Effect of several compounds on biliary excretion of paclitaxel and its metabolites in guinea-pigs

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The objective of this study was to evaluate the *in vivo* metabolic profile of paclitaxel and to examine the effect of potential co-administered drugs on the biliary secretion of paclitaxel and its metabolites in guinea-pigs. We first investigated *in vitro* paclitaxel metabolism using liver microsomes obtained from various species to identify the most suitable animal model with a similar metabolism to humans. Then, *in vivo* paclitaxel metabolism was investigated in male guinea-pigs. The levels of paclitaxel and its metabolites were measured by high-performance liquid chromatography in bile samples from guinea-pigs after paclitaxel i.v. injection (6 mg/kg). We further evaluated the effects of various drugs (quercetin, ketoconazole, dexamethasone, cotrimoxazole) on the biliary secretion of paclitaxel and its metabolites in guinea-pigs. This work demonstrated significant *in vitro* interspecies differences in paclitaxel metabolism. Our findings showed both *in vitro* and *in vivo* similarities between human and guinea-pig biotransformation of paclitaxel. 6α-Hydroxypaclitaxel, the main human metabolite of paclitaxel, was found in guinea-pig bile. After paclitaxel combination with ketoconazole or quercetin in guinea-pigs, the cumulative biliary excretion of paclitaxel and its metabolites up to 6 h was significantly decreased by 62 and 76%, respectively. The co-administration of cotrimoxazole or pretreatment with dexamethasone did not alter significantly cumulative biliary excretion. The guinea-pig is a suitable model to study metabolism and biliary excretion of paclitaxel, and to investigate *in vivo* drug interactions. *Anti-Cancer Drugs* 16:675–682 © 2005 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2005, **16**:675–682

Keywords: biliary elimination, drug–drug interactions, guinea-pigs, paclitaxel metabolism

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Received 17 January 2005 **Revised form accepted** 14 March 2005

Introduction

Paclitaxel is widely used in the treatment of many types of cancer [1]. Hepatic metabolism and biliary clearance are the major means of paclitaxel elimination. In human liver, paclitaxel is reported to be mainly metabolized by CYP2C8 to 6α-hydroxypaclitaxel and by CYP3A4 to 3′*p*-hydroxypaclitaxel. The action of these two isoforms (CYP2C8 and CYP3A4) leads to the formation of a dihydroxylated metabolite: 6α,3′*p*-dihydroxypaclitaxel [2,3] (Fig. 1). These known metabolites are either inactive or less potent than the parent compound [4,5]. Any alteration in paclitaxel biliary elimination will lead to an erratic pharmacokinetic profile with subsequent modification in the clinical outcome. In this respect, developing an animal model for screening and predicting pharmacokinetic interactions could be of interest.

To date, species differences in paclitaxel metabolism have been described. *In vitro* metabolism of paclitaxel was different in rat and human liver microsomes [6,7]. The elimination of paclitaxel in bile was extensively investigated in rats [8–10]. However, the human major metabolite was not found in rat bile samples, thus suggesting that this species is not a good animal model for studying paclitaxel metabolism. Therefore, we carried out an *in vitro* comparative study of paclitaxel metabolism. We aimed to identify the species that would be the most suitable to study *in vivo* paclitaxel metabolism. Our findings showed similarities between human and guinea-pig biotransformation of paclitaxel. Thus, we carried out a study of the biliary disposition profile of paclitaxel and its metabolites in guinea-pigs after i.v. administration of 6 mg/kg paclitaxel. As paclitaxel is used in combination and is metabolized by the cytochrome P450 system, we further investigated potential drug–drug interactions on paclitaxel metabolism in guinea-pigs. Because hepatobiliary excretion is the major pathway of paclitaxel elimination, our study focused on the possible impairment of this elimination pattern.

Materials and methods

Chemicals

Paclitaxel, baccatin III (internal standard), quercetin, ketoconazole and NADPH (type I, sodium salt) were purchased from Sigma Aldrich (St Quentin Fallavier, France). Paclitaxel (Taxol) 6 mg/ml formulated in a

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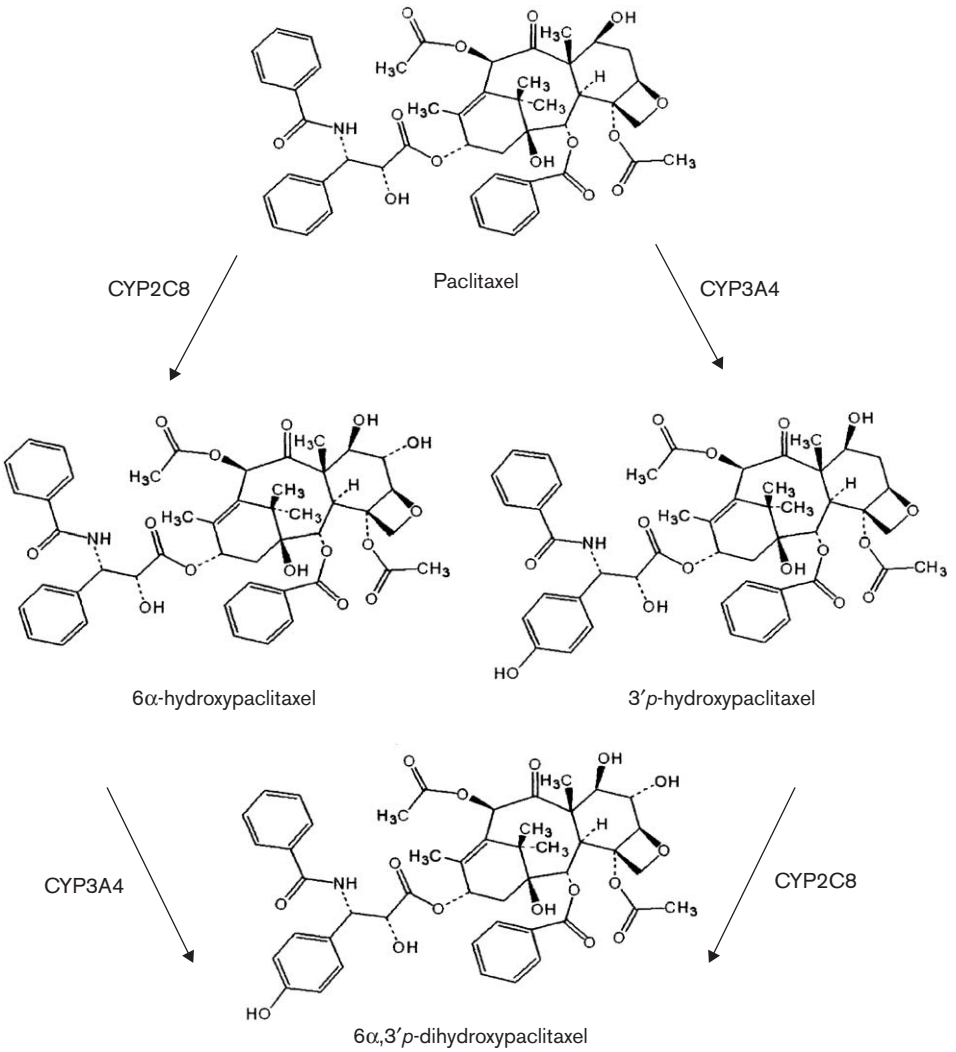
Fig. 1



Human metabolic pathway of paclitaxel.

mixture of ethanol and Cremophor EL was used for animal experiments, and was obtained from Bristol-Myers Squibb (Puteaux, France). Dexamethasone 20 mg/5 ml (Merck, Paris, France) and cotrimoxazole (Bactrim; Roche, Neuilly-sur-Seine, France) were obtained from commercial sources. Isoflurane (Forene) was purchased from Abbott (Rungis, France). Paclitaxel metabolites were kindly provided by Dr Monsarrat (Toulouse, France). All chemicals and solvents were of high-performance liquid chromatography (HPLC) grade or higher.

Microsomal fractions

Male and female Hairless and Sprague-Dawley rats, BALB/c and OF1 mice, male Dunkin Hartley albino guinea-pigs, and male New Zealand white rabbits were obtained from Charles River (L'Arbresle, France). After sacrifice of animals, livers were removed, pooled and immediately stored at −80°C until microsome preparation. Microsomal fractions were prepared in Tris–HCl buffer (100 mM; pH 7.4) by differential ultracentrifugation as previously described [11]. Male Beagle dog and Cynomolgus monkey liver microsomes were obtained from Iffa Credo (L'Arbresle, France). Human liver microsomes were obtained as described previously [12].

In vitro interspecies variability of paclitaxel metabolism

The incubation of 1 mg/ml liver microsomal proteins and 25 μM paclitaxel in methanol was performed for 1 h at 37°C with 1 mM NADPH as previously described [12]. Preliminary experiments indicated that the formation of paclitaxel metabolites was linear up to 1 mg/ml of microsomal proteins and up to 1 h of incubation time with human liver microsomes. To investigate the

comparative study of paclitaxel metabolism, we applied the same incubation conditions for animal liver microsomes. The reaction was stopped by addition of 2.5 ml of ethyl acetate and 10 µl of internal standard baccatin III (1 mM in methanol) was added. The tubes were then gently shaken for 5 min on an Ika Vibrax VXR shaker at 1600 vibrations/min. After a 5-min centrifugation at 2500 r.p.m., the organic layer was removed to a clean tube and evaporated until dry under a stream of nitrogen. The residue was reconstituted with 100 µl of methanol and 80 µl was injected for HPLC analysis. All experiments were performed in triplicate.

