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Pilot study of rosiglitazone as an *in vivo* probe of paclitaxel exposure

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Paclitaxel and rosiglitazone are primarily metabolized by CYP2C8 and their *in vitro* metabolism by human liver microsomes is correlated. Probe assays that quantify the *in vivo* activity of CYP enzymes which are important in drug metabolism have been developed for use in clinical pharmacology research. A probe of CYP2C8 that is easy to administer and interpret may be valuable for individualized dosing of paclitaxel.

WHAT THIS STUDY ADDS

- This pilot study demonstrates for the first time that there is an *in vivo* correlation between paclitaxel and rosiglitazone exposure. The finding, that a single rosiglitazone plasma concentration after oral dosing may explain significant variance in paclitaxel exposure, suggests that rosiglitazone may satisfy the requirements of a clinically useful *in vivo* probe. However, it is acknowledged that there is a need for further studies evaluating the use of rosiglitazone as a CYP2C8 probe and quantifying the relationship, in order to guide dosing of narrow therapeutic index drugs metabolized primarily by CYP2C8, such as paclitaxel.

AIMS

To evaluate the use of rosiglitazone and the erythromycin breath test (ERMBT), as probes of CYP2C8 and CYP3A4, respectively, to explain inter-individual variability in paclitaxel exposure.

METHODS

The concentration of rosiglitazone at 3 h and ERMBT results were included in a regression model to explain the variability in paclitaxel exposure in 14 subjects.

RESULTS

Rosiglitazone concentration was significantly correlated with paclitaxel exposure ($P = 0.018$) while ERMBT had no predictive value ($P = 0.47$).

CONCLUSIONS

The correlation between the exposure of rosiglitazone and paclitaxel likely reflects mutual dependence on the activity of CYP2C8. Rosiglitazone or similar agents may have value as *in vivo* probes of CYP2C8 activity.

Introduction

Interpatient variability in toxic and therapeutic response to chemotherapy, partially caused by differences in the activity of the CYP450 enzyme system, is a substantial problem in cancer treatment. One emerging approach to characterize a drug's metabolism is through the use of a probe, a marker agent that shares the drug's metabolic pathway. Probe-based tests to measure CYP450 phenotype that are safe, easy to administer and quickly interpretable have been developed for some enzymes [1] relevant to anti-cancer therapy but not all.

Paclitaxel is one of the most effective chemotherapeutic agents used in the treatment of solid tumour malignancies. It is metabolized to inactive metabolites primarily by CYP2C8 with a contribution from CYP3A4 [2]. A probe assay that could explain the significant interpatient variability in paclitaxel exposure might help clinicians select more appropriate doses for an individual patient to optimize efficacy and limit toxicity.

The erythromycin breath test (ERMBT) has been widely implemented to measure hepatic CYP3A4 activity [3]. However, there is no such probe for CYