Gynotek, Germering, Germany); a model Midas autosampler provided with a 500 μl loop (Spark Holland, Emmen, The Netherlands) used in the sample pick-up mode and a Spectroflow SF757 absorbance detector (Kratos, Ramsey, NY) set at 227 nm. Separation was performed using a stainless steel analytical column (150 × 4.6 mm) packed with 5 μm APEX-octyl material (Jones Chromatography, Littleton, CO).

HPLC system I. The mobile phase consisted of acetonitrile:methanol:0.01 M ammonium acetate buffer pH 5.0 (4:1:5.5, v/v/v) and was delivered at a flow rate of 1.0 ml/min. The detector outlet was connected to a model 2112 Redirac fraction collector (LKB Bromma, Uppsala, Sweden) and mobile phase fractions were collected every 0.4 min.

HPLC system II. Elution was performed using a 60 min linear gradient from 10 to 48% (v/v) of acetonitrile in 0.01 M ammonium acetate buffer, pH 5. The mobile phase was delivered at a flow rate of 1.0 ml/min. The column outlet was connected to a fraction collector (LKB Bromma). Mobile phase fractions were collected every 0.3 min.

Measurement of ^{3}H levels

Volumes of 200 μl of feces homogenate and 1 ml of Soluene (Packard Bioscience, Groningen, The Netherlands) were incubated for 1.5 h at 50°C in a 20 ml polyethylene liquid scintillation vial. After adding 500 μl of 2-propanol the mixture was incubated for 2 h at 50°C. Finally, 200 μl of 30% hydrogen peroxide solution was added and after 10 min 10 ml Hionic Fluor scintillation cocktail (Packard Bioscience). To determine the radioactivity in HPLC fractions, 50 μl of each fraction was mixed with 2 ml of Ultima Gold scintillation cocktail (Packard Bioscience). Radioactivity was counted using a Packard model Tricarb β-scintillation counter.

MS

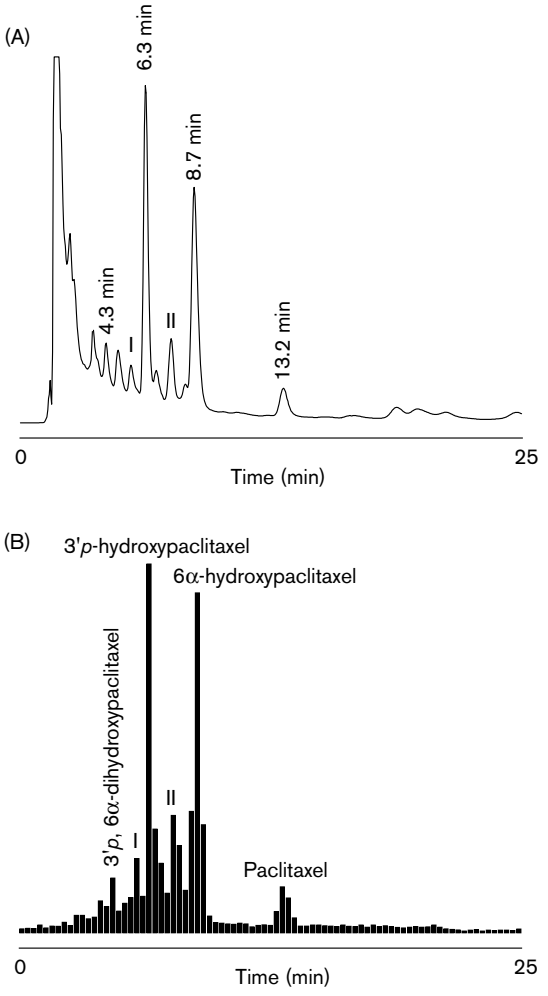
Reference solutions of paclitaxel and 6α-hydroxypaclitaxel and the HPLC fractions selected on their levels of radioactivity were introduced into a model API 365 triple quadrupole mass spectrometer by a TurbolonSpray sample inlet (Sciex, Thornhill, Ontario, Canada) without splitting. Ions were created at atmospheric pressure. A mass spectrum was recorded of each sample using the first quadrupole (Q1 scan). Next, the (putative) parent ($M\text{-}H^+$) ion was selected and further fragmented in the collision cell (Q2), using various conditions, and the MS/MS spectrum was recorded [product ion (PI) scan]. Deviations of 0.5 mass units from the theoretical molecular mass values were accepted.

Results

Lipophilic metabolites

Within 24 h about 70% of the administered dose was excreted in the feces as [^{3}H]paclitaxel. Since the 0–8 h feces fraction contained the largest concentration of radioactivity per gram feces (in total about 40% of the administered dose), this sample was used for isolation and identification of new metabolites. The lipophilic sample, which was obtained after diethyl ether extraction, contained about 70% of the radioactivity present in the feces sample. Upon injection into HPLC system I, the chromatogram showed several peaks at 227 nm, which is the absorption maximum of paclitaxel and its metabolites (Fig. 2A). The results of the ^{3}H measurements of the HPLC fractions revealed that six of these peaks were paclitaxel related (Fig. 2B). Based on their retention times, the peaks on 4.3, 6.3, 8.7 and 13.2 min were identified as 3′*p*,6α-dihydroxypaclitaxel, 3′*p*-hydroxypaclitaxel, 6α-hydroxypaclitaxel and unchanged

Fig. 2



(A) UV chromatogram of the lipophilic feces extract. (B) Radioactivity pattern of the HPLC fractions obtained after injection of the lipophilic extract.

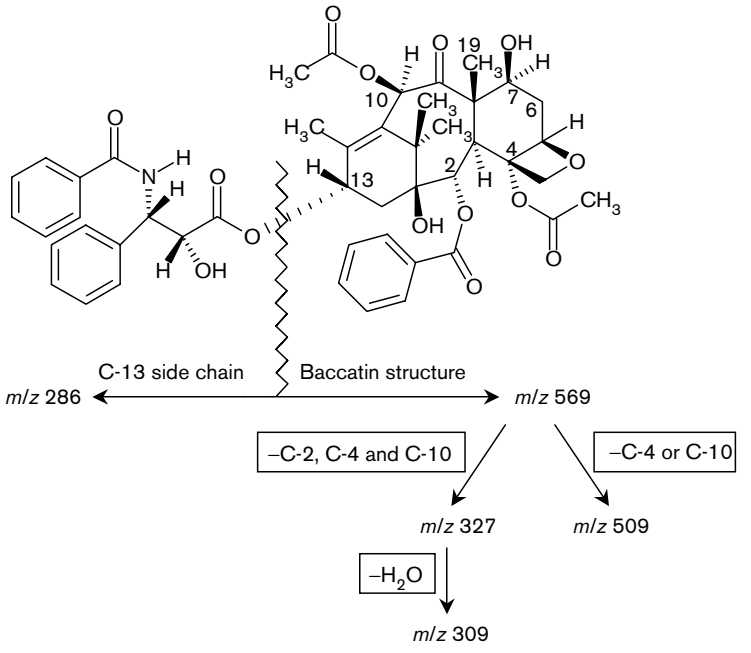
paclitaxel, respectively, and together represented about 65% of the radioactivity present in the lipophilic sample. The identity of the peaks on 8.7 and 13.2 min were confirmed by MS. The peak on 13.2 min displayed the same mass spectrum as the paclitaxel reference compound, with *m/z* 854 as $M\text{-}H^+$ (protonated paclitaxel) and diagnostic fragment ions at *m/z* 569 ($M\text{-}H^+$ without side chain at C-13: the baccatin structure), 509 (fragment 569 without side chains at C-4 or C-10), 327 (fragment 569 without side chains at C-2, C-4 and C-10; the taxane ring), 309 (taxane ring without H_2O) and 286 (side chain at C-13) (Fig. 3 and Table 1). Characteristic fragments of 6α-hydroxypaclitaxel (the peak with retention time 8.7 min) that were found using mass spectrometry were *m/z* 870 ($M\text{-}H^+$), 585 ($M\text{-}H^+$ without side chain at C-13), 525 (fragment 585 without side chain C-4 or C-10), 343 (fragment 585 without C-2, C-4 and C-10 = taxane ring with hydroxyl function) and 286 (side chain at C-13) (Fig. 3 and Table 1). 3′*p*-Hydroxypaclitaxel typically showed a parent ion at *m/z* 870, ions similar to paclitaxel at *m/z* 569 and 509, and an ion at *m/z* 302 (side chain at C-13). In most cases satellite peaks were found next to the parent ion that could be attributed to conjugates with ammonium ions (M + 17) and sodium ions (M + 23). The unknown metabolites, which had retention times of 5.6 min (metabolite I) and 7.6 min (metabolite II), represented about 6 and 12% of the radioactivity present in the lipophilic sample, respectively. The mass spectra of the unknown metabolites showed both *m/z* 870 as $M\text{-}H^+$ (Table 1), thus suggesting that these metabolites were also monohydroxylated. The finding of the fragments with *m/z* 585 ($M\text{-}H^+$ without side chain at C-13) and 286 (side chain at C-13) in the positive PI scan of both metabolites, indicates that the hydroxy group is not attached to the side chain at C-13, but to the baccatin structure, as in 6α-hydroxypaclitaxel. The mass spectrum of metabolite I showed a fragment with *m/z* 137 in the negative ion scan mode. This fragment was not found in the mass spectra of paclitaxel or 6α-hydroxypaclitaxel and is probably the benzoate side chain at C-2 (typically giving a signal at *m/z* 121) with an additional hydroxyl function attached to it (resulting in a signal at *m/z* 137). Moreover, similar to the mass spectrum of paclitaxel, a fragment at *m/z* 327 was found, instead of *m/z* 343 which is found in the mass spectrum of 6α-hydroxypaclitaxel, further indicating that the hydroxyl function was not attached to the taxane ring.

The mass spectrum of metabolite II showed substantial similarities with that of 6α-hydroxypaclitaxel, with a very characteristic fragment at *m/z* 343 representing the taxane ring with an additional hydroxyl function attached to it. In the negative ion scan mode *m/z* 121 was found, which is assigned to the benzoate side chain at C-2, indicating that the hydroxyl function is not attached to this side chain.

Hydrophilic metabolites

The aqueous residue remaining after diethyl ether extraction contained about 30% of the radioactivity in the 0–8 h feces sample. HPLC analysis and subsequent measurement of the radioactivity in the collected HPLC fractions resulted in five putative metabolite peaks which represented about 40% of the radioactivity present in the aqueous sample (Fig. 4). Due to the presence of large amounts of endogenous compounds, the chromatographic traces obtained by UV detection did not provide useful information. Based on their relative abundance, only metabolites A and E in the HPLC fractions were further analyzed by mass spectrometry. The Q1 mass spectrum and/or subsequent PI scans of metabolite A showed no taxane-related fragments (Table 1). The Q1 mass spectrum of metabolite E only showed a fragment at *m/z* 327 that could somehow be related to paclitaxel.

Fig. 3



Characteristic fragmentation pattern of paclitaxel.

Table 1 Mass spectra data of paclitaxel and metabolites

Compound	Scan type	Prominent ions (*m/z*)
Paclitaxel	Q1	854.3, 569.4, 509.2, 327.1
	PI (*m/z* 854)	569.1, 509.2, 327.0, 309.0, 285.9
6α-hydroxypaclitaxel	Q1	870.5, 585.3, 525.5, 507.2
	PI (*m/z* 870)	870.1, 585.2, 525.2, 343.3, 285.9
Metabolite I	Q1	870.6, 585.3
	PI (*m/z* 870)	585.3, 525.4, 327.7, 308.9, 286.0
Metabolite II	Q1	870.5, 585.3, 525.6
	PI (*m/z* 870)	870.3, 851.9, 810.8, 792.3, 585.2, 567.5, 524.8, 507.0, 343.3, 285.7
Metabolite A	Q1	1078.3, 915.0, 892.9, 870.9, 848.7, 840.6, 826.4, 602.0, 566.5
Metabolite E	Q1	1053.7, 991.6, 932.5, 872.8, 817.8, 740.6, 593.6, 503.5, 373.2, 355.4, 327.4, 309.9, 149.2
	PI (*m/z* 991)	991.5, 973.3, 913.8, 893.9, 853.9, 792.0, 569.1, 551.3, 509.0, 449.1, 405.0, 326.9, 285.9, 283.7

Whereas only the characteristic ions of paclitaxel and 6α-hydroxypaclitaxel are mentioned, all found ions of metabolites I, II, A and B are mentioned.

