NCT01110291: induction of CYP3A activity and lowered exposure to docetaxel in patients with primary breast cancer

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Abstract

Purpose: To study the CYP3A activity before and after docetaxel administration. Furthermore, it was investigated whether peroral midazolam could predict docetaxel exposure and adverse events. Methods: Twenty patients with primary high risk breast cancer were given docetaxel as a 1-hour infusion 80 mg/m^2 in a 21-day cycle in three cycles followed by three cycles of cyclophosphamide, epirubicin and fluorouracil. CYP3A activity was assessed a day before and a day after docetaxel by 7.5 mg oral midazolam. All patients were given peroral dexamethasone a total dose of 45 mg, of which 15 mg was given before docetaxel infusion and 30 mg before the latter assessment of CYP3A activity. All except one patient were given 11-19 mg of intravenous dexamethasone before docetaxel infusion. **Results:** CYP3A activity was clearly induced when assessed a day after docetaxel administration as shown by lower midazolam AUC ($p<0.0001$) and higher AUC ratio (1-OH-midazolam/midazolam, $p=0.018$). The mean docetaxel AUC was about a half of that previously reported in the literature. Incidence of febrile neutropenia was smaller (15%) than reported in literature with comparable docetaxel doses and seemed to associate with slower metabolism. No correlation between pharmacokinetics of midazolam and docetaxel were found at baseline. **[text deleted] Conclusions:** We show here a markedly reduced docetaxel exposure followed by CYP3A induction by, most likely, dexamethasone. Peroral midazolam seemed not to predict docetaxel exposure. Slow CYP3A-mediated metabolism might predispose patients to adverse events of docetaxel.

Keywords: docetaxel, CYP3A activity, **[text deleted]** peroral midazolam, dexamethasone, induction

Introduction

Docetaxel is a semisynthetic taxane shown to improve survival in metastatic breast cancer both as single therapy and in combination regimens [1]. According to data from two adjuvant trials, docetaxel is recommended for patients with early breast cancer at high risk for recurrence [1, 2, 3]. The use of docetaxel is, however, associated with severe toxicity, especially, in adjuvant settings [4]. The most common and severe adverse events are neutropenia and neutropenic fever [2, 4]. Incidence of adverse events increases along with higher docetaxel exposure [5]. Docetaxel clearance is highly variable ranging about 6-fold between individuals (from 5.4 to 29.1 liters/h/m^2) [6]. This interindividual variability is explained by variations in docetaxel elimination rate. Docetaxel is mainly inactivated in the liver via cytochrome P450 (CYP) 3A4 and 3A5 enzymes [7]. In addition, the intestinal P-glycoprotein (P-gp) plays a key role in the fecal elimination of docetaxel by modulating its reabsorption after hepatobiliary secretion [8]. P-gp has been shown to have no effect, however, on the disposition of docetaxel in the systemic circulation [8]. **Also, the plasma concentration of alpha-1-acid glycoprotein, which has been suggested to be the main determinant of docetaxel plasma binding variability, has been shown to affect docetaxel clearance [9, 10].**

[text deleted]

Hepatic CYP3A4 activity measured by erythromycin breath test or clearance of intravenously administered midazolam has been shown to correlate with docetaxel clearance [5, 6]. Both of these assessments require intravenous administration and, erythromycin breath test, additionally, radioactive labelling. Thus, application of these

methods into clinical setting is laborious. We wanted to study the association between CYP3A activity and docetaxel concentration using oral administration of midazolam, which could be easily applied into clinical practice. **[text deleted] In addition,** the possible changes in the CYP3A activity during the treatment cycles were investigated. Furthermore, the relationship between **[text deleted]** docetaxel exposure and patient outcome was assessed.

Materials and methods

Patients

Altogether 20 patients aged 32-58 years with histologically verified high risk breast cancer, for which docetaxel was indicated, were included in this study conducted in the University Hospital of Turku, Finland, between years 2003-2004. High risk for recurrence was predicted by positive lymph nodes (17 patients) or, if node negative, by a histological grade 3 of a T2 tumour (3 patients; table 1). None of the patients had evidence of distant metastases. For eligibility, the patients had to have a performance status of 0 or 1 according to the World Health Organization's (WHO) performance scale. They had to have adequate bone marrow reserve defined as haemoglobin greater than 100 g/l, a leukocyte count greater than 3.0 x 10^9 /l (or a neutrophil count greater than 1.5 x 10^9 /l) and a platelet count greater than 120 x 10^9 /l. Their liver function had to be adequate, which was defined as an alanine aminotransferase level no greater than 1.5 times the normal level, an alkaline phosphatase level no greater than 2.5 times the normal level and a normal bilirubin level. Severe physical or psychiatric disease, pregnancy or lactation and substance abuse were criteria for exclusion. Also, concomitant medication with a known CYP3A substrate, inducer or inhibitor was not allowed. The patients were informed on the study both verbally and in writing, and a written informed consent was obtained. The study protocol was approved by the Ethics Committee of the Hospital District of Varsinais-Suomi, Finland, and the National Agency of Medicines, Finland.

Study plan

Chemotherapy

Docetaxel 80 mg/m^2 of body surface area (BSA) was given as an i.v. infusion during 60 minutes on day 0 repeated three times in a 21-day schedule. The docetaxel dosage was reduced by 20% in altogether 11 patients (in 9 patients during the second and in 2 patients during the third cycle) due to grade 3-4 toxicity (excluding nausea). The cycle was postponed for 2-7 days in altogether 5 patients due to low neutrophil count (2 patients) or practical reasons (3 patients). The docetaxel concentration was measured from blood samples collected on day 0 in cycles 1 and 3. Blood samples were drawn into heparinised tubes 2-4 minutes before the end of the infusion and 15 and 45 minutes, as well as 3, 6, 12, and 24 hours after the end of the infusion. The blood samples were centrifuged for 10 min at 2000 g, and the separated plasma was stored at -20 °C until analyzed.

Three weeks after the last docetaxel regimen, the patients were additionally given a standard combination treatment, composed of cyclophosphamide, epirubicin and fluorouracil (CEF), which was repeated three times.

Pre-medications

All patients were pre-medicated with dexamethasone to reduce the incidence and severity of fluid retention and hypersensitivity reactions. The patients received dexamethasone 7.5 mg p.o. altogether six times, in the evening on day -1, one hour before the docetaxel infusion and in the evening on day 0, in the morning and evening

on day 1 and in the morning on day 2. In addition, the patients were given dexamethasone 0.2 mg/kg i.v. and tropisetron 5 mg i.v. just before the docetaxel infusion to reduce nausea. One patient with unstable diabetes was not given the intravenous dexamethasone, but tropisetron was given to all patients.

CYP3A phenotyping with midazolam

Midazolam phenotyping was done a day before (day -1) and a day after (day +1) the docetaxel infusion in cycles 1 and 3. On day -1 the midazolam phenotyping was completed before the first dose of dexamethasone. The patients were given midazolam 7.5 mg p.o. in the morning, and repeated blood samples for analysis of midazolam and its CYP3A-dependent metabolite 1-OH-midazolam were drawn into EDTA tubes just before as well as 1, 2, 4, 6 and 8 hours after midazolam administration. The blood samples were centrifuged for 10 min at 2000 g, and the separated plasma was stored at -20 °C until analyzed. **[text deleted]**

Evaluations of safety and toxicity

For safety and practical reasons the patients were hospitalized from the morning of day -1 until the evening of day +1 of each cycle. The following laboratory tests were performed no more than 7 days prior to the first docetaxel infusion: blood haemoglobin, white blood cell (WBC) count, neutrophil count, platelet count and serum creatinine, alanine aminotransferase, alkaline phosphatase, bilirubin, albumin and ionized calcium level. These tests (excluding the ionized calcium) were also repeated on day 0 before the docetaxel infusion and on days 7 and 14 of each

docetaxel cycle and three weeks after the last docetaxel infusion. Blood haemoglobin, WBC, neutrophil and platelet counts were also performed prior to the start of chemotherapy of CEF cycles.

The evaluation of toxicity was done before the start of each docetaxel cycle and three weeks after the last docetaxel cycle by using a validated scoring system following the WHO's instructions (National Cancer Institute/National Institutes of Health Common Toxicity Criteria, available online at http://ctep.cancer.gov/reporting/ctc.html). Of all the recorded evaluations, the clinically most significant adverse events associated with docetaxel therapy were included in statistical analysis. These were hospitalization due to febrile neutropenia and low neutrophil count. The neutrophil counts, which were measured three times between the docetaxel cycles, were graded according to WHO and the lowest grading of cycles 1 and 3 were used in the analysis. In addition, the lowest grading of each of the three cycles was added together and this sum was used in the analysis (table 2).

Analytical methods

Assessment of midazolam and 1-OH-midazolam concentrations

Samples for analysis of midazolam and 1-OH-midazolam were prepared for analysis with liquid-liquid extraction. After the addition of 50 μl of internal standard solution (100 ng/ml of nitrazepam in 5 % methanol/water) the mixture was extracted with 5 ml of ethyl acetate. About 4.5 ml of the organic layer was evaporated to dryness in PP-tubes under a gentle stream of nitrogen at about 40 ^{o}C and the residue was dissolved

in 100 μl of a solution containing 50 % methanol / 50 % 5 mM ammonium formate (v/v). The sample was transferred into an autosampler vial from which 22 μl was injected into the high-performance liquid chromatography (HPLC) - mass spectrometer (MS) / MS system.

Separations for analysis of the content of midazolam and 1-OH-midazolam were performed with a Symmetry C_{18} 100 x 2.1 mm i.d. (3.5 μM) column coupled with an integrated Symmetry C_{18} guard column (Waters Corp). The mobile phase consisted of eluents A (methanol) and B (5 mM ammonium formate buffer). The eluent system was isocratic: 68 % of A and 32 % of B. The eluent B was filtered before use through a 0.45 μm HV filter (Millipore). The flow rate was 0.2 ml/min.

Mass spectrometric detection was carried out using a PE SCIEX API 365 triple-quadruple instrument using positive ISI (ion spray ionisation) and MRM (multiple reaction monitoring) mode. The needle potential was set to 3.5 kV and the temperature of the heated nitrogen gas was 380 ^{o}C. The declustering potential was set to 25 V, focusing potential to 100 V, and entrance potential to 8.0 V for midazolam, to 4.0 V for 1-OH-midazolam and to 5.0 V for internal standard. The collision energy was set to 33 V for midazolam and to 29 V for 1-OH-midazolam and internal standard. Nebulizer gas (nitrogen) was set to value 12, curtain gas (nitrogen) was set to value 12 and collision gas (nitrogen) was set to value 4.0. The parent ion – splitter ion pairs detected were *m/z* 326.1 – 291.0 (midazolam), *m/z* 342.1 – 324.0 (1-OH-midazolam) and *m/z* 282.1 – 236.0 (internal standard).

The quantitation of the analytes was accomplished with HPLC-MS/MS. The calculations of quantitations were based on peak areas of the analytes and the internal standard. The data from the HPLC-MS/MS analyses were collected using Applied Biosystems Analyst 1.2 software. The peak integrations, calibration curves and quantitations were generated with the same software. The standard curves (concentration range 0.20-50 ng/ml) were generated using weighted (1/x) linear regression. The lower limits of quantification were 1.0 ng/ml and 0.2 ng/ml for midazolam and 1-OH-midazolam, respectively. The interday coefficient of variation at 5 ng/ml concentration was 8.3% for midazolam and 10.7% for 1-OH-midazolam.

Assessment of docetaxel concentrations

Docetaxel concentrations were determined by a sensitive and specific assay transferred from sanofi-aventis and partially validated at Parexel International Bioanalytical Laboratories. Docetaxel and the internal standard were separated from human plasma by a solid-phase extraction (SPE) using an Empore^{TM} 96 well extraction disk plate C18 system. The combined extracts were transferred into HPLC injection vials and placed in the pre-cooler injector. 10 μl of sample extracts were injected into the LC-MS/MS system.

Liquid chromatography was performed using a HP1100 system from Agilent. The analytical column (100 x 2.1 mm i.d.) was packed with a 5 μm Hypersil BDS C18 stationary phase. The elution was carried out with a mobile phase made of a mixture of methanol and 0.1 % of formic acid (60/40; v/v) filtered and degassed by a 0.2 μm solvent filter before use. The mobile phase was used at a flow rate of 0.25 ml/min.

The HPLC system was connected to a PE Sciex API 3000 MS/MS system, operating in the Turbo Ion Spray positive mode. The MS/MS system was focused in the MRM mode to monitor the ion transitions: 808.5 – 527.2 (docetaxel) and 832.4 – 491.1 (internal standard). The API 3000 MS/MS system was remotely controlled by a Power Macintosh model G3 using a MAC OS 7 (or higher revision) operating system. A plasma standard curve was daily elaborated between 1 and 500 ng/ml of docetaxel in heparinised human plasma. The concentrations of pharmacokinetic samples were calculated from calibration curves obtained by a $1/x^2$ weighted linear regression analysis. A method set-up was performed before the start of study sample analysis and all method set-up results were in the predefined acceptance criteria (relative standard deviation (RSD) ≤15 %, except ≤20 % at the lower limit of quantitation (LLQ); bias ± 15 %, except ± 20 % at LLQ for calibration standards and quality control samples).

[text deleted]

Pharmacokinetic analysis

The ratio of the area under the concentration-time curve ($AUC_{0-\infty}$) values of 1-OH-midazolam and midazolam (*i.e.* AUC ratio) was determined and used as a marker for CYP3A activity. In six patients the midazolam or the 1-OH-midazolam concentration was below the lower limit of quantification in samples taken 1, 6 or 8 hours after drug intake. For these values, half of the quantification limit value was used in the analysis. One patient had some detectable midazolam in the baseline (0 h) sample taken on day 1 in the cycle 3. This baseline concentration was reduced from the measurements prior to pharmacokinetic analysis. The pharmacokinetics of docetaxel was described

by $AUC_{0-\infty}$, the peak plasma concentration (C_{max}), the time from drug intake to peak concentration (t_{max}), the mean elimination half-life ($t_{1/2}$) and clearance (Cl). The pharmacokinetic analyses were performed using the WinNonlin Professional program, version 4.1 (Pharsight Corporation, Mountain View, California, USA).

Statistical analysis

The analysis of variance for repeated measures was used to study the changes in drug concentrations. The analysis of variance (ANOVA) or the Kruskall-Wallis (Mann Whitney U) test and correlation analysis (Pearson or Spearman) were used in comparisons between adverse events and drug concentrations/genetics. The Cox proportional hazard analysis was used to study survival. P values of 5 % or less were regarded as significant. The data which failed to fit the normal distribution was log-transformed prior to analysis. The non-parametric methods were used if the data failed to fit the normal distribution after log-transformation. The data was analysed using the SAS Enterprise Guide for Windows, version 3.0 (SAS Institute Inc., Cary, NC, USA).

Results

Docetaxel pharmacokinetics

The mean docetaxel pharmacokinetic parameters after a dose of 80 mg/m^2 are presented in table 3. Eleven patients had a dose reduction to 60 mg/m^2 after cycle 1 or 2. The mean docetaxel AUC was about a half of that previously reported in the literature in European populations (table 3). Docetaxel clearance was accordingly clearly greater than what has been seen in other studies with corresponding doses (table 3).

CYP3A activity before and after docetaxel infusion

The mean midazolam AUC was significantly lower on days +1 than on days -1 (mean 58.5 h*ng/ml vs. 95.6 h*ng/ml, $p<0.0001$, cycle 1; mean 65.0 h*ng/ml vs. 113.6 h*ng/ml, $p<0.0001$, cycle 3) (figure 1, table 4). There were no significant difference between the day -1 values ($p=0.069$) or day +1 values ($p=0.188$) of cycles 1 and 3 (figure 1, table 4). The AUC ratio 1-OH-midazolam/midazolam was also significantly changed during the cycles ($p=0.018$). The mean AUC ratio was higher on days +1 (mean 0.32 and 0.30) than on days -1 (mean 0.27 and 0.26; table 4), but statistically the difference was significant only between the day +1 of cycle 1 and the day -1 of cycle 3 ($p=0.017$, figure 1, table 4).

Association between docetaxel and midazolam concentrations

Docetaxel AUC or clearance did not correlate with midazolam concentrations on day -1 in cycle 1 (figure 2). The studied parameters for midazolam concentration were midazolam AUC calculated to the last measuring point (AUC_{last}) (p=0.22 and p=0.42) and to infinity ($AUC_{0-\infty}$) (p=0.54 and p=0.63), midazolam weight-adjusted clearance (p=0.89 and p=0.67), AUC ratio (1-OH-midazolam/midazolam) (p=0.88 and p=0.76), midazolam concentration at 4 hours (p=0.38 and p=0.50) and at 6 hours (p=0.89 and p=0.93), and AUC ratio at 4 hours (p=0.88 and p=0.97) and 6 hours (p=0.86 and p=0.56). In cycle 3, docetaxel AUC correlated positively with midazolam AUC ratio (r=0.50, p=0.025), and midazolam AUC ratio at 4 hours (r=0.67, p=0.0012) and 6 hours (r=0.65, p=0.0017). Docetaxel clearance correlated negatively with midazolam AUC ratio (r=-0.38, p=0.094), AUC ratio at 4 hours (r=-0.53, p=0.017) and AUC ratio at 6 hours (r=-0.52, p=0.018) in cycle 3.

Docetaxel concentration and adverse events

Altogether three patients (15 %) were hospitalized due to febrile neutropenia during the docetaxel treatments, two in cycle 1 and one in cycle 2. The patient hospitalized in cycle 2 had first only fever and neutropenia developed later. She was diagnosed with influenza A. Two of the hospitalized patients received leukocyte growth factors. All the patients had grade 3 or 4 neutropenia at some point during the docetaxel treatment. The neutrophil count gradings are shown in table 2. Docetaxel AUC correlated with the given docetaxel dose (based on body surface area) in cycle 3 (r=0.53, p=0.016), but only weakly in cycle 1 (r_s=0.38, p=0.097). Docetaxel clearance did not correlate with the given dose in either cycle (p=0.49 and p=0.67). There were no associations between the given docetaxel dose and adverse events (low neutrophil

count, p=0.47 and p=0.50 in cycle 1 and 3, respectively, or febrile neutropenia, p=0.95 in cycle 1 only).

The docetaxel AUC (p=0.87) and clearance (p=0.37) of the three patients with neutropenic fever were not significantly different from the other patients' values, although the patients with febrile neutropenia did have somewhat lower mean docetaxel clearance than the other patients (mean 76 l/h vs. 88 l/h). The patients with febrile neutropenia also tended to have higher docetaxel C_{max} values than the others (mean 2029 ng/ml vs. 1566 ng/ml, p=0.064, figure 3). There were no associations between the docetaxel AUC (p=0.16, Kruskall-Wallis test or r_s=-0.24, p=0.30, cycle 1; p=0.32, ANOVA or r=-0.35, p=0.13, cycle 3), clearance (p=0.91, Kruskall-Wallis test or r_s=0.18, p=0.45, cycle 1; p=0.74, ANOVA or r=0.29, p=0.21, cycle 3) or C_{max} (p=0.14, Kruskall-Wallis test or r_s=-0.17, p=0.47, cycle 1; p=0.32, ANOVA or r=-0.32, p=0.17, cycle 3) and the low neutrophil count (nadir or sum of nadirs).

CYP3A activity and adverse events

CYP3A activity defined as the AUC ratio of 1-OH-midazolam and midazolam showed no association with neutrophil count (data not shown). There was a trend towards lower AUC ratio among the three patients with neutropenic fever compared with the other patients (mean 0.19 vs. 0.29, p=0.088, figure 3).

[text deleted]

Survival analysis

The mean follow-up time of the patients after the initiation of the study treatments was 4.4 years (range 0.75 - 5.8 years). Four patients died during the follow-up period, 9 (two patients), 12 or 23 months after initiation of therapy, respectively. Midazolam AUC or AUC ratio (assessed before docetaxel infusion), docetaxel AUC or clearance, low neutrophil count or febrile neutropenia was not associated with survival after treatment. Also, known prognostic factors lymph node metastases, Her2, Ki-67, estrogen and progesterone receptor status were not predictors of survival (data not shown).

Discussion

Although docetaxel clearance has been shown to be highly variable, the docetaxel concentrations in this study were generally clearly lower than what has been reported in the literature. The mean docetaxel AUC was about a half of and the mean clearance more than double to that reported with corresponding doses of docetaxel in European populations (table 3). Only **Caucasians** were included in this comparison to avoid the possible influence of race on docetaxel metabolism. It has been suspected, for example, that Asians may have lower CYP3A activity than Caucasians [11].

Docetaxel metabolism has been shown to be inducible by CYP3A inducers [12] and, therefore, other drugs and herbal products affecting CYP3A activity were forbidden during the study. We found that the CYP3A activity was markedly and rapidly induced when assessed a day after docetaxel infusion as evidenced by significantly lower midazolam AUC values and higher 1-OH-midazolam/midazolam AUC ratios on day +1 and by low docetaxel exposure. By the point of assessment of CYP3A activity, the patients had received dexamethasone 7.5 mg perorally altogether four times (30 mg) and all except one patient also 0.2 mg/kg intravenously (11-19 mg). Dexamethasone is a known inducer of CYP3A activity [13, 14]. However, Goh et al. have shown that peroral dexamethasone 8 mg x 6 administered every 12 hours, beginning either 24 hours before or after docetaxel treatment, did not significantly alter docetaxel pharmacokinetics in Asian patients with solid tumours [5]. Docetaxel has been shown to induce CYP3A4 gene expression *in vitro* in peripheral mononuclear cells of 16 previously untreated lung cancer patients [15]. Also, in another *in vitro* study, another taxane, paclitaxel, has been shown to increase

CYP3A4 mRNA in human hepatocytes [16]. Hence, taxanes seem to autoinduce their own metabolism at least in *in vitro* conditions. The CYP3A induction we found here in patients could have been caused by either dexamethasone or docetaxel itself or both. **However, the inductive effect of docetaxel has been present in earlier studies as well, so that dexamethasone remains the most likely candidate for the induction.** As a comparison to the before mentioned study by Goh et al. [5], we used a different dosing schedule and route of administration in a genetically different population. In table 3 there are three studies where dexamethasone has been used as premedication in European populations [17, 18, **19]**. As a comparison to these studies, we used a larger total dose of dexamethasone of which about a half was administered intravenously. **In addition,** in our study the dexamethasone premedication was administered during a shorter time period before docetaxel infusion than in the other three studies (in 12 hours vs. in 24 hours). **[text deleted]**

Although the docetaxel exposure in this study was smaller than expected, grade 3 or 4 neutropenia occurred in all patients at some point of docetaxel treatment. As a comparison, in a large adjuvant setting with primary breast cancer patients in comparable performance status, and with peroral dexamethasone premedication 8mg x 6 every 12 hours beginning 24 hours before chemotherapy, (n=744) the incidence of grade 3 or 4 neutropenia was 65.5% [2]. On the other hand, febrile neutropenia occurred in 25-29% of those patients, whereas its incidence was only 15% in our study [2]. In another study in Asian patients receiving docetaxel alone, grade 3 or 4 neutropenia occurred in 17 of 23 (74%) and febrile neutropenia in 6 of 23 (26%) of patients after 75 mg/m^2 of docetaxel [5]. These patients received peroral dexamethasone 8mg x 6 every 12 hours beginning 24 hours after docetaxel treatment

in the first treatment cycle and 24 hours before docetaxel treatment in the second treatment cycle [5]. Interestingly, after an approximate follow-up period of 5 years, 80% of the patients in our study were alive and free of disease. In the study by Martin et al. [2] estimated rates of disease-free survival at five years was 75 % in patients who received adjuvant docetaxel. Thus, although the docetaxel exposure was smaller than expected, it did not seem to affect survival. Naturally, firm conclusions of the incidence of adverse events or survival cannot be made based on the quite small sample size of our study. For the same reason, known prognostic factors of the primary tumour failed to associate with survival in this material.

