The effect of *Echinacea purpurea* on the pharmacokinetics of docetaxel

Andrew K. L. Goey,¹ Irma Meijerman,² Hilde Rosing,³
Jacobus A. Burgers,⁴ Marja Mergui-Roelvink,⁵ Marianne Keessen,⁵
Serena Marchetti,⁵ Jos H. Beijnen^{1,3} & Jan H. M. Schellens^{1,5}

¹Department of Pharmaceutical Sciences, Division of Pharmacoepidemiology & Clinical Pharmacology and ²Department of Pharmaceutical Sciences, Division of Pharmacology, Utrecht University, Utrecht, ³Department of Pharmacy & Pharmacology, Slotervaart Hospital/The Netherlands Cancer Institute, Amsterdam and ⁴Department of Thoracic Oncology and ⁵Department of Clinical Pharmacology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- The herbal immunostimulant *Echinacea purpurea* (*E. purpurea*) is widely used among cancer patients.
- *E. purpurea* has been shown to induce cytochrome P450 3A4 (CYP3A4) *in vitro* and in clinical studies.
- CYP3A4 is extensively involved in the metabolism of many anticancer drugs, such as docetaxel.

WHAT THIS STUDY ADDS

- This is the first clinical study to investigate the pharmacokinetic interaction between *E. purpurea* and an anticancer drug metabolized by CYP3A4.
- The commercially available *E. purpurea* extract did not significantly alter the pharmacokinetics of docetaxel.
- The applied *E. purpurea* formulation at the recommended dose may be combined safely with docetaxel and presumably also with other anticancer drugs primarily metabolized by CYP3A4.

Correspondence

Dr Andrew K. L. Goey PharmD,
Department of Pharmaceutical Sciences,
Division of Pharmacoepidemiology &
Clinical Pharmacology, Utrecht University,
Universiteitsweg 99, 3584 CG Utrecht, The
Netherlands.
Tel.: +31 6 2025 0137
Fax: +31 3 0253 9166
E-mail: andrewgoey@hotmail.com

Keywords

clinical trial, CYP3A4, docetaxel, *Echinacea purpurea*, herb–drug interactions, pharmacokinetics

Received

12 December 2012

Accepted

10 May 2013

Accepted Article Published Online

23 May 2013

AIMS

The herbal medicine *Echinacea purpurea* (*E. purpurea*) has been shown to induce cytochrome P450 3A4 (CYP3A4) both *in vitro* and in humans. This study explored whether *E. purpurea* affects the pharmacokinetics of the CYP3A4 substrate docetaxel in cancer patients.

METHODS

Ten evaluable cancer patients received docetaxel (135 mg, 60 min IV infusion) before intake of a commercially available *E. purpurea* extract (20 oral drops three times daily) and 3 weeks later after a 14 day supplementation period with *E. purpurea*. In both cycles, pharmacokinetic parameters of docetaxel were determined.

RESULTS

Before and after supplementation with *E. purpurea*, the mean area under the plasma concentration–time curve of docetaxel was 3278 ± 1086 and 3480 ± 1285 ng ml⁻¹ h, respectively. This result was statistically not significant. Nonsignificant alterations were also observed for the elimination half-life (from 30.8 ± 19.7 to 25.6 ± 5.9 h, $P = 0.56$) and maximum plasma concentration of docetaxel (from 2224 ± 609 to 2097 ± 925 ng ml⁻¹, $P = 0.30$).

CONCLUSIONS

The multiple treatment of *E. purpurea* did not significantly alter the pharmacokinetics of docetaxel in this study. The applied *E. purpurea* product at the recommended dose may be combined safely with docetaxel in cancer patients.

Introduction

The use of complementary and alternative medicines among cancer patients and the associated risk of herb–drug interactions have increased over recent years [1, 2]. Especially for anticancer drugs, which usually have narrow therapeutic windows, these interactions could have serious consequences, such as an increased risk of toxicities or undertreatment.

Among cancer patients, *Echinacea* is a widely used herbal supplement. In a survey including 318 cancer patients, *Echinacea* was the most popular herbal medicine, used by 21% of all users of complementary and alternative medicines [3]. *Echinacea* was also reported to be the second-most popular pharmacological complementary and alternative medicines agent among cancer patients enrolled into phase I clinical trials [4].

Echinacea is generally used to stimulate the immune system and to prevent the common cold and upper respiratory infections [5, 6]. The most common species of *Echinacea* are *Echinacea angustifolia*, *Echinacea pallida* and *Echinacea purpurea* (*E. purpurea*). The components of *Echinacea* responsible for the pharmacological effects are caffeic acid derivatives, alkylamides, polysaccharides and glycoproteins [7]. Of these components, caffeic acid derivatives and the more bioavailable alkylamides are found in ethanolic liquid extracts for medicinal use [8].

The use of *Echinacea* by cancer patients may interfere with their conventional chemotherapy via interactions with the cytochrome P450 (CYP) 3A4 isoenzyme system. This enzyme system is involved in the metabolism of many anticancer drugs. Both in supersomes and in hepatocytes, it has been shown that *Echinacea* extracts have the potential to inhibit CYP3A4 *in vitro* [9, 10]. There are indications that *Echinacea* is also capable of inducing CYP3A4. Induction of CYP3A4 by *E. purpurea* has been shown in healthy volunteers, in whom the systemic exposure to the CYP3A4 probe midazolam was significantly decreased after supplementation with *E. purpurea* for 28 days [11]. In another clinical study with midazolam in healthy volunteers, *E. purpurea* also affected CYP3A4 function [12]. In this study on volunteers, the systemic clearance of intravenous (IV) midazolam was significantly increased, which reflects induction of hepatic CYP3A4 activity, while intestinal CYP3A4 was not significantly affected, as shown by the lack of significant alterations in oral clearance of orally administered midazolam [12]. In a third clinical study, no significant effect of *E. purpurea* on midazolam pharmacokinetics was reported in healthy volunteers [13]. Thus, both *in vitro* and clinical results showed that *E. purpurea* has the potential to affect CYP3A4, but results concerning inhibition and induction are inconsistent.

An anticancer drug for which systemic exposure may be affected via CYP3A4 modulation by *E. purpurea* is docetaxel. Docetaxel has, among other indications, been approved for the treatment of locally advanced or meta-

static breast cancer, nonsmall cell lung cancer and hormone-refractory metastatic prostate cancer at doses ranging from 75 to 100 mg m⁻², administered as a 1 h IV infusion every 3 weeks. Pharmacokinetic interactions between docetaxel and *E. purpurea* could be expected, because docetaxel is extensively metabolized by CYP3A4. As docetaxel is administered intravenously, hepatic CYP3A4 is mainly involved in its metabolism. Expected induction of hepatic CYP3A4 by *E. purpurea* may lead to decreased plasma levels of docetaxel. For docetaxel, systemic exposure has shown to be a good predictor for its efficacy and toxicity [14]. Thus, CYP3A4 induction by *E. purpurea* could lead to undertreatment in patients receiving docetaxel chemotherapy.

Currently, no clinical studies concerning pharmacokinetic interactions between *E. purpurea* and anticancer drugs have been reported. Results of the present study may provide valuable information about the safety of concomitant use of *E. purpurea* with other anticancer agents metabolized by CYP3A4. In the present study, the primary objective was to determine the effect of *E. purpurea* on the pharmacokinetics of docetaxel. The secondary objective was to assess the effect of *E. purpurea* supplementation on safety parameters, such as grade 3 and 4 toxicities induced by docetaxel [according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE, version 3.0)].

Methods

Patients

This clinical study was performed at the Netherlands Cancer Institute (NKI, Amsterdam, The Netherlands). Patients with histological or cytological proof of cancer for whom treatment with docetaxel was considered to be of therapeutic benefit (e.g. advanced breast, gastric, oesophagus, bladder, prostate, ovarian, nonsmall cell lung, head and neck cancer) were included. Other inclusion criteria were as follows: age ≥ 18 years, performance status ≤ 2 according to the World Health Organization scale, life expectancy > 3 months, absolute neutrophil count $\geq 1.5 \times 10^9$ l⁻¹, platelet count $\geq 100 \times 10^9$ l⁻¹, haemoglobin level ≥ 6.0 mmol l⁻¹, hepatic function as defined by serum bilirubin ≤ 1.5 times the upper limit of normal and alanine aminotransferase and aspartate aminotransferase ≤ 2.5 times the upper limit of normal, renal function as defined by serum creatinine ≤ 1.5 times the upper limit of normal or creatinine clearance ≥ 50 ml min⁻¹, able and willing to swallow and retain oral medication, to comply with the protocol procedures and to follow dietary restrictions.

Patients were excluded in the event of any treatment with investigational drugs within 30 days before the start of the study or the use of herbal supplements within 6 weeks prior to study treatment. Other exclusion criteria

were as follows: alcoholism, drug addiction, psychotic disorders leading to non-adequate follow-up, concomitant use of multidrug resistance and CYP3A-modulating drugs, uncontrolled infectious disease, HIV-1 or HIV-2 type patients, unresolved (>grade 1) toxicities of previous chemotherapy, bowel obstruction or motility disorders that may influence the absorption of drugs, pregnancy, chronic use of H₂-receptor antagonists or proton pump inhibitors, neurological disease that may render a patient at increased risk for peripheral or central neurotoxicity and presence of symptomatic cerebral or leptomeningeal metastases.

The study (EudraCT number: 2008-000886-41) has been approved by the Medical Ethical Committee of the NKI, and all patients provided written informed consent prior to study entry. All patients were treated between April 2009 and March 2010.

Drug administration

Docetaxel (Taxotere®; Aventis Pharma SA, Antony Cedex, France) was administered intravenously and was supplied in a 15 ml clear glass vial containing 2 ml of a 40 mg ml⁻¹ docetaxel solution in polysorbate 80. Standard docetaxel pretreatment consisted of oral dexamethasone 8 mg two times daily for three consecutive days: 1 day before, on the day of docetaxel administration and 1 day after. Furthermore, commercially available *E. purpurea* drops were used (A. Vogel Echinaforce®, batch 08 K0302; Biohorma BV, Elburg, The Netherlands). These drops were labelled to contain 95% aerial parts and 5% roots of *E. purpurea*.

Study design and procedures

On day 1, all patients received docetaxel at an absolute dose of 135 mg given as a 60 min IV administration (cycle 1). The dose of 135 mg was based on a safe dose of 75 mg m⁻² and a mean body surface area of 1.8 m². From day 7 until the morning of day 22, the patients ingested 20 drops of the *E. purpurea* extract three times daily. On day 22, the second cycle of docetaxel was administered according to the same dosing schedule as on day 1 (Figure 1). The

follow-up of each patient ended with an end-of-treatment visit 3 weeks after day 22.

Docetaxel analysis

Blood samples for assessment of docetaxel pharmacokinetics were drawn at predose, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 7, 10, 24 and 48 h after the start of the docetaxel infusion. Blood was collected in heparinized tubes and centrifuged at 1500g for 10 min at 4°C. Subsequently, plasma was separated and stored at -20°C until analysis.

Docetaxel plasma levels were quantified using a validated liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) assay with a lower limit of quantification of 0.25 ng ml⁻¹ [15].

Analysis of *E. purpurea* constituents: alkylamides

In order to check the compliance to *E. purpurea* intake, patients had to keep diaries. In addition, they were called at regular intervals by the research team, and single blood samples were collected in heparinized tubes on days 7, 14 and 22. In a subset of four patients, the pharmacokinetics of alkylamides were studied by collection of blood samples at $t = 0, 0.5, 1$ and 2 h after administration of *E. purpurea*. Plasma was separated after centrifugation at 1500g for 10 min at 4°C and stored at -20°C until quantitative analysis of dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutylamides (DTAI) using a validated LC-MS/MS assay [16].

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated using noncompartmental analysis with R software (version 2.10.1; R Development Core Team, Vienna, Austria) by employing validated scripts.

The following pharmacokinetic parameters of docetaxel were calculated: area under the plasma concentration-time curve from time zero to infinity (AUC_{0-∞}), elimination half-life ($t_{1/2}$) and maximum plasma concentration (C_{max}).

Statistical analysis

For each patient, the values of AUC_{0-∞}, $t_{1/2}$ and C_{max} of docetaxel in cycle 1 (before *E. purpurea*) were compared

Figure 1

Study design. Abbreviation is as follows: PK, pharmacokinetics

with values obtained in cycle 2 (after *E. purpurea*). After logarithmic transformation of these parameters, Student's paired *t*-test ($\alpha = 0.05$) was performed by use of *R*.

Docetaxel-related adverse events

Docetaxel-related adverse events during cycles 1 and 2 were registered according to NCI CTCAE version 3.0. Adverse events were considered as docetaxel related when rated as 'possibly', 'probably' or 'definitely' related by the investigator.

Results

Patients

Eleven patients were included (Table 1), of whom one patient needed to be replaced because the second docetaxel course was not administered due to her deteriorated physical condition. Hence, in total 10 patients were eligible for evaluation.

Effect of *E. purpurea* on the pharmacokinetics of docetaxel

In Figure 2, the mean plasma concentration–time curves of docetaxel in the absence and presence of *E. purpurea* are presented. The individual differences in docetaxel $AUC_{0-\infty}$ are depicted in Figure 3. In five patients, an increase of the $AUC_{0-\infty}$ of docetaxel was observed after supplementation with *E. purpurea*, while in the other five patients the $AUC_{0-\infty}$ decreased. The mean values of pharmacokinetic parameters of docetaxel are shown in Table 2. Intake of *E. purpurea* did not result in statistically significant changes in $AUC_{0-\infty}$, C_{max} and $t_{1/2}$ of docetaxel.

