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ORIGINAL ARTICLE

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Metabolism of docetaxel in mice

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Abstract Previous studies have shown by quantification of the parent drug and the known metabolites M-1, M-2, M-3 and M-4 that the mass balance of docetaxel in mice and humans is not complete. We therefore used reversed-phase high-performance liquid chromatography (HPLC) with photodiode array (PDA) detection and tandem mass spectrometry to trace and identify putative metabolites in the feces and bile of mice injected intravenously with docetaxel. HPLC-PDA revealed two metabolic products in the feces and more than ten potential new metabolites in the bile. Mass spectrometry was performed on docetaxel reference compound, on the known metabolites M-1, M-2, M-3 and M-4, and on HPLC eluate fractions containing metabolic products, six fractions originating from the bile and two from the feces. The mass spectra of the most abundant unknown metabolite in the bile and the feces were identical, and indicated that this structure contained a carboxyl moiety at the *tert*-butyl group. Under the conditions of storage this product degraded to metabolite M-4. All other unknown metabolites found in the bile samples were oxidized products, with the oxidations in both the C-13 side chain and the baccatin structure, the latter being a new finding.

Keywords Docetaxel · Metabolites · Mice · Feces · Bile

Introduction

Since its introduction almost a decade ago, docetaxel has been shown to be one of the most promising anticancer agents known today. It is active against a wide variety of solid tumours such as ovarian, lung, breast, and head and neck cancer (reviewed in references 2, 3 and 14). Docetaxel is a semisynthetic taxane analog prepared from 10-deacetyl baccatin III, a noncytotoxic precursor isolated from the needles of the European yew tree (*Taxus baccata* L.). It inhibits cell replication by blocking the cells in the late G_2-M phase of the cell cycle due to inhibition of the microtubule dynamics. Studies in cancer patients have shown that the drug exhibits linear pharmacokinetics over a wide range of dose levels after intravenous administration [5] and that it is mainly excreted from the body via the hepatobiliary route, whereas less than 10% of the dose is excreted in the urine [4, 6, 7]. So far, four metabolites, usually called M-1, M-2, M-3 and M-4 based on their elution profile in high-performance liquid chromatography (HPLC), have been identified in human feces (Fig. 1) [6, 9]. The metabolic pathway is postulated to be the following: the *tert*-butyl group on the side chain at C-13 of docetaxel is first oxidized into a primary alcohol (M-2), which leads after a second oxidation probably via an intermediate aldehyde to the two cyclic hydroxyoxazolidinones which are diastereomers (M-1 and M-3). It is postulated that a further oxidation of the intermediate aldehyde would lead to the formation of an unstable carboxylic acid derivative, from which the oxazolidinedione (M-4) is formed by cyclization [9]. The oxidative steps most probably result from involvement of the cytochrome P_{450} isoenzyme 3A subfamily (CYP3A) as has been shown by inhibition and induction studies using typical CYP3A inhibitors and inducers such as erythromycin, ketoconazole and toleandomycin and dexamethasone and rifampicin [8]. The same metabolic pathway has also been reported to occur in rats [6].

Mass balance studies in mice performed in our laboratory have shown that the peaks matching the metabolites M-1, M-2, M-3 and M-4 isolated from human feces [13] are also present in the feces of mice treated with oral or intravenous [^{3}H]-docetaxel [1]. This indicates that metabolism of the drug in this species

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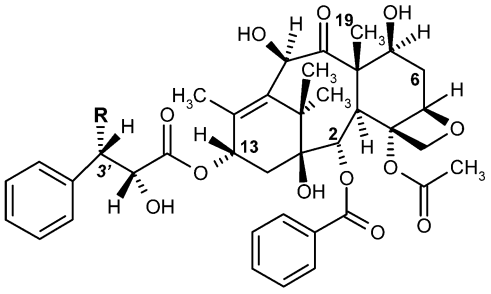


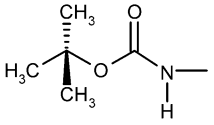
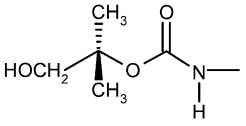
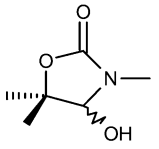
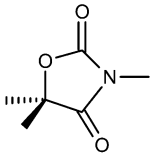
Compound	R
Docetaxel	
M-2	
M-1 & M-3	
M-4	

Fig. 1 Molecular structures of docetaxel and its metabolites M-1, M-2, M-3 and M-4

occurs via pathways comparable to those shown for humans and rats. However, the total amount of unchanged drug and known metabolites represented only about 50% and 70% of the administered dose after intravenous and oral administration, respectively, whereas almost the entire radiolabelled dose was recovered. Obviously, the missing 30–50% of the dose consists of unknown metabolites.

To perform a qualitative search for unknown metabolites of docetaxel, we administered the drug intravenously to a small number of Mdr1a/1b(−/−) P-glycoprotein knock-out mice and collected the feces and bile. These mice were used because the excretion of unchanged docetaxel in Mdr1a/1b(−/−) mice is very low due to the absence of P-glycoprotein and hence metabolism is an even more important pathway of elimination [1]. A range of putative new metabolites was detected in the excretion products by using HPLC with UV detection. Information about the structure of six of these products was obtained by tandem mass spectrometry.

Materials and methods

Animals

Male and female FVB Mdr1a/1b(−/−) mice, 11–18 weeks of age, were used in all experiments. They were allowed food and acidified water ad libitum and were maintained according to institutional guidelines, which are based on Dutch law.

Drugs and chemicals

The clinical formulation of docetaxel (Taxotere, 10 mg/ml in Tween 80/ethanol/saline 20:13:67, v/v/v) originated from Aventis (Antony Cedex, France) and [^{3}H]-docetaxel (1.0 mCi/ml in ethanol, specific activity 7.3 Ci/mmol) was prepared by Moravek Biochemicals (Brea, Calif.). The docetaxel metabolites M-1, M-2, M-3 and M-4 were available as purified products from human feces [13]. A mixture of metabolites M-1, M-2, M-3 and M-4 separated from murine feces was available in ethanol. Lyophilized bovine serum albumin (fraction V) was obtained from Roche Diagnostics (Mannheim, Germany) and saline from Braun (Emmer-Compascuum, The Netherlands). All other chemicals were purchased from E. Merck (Darmstadt, Germany) and were of analytical or Lichrosolv gradient grade. Water purified by a Milli-Q Plus system (Millipore, Milford, Mass.) was used in all aqueous solutions.

Fecal excretion experiment

A docetaxel drug solution was prepared containing 3.0 mg/ml of docetaxel and 37.3 μCi/ml of [^{3}H]-docetaxel by appropriate dilution of the clinical docetaxel solution and the [^{3}H]-docetaxel stock solution with saline. Two mice were placed in Ruco metabolic cages (Valkenswaard, The Netherlands) 3 days before the start of the experiments to allow them to acclimate. The docetaxel drug solution was administered as an intravenous bolus injection via the tail vein, at a dose level of 30 mg/kg of docetaxel and 373 μCi/kg of [^{3}H]-docetaxel. Feces were collected during the time periods 0–8, 8–24 and 24–48 h after drug administration. The feces samples were homogenized 1:10 (g/v) in 4% of bovine serum albumin in water (g/v) using a PT 1200 Polytron homogenizer (Kinematika, Switzerland) and stored at −20°C until analysis.

Gall bladder cannulation experiment

The gall bladders of three mice were cannulated as described previously [12]. After 15 min collection of a blank bile sample, 20 mg/kg of docetaxel was injected intravenously via the tail vein, and the bile was collected for another 1.5 h. Animals were then killed by cervical

dislocation and bile samples were stored at −20°C until further processing.

Analytical procedures

Lipophilic metabolites

Aliquots of 200 µl feces homogenate were extracted twice with 4 ml diethyl ether. After centrifugation for 5 min at 2000 *g*, the aqueous layers were frozen in a bath of dry-ice/ethanol. The supernatant diethyl ether layers were decanted and evaporated at 37°C under nitrogen. The residues were redissolved in 200 µl acetonitrile/water (3:7, v/v) and subjected to HPLC analysis using an isocratic HPLC system.

Hydrophilic metabolites

The aqueous residues remaining after diethyl ether extraction of the feces samples were extracted with 0.5 ml methanol. After centrifugation for 5 min at 2000 *g* the aqueous layers were frozen in a bath of dry-ice/ethanol and the supernatant methanol layers were decanted and evaporated to dryness using a Savant FDC206 freeze drying chamber connected to a Trivac D4B vacuum pump (Leybold) and a Savant RVT 4104 refrigerated vapour trap. The residue was redissolved in 400 µl methanol/water (3:1, v/v), centrifuged for 10 min at 20,000 *g* and 200–250 µl of the clear supernatant was injected into the gradient HPLC system.

HPLC systems

The HPLC equipment consisted of a model 480 ternary gradient pump (Gyncotek, Germering, Germany), a Midas autosampler (Spark Holland, Emmen, The Netherlands), a model 996 photodiode array detector (Waters, Milford, Mass.) and a model 2112 Redirac fraction collector (LKB Bromma, Uppsala, Sweden). Chromatography was performed on a stainless steel column (150×4.6 mm) packed with 5 µm APEX octyl material (Jones Chromatography, Littleton, Colo.) using different separation modes.

Isocratic separation was done using a mobile phase consisting of acetonitrile/methanol/0.01 *M* ammonium acetate buffer, pH 5.0, 35:10:55 (v/v/v) delivered at a flow rate of 1 ml/min. Gradient separation was performed using mobile phase A (acetonitrile/water 80:20, v/v) and mobile phase B (0.01 *M* ammonium acetate buffer, pH 5.0). Initial conditions were 25% A and 75% B from 0 to 7 min. From 7 to 15 min a linear gradient was applied to a final ratio of 63% A and 37% B, which was maintained for another 10 min.

Radioactivity determinations

Volumes of 200 µl feces homogenate and 1 ml Soluene (Packard Bioscience, Groningen, The Netherlands) were incubated for 1.5 h at 50°C in 20 ml polyethylene liquid scintillation vials. Next, 500 µl 2-propanol was added and the incubation was continued for 2 h at 50°C. Finally, 200 µl 30% hydrogen peroxide solution was added, followed by 10 ml Hionic Fluor scintillation cocktail (Packard Bioscience). The radioactivity in the aqueous residues remaining after diethyl ether extraction and subsequent methanol extraction were measured as described above. Samples (50 µl) from HPLC fractions were mixed with 2 ml UltimaGold scintillation cocktail. Radioactivity was determined using a Tricarb β-scintillation counter (Packard Bioscience).

Mass spectrometry

A Sciex model API 365 triple quadrupole mass spectrometer (Thornhill, Ont., Canada) was used. Specimens were introduced into the mass spectrometer by a TurbolonSpray sample inlet (Sciex) without splitting. Ions were created at atmospheric pressure. Positive and negative mass spectra were recorded of each sample using the first quadrupole (Q1 scan). Next, the (putative) parent (MH^+) ion was selected and further fragmented in the collision cell (Q2), under various conditions, and the MS–MS spectrum was recorded (Product ion scan; PI-scan).

Results

HPLC analysis of feces samples

Measurement of the radioactivity in the feces samples obtained from the excretion experiment revealed that 91–97% of the total excreted radioactivity was recovered within 24 h. Therefore, the 0–8 and 8–24 h feces homogenates were pooled and used for the isolation of metabolites. Isocratic HPLC analysis of the diethyl ether extract of the feces homogenate showed no other peaks besides unchanged docetaxel and the known metabolites M-1, M-2, M-3 and M-4. Since the aqueous fraction contained 20–25% of the radioactivity, this fraction was further analyzed using the gradient HPLC system for unknown metabolic products. Two peaks with retention times of 15.1 min (metabolite A) and 20.0 min (metabolite B), respectively, were detected in the UV chromatogram as well as in the corresponding radioactivity pattern of the HPLC fractions (Fig. 2). Metabolites A and B represented about 50% and 5–10%, respectively, of the total radioactivity in the sample subjected to HPLC.

HPLC analysis of bile samples

Since large amounts of interfering endogenous substances eluted between 12 and 20 min after injection of the aqueous feces fraction into the gradient HPLC system, we decided to use bile as biological matrix. This

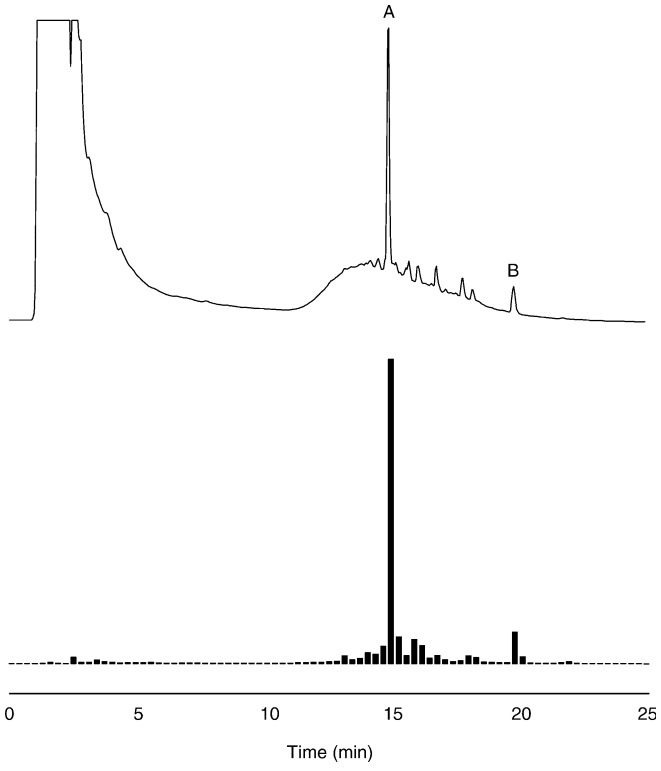


Fig. 2 UV chromatogram recorded at 231 nm of the aqueous extract of feces obtained from mice after administration of 30 mg/kg of [^{3}H]-docetaxel (373 μCi/kg) by intravenous bolus injection injected into the hydrophilic HPLC system (*A*) and the subsequent radioactivity pattern of the HPLC fractions, which were collected every 0.3 min (*B*), Peaks *A* and *B* are previously unknown metabolic products of docetaxel

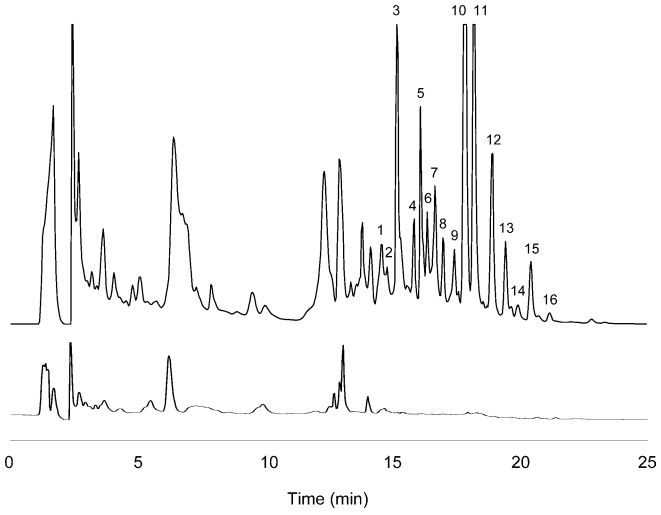


Fig. 3 UV chromatograms recorded at 231 nm of 50 µl bile obtained from a mouse after intravenous bolus injection of 20 mg/kg of docetaxel and of 10 µl of blank mouse bile, respectively. The bile samples were injected into the hydrophilic HPLC system. Peaks 10 and 11 are the known metabolites M-1, M-2 and M-3, peak 15 represents docetaxel, and peaks 3 and 12 are represent metabolites A and B found in the aqueous extract of feces. All other peaks had UV absorption spectra similar to those of docetaxel

matrix is much cleaner then feces, thus allowing injection of samples into the HPLC without sample clean-up. Moreover, it contains the substances excreted by the liver in a higher concentration than present in feces. Bile was collected for 1.5 h after intravenous bolus injection of 20 mg/kg of docetaxel to mice with a gall bladder cannula. Upon injection of 50 µl of bile sample into the hydrophilic HPLC system, 16 peaks with a UV spectrum similar to that of docetaxel were detected (Fig. 3) and were collected for further MS analysis. No peaks were detected in blank bile between 15 and 22.5 min after injection (Fig. 3). Based on the retention times obtained after injection of standard solutions containing the known metabolites M-1, M-2 and M-3 and docetaxel into the hydrophilic HPLC system, it appeared that peaks 10 and 11 were metabolites M-1, M-2 and M-3 and that peak 15 was docetaxel. Furthermore, peak 3 appeared to have the same retention time as metabolite A found in feces homogenate (Fig. 2).

When 50 µl bile was injected into the isocratic HPLC system, M-1, M-2 and M-3 were eluted in three peaks that were not completely baseline resolved. Their retention times were between 7 and 9 min, whereas docetaxel eluted at 20.5 min (Fig. 4). Furthermore, several other peaks with UV spectra similar to that of docetaxel were detected. The retention times of two peaks at 14.8 (peak 17) and 15.4 min (peak 18) were very close to those obtained after injection of a reference solution of metabolite M-4, and MS of eluate fractions was used to verify that one of these represented M-4 (see below). In order to obtain baseline resolved metabolites M-1, M-2 and M-3, 50 µl bile was injected into the isocratic HPLC system with a slightly modified mobile phase (acetonitrile/methanol/water 29:8.5:61, v/v/v). These peaks were also collected for MS analysis.

MS analysis of docetaxel and M-1, M-2 and M-3

We started with the MS analysis of a reference solution of docetaxel and the HPLC fractions containing the known metabolites M-1, M-2 and M-3 separated from bile samples. The Q1 scan of docetaxel revealed the protonated parent ion (MH^+) at *m*/*z* 808.4 and a characteristic fragment at *m*/*z* 527.1, being docetaxel without the C-13 side chain. A PI scan of the MH^+ ion showed typical fragments at *m*/*z* 527.2 and *m*/*z* 282.1, representing cleavage of the C-13 side chain (Table 1, Fig. 5). Moreover, products at *m*/*z* 790.5 ($MH^+ - H_2O$) and 690.1 ($MH^+ - H_2O$–*tert*-butyl ester) were formed (Fig. 5). Interestingly, this latter fragment, which was also found in the negative PI scan, is very characteristic since it was only found in structures where the tertiary nitrogen atom was not part of a ring structure, as is found in M-1 and M-3 (Fig. 1). The PI scan of the MH^+ ion of M-2 (*m*/*z* 824.4) also showed these fragments at *m*/*z* 690.4, next to fragments at *m*/*z* 527.5 and at *m*/*z* 298.3. This pattern reflects the hydroxylation of the

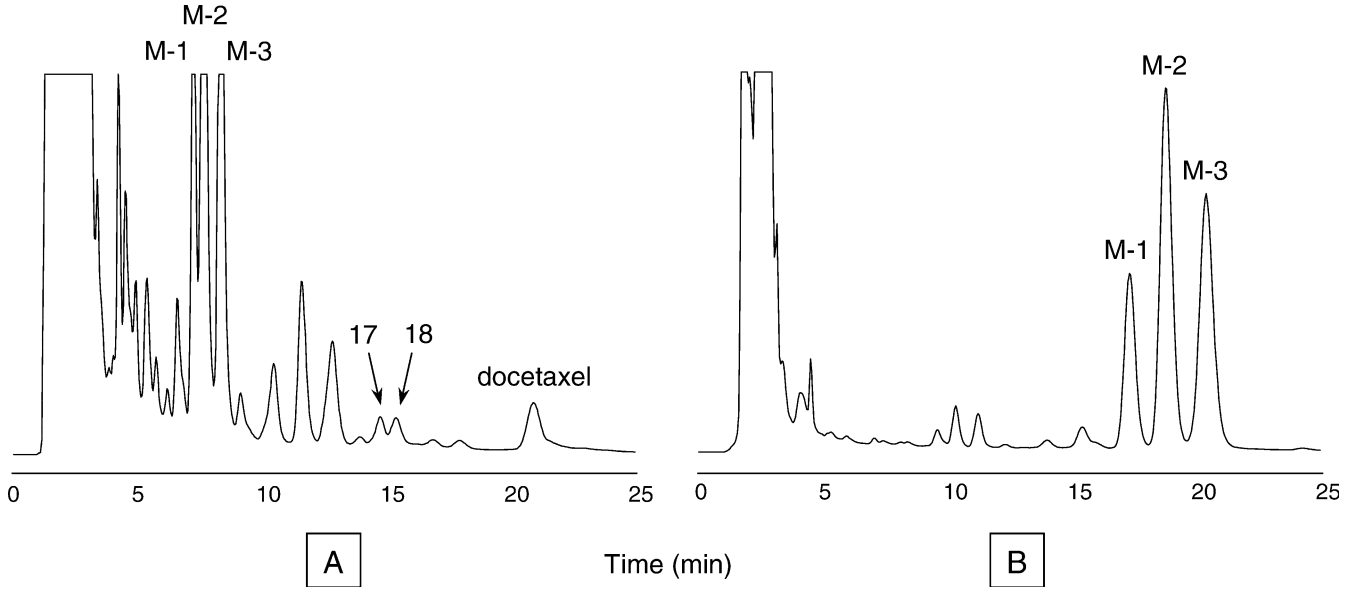


Fig. 4 UV chromatogram recorded at 231 nm of 50 µl bile obtained from a mouse after intravenous bolus injection of 20 mg/kg of docetaxel injected into the hydrophobic HPLC system (**A**). Peak 17 and 18 represent previously unknown metabolic products of docetaxel and the metabolites M-1, M-2 and M-3 are not completely baseline separated on this system. UV chromatogram recorded at 231 nm of 50 µl bile obtained from a mouse after intravenous bolus injection of 20 mg/kg of docetaxel injected into the hydrophobic HPLC system supplied with a mobile phase containing acetonitrile/methanol/water 29:8.5:61 (v/v/v) (*B*s). The metabolites M-1, M-2 and M-3 are well-separated on this system

tert-butyl group of the C-13 side chain. The enantiomers M-1 and M-3 in the Q1 scan showed MH^+ ions at *m*/*z* 822.4 and 822.3, respectively. The subsequent PI scans showed characteristic ions at *m*/*z* 527.3 and *m*/*z* 295.9 for M-1 and 527.2 and 296.4 for M-3, but not at *m*/*z* 690, which thus represented the C-13 side chain after reduction and cyclization of the *tert*-butyl group of M-2.

MS of peaks 17 and 18 and human and murine M-4

The Q1 scans of the HPLC fractions containing peaks 17 and 18, which were derived from bile samples, both showed MH^+ ions at *m*/*z* 822 similar to metabolite M-1 and M-2. This fragment does not correspond to metabolite M-4 with a molecular weight of 820. The PI scan of peak 17 showed fragments at *m*/*z* 526.9 and *m*/*z* 509.1, indicative of an unchanged baccatin structure. Furthermore, fragments at *m*/*z* 750.1 and *m*/*z* 687.9 suggests oxidation of the *tert*-butyl group as in M-2 followed by a reduction at the C-2′ and/or C-3′ position.

Table 1 Selected MS data of docetaxel and metabolites detected after HPLC separation of bile samples

Compound	Scan type	Prominent ions (*m*/*z*)
Docetaxel reference	Q1	808.4, 527.1
	PI (808)	790.5, 690.1, 527.2, 282.1
M-2 bile	Q1	824.4
	PI (824)	690.4, 527.5, 298.3
M-1/M-3 bile	Q1	822.4/822.3
	PI (822)	527.3/527.2, 295.9/296.4
Peak 17	Q1	822.3
	PI (822)	750.1, 687.9, 526.9, 509.1
Peak 18	Q1	822.4
	PI (822)	525.1, 507.4, 297.9
M-4 human feces	Q1	820.5
	PI (820)	527.3, 509.3, 294.1
M-4 murine feces	Q1	820.3
	PI (820)	527.2, 509.3, 294.0
Peak 3	Q1	855.4, 837.1, 820.4
	PI (855)	690.0, 527.5, 509.3, 312.2
Peak 5	Q1	855.4
	PI (855)	838.4, 557.1, 282.1
Peak 7	Q1	840.4, 822.6
	PI (840)	543.2, 298.2
Peak 12	Q1	824.1, 527.2
	PI (824)	690.1, 543.2, 525.4, 509.3, 297.8, 282.3

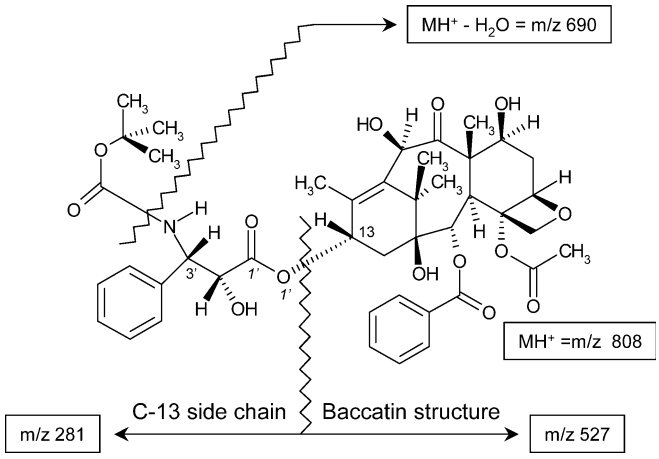


Fig. 5 Mass spectrometric fragmentation of docetaxel, showing the formation of the characteristic fragments at *m/z* 527 (the baccatin structure), *m/z* 281 (the C-13 side chain) and *m/z* 690 (docetaxel without a water molecule and the group attached to the tertiary nitrogen in the C-13 side chain)

The PI scan of peak 18 showed a fragment at *m/z* 297.9 indicating monohydroxylation of the C-13 side chain as has occurred in metabolite M-2. Other fragments at *m/z* 525.1 and *m/z* 507.4 indicate loss of H_2 and introduction of a double bond in the baccatin structure. Next, we injected a reference solution containing metabolite M-4 isolated previously from human feces [13]. In the Q1 scan a peak at *m/z* 820.5 was detected, which was further fragmented in the PI scan to typical fragments at *m/z* 527.3 and *m/z* 509.3 (characteristic of the unchanged baccatin structure) and *m/z* 294.1 (C-13 side chain of M-4). A similar fragmentation pattern was found upon analyzing metabolite M-4 separated from a crude metabolite mixture obtained previously by extraction of feces from mice treated with docetaxel. These findings indicate that metabolite M-4 is not excreted in the bile, but is formed in the intestinal contents or in the feces.

MS of peaks 3, 5, 7 and 12 of unknown metabolites

The Q1 spectrum of peak 3 (corresponding to peak A from feces), which was the largest peak next to the peaks from M-1, M-2 and M-3, contained three fragments at *m/z* 855.4, 837.1 and 820.4, which all showed a similar fragmentation pattern in subsequent PI scans (Fig. 6). Fragments at *m/z* 527.5 and *m/z* 509.3 (the unchanged baccatin structure) were found, as well as a fragment at *m/z* 312.2, which could be the C-13 side chain that has undergone a reduction and is carrying two additional oxygen atoms. Although the fragment at *m/z* 690 was not observed in the positive PI scan, it was found in the negative PI scan, suggesting that the tertiary nitrogen was not part of a ring structure.

In the Q1 spectrum of peak 5 a fragment at *m/z* 855.4 was found. The PI scan of this fragment showed a fragment at *m/z* 838.4, indicating that the ion at *m/z* 855.4 is the parent associated with an ammonium ion

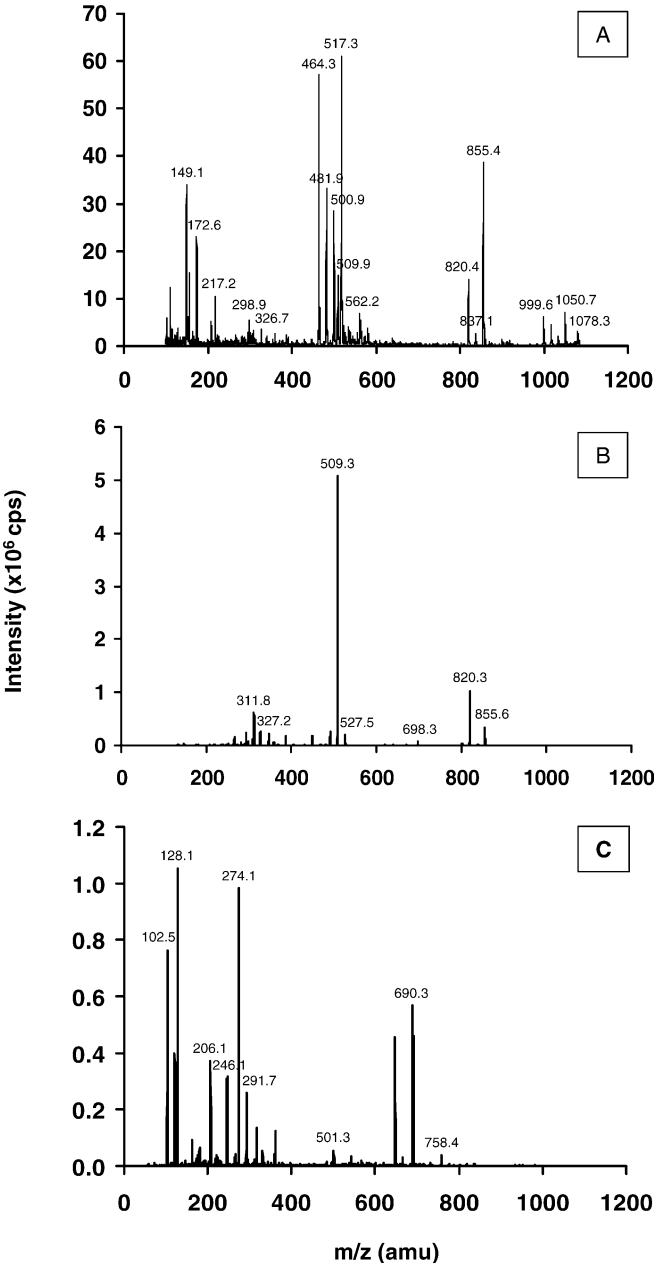


Fig. 6 Q1 scan (**A**), and positive (**B**) and negative PI scans (**C**) of the metabolic product in peak 3

($MH^+ + 17$). Other fragments found were *m/z* 282.1, representing the unchanged C-13 side chain and *m/z* 557.1, which may be the baccatin structure following the loss of H2 and the introduction of a double bond and the introduction of two additional oxygen atoms.

Two prominent fragment peaks at *m/z* 840.4 and 822.6 appeared in the Q1 scan of peak 7. Both showed the same fragmentation pattern in their PI scans, suggesting that the peak at *m/z* 840.4 represents the MH^+ ion, whereas the fragment at *m/z* 822.6 represents the parent ion after the loss of water ($MH^+ - 18$). The most characteristic fragments in the PI scan of the ion at m/z 840.4 were fragments at *m/z* 543.2 and 298.2. The latter

fragment was also present in the PI scan of M-2 and is the C-13 side chain with an additional oxygen atom. The fragment at m/z 543.2 likely presents the baccatin structure, which is also supplemented with an additional oxygen atom.

A peak with a mass at m/z 824.1 is most probably the MH^+ ion of peak 12, indicating the presence of one additional oxygen atom relative to docetaxel. Remarkably, three fragment pairs at m/z 525.4/509.3, m/z 543.2/527.2 and m/z 297.8/282.3 were found in the PI scan. This finding suggests that both the baccatin structure and the C-13 side chain carry an additional oxygen atom, whereas both are also found without the oxygen atom (as in unchanged docetaxel). Moreover, the fragment at m/z 690.1 was also found, indicating that the tertiary nitrogen is not included in a ring structure.

MS analysis of metabolite A and its degradation product

Metabolite A was the largest peak found in the chromatogram of the aqueous fraction of feces. MS analysis of the HPLC fraction containing metabolite A showed the same mass spectra as metabolite 3 separated from the bile samples, indicating that these products are identical. During the experiments it appeared that metabolite A degraded into metabolite B when the HPLC fraction containing the metabolite was stored at −20°C. MS analysis indicated that the degradation product was equal to metabolite M-4.

Discussion

By using HPLC analysis more than ten docetaxel-related metabolic products were found in the bile of mice receiving docetaxel by intravenous bolus injection. Two metabolites were also found in the aqueous fraction of feces left after previous diethyl ether extraction. All putative metabolites, except the metabolite represented by peak 16, were more hydrophilic then docetaxel and most were more hydrophilic than the previously found metabolites M-1, M-2, M-3 and M-4. Comprehensive tandem MS analyses yielded more insight into the molecular structures of six metabolic products. Whereas the previously described metabolites of docetaxel all result from modifications of the C-13 side chain [6, 9] (Fig. 1), the baccatin structure was modified in four of the six new metabolic products analyzed by MS. All modifications involved oxidations of the baccatin moiety that will probably be catalyzed by CYP450 isoenzymes.

The most abundant unknown metabolites found in the aqueous fraction of feces and in the bile samples were metabolite A and peak 3, respectively. MS analysis showed that these metabolites had identical molecular structures. The mass spectra showed that the metabolite carried two additional oxygen atoms at the C-13 side chain and that the baccatin structure was unaffected. The peak at m/z 838 found in the PI scan of a fragment at m/z 855 most probably represents the MH^+ ion, whereas m/z 855 itself exists as the parent associated with an ammonium ion. A fragment at m/z 690 was found in the negative PI scans, indicating that the tertiary nitrogen was not included in a ring structure. This was also found in the negative PI scans of docetaxel but not of M-1 and M-3. During the experiments it appeared that metabolite A degraded into metabolite B upon storage at −20°C in mobile phase. MS analysis showed that metabolite B was equal to the known metabolite M-4. Monegier et al. [9] have described a metabolite with m/z 838 which they believed to be the intermediate from which metabolite M-4 is formed and postulated that it carries a reactive carboxyl group at the *tert*-butyl group (Fig. 7). Peak 3/metabolite A also had a molecular mass of m/z 838 and was one of the most hydrophilic compounds found, suggesting that it is the carboxylic acid derivative also found by Monegier et al. Moreover, our data clearly showed that peak 3/metabolite A is a precursor of metabolite M-4. Our HPLC-UV data showed that whereas metabolite M-4 was present in the feces of the mice used in the excretion experiment, it was not present in the bile samples. A similar observation has previously been reported in rats [6]. Probably, metabolite M-4 is exclusively formed in the intestinal contents or in the feces.

A parent mass at m/z 838 was found for peak 5. The results of the PI scan showed that the C-13 side chain was not changed but that two additional oxygen atoms were attached to the baccatin structure. Oxidation of the baccatin structure is a common metabolic modification of paclitaxel, resulting in metabolic products such as 6α-hydroxypaclitaxel [10, 15], 2*m*-hydroxypaclitaxel [10, 11] and 19-hydroxypaclitaxel [10]. However, the MS data do not provide information about the positions of the oxygen atoms in the molecule. The mass spectrum of peak 7 showed a MH^+ ion at m/z 840, indicating the presence of two additional oxygen atoms in the structure. The C-13 side chain showed the same fragmentation scheme as found for metabolite M-2 indicative for an oxidation of the *tert*-butyl group. The second oxygen

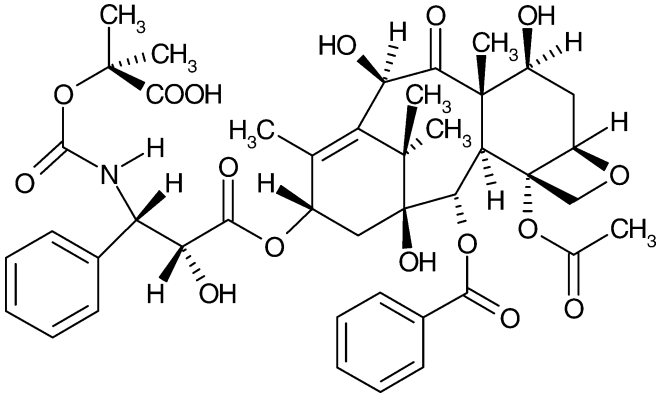


Fig. 7 Proposed molecular structure of peak 3

atom was attached to the baccatin structure, but its exact position is not clear. The parent ion of peak 12 was *m*/*z* 824, indicating the addition of one oxygen atom relative to docetaxel. The Q1 scan also showed a fragment at *m*/*z* 527 as in unchanged docetaxel, M1–M4 and other structures where the additional oxygen is located at the C-13 side chain. Most likely, oxidation has occurred at the C-3′ position or at the phenyl moiety attached to the C-3′ position. Apparently, the presence of the oxygen at this location has a remarkable effect on the fragmentation in the PI scan.

Cleavage of the baccatin structure results in peaks at *m*/*z* 525 and 543, the latter being found only in case of oxidation of the baccatin structure. Similarly, next to the fragment at *m*/*z* 298 (C-13 side chain + oxygen) a second signal at *m*/*z* 282 (C-13 side chain as in unchanged docetaxel) was observed. The most likely explanation is that whereas cleavage between the baccatin structure usually occurs between the C-13 and O, it now also occurs between C-1 and O. In this latter case the mass of the leaving C-13 side chain appears to be the same as in unchanged docetaxel as the mass of the extra oxygen in the side chain has been lost to the baccatin structure. The exact location of the extra oxygen atom could not be determined. However, a probable location may be at the *para* position of the phenyl moiety, yielding a metabolic product that is also formed from paclitaxel (viz., 3 *p*-hydroxypaclitaxel) [10, 11].

Two small peaks (peaks 17 and 18 in Fig. 4) from the bile samples were observed in the UV chromatogram that eluted very close to metabolite M-4. They were further characterized by MS, although their relatively low concentrations made the interpretation of the data more difficult. The metabolic product peak 17 had a parent mass at *m*/*z* 822 as in metabolite M-1 and M-3. Whereas no characteristic fragments of the C-13 side chain were found, the baccatin structure appears to be unchanged, suggesting oxidation of the C-13 side chain. Peak 18 also showed a parent mass at *m*/*z* 822. Its fragmentation pattern indicated a C-13 side chain similar to metabolite M-2 and the reduction of the baccatin structure. The location of this reduction (e.g. the introduction of a double bond in the molecule) could not be established.

In conclusion, our study showed that as well as the previously identified metabolites M-1 to M-4 in humans, a large range of degradation products of docetaxel are present in the bile or feces of mice. We obtained information on the molecular structure of six of these products using tandem MS analysis. The putative carboxylic acid metabolite was the most abundant product. This metabolite was shown to be a precursor of metabolite M-4. All other unknown metabolites that were found were oxidation products of docetaxel.

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