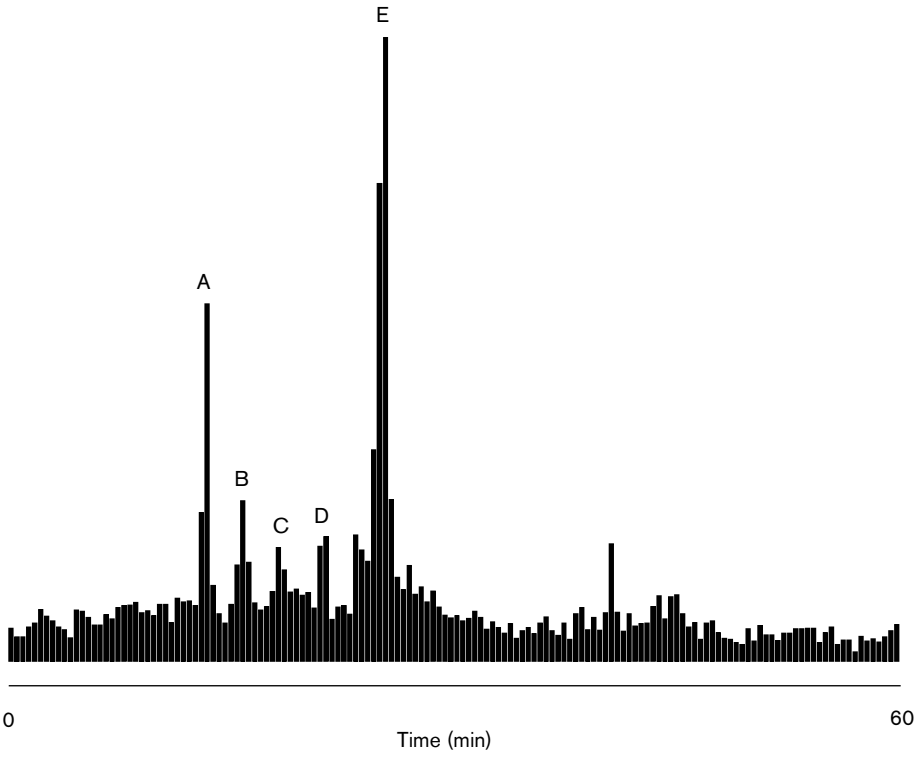
However, the positive PI scan of an ion at *m/z* 991 resulted in a fragment at *m/z* 854 together with other potentially paclitaxel-related fragments at *m/z* 569, 551, 509, 327 and 286 (Fig. 5). The PI scans of other ions from the Q1 scan of metabolite E did not reveal any taxane-related fragmentation patterns.

Discussion

In addition to paclitaxel and the known metabolites 6α-hydroxypaclitaxel, 3′*p*-hydroxypaclitaxel and 3′*p*,6α-dihydroxypaclitaxel, seven radiolabeled metabolic products were found in the feces of a mouse that received [^{3}H]paclitaxel by i.v. bolus injection. Two metabolites were detected in the lipophilic fraction following diethyl ether extraction of feces homogenate and after subsequent methanol extraction of the aqueous fraction, five other more hydrophilic metabolites were found.

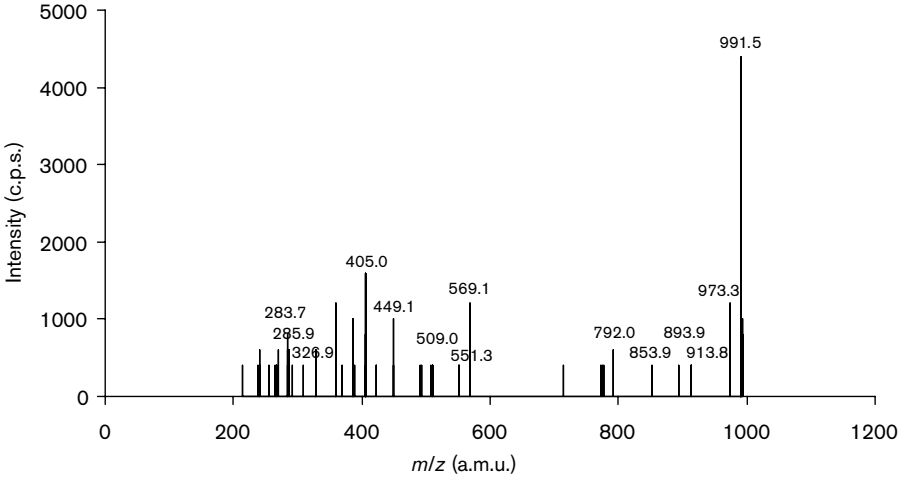
The lipophilic metabolites had retention times in the same range as the three known hydroxylated metabolites 6α-hydroxypaclitaxel, 3′*p*-hydroxypaclitaxel and 3′*p*,6α-dihydroxypaclitaxel. By using MS it was shown that both metabolites were monohydroxylated. The hydroxyl function was attached to the benzoate side chain in case of metabolite I and to the taxane ring like in 6α-hydroxypaclitaxel in case of metabolite II; however, on the basis of these mass spectra it was not possible to locate the exact positions of the hydroxyl functions. In rats, a metabolite with a hydroxyl function located at the *m* position of the benzoate moiety has been identified (i.e. 2*m*-hydroxypaclitaxel; Fig. 1) [3]. Another metabolite carrying a hydroxyl group at the C-19 position of the