Table 1

Patient characteristics (*n* = 11)

Gender	
Female	2
Male	9
Age (years)	
Median	58
Range	42–67
Race	
Caucasian	11
World Health Organization performance status	
0	5
1	3
2	3
Primary tumour	
Ovarian	5
Nonsmall cell lung carcinoma	2
Endometrium	1
Unknown primary tumour	2
Oesophageal	1

Docetaxel-related adverse events

The incidence of docetaxel-related adverse events differed between the two docetaxel courses. During the course without *E. purpurea*, 24 adverse events (grade 1–2, 22

Figure 2

Mean ($\pm$ SD) plasma concentration–time curves for docetaxel alone and after *E. purpurea* supplementation (*n* = 10). $\cdots\diamond\cdots$, docetaxel 135 mg; $\text{---}\blacksquare\text{---}$, docetaxel 135 mg + *E. purpurea*

Figure 3

Individual values of the area under the plasma concentration–time curve extrapolated to infinity ($AUC_{0-\infty}$) for docetaxel before and after *E. purpurea* supplementation (*n* = 10)

Table 2

Summary of docetaxel pharmacokinetic parameters

Parameter	Day 1: docetaxel alone	Day 22: with pretreatment of <i>E. purpurea</i>	<i>P</i> value
$AUC_{0-\infty}$ (ng ml ⁻¹ h)	3278 $\pm$ 1086	3480 $\pm$ 1285	0.51
C_{max} (ng ml ⁻¹)	2224 $\pm$ 609	2097 $\pm$ 925	0.30
$t_{1/2}$ (h)	30.8 $\pm$ 19.7	25.6 $\pm$ 5.9	0.56

Pharmacokinetic data are given as means $\pm$ SD and were obtained on day 1 (docetaxel alone) and day 22 (14 days after start of *Echinacea purpurea*). The *P* values were obtained from Student's paired *t* test. Abbreviations are as follows: $AUC_{0-\infty}$, area under the docetaxel plasma concentration–time curve extrapolated to infinity; C_{max} , peak plasma concentration; and $t_{1/2}$, half-life of the terminal disposition phase.

events; and grade 3–4, two events) were reported in nine patients, while 16 adverse events (grade 1–2, 15 events; and grade 3–4, one event) occurred in six patients in the course after *E. purpurea* supplementation. However, in the majority of the patients the incidence of docetaxel-related adverse events did not correlate with changes in the $AUC_{0-\infty}$ of docetaxel.

The most common adverse events were fatigue (cycle 1, five events; and cycle 2, four events), alopecia (five events in both cycles), rash (cycle 1, three events; and cycle 2, one event) and allergic reaction (cycle 1, two events; and cycle 2, one event), and were mostly of grade 1–2.

Adherence to *E. purpurea* intake

Adherence was confirmed by inspection of patients' diaries, telephone calls and inspection of returned bottles. Bioanalysis of alkylamides in plasma samples was less applicable to demonstrate adherence. Several bioanalytical assays have been developed for quantification of alkylamides such as undeca-2-ene-8,10-diynoic acid isobutylamide [17] and DTAI [16]. In the present study, DTAI, the most abundant alkylamides in *E. purpurea*, were determined. Unfortunately, in the majority of the patients' samples collected at random time points on days 14 and 22, DTAI plasma levels were below the lower limit of quantification of 0.01 ng mL^{-1} . Pharmacokinetic blood sampling from 0 until 2 h after ingestion of *E. purpurea* revealed that DTAI had already reached the lower limit of quantification after 2 h [16].

Discussion

Based on significant induction of CYP3A4 by *E. purpurea* in previous clinical studies with midazolam [11, 12], the pharmacokinetic interaction between *E. purpurea* and docetaxel was investigated in the present clinical study. In contrast to the significant interaction in our clinical study with the herbal antidepressant St John's wort and docetaxel [18], *E. purpurea* did not significantly affect systemic exposure to docetaxel in the present study. Intraindividual changes in AUC (Figure 3) were in line with an estimated intraindividual variability in docetaxel clearance of 25% [19]. There are no clear explanations for the more remarkable 93% increase in $AUC_{0-\infty}$ in one patient. The incidence of docetaxel-related adverse events, however, did not increase in this patient (data not shown).

Compared with the clinical studies in which significant induction of CYP3A4 by *E. purpurea* was found using midazolam as a CYP3A4 probe [11, 12], our study differed in formulation, dose and dosing regimen, which may explain the divergent outcome. First, in the study of Penzak *et al.* [11], *E. purpurea* was administered for a longer time period (28 days). In the study of Gorski *et al.* [12], however,

a shorter supplementation period (8 days) was applied, but the dosing frequency was higher (four times daily). Second, comparison between the contents of the *E. purpurea* formulations used in the midazolam studies [11, 12] and in the present study is complicated. The formulations of Penzak *et al.* [11] and Gorski *et al.* [12] contained 500 and 400 mg *E. purpurea* extract, respectively. Our commercial product was only labelled to contain 95% aerial parts and 5% roots of *E. purpurea*, and no information was provided about the total amount of extract used. Previously, this formulation has been shown to contain $18\text{--}34 \mu\text{g mL}^{-1}$ DTAI [5, 9]. In contrast, contents of DTAI or other alkylamides in the extracts used in the midazolam studies [11, 12] were not specified. Due to these differences in specifications and the absence of clinical interaction studies with Echinaforce® and midazolam, the effects of Echinaforce® on midazolam pharmacokinetics remain unknown.

Besides differences in the amounts and phytochemical content of *E. purpurea* extracts, differences in their origin may also have contributed to the conflicting clinical outcomes. For example, alkylamide content is known to vary considerably across different parts of *E. purpurea* plants [20], and DTAI are more abundant in roots than in leaves. Consequently, the root extract used by Gorski *et al.* [12] was likely to contain more DTAI than our product and could exert a more potent effect on CYP3A4. The ability of alkylamides and *E. purpurea* extracts to induce CYP3A4, however, is inconclusive according to *in vitro* data. Recently, Modarai *et al.* have shown that *E. purpurea* extract and isolated alkylamides did not significantly induce CYP3A4 in HepG2 cells [21]. However, the lack of an effect on CYP3A4 could be explained by the use of HepG2 cells. It has been shown previously that LS180 cells are to be preferred over HepG2 cells to study CYP3A4 induction, because LS180 cells show higher CYP3A4 expression [22]. In LS180 cells, our group has shown significant induction of CYP3A4 by isolated alkylamides and *E. purpurea* extracts using a gene reporter assay, which is a reliable method to assess the CYP3A4 induction potential of compounds [23]. Induction of CYP3A4 became significant ($P < 0.05$) at relatively high concentrations of 10 and $100 \mu\text{g mL}^{-1}$ alkylamides and *E. purpurea* extract (data not shown).

In addition to the moderate CYP3A4-inducing properties of *E. purpurea*, its systemic exposure could be insufficient to induce hepatic CYP3A4 significantly in the present study. For example, pharmacokinetic analysis of DTAI indicated that plasma levels of these major alkylamides were undetectable or in the lower range of the calibration curve ($<0.08 \text{ ng mL}^{-1}$) halfway through the supplementation period. In addition, DTAI were also rapidly eliminated within 2 h after intake. This finding indicates that the absence of DTAI in plasma samples collected during the study was caused by low systemic absorption and rapid elimination of DTAI. As plasma levels of DTAI

were not quantifiable or hardly quantifiable throughout the study period on days 14 and 22, compliance with *E. purpurea* supplementation could not be checked by pharmacokinetic analysis of DTAI. However, inspection of patient diaries and returned bottles of *E. purpurea* indicated that patients ingested their drops according to the schedule.

Besides the dosing regimen and content of the applied *E. purpurea* product, docetaxel pretreatment with dexamethasone may also have contributed to the lack of a significant effect of *E. purpurea* on the pharmacokinetics of docetaxel. Dexamethasone is a known inducer of CYP3A4 [24]. Assuming induction of CYP3A4 by dexamethasone, systemic exposure to docetaxel could have been decreased already in both courses, thus making the inductive effect of *E. purpurea* during the second course less noticeable. However, results regarding clinical effects of dexamethasone on CYP3A4 are conflicting. A significant pharmacodynamic interaction has been shown between dexamethasone and the CYP3A4 substrate lapatinib [25], while dexamethasone did not significantly alter docetaxel pharmacokinetics in Asian patients [26]. Presumably, these differences in outcomes resulted from differences in exposure to dexamethasone. In the lapatinib study, the median duration of treatment with dexamethasone was 11 days [25], which was substantially longer than the 3 day treatment period with dexamethasone in the study with docetaxel in Asians [26]. These data suggest that treatment with dexamethasone for 3 days in the present study would have had only a modest inductive effect on CYP3A4.

While this study focused on CYP3A4, the drug efflux transporter P-glycoprotein (P-gp, ABCB1) is also involved in the pharmacokinetics of docetaxel. In accordance with CYP3A4, P-gp is also regulated by the nuclear pregnane X receptor. Consequently, upregulation of P-gp by *E. purpurea* could have resulted in decreased plasma levels of docetaxel. In clinical practice, however, the role of P-gp in docetaxel pharmacokinetics does not seem to be relevant. For example, the potent P-gp inhibitors R101933 [27, 28] and zosuquidar [29] did not significantly alter plasma levels of docetaxel in cancer patients. Furthermore, there were no significant associations between several P-gp polymorphisms and docetaxel clearance [30]. Moreover, *E. purpurea* is unlikely to affect P-gp function, because no significant interactions were found in clinical studies with *Echinacea* extracts and the sensitive P-gp substrates fexofenadine [11] and digoxin [31].

Significant clinical interactions between *E. purpurea* and the CYP3A4 and CYP3A5 substrate midazolam indicate that *E. purpurea* also has the potential to interact with CYP3A5. Corresponding to CYP3A4, the polymorphic CYP3A5 enzyme is also regulated by pregnane X receptor and is involved in the metabolism of docetaxel [32]. However, potential CYP3A5 induction is not likely to affect docetaxel pharmacokinetics significantly, because the

affinity of docetaxel for CYP3A4 is approximately 10 times higher than for CYP3A5 [33]. In agreement with this finding, no significant correlation was observed between the inactive CYP3A5*3 genotype, which is present in the majority of Caucasians, and docetaxel clearance in cancer patients [26, 30].

This study was not planned in a randomized crossover design. Considering the risk of tumour progression, randomization was not in the interest of patients with advanced cancer. Patients randomized to the group starting with *E. purpurea* intake would then have to wait for 14 days prior to receiving their first cycle of docetaxel. The absence of randomization may be seen as a limitation of this study. However, the fixed treatment sequence in this study is not likely to introduce substantial bias to the pharmacokinetic results.

No significant period effect of docetaxel exposure is expected based on data published in the literature [34, 35]. A modest inpatient variability of the AUC_{0-24h} of docetaxel (mean ratio of cycle 2 to cycle 1 was 1.11 ± 0.14) was reported after repeated administration of docetaxel administered over 1 h at a dose of 55 mg m^{-2} every 3 weeks [34]. Accordingly, a similar inpatient variability was reported after repeated 3 weekly administration of docetaxel dosed at 100 mg m^{-2} over 1 h [35]. In addition, the pharmacokinetic end-points in the present study are objective outcomes; therefore, biased results by learning effects are very unlikely. Furthermore, before the start of the second docetaxel treatment, patients underwent physical examination, and laboratory values were checked to ensure that inclusion and exclusion criteria were still met. It can thus be assumed that patients' basic medical conditions were comparable between the two cycles.

Carryover effects were also not likely to affect the pharmacokinetic results, because docetaxel levels were not quantifiable in the predose plasma samples of cycle 2. Thus, the washout period of 3 weeks was adequate.

Furthermore, a validated LC-MS/MS assay for docetaxel analysis was used, and for every patient the plasma samples of both cycles were analysed within the same analytical run. The sequence of treatment was therefore not likely to affect the bioanalysis of docetaxel.

It should be noted that the outcome of this study applies only to the specific *E. purpurea* formulation and dose used in the present study. As stated above, alkylamide distribution varies in different parts of *E. purpurea* plants [20] and also in several liquid *E. purpurea* preparations [36]. Thus, the risk of CYP3A4-mediated interactions may be product dependent.

In conclusion, our findings showed that at the recommended dose and schedule of a commercially available *E. purpurea* extract no statistically significant interference with docetaxel pharmacokinetics could be demonstrated. This result indicates that the applied *E. purpurea* formulation may be combined safely with docetaxel.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: support from the Dutch Cancer Society for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

This work was supported by the Dutch Cancer Society [UU 2007–3795]. We would like to thank Roel Maas-Bakker (Department of Pharmaceutical Sciences, Division of Pharmacoepidemiology & Clinical Pharmacology, Utrecht University) for his technical assistance in the in vitro experiments performed at Utrecht University.

REFERENCES

- Engdal S, Klepp O, Nilsen OG. Identification and exploration of herb-drug combinations used by cancer patients. *Integr Cancer Ther* 2009; 8: 29–36.
- Ulbricht C, Chao W, Costa D, Rusie-Seamon E, Weissner W, Woods J. Clinical evidence of herb-drug interactions: a systematic review by the natural standard research collaboration. *Curr Drug Metab* 2008; 9: 1063–120.
- Werneke U, Earl J, Seydel C, Horn O, Crichton P, Fannon D. Potential health risks of complementary alternative medicines in cancer patients. *Br J Cancer* 2004; 90: 408–13.
- Dy GK, Bekele L, Hanson LJ, Furth A, Mandrekar S, Sloan JA, Adjei AA. Complementary and alternative medicine use by patients enrolled onto phase I clinical trials. *J Clin Oncol* 2004; 22: 4810–5.
- Woelkart K, Marth E, Suter A, Schoop R, Raggam RB, Koidl C, Kleinhapfl B, Bauer R. Bioavailability and pharmacokinetics of *Echinacea purpurea* preparations and their interaction with the immune system. *Int J Clin Pharmacol Ther* 2006; 44: 401–8.
- Colalto C. Herbal interactions on absorption of drugs: mechanisms of action and clinical risk assessment. *Pharmacol Res* 2010; 62: 207–27.
- Woelkart K, Bauer R. The role of alkamides as an active principle of *echinacea*. *Planta Med* 2007; 73: 615–23.
- Matthias A, Blanchfield JT, Penman KG, Toth I, Lang CS, De Voss JJ, Lehmann RP. Permeability studies of alkylamides and caffeic acid conjugates from *echinacea* using a Caco-2 cell monolayer model. *J Clin Pharm Ther* 2004; 29: 7–13.
- Modarai M, Gertsch J, Suter A, Heinrich M, Kortenkamp A. Cytochrome P450 inhibitory action of *Echinacea* preparations differs widely and co-varies with alkylamide content. *J Pharm Pharmacol* 2007; 59: 567–73.
- Hellum BH, Hu Z, Nilsen OG. The induction of CYP1A2, CYP2D6 and CYP3A4 by six trade herbal products in cultured primary human hepatocytes. *Basic Clin Pharmacol Toxicol* 2007; 100: 23–30.
- Penzak SR, Robertson SM, Hunt JD, Chairez C, Malati CY, Alfaro RM, Stevenson JM, Kovacs JA. *Echinacea purpurea* significantly induces cytochrome P450 3A activity but does not alter lopinavir-ritonavir exposure in healthy subjects. *Pharmacotherapy* 2010; 30: 797–805.
- Gorski JC, Huang SM, Pinto A, Hamman MA, Hilligoss JK, Zaheer NA, Desai M, Miller M, Hall SD. The effect of *echinacea* (*Echinacea purpurea* root) on cytochrome P450 activity in vivo. *Clin Pharmacol Ther* 2004; 75: 89–100.
- Gurley BJ, Gardner SF, Hubbard MA, Williams DK, Gentry WB, Carrier J, Khan IA, Edwards DJ, Shah A. In vivo assessment of botanical supplementation on human cytochrome P450 phenotypes: citrus aurantium, *Echinacea purpurea*, milk thistle, and saw palmetto. *Clin Pharmacol Ther* 2004; 76: 428–40.
- Bruno R, Hille D, Riva A, Vivier N, ten Bokkel Huinink WW, van Oosterom AT, Kaye SB, Verweij J, Fossella FV, Valero V, Rigas JR, Seidman AD, Chevallier B, Fumoleau P, Burris HA, Ravdin PM, Sheiner LB. Population pharmacokinetics/pharmacodynamics of docetaxel in phase II studies in patients with cancer. *J Clin Oncol* 1998; 16: 187–96.
- Kuppens IE, van Maanen MJ, Rosing H, Schellens JH, Beijnen JH. Quantitative analysis of docetaxel in human plasma using liquid chromatography coupled with tandem mass spectrometry. *Biomed Chromatogr* 2005; 19: 355–61.
- Goey AK, Rosing H, Meijerman I, Sparidans RW, Schellens JH, Beijnen JH. The bioanalysis of the major *Echinacea purpurea* constituents dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutylamides in human plasma using LC-MS/MS. *J Chromatogr B Analyt Technol Biomed Life Sci* 2012; 902: 151–6.
- Goey AK, Sparidans RW, Meijerman I, Rosing H, Schellens JH, Beijnen JH. A sensitive LC-MS/MS method for the quantitative analysis of the *Echinacea purpurea* constituent undeca-2-ene-8,10-diynoic acid isobutylamide in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 2011; 879: 41–8.
- Goey AKL, Meijerman I, Rosing H, Keessen M, Beijnen JH, Schellens JHM. Abstract 761: phase I interaction study of docetaxel with supplementation of St. John's wort. *Cancer Res* 2012; 72: (Suppl. 1): 184.
- Launay-Iliadis MC, Bruno R, Cosson V, Vergniol JC, Oulid-Aissa D, Marty M, Clavel M, Aapro M, Le Bail N, Iliadis A. Population pharmacokinetics of docetaxel during phase I studies using nonlinear mixed-effect modeling and nonparametric maximum-likelihood estimation. *Cancer Chemother Pharmacol* 1995; 37: 47–54.
- Perry NB, van Klink JW, Burgess EJ, Parmenter GA. Alkamide levels in *Echinacea purpurea*: a rapid analytical method revealing differences among roots, rhizomes, stems, leaves and flowers. *Planta Med* 1997; 63: 58–62.
- Modarai M, Silva E, Suter A, Heinrich M, Kortenkamp A. Safety of Herbal Medicinal Products: *echinacea* and Selected Alkylamides Do Not Induce CYP3A4 mRNA Expression. *Evid Based Complement Alternat Med* 2011; 2011: 213021.

- 22** Harmsen S, Koster AS, Beijnen JH, Schellens JH, Meijerman I. Comparison of two immortalized human cell lines to study nuclear receptor-mediated CYP3A4 induction. *Drug Metab Dispos* 2008; 36: 1166–71.
- 23** Luo G, Cunningham M, Kim S, Burn T, Lin J, Sinz M, Hamilton G, Rizzo C, Jolley S, Gilbert D, Downey A, Mudra D, Graham R, Carroll K, Xie J, Madan A, Parkinson A, Christ D, Selling B, LeCluyse E, Gan LS. CYP3A4 induction by drugs: correlation between a pregnane X receptor reporter gene assay and CYP3A4 expression in human hepatocytes. *Drug Metab Dispos* 2002; 30: 795–804.
- 24** Pascucci JM, Drocourt L, Fabre JM, Maurel P, Vilarem MJ. Dexamethasone induces pregnane X receptor and retinoid X receptor- α expression in human hepatocytes: synergistic increase of CYP3A4 induction by pregnane X receptor activators. *Mol Pharmacol* 2000; 58: 361–72.
- 25** Teo YL, Saetaew M, Chanthawong S, Yap YS, Chan EC, Ho HK, Chan A. Effect of CYP3A4 inducer dexamethasone on hepatotoxicity of lapatinib: clinical and in vitro evidence. *Breast Cancer Res Treat* 2012; 133: 703–11.
- 26** Goh BC, Lee SC, Wang LZ, Fan L, Guo JY, Lamba J, Schuetz E, Lim R, Lim HL, Ong AB, Lee HS. Explaining interindividual variability of docetaxel pharmacokinetics and pharmacodynamics in Asians through phenotyping and genotyping strategies. *J Clin Oncol* 2002; 20: 3683–90.
- 27** van Zuylen L, Sparreboom A, van der Gaast A, Nooter K, Eskens FA, Brouwer E, Bol CJ, de Vries R, Palmer PA, Verweij J. Disposition of docetaxel in the presence of P-glycoprotein inhibition by intravenous administration of R101933. *Eur J Cancer* 2002; 38: 1090–9.
- 28** van Zuylen L, Sparreboom A, van der Gaast A, van der Burg ME, van Beurden V, Bol CJ, Woestenborghs R, Palmer PA, Verweij J. The orally administered P-glycoprotein inhibitor R101933 does not alter the plasma pharmacokinetics of docetaxel. *Clin Cancer Res* 2000; 6: 1365–71.
- 29** Fracasso PM, Goldstein LJ, de Alwis DP, Rader JS, Arquette MA, Goodner SA, Wright LP, Fears CL, Gazak RJ, Andre VA, Burgess MF, Slapak CA, Schellens JH. Phase I study of docetaxel in combination with the P-glycoprotein inhibitor, zosuquidar, in resistant malignancies. *Clin Cancer Res* 2004; 10: 7220–8.
- 30** Puisset F, Chatelut E, Dalenc F, Busi F, Cresteil T, Azema J, Poublanc M, Hennebelle I, Lafont T, Chevreau C, Roche H. Dexamethasone as a probe for docetaxel clearance. *Cancer Chemother Pharmacol* 2004; 54: 265–72.
- 31** Gurley BJ, Swain A, Williams DK, Barone G, Battu SK. Gauging the clinical significance of P-glycoprotein-mediated herb-drug interactions: comparative effects of St. John's wort, Echinacea, clarithromycin, and rifampin on digoxin pharmacokinetics. *Mol Nutr Food Res* 2008; 52: 772–9.
- 32** van Schaik RH. CYP450 pharmacogenetics for personalizing cancer therapy. *Drug Resist Updat* 2008; 11: 77–98.
- 33** Shou M, Martinet M, Korzekwa KR, Krausz KW, Gonzalez FJ, Gelboin HV. Role of human cytochrome P450 3A4 and 3A5 in the metabolism of taxotere and its derivatives: enzyme specificity, interindividual distribution and metabolic contribution in human liver. *Pharmacogenetics* 1998; 8: 391–401.
- 34** Van Veldhuizen PJ, Reed G, Aggarwal A, Baranda J, Zulfikar M, Williamson S. Docetaxel and ketoconazole in advanced hormone-refractory prostate carcinoma: a phase I and pharmacokinetic study. *Cancer* 2003; 98: 1855–62.
- 35** Brunsvig PF, Andersen A, Aamdal S, Kristensen V, Olsen H. Pharmacokinetic analysis of two different docetaxel dose levels in patients with non-small cell lung cancer treated with docetaxel as monotherapy or with concurrent radiotherapy. *BMC Cancer* 2007; 7: 197.
- 36** Modarai M, Yang M, Suter A, Kortenkamp A, Heinrich M. Metabolomic Profiling of Liquid Echinacea Medicinal Products with In Vitro Inhibitory Effects on Cytochrome P450 3A4 (CYP3A4). *Planta Med* 2009; 76: 378–85.