Enzymatic kinetics were performed with human and guinea-pig liver microsomes to determine Michaelis–Menten constants for paclitaxel hydroxylases. The velocity of paclitaxel metabolism was determined by paclitaxel incubation, as described above, at various concentrations ranging from 5 to 50 µM. All microsomal incubations of paclitaxel were performed in triplicate. Apparent K_m (Michaelis constant) and V_m (maximum velocity) were estimated with Lineweaver–Burk representations by non-linear regression in Microsoft Excel software.

Animals

Male Dunkin Hartley albino guinea-pigs (Harlan, Gannat, France) with an average weight of 400 g were used. Animals were housed in a room maintained at 22°C with a 12-h cycle of light and darkness for at least 1 week before experiments, with tap water *ad libitum*. All experiments involving animals adhered to the Principles of Laboratory Animal Care (NIH publ. 85-23, revised in 1985). The protocols employed were approved by the local committee for animal experiments.

Animal experiments

For anesthesia, guinea-pigs were first induced in a plexiglas chamber with 3% isoflurane and maintained to effect with 1–2% isoflurane via a mask with a mixture of O_2/N_2O. The common bile duct was cannulated with a 20-gauge catheter (Becton Dickinson, Rungis, France) attached to tubing for bile collection. A control bile was collected prior to paclitaxel administration for 10 min. The commercial formulation of paclitaxel (6 mg/kg; 7 µM/kg) dissolved in 0.9% saline was injected as a 1-min bolus into the jugular vein ($n = 4$). Bile was collected hourly for 6 h after paclitaxel administration as a continuous sample flow with pre-weighed tubes changed hourly. The bile flow was determined gravimetrically assuming 1 ml of bile to have a density of 1 g. All bile samples were stored at −20°C until analysis within 1 week. Preliminary assays have shown paclitaxel stability in rat bile for 1 month (data not shown). During the procedure, body temperature was monitored and maintained at 37°C with a standard heating pad and a lamp to prevent hypothermia, and the abdomen was maintained moist with saline-soaked gauze.

The effects of various drugs (quercetin, ketoconazole, dexamethasone, cotrimoxazole) on the biliary excretion of paclitaxel and its metabolites were investigated using male guinea-pigs. Doses used for the co-administered drugs were selected according to data found in the literature for guinea-pigs. Quercetin (25 mg/kg) suspended in 0.5% carboxymethylcellulose solution was given i.p. to four guinea-pigs prior to paclitaxel (6 mg/kg). Dexamethasone (20 mg/kg) was given i.p. daily for 3 consecutive days before paclitaxel i.v. bolus administration. Ketoconazole (10 mg/kg) dissolved in 1 ml of 0.05 M HCl was administered i.p. before paclitaxel. Cotrimoxazole (20 mg/kg of trimethoprim and 100 mg/kg of sulfamethoxazole) was given i.p. before paclitaxel administration. Bile samples collected hourly for 6 h after paclitaxel administration were stored at −20°C until analysis within 1 week.

Bile sample analysis

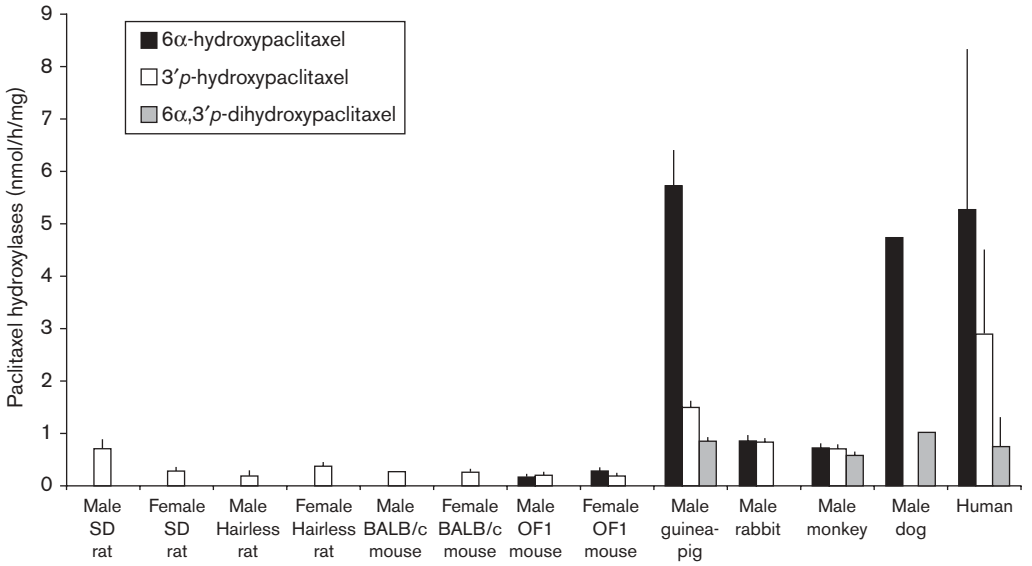
Bile samples were allowed to warm at room temperature prior to analysis. A 1-ml bile sample was spiked with 10 µl of 1 mM internal standard (baccatin III), extracted twice with 3 ml of ethyl acetate, shaken for 10 min on an Ika Vibrax VXR shaker and centrifuged at 2500 r.p.m. for 10 min. The organic layer was separated and evaporated under a gentle stream of nitrogen. The residue was reconstituted in 100 µl of methanol and 80 µl was injected for HPLC. The biliary excretion rates of paclitaxel or its metabolites were calculated as the product of their concentration in bile and the biliary flow.