Fig. 4



Radioactivity pattern of the HPLC fractions obtained after injection of the aqueous feces extract.

Fig. 5



The PI scan of metabolite E (*m/z* 991).

taxane ring (19-hydroxypaclitaxel; Fig. 1) was also found previously in rats [6–10]. The chromatographic performance of both metabolites relative to 3′*p*-hydroxypaclitaxel and 6α-hydroxypaclitaxel was comparable to that found in our study [6–10]. Although the metabolism of paclitaxel in mice and humans differs from rats, it is possible that metabolites I and II possess the same structures as the above-mentioned rat metabolites.

Five paclitaxel-related peaks were found after methanol extraction of the aqueous sample that remained after diethyl ether extraction. The two most abundant metabolites were further analyzed by MS. Although the Q1 mass spectrum of metabolite E by itself did not reveal clear paclitaxel-related fragments, the PI scan of the peak at *m*/*z* 991 showed fragments that were diagnostic for paclitaxel. As none of the other peaks found in the Q1 scan showed paclitaxel-related fragments in the PI scan, the ion at *m*/*z* 991 may represent the protonated parent. However, unlike most other parent ions, this one was not accompanied by peaks of the parent ion supplemented with an ammonium or sodium ion. The metabolites remaining after diethyl ether extraction represent the hydrophilic species compared to the known hydroxylated metabolites. Consequently, they could be products of phase II metabolic reactions resulting in polar conjugates such as glucuronide, sulfates, mercapturate or glutathionate conjugates. However, there are no obvious candidate conjugates that would explain the mass difference of 137 relative to paclitaxel (*m*/*z* 854). Formation of conjugates has not been reported in earlier studies [3,4,12,13].

In conclusion, seven previously unknown metabolites of paclitaxel were detected in the feces of mice. Two metabolites were monohydroxylated structures, which may be identical to 2*m*-hydroxypaclitaxel and 19-hydroxypaclitaxel. Including these metabolites about 60% of the mass balance of paclitaxel could be quantified.

References

1 Huizing MT, Misser VH, Pieters RC, ten Bokkel Huinink WW, Veenhof CH, Vermorken JB, *et al.* Taxanes: a new class of antitumor agents. *Cancer Invest* 1995; **13**:381–404.

2 Rowinsky EK, Donehower RC. Paclitaxel (Taxol). *N Engl J Med* 1995; **332**:1004–1014.

3 Monsarrat B, Mariel E, Cros S, Gares M, Guenard D, Gueritte-Voegelein F, *et al.* Taxol metabolism. Isolation and identification of three major metabolites of taxol in rat bile. *Drug Metab Dispos* 1990; **18**:895–901.

4 Walle T, Walle UK, Kumar GN, Bhalla KN. Taxol metabolism and disposition in cancer patients. *Drug Metab Dispos* 1995; **23**:506–512.

5 Malingre MM, Schellens JH, van Tellingen O, Rosing H, Koopman FJ, Duchin K, *et al.* Metabolism and excretion of paclitaxel after oral administration in combination with cyclosporin A and after i.v. administration [In process citation]. *Anti-Cancer Drugs* 2000; **11**:813–820.