There were no associations between docetaxel and midazolam concentrations in cycle 1. An association was found in cycle 3, but it was inverse. Dexamethasone is also a known inducer of P-gp [20]. A P-gp inhibitor has been shown not to alter docetaxel clearance. However, the fecal excretion of docetaxel was markedly reduced by a P-gp inhibitor, but levels of the major CYP3A4-mediated metabolites of docetaxel in feces were significantly increased [8]. Thus, docetaxel pharmacokinetics may have been modified by multiple mechanisms which have led to a random correlation between docetaxel and midazolam in cycle 3. However, at baseline with no prior inducers, no correlations were found. **While oral midazolam is a well-established and sensitive marker for CYP3A activity, the difference in the mode of administration vs. docetaxel (i.v.) may at least partly explain the lack of correlation between midazolam and docetaxel exposure.** Also, there were no clear associations between CYP3A activity or docetaxel exposure and adverse events. Thus, it is concluded that oral midazolam cannot be used as a predictor of docetaxel exposure at least when dexamethasone is given. **[text deleted]** The weak associations of occurrence of febrile

neutropenia and higher docetaxel C_{max} values and lower midazolam AUC ratio suggest that slow docetaxel metabolism may predispose to adverse events. **[text deleted]**

To our knowledge, we are the first to report CYP3A induction followed by dexamethasone premedication and docetaxel infusion by investigating CYP3A activity using midazolam as a probe drug. However, since we did not have a control group in which the docetaxel pharmacokinetics would have been measured without prior dexamethasone administration, we cannot definitely conclude dexamethasone to be responsible for the induction. Also, we did not investigate the role of alpha-1-acid glycoprotein in docetaxel metabolism. The sample size in this study was too small to draw any firm conclusions, especially on survival. However, the low docetaxel exposure in this study may have contributed to the low incidence of febrile neutropenia and should have affected survival, as well. Studies with larger patient populations and with direct assessment of docetaxel pharmacokinetics both before and after dexamethasone are needed to confirm these results. Until that it is recommended to give dexamethasone as a supplemental drug according to regimens found not to influence docetaxel clearance, *i.e.* by keeping the pre-infusion dose as little as possible, avoiding intravenous administration of dexamethasone and managing the adverse effects of docetaxel by continuing dexamethasone administration after docetaxel infusion.

Conclusions

In conclusion, CYP3A activity was clearly and rapidly induced after administration of dexamethasone 30-49 mg and docetaxel 60-80 mg/m^2. This induction was disappeared after a 21-day wash-out period. The docetaxel exposure was markedly smaller than expected based on literature, but this did not have influence on survival or occurrence of neutropenia. Incidence of febrile neutropenia was, however, smaller than reported in other studies with comparable docetaxel dosage. Moreover, peroral midazolam did not seem to have value as a predictor of docetaxel pharmacokinetics in this setting. According to results from this and other studies, slow CYP3A-mediated metabolism might predispose patients to adverse events of docetaxel.

Competing interests

This study has received financial support from sanofi-aventis.

Authors' contributions

JH gathered and interpreted the results, performed the statistical analysis and drafted the manuscript. LS participated in the design of the study and carried out the clinical phase of the study. SJ participated in the design, coordination and clinical phase of the study. SP participated in the study design and coordination. KL participated in the design and conduction of the study, in interpretation of the results and acquired funding for the study. All authors read and approved the final manuscript.

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Figure legends

Figure 1. Midazolam AUC **(A)** and AUC ratio of 1-OH-midazolam/midazolam **(B)** after a 7.5 mg oral midazolam a day before (day -1) and a day after (day +1) docetaxel infusion. Docetaxel was given in three cycles, with a 20- day wash-out period between the cycles. The mean values of the groups are marked with a line.

Figure 2. At baseline, there were no correlations between docetaxel clearance and midazolam AUC_{inf} (*i.e.* $AUC_{0-\infty}$) (r_s=0.11, p=0.63) **(A)** or 1-OH-midazolam/midazolam AUC ratio (r_s=-0.07, p=0.76) **(B)**.

Figure 3. Docetaxel C_{max} values after docetaxel 80 mg/m^2 and midazolam AUC ratios (means + SD) assessed a day before docetaxel infusion in patients with febrile neutropenia (n=3, mean C_{max} 2029 ng/ml, mean AUC ratio 0.19) and in patients with no febrile neutropenia (n=17, mean C_{max} 1566 ng/ml, p=0.064; mean AUC ratio 0.29, p=0.088).

Table 1. Patient and tumour characteristics (n=20)

Characteristic		**Data**
Age (years); mean (range)		47 (32-58)
Survival after therapy (days); median (range)		1854 (270-2094)
CYP3A5 SNP g.6986A>G, *1→*3 (n)	*1*1	1
	*1*3	1
	*3*3	18
ABCB1 SNP c.3435C>T (n)	CC	6
	CT	12
	TT	2
Characteristics of the primary tumour		**n**
Tumour	T1	2
	T2	16[1]
	T3	1
	Unknown	1
Node	N0	3
	N1	17
Histology	Ductal	15[2]
	Lobular	5
	Other	1
Grade	2	8
	3	12
Her2	Positive	4
	Negative	14
	Unknown	2
Ki-67	Positive[3]	15
	Negative	4
	Unknown	1
Estrogen receptors	Positive[4]	9
	Negative	10
	Unknown	1
Progesterone receptors	Positive[4]	7
	Negative	12
	Unknown	1

[1]largest diameter of a multifocal tumour
[2]multifocal tumour both ductal and lobular
[3]>17% of tumour cells positive
[4]>20% of tumour cells positive

Table 2. Incidence of adverse events during the three docetaxel treatment cycles.

	The lowest grading of neutrophil count according to WHO, i.e. the neutrophil nadir			
Patient ID	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Sum of nadirs**
2	4*	4	3	11
3	4*	3	3	10
4	3	4	3	10
5	3	3	3	9
6	3	2	3	8
7	3	2	2	7
8	4	3	2	9
9	4	2	2	8
10	3	4	3	10
11	3	3	3	9
12	3	2	3	8
13	4	4	4	12
14	3	1	3	7
15	4	3	3	10
16	3	4*	3	10
17	3	4	2	9
18	4	0	2	6
19	4	3	4	11
20	4	4	3	11
21	3	3	2	8
Sum	**69**	**58**	**56**	**183**
Mean	**3.45**	**2.9**	**2.8**	**9.15**

*hospitalized due to neutropenic fever

Table 3. Pharmacokinetics of docetaxel after 1-hour infusion in high risk breast cancer patients (current study) and in European patients with various malignancies.

Docetaxel dose (mg/m^2)	**AUC (h*ng/ml)**	**Cl (l/h)**	**C_{max} (ng/ml)**	**$t_{1/2}$ (h)**	**N**	**Reference**
CYCLE 1						Current study
80 (range 76.5-81.3)	1737 ± 371	87 ± 27	1635 ± 512	17 ± 5.8	20	
CYCLE 3	p=0.36*	p=0.45*	p=0.31*	p=0.10*		
80 (range 78.5-81.3)	1905 ± 468	79 ± 23	1852 ± 538	15 ± 3.2	9	
60 (range 58.8-65)	1251 ± 379	100 ± 41	1135 ± 453	16 ± 4.1	11	
All patients	1545 ± 529	90 ± 35	1458 ± 604	15 ± 3.7	20	
Data are given as arithmetic mean ± arithmetic SD *p-values between docetaxel pharmacokinetic parameters of cycle 1 and 3 after 80 mg/m^2 of docetaxel Premed.: dexamethasone 7.5 mg p.o. 12 h and 1 h before infusion plus 11-19 mg i.v. just before infusion						
100	5000	43.9			69	[17]
75	3410	43.7				
60	2710	45.1				
Premed.: dexamethasone 8 mg p.o. 24 h, 12 h, just before infusion						
75/100		41.8			69	[18]
Premed.: dexamethasone 8 mg p.o. 24 h, 12 h, just before infusion						
	5340	36.9			25	[19]
Premed.: dexamethasone 8mg p.o. 24 h, 12 h, possibly just before infusion						
70/75/85/100		38.5			57	[21]
Premed.: methylprednisolone 60 mg i.v. 60 min before infusion						
70-75/85/100	4400	38.5			56	[22]
Premed.: methylprednisolone 60 mg i.v. 60 min before infusion						
100	5930	18	5450	9.59	5	[23]
75	3744	22	3128	12.7	6	
60	1838	33	1642	16.9	6	
Premed.: not available						
70/75/85/100	4100	40.0			92	[24]
Premed.: not available						

[table 3 revised]

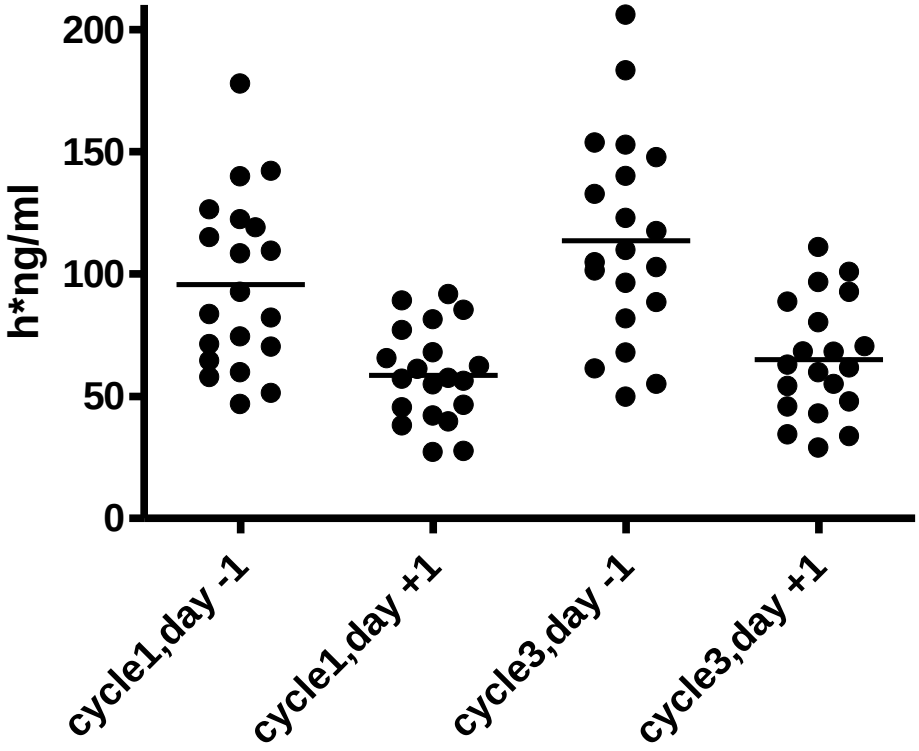
Table 4. Pharmacokinetics of midazolam and its main metabolite 1-OH-midazolam after 7.5 mg oral midazolam in patients with high risk breast cancer. These pharmacokinetic parameters were measured a day before and a day after docetaxel infusion in two treatment cycles.

Variables	**Cycle 1**		**Cycle 3**	
	Day -1	**Day +1**	**Day -1**	**Day +1**
Midazolam				
AUC (h*ng/ml)	96 ± 35	59 ± 19	114 ± 42	65 ± 24
C_{max} (ng/ml)	24 ± 6.9	22 ± 6.6	29 ± 9.0	23 ± 9.7
t_{max} (h)	1.7 (1-8)	1.4 (1-2)	1.5 (1-4)	1.4 (1-2)
$t_{1/2}$ (h)	3.7 ± 1.0	2.7 ± 0.8	3.6 ± 1.0	3.4 ± 1.3
Difference between midazolam AUC values (overall, p<0.0001)				
day -1 vs. day +1	P<0.0001		P<0.0001	
day -1 vs. day -1	P=0.069			
day +1 vs. day +1			P=0.188	
1-OH-midazolam				
AUC (h*ng/ml)	24 ± 7.4	17 ± 5.9	27 ± 10	18 ± 4.8
C_{max} (ng/ml)	8.1 ± 3.0	7.7 ± 4.2	9.2 ± 4.6	7.5 ± 2.6
t_{max} (h)	1.7 (1-8)	1.4 (1-2)	1.5 (1-4)	1.3 (1-2)
$t_{1/2}$ (h)	3.1 ± 0.9	2.9 ± 1.1	2.9 ± 1.0	3.3 ± 1.0
AUC ratio (1-OH-midazolam/midazolam)	0.27 ± 0.09	0.32 ± 0.11	0.26 ± 0.11	0.30 ± 0.09
Difference between midazolam AUC ratios (overall, p=0.018)				
day -1 vs. day +1	P=0.067		P=0.165	
day -1 vs. day -1	P=1.00			
day +1 vs. day +1			P=1.00	
day +1 of cycle 1 vs. day -1 of cycle 3		P=0.017		

Data are given as arithmetic mean ± arithmetic SD except for t_{max} data, which are given as mean and range

Figure 1.

A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100
50
0
h*ng/ml
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cycle1,day +1
cycle3,day -1
cycle3,day +1

B

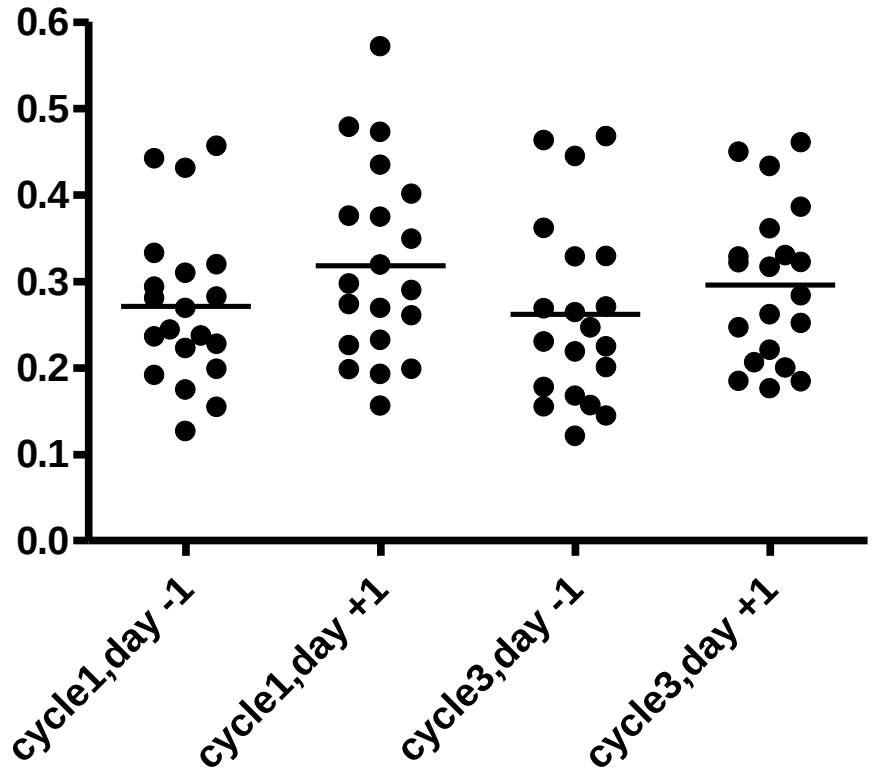
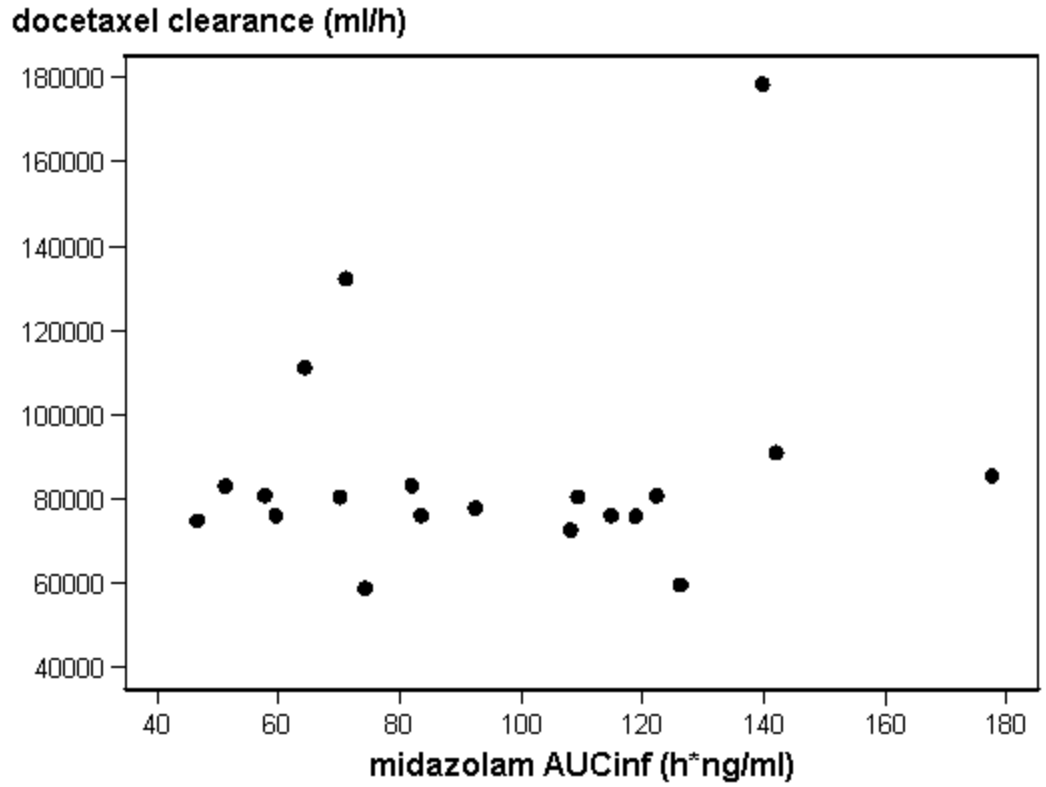
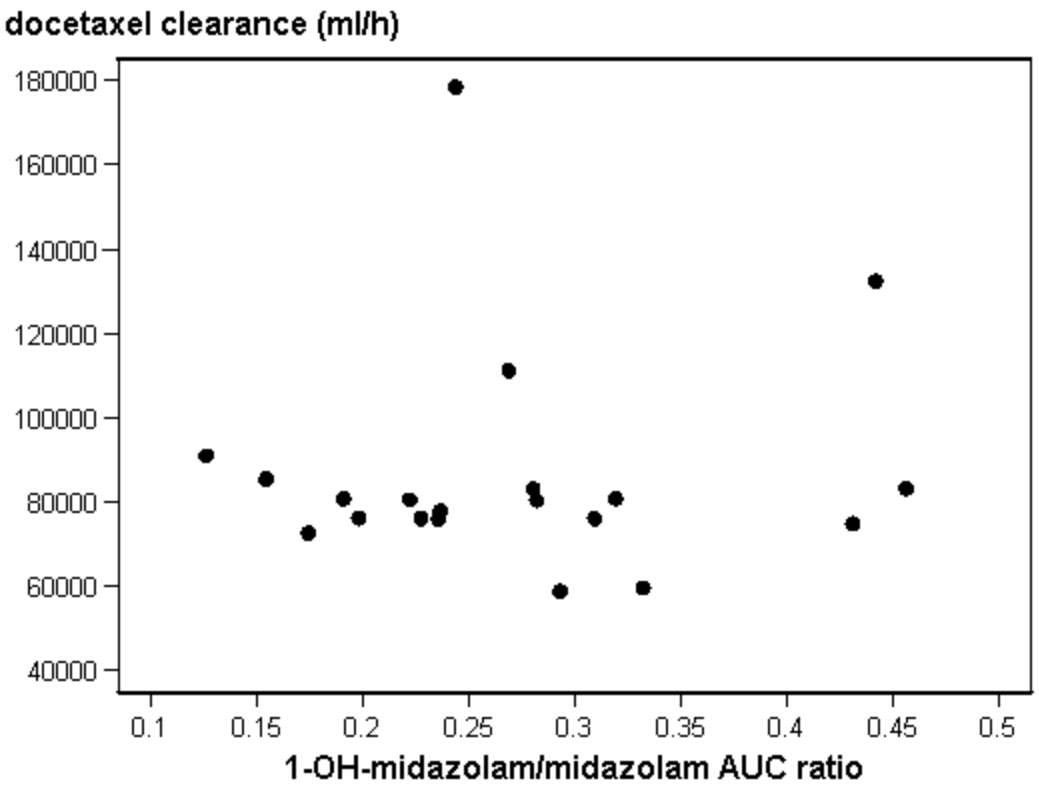

0.6
0.5
0.4
0.3
0.2
0.1
0.0
cycle1,day -1
cycle1,day +1
cycle3,day -1
cycle3,day +1

Figure 2.

A


docetaxel clearance (ml/h)
180000
160000
140000
120000
100000
80000
60000
40000
40
60
80
100
120
140
160
180
midazolam AUCinf (h*ng/ml)

B


docetaxel clearance (ml/h)
180000
160000
140000
120000
100000
80000
60000
40000
0.1
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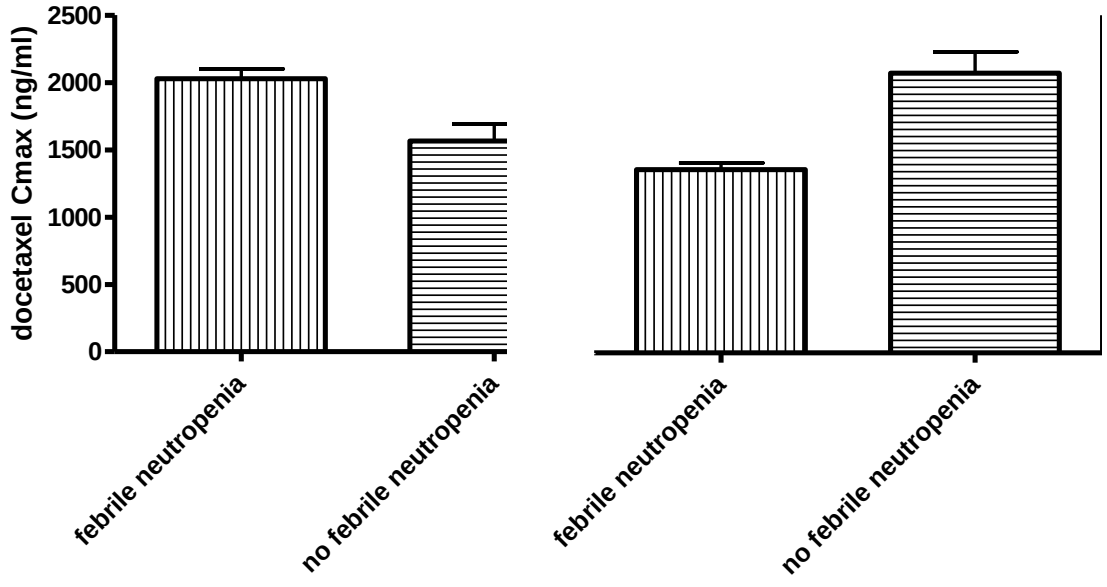
0.2
0.25
0.3
0.35
0.4
0.45
0.5
1-OH-midazolam/midazolam AUC ratio

Figure 3.


docetaxel Cmax (ng/ml)
2500
2000
1500
1000
500
0
febrile neutropenia
no febrile neutropenia
febrile neutropenia
no febrile neutropenia