HPLC analysis

Paclitaxel and metabolites were quantified by HPLC. The analytical procedure was adapted from Huizing *et al.* [13]. Reversed-phase HPLC analysis was performed on a Hewlett Packard 1090 system equipped with an automatic injector (HP 1050), a ternary solvent delivery system and a multiple-wavelength detector (HP 1110). Data analysis was performed using Hewlett-Packard HPLC^{2D} ChemStation software. Samples were analyzed on a Symmetry Shield C_{18} column (4.6 × 250 mm) with 5-µm particles (Waters, St Quentin). Isocratic elution was conducted with ammonium acetate buffer 20 mM (pH 5)/acetonitrile/methanol (48/37/15, v/v/v) at a flow rate of 1 ml/min and a detection wavelength of 230 nm.

Paclitaxel concentration was calculated by determining the ratio of paclitaxel peak height to that of the internal standard. The hydroxylated metabolites were quantified using a paclitaxel standard curve due to the lack of sufficient quantities of paclitaxel metabolites as reference standards. Previous studies have shown nearly identical molar extinction coefficients at 230 nm for

Fig. 2



Interspecies variability of paclitaxel metabolism. Liver microsomes (1 mg/ml) from various species were incubated with 25 μM of paclitaxel for 1 h at 37°C. Data are the mean ± SD of three experiments and expressed as nmol metabolites formed/h/mg microsomal protein. Human data are the mean of 22 microsomes samples.

paclitaxel and its metabolites [4,13]. The standard curves for paclitaxel were linear ($r^2 > 0.99$) over the concentration range 0.25–50 μM. Extraction recoveries for bile samples ranged from 80 to 91%. The metabolites were identified by comparison of their retention times with authentic standards.

Statistical analysis

All results are expressed as the mean ± SD for four animals in each treatment group. Statistical analysis was performed by using Student's *t*-test found in SigmaStat (version 2.03). $p < 0.05$ was considered statistically significant.

Results

In vitro interspecies variability of paclitaxel metabolism

Paclitaxel metabolism was investigated using liver microsomes obtained from different species (Fig. 2). Human data were obtained from our previous work on the *in vitro* interindividual variability of paclitaxel metabolism [12]. The present study showed that paclitaxel metabolism was species dependent. There was considerable interspecies variation in both quantitative and qualitative formation of metabolites. 6α-Hydroxypaclitaxel, the main human metabolite, was not detected in rat and BALB/c mice microsomes. 3′*p*-Hydroxypaclitaxel was not found in the male dog. The three metabolites detected in human liver microsomes were formed in the male guinea-pig and monkey. The pattern of paclitaxel metabolism was similar for both human and guinea-pig liver microsomes, with 6α-hydroxypaclitaxel being the major metabolite.

Table 1 Enzymatic parameters of paclitaxel hydroxylases for human and guinea-pig liver microsomes

Microsomes	Paclitaxel 6α-hydroxylase	Paclitaxel 3′*p*-hydroxylase
Human liver (HL18)	K_m = 21.9 μM	K_m = 63.2 μM
	V_m = 9.8 nmol/h/mg	V_m = 9.6 nmol/h/mg
Human liver (HL29)	K_m = 14.1 μM	K_m = 23.0 μM
	V_m = 7.6 nmol/h/mg	V_m = 4.9 nmol/h/mg
Human liver (HL32)	K_m = 30.4 μM	K_m = 59.1 μM
	V_m = 7.6 nmol/h/mg	V_m = 7.6 nmol/h/mg
Male guinea-pig	K_m = 20.7 μM	K_m = 62.7 μM
	V_m = 8.2 nmol/h/mg	V_m = 3.2 nmol/h/mg

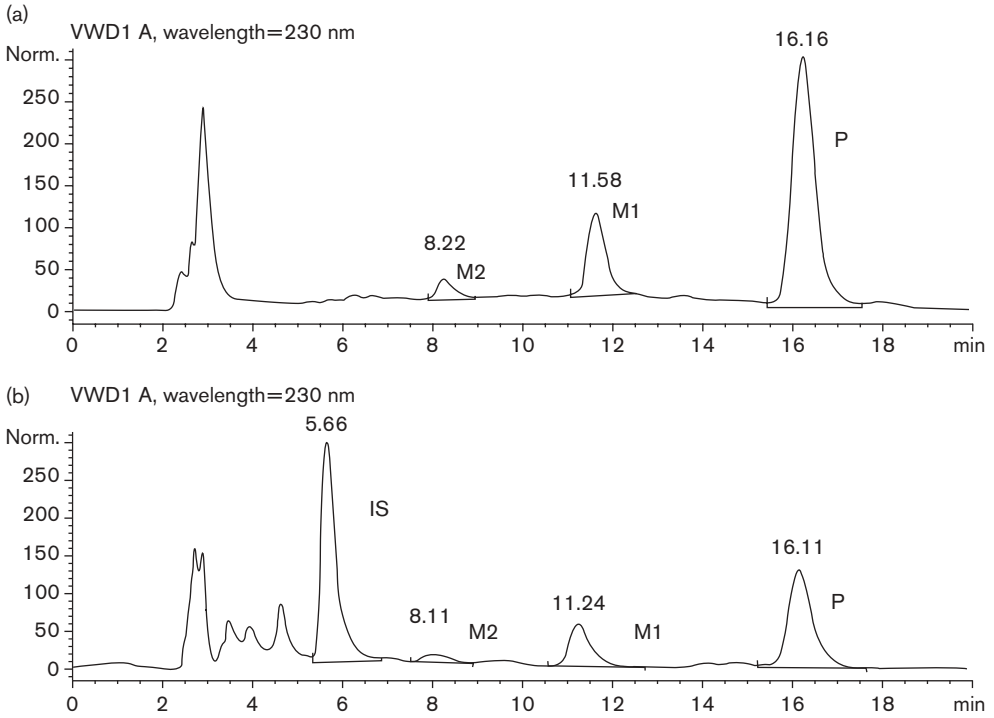
Apparent K_m and V_m were estimated by non-linear regression using Lineweaver–Burk representations after incubation of paclitaxel at various concentrations ranging from 5 to 50 μM with 1 mg/ml of human or guinea-pig liver microsomes for 1 h at 37°C. The formation rates of paclitaxel metabolites were expressed as nmol product/mg microsomal protein/h.

Apparent Michaelis–Menten constants for paclitaxel 6α-hydroxylase were comparable for human and guinea-pig liver microsomes (Table 1).

Biliary excretion of paclitaxel and its metabolites in guinea-pigs

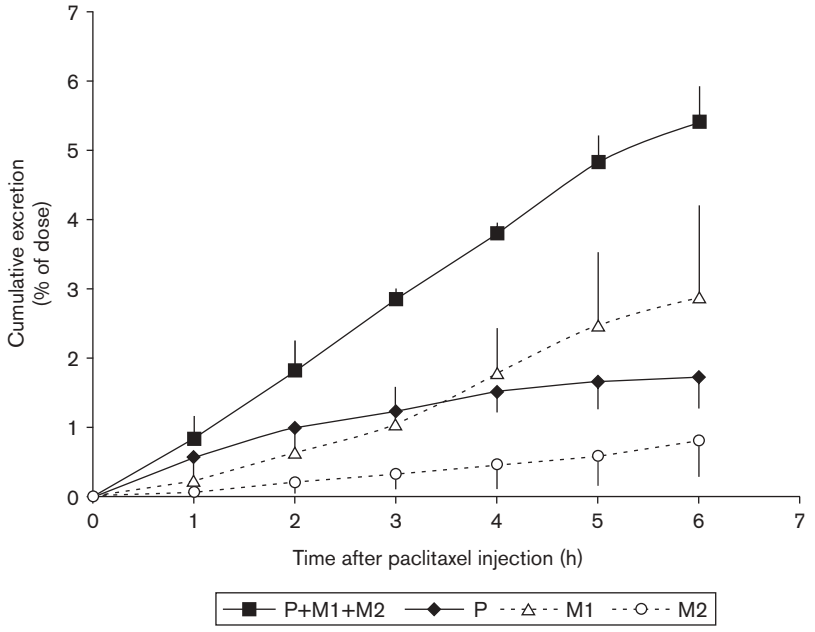
The main biliary metabolite was 6α-hydroxypaclitaxel in male guinea-pigs. Lower quantities of 3′*p*-hydroxypaclitaxel were also found in guinea-pig bile. The dihydroxylated metabolite was observed in only a few samples and its levels were below the limit of detection. The typical HPLC-UV chromatograms of human liver microsomes and guinea-pig bile are presented in Figure 3. The cumulative biliary elimination curves of paclitaxel and its

Fig. 3



Typical HPLC-UV chromatograms obtained after extraction by ethyl acetate of (a) paclitaxel incubation (25 μM) for 1 h with human liver microsomes and (b) bile 1 h after i.v. paclitaxel administration (6 mg/kg) to male guinea-pigs. Peaks IS (5.7 min), M2 (8.1 min), M1 (11.2 min) and P (16.1 min) represent, respectively, internal standard as baccatin, 3′*p*-hydroxypaclitaxel, 6α-hydroxypaclitaxel and paclitaxel.

Fig. 4



Cumulative biliary excretion of paclitaxel and its metabolites in male guinea-pigs. The cumulative amount excreted in bile is expressed as the percentage of the initial injected dose. Each data point represents the mean ± SD of four animals. (■) Unchanged paclitaxel (P) and its hydroxylated metabolites (M1, M2), (♦) Unmetabolized paclitaxel (P). (△) 6α-Hydroxypaclitaxel (M1). (○) 3′*p*-Hydroxypaclitaxel (M2).

metabolites are depicted in Figure 4. Male guinea-pigs excreted 5.4 ± 0.5% of the injected dose into bile over a 6-h collection. 6α-Hydroxypaclitaxel accounted for 53% of total biliary recovery as the main fraction.

Effects of co-administered compounds on the biliary excretion of paclitaxel and its metabolites in guinea-pigs

The effects of quercetin, cotrimoxazole, dexamethasone and ketoconazole on the biliary excretion of paclitaxel up to 6 h after paclitaxel administration were investigated using male guinea-pigs (Tables 2 and 3). In each subset, biliary flow was unaffected at each time point compared to that of control group receiving paclitaxel alone.

The percentage of cumulative biliary excretion of paclitaxel and its metabolites in the quercetin co-treated group (1.3 ± 0.6%; mean ± SD) was significantly lower than that in the control group receiving paclitaxel alone (5.4 ± 0.5%; mean ± SD) ($p < 0.001$). Quercetin co-administration reduced significantly the cumulative biliary excretions of unchanged paclitaxel and 6α-hydroxypaclitaxel, whereas that of 3′*p*-hydroxypaclitaxel was not significantly affected.

Table 2 Effect of several compounds on the biliary excretion of paclitaxel and its metabolites in male guinea-pigs

	Percentage of dose excreted into bile			
	Unchanged paclitaxel	6α-Hydroxypaclitaxel	3′*p*-Hydroxypaclitaxel	Total
Paclitaxel	1.72 ± 0.44	2.88 ± 1.31	0.81 ± 0.52	5.42 ± 0.50
Paclitaxel/quercetin	0.38 ± 0.05[a]	0.68 ± 0.51[a]	0.23 ± 0.17	1.29 ± 0.60[b]
Paclitaxel/ketoconazole	1.34 ± 0.24	0.72 ± 0.26[a]	0.02 ± 0.02[a]	2.08 ± 0.50[b]
Paclitaxel/dexamethasone	2.09 ± 0.62	5.03 ± 2.83	1.27 ± 0.36	8.39 ± 2.08
Paclitaxel/cotrimoxazole	1.90 ± 0.56	4.91 ± 2.96	1.63 ± 0.65	8.44 ± 1.99

The results are expressed as the mean percent ± SD of cumulative biliary excretion of paclitaxel and its metabolites 6 h after paclitaxel administration for four animals in each subset. The significant values are indicated by [a]$p < 0.05$ and [b]$p < 0.001$.

Table 3 Amounts of paclitaxel and metabolites recovered into the bile

Time after paclitaxel injection (h)	Bile flow (ml)	Unchanged paclitaxel (nmol)	6α-Hydroxypaclitaxel (nmol)	3′*p*-Hydroxypaclitaxel (nmol)
Paclitaxel				
1	3.8 ± 0.9	14.5 ± 4.6	5.6 ± 2.0	2.1 ± 1.8
2	4.4 ± 0.5	11.5 ± 5.0	10.4 ± 3.1	5.0 ± 1.5
3	3.2 ± 1.1	6.2 ± 2.8	10.1 ± 6.3	4.1 ± 3.0
4	3.5 ± 1.0	5.8 ± 3.1	14.0 ± 3.9	6.6 ± 2.2
5	3.7 ± 0.9	5.5 ± 0.1	17.0 ± 7.9	5.2 ± 1. 5
6	2.9 ± 0.6	2.7 ± 1.4	9.7 ± 5.6	4.1 ± 2.3
Paclitaxel/quercetin				
1	2.9 ± 0.5	2.8 ± 1.4	2.1 ± 1.1	0.7 ± 0.8
2	2.9 ± 0.9	1.9 ± 0.9	2.6 ± 1.2	1.3 ± 1.5
3	2.7 ± 0.8	2.2 ± 0.3	3.5 ± 2.5	1.4 ± 0.9
4	2.5 ± 0.7	1.8 ± 0.7	3.7 ± 2.6	1.4 ± 1.0
5	2.3 ± 0.8	1.4 ± 0.9	4.7 ± 4.5	1.4 ± 1.1
6	2.3 ± 1.1	1.5 ± 0.9	3.3 ± 2.5	1.6 ± 1.8
Paclitaxel/ketoconazole				
1	4.8 ± 1.5	11.0 ± 3.0	1.4 ± 1.1	0
2	4.6 ± 1.2	7.7 ± 2.3	3.2 ± 0.8	0
3	4.5 ± 0.9	7.2 ± 2.0	4.8 ± 1.6	0.1 ± 0.2
4	4.2 ± 0.9	5.8 ± 1.5	5.2 ± 1.9	0.1 ± 0.1
5	3.7 ± 0.7	5.3 ± 1.9	5.1 ± 2.3	0.2 ± 0.4
6	3.4 ± 0.7	3.6 ± 1.3	4.3 ± 1.3	0.1 ± 0.2
Paclitaxel/dexamethasone				
1	3.5 ± 1.4	10.8 ± 2.9	8.5 ± 9.3	2.2 ± 2.7
2	3.6 ± 1.2	10.7 ± 1.7	23.5 ± 18.1	6.0 ± 1.3
3	3.3 ± 1.1	8.6 ± 1.0	25.6 ± 18.4	5.4 ± 1.1
4	3.5 ± 1.2	8.2 ± 1.5	28.1 ± 19.4	6.0 ± 1.8
5	3.5 ± 1.2	7.9 ± 2.0	29.4 ± 22.2	6.4 ± 2.2
6	3.2 ± 0.9	6.6 ± 1.8	27.3 ± 15.3	5.8 ± 2.7
Paclitaxel/cotrimoxazole				
1	4.9 ± 0.5	17.4 ± 7.2	12.2 ± 6.9	5.1 ± 2.2
2	4.5 ± 0.4	12.1 ± 8.8	19.9 ± 8.0	8.5 ± 5.4
3	4.4 ± 0.6	13.0 ± 10.2	24.3 ± 6.4	12.7 ± 12.0
4	4.3 ± 0.5	8.6 ± 3.1	41.3 ± 32.5	10.6 ± 4.7
5	4.0 ± 0.3	4.3 ± 1.5	22.7 ± 11.1	7.7 ± 3.9
6	3.7 ± 1.4	2.2 ± 1.1	19.3 ± 17.5	5.4 ± 0.6

The results are expressed as the mean ± SD of four animals. The means ± SD of paclitaxel administered in each subset were 2.6 ± 0.3, 3.1 ± 0.7, 2.9 ± 0.2, 2.6 ± 0.5 and 3.0 ± 0.4 μM, respectively, for paclitaxel, quercetin co-treated, ketoconazole co-treated, dexamethasone pretreated and cotrimoxazole co-administered groups.

Total cumulative biliary excretion was markedly reduced after combination with ketoconazole ($2.1 \pm 0.5\%$; mean ± SD) ($p < 0.001$). The ketoconazole co-treated group displayed a significant decrease in the biliary secretion of the two hydroxylated metabolites, whereas the relative proportion of unchanged paclitaxel recovered into bile increased.

In the dexamethasone-pretreated group, total biliary secretion was not significantly increased than that in the control receiving paclitaxel alone ($p = 0.064$).

There were no significant quantitative differences in the biliary excretion of unmetabolized paclitaxel and its metabolites between guinea-pigs co-treated with cotrimoxazole and the control group ($p = 0.054$).

Discussion

In vitro interspecies variability of paclitaxel metabolism

Previous studies have investigated *in vivo* paclitaxel metabolism, biliary secretion and disposition in rats or mice [8–10,14]. Our *in vitro* results showed that 6α-hydroxypaclitaxel was not found in rats and BALB/c mice. The results of the present study are consistent with previous data reporting wide interspecies variability in paclitaxel metabolism, that might be explained by interspecies differences in the expression and activity of enzymes involved in paclitaxel metabolism. The *in vitro* metabolic profile of paclitaxel in guinea-pigs was similar to that in humans [2,3,12]. The present study reports for the first time that the guinea-pig is a convenient model for paclitaxel metabolism studies. Our results are in line with a recent study reporting that the isolated guinea-pig liver is a suitable model for the study of hepatic metabolism. Indeed, the physiology of the guinea-pig is closer to human physiology compared with rats [15].

Biliary excretion of paclitaxel and its metabolites in guinea-pigs

We have therefore used guinea-pigs receiving i.v. paclitaxel to study the *in vivo* metabolic fate of this anti-neoplastic agent. Thus, we have studied the biliary secretion of paclitaxel and its hydroxylated metabolites in guinea-pigs. HPLC-UV analysis in this study enabled us to detect important amounts of 6α-hydroxypaclitaxel, the main human metabolite. In this present study, 6α-hydroxypaclitaxel represented the large majority of biliary paclitaxel derivatives in guinea-pigs. As CYP2C8 catalyses paclitaxel 6α-hydroxylation and plays an emerging role in the metabolism of drugs and endogenous compounds [16], guinea-pigs could represent a convenient animal model to study these drugs.

The biliary elimination of paclitaxel in guinea-pigs was compared to that in humans [9,17]. More than 5% of administered dose was excreted into the bile of male guinea-pigs 6 h after bolus injection, whereas approximately 15% was eliminated into human bile 6 h after paclitaxel infusion. The percentage of unchanged paclitaxel excreted into bile was comparable in guinea-pigs (1.7%) and humans (2.6%) [9]. This discrepancy in results might be partly due to different doses and administrations, and more important tissue distribution of paclitaxel in guinea-pigs. The sensitivity of our analytical procedure might be insufficient to quantify all metabolites of paclitaxel in guinea-pig bile.

Effects of co-administered drugs on the biliary excretion of paclitaxel in guinea-pigs

As paclitaxel is administered in combination therapy and its metabolism is CYP dependent, drug interactions are likely to occur. Inhibition and induction of CYPs are among the most common causes of drug interactions. Investigating *in vivo* drug–drug interactions in an animal model that presents nearby similar metabolic pathways of paclitaxel to humans could help to refine paclitaxel clinical use.

Assessing *in vivo* association of quercetin and paclitaxel in guinea-pigs is relevant as quercetin is a dietary compound, known as a CYP2C8 inhibitor [7] and has therapeutic potential as an anti-cancer drug [18,19]. A significant decrease in the biliary excretion of unchanged paclitaxel and 6α-hydroxypaclitaxel was observed after co-treatment with quercetin in our investigation. The concurrent use of a natural compound such as quercetin could considerably alter paclitaxel metabolism and disposition. Recent studies have investigated *in vivo* association of paclitaxel and flavonoids including quercetin in rats in order to improve paclitaxel biodisponibility after oral administration [20,21]. Therefore, it seemed to be of interest to perform these studies in guinea-pigs.

Ketoconazole, a known CYP3A4 inhibitor, can inhibit 6α-hydroxypaclitaxel formation at high concentration and has been described as a non-competitive inhibitor of CYP2C8 [22]. *In vivo* drug interaction between ketoconazole and rosiglitazone has been recently reported, probably due to the inhibition of CYP2C8 by ketoconazole [23]. A significant decrease in the biliary excretion of both hydroxylated metabolites was observed after association of ketoconazole with paclitaxel in our investigation.

Although a few inhibitors of CYP2C8 used in the clinical situation are described to date, it seems to be of interest to investigate *in vivo* metabolic interactions of paclitaxel with inhibitors of this isoenzyme. Trimethoprim frequently combined with sulfamethoxazole and cotrimoxazole have been recently reported as *in vitro* inhibitors of CYP2C8 and CYP2C9, respectively [24]. A recent study has described a clinical interaction between trimethoprim and repaglinide, probably by CYP2C8 inhibition due to trimethoprim [25]. Cotrimoxazole, used as an anti-bacterial agent, can be

potentially co-administered with paclitaxel in the clinical situation. No significant alteration in the biliary excretion of unchanged drug or metabolites was observed with co-administration of cotrimoxazole. According to our results, cotrimoxazole might not alter paclitaxel metabolism in the clinical situation.

Patients receive premedication including dexamethasone to prevent hypersensitivity reactions due to paclitaxel administration. Dexamethasone is also a well-known inducer of CYP3A4 and is reported to induce the CYP2C8 isoform *in vitro* [26]. Paclitaxel metabolism was not significantly altered *in vivo* by dexamethasone pretreatment in guinea-pigs.

The present study has shown decreased cumulative biliary excretion of paclitaxel and its metabolites in guinea-pigs after co-administration of ketoconazole or quercetin, suggesting a possible higher pharmacological activity of paclitaxel in the clinical outcome. To conclude, relevant metabolic and drug–drug interaction studies of paclitaxel can be performed in guinea-pigs.

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