6 Monsarrat B, Alvinerie P, Wright M, Dubois J, Gueritte-Voegelein F, Guenard D, *et al.* Hepatic metabolism and biliary excretion of Taxol in rats and humans. *J Natl Cancer Inst Monogr* 1993; 39–46.

7 Klecker RW, Jamis-Dow CA, Egorin MJ, Erkmen K, Parker RJ, Stevens R, *et al.* Effect of cimetidine, probenecid, and ketoconazole on the distribution, biliary secretion, and metabolism of [^{3}H]taxol in the Sprague-Dawley rat. *Drug Metab Dispos* 1994; **22**:254–258.

8 Walle T, Kumar GN, McMillan JM, Thornburg KR, Walle UK. Taxol metabolism in rat hepatocytes. *Biochem Pharmacol* 1993; **46**:1661–1664.

9 Jamis-Dow CA, Klecker RW, Katki AG, Collins JM. Metabolism of taxol by human and rat liver *in vitro*: a screen for drug interactions and interspecies differences. *Cancer Chemother Pharmacol* 1995; **36**:107–114.

10 Anderson CD, Wang J, Kumar GN, McMillan JM, Walle UK, Walle T. Dexamethasone induction of taxol metabolism in the rat. *Drug Metab Dispos* 1995; **23**:1286–1290.

11 Royer I, Alvinerie P, Armand JP, Ho LK, Wright M, Monsarrat B. Paclitaxel metabolites in human plasma and urine: identification of 6 alpha-hydroxytaxol, 7-epitaxol and taxol hydrolysis products using liquid chromatography/atmospheric-pressure chemical ionization mass spectrometry. *Rapid Commun Mass Spectrom* 1995; **9**:495–502.

12 Monsarrat B, Chatelut E, Royer I, Alvinerie P, Dubois J, Dezeuse A, *et al.* Modification of paclitaxel metabolism in a cancer patient by induction of cytochrome P450 3A4. *Drug Metab Dispos* 1998; **26**:229–233.

13 Cresteil T, Monsarrat B, Alvinerie P, Treluyer JM, Vieira I, Wright M. Taxol metabolism by human liver microsomes: identification of cytochrome P450 isozymes involved in its biotransformation. *Cancer Res* 1994; **54**:386–392.

14 Harris JW, Rahman A, Kim BR, Guengerich FP, Collins JM. Metabolism of taxol by human hepatic microsomes and liver slices: participation of cytochrome P450 3A4 and an unknown P450 enzyme. *Cancer Res* 1994; **54**:4026–4035.

15 Rahman A, Korzekwa KR, Grogan J, Gonzalez FJ, Harris JW. Selective biotransformation of taxol to 6 alpha-hydroxytaxol by human cytochrome P450 2C8. *Cancer Res* 1994; **54**:5543–5546.

16 Kumar GN, Walle UK, Walle T. Cytochrome P450 3A-mediated human liver microsomal taxol 6 alpha-hydroxylation. *J Pharmacol Exp Ther* 1994; **268**:1160–1165.

17 Sonnichsen DS, Liu Q, Schuetz EG, Schuetz JD, Pappo A, Relling MV. Variability in human cytochrome P450 paclitaxel metabolism. *J Pharmacol Exp Ther* 1995; **275**:566–575.

18 Desai PB, Duan JZ, Zhu YW, Kouzi S. Human liver microsomal metabolism of paclitaxel and drug interactions. *Eur J Drug Metab Pharmacokinet* 1998; **23**:417–424.

19 Sparreboom A, van Tellingen O, Nooijen WJ, Beijnen JH. Determination of paclitaxel and metabolites in mouse plasma, tissues, urine and faeces by semi-automated reversed-phase high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 1995; **664**:383–391.

20 Sparreboom A, Huizing MT, Boesen JJ, Nooijen WJ, van Tellingen O, Beijnen JH. Isolation, purification, and biological activity of mono- and dihydroxylated paclitaxel metabolites from human feces. *Cancer Chemother Pharmacol* 1995; **36**:299–304.

21 Schinkel AH, Mayer U, Wagenaar E, Mol CA, van Deemter L, Smit JJ, *et al.* Normal viability and altered pharmacokinetics in mice lacking mdr1-type (drug-transporting) P-glycoproteins. *Proc Natl Acad Sci USA* 1997; **94**:4028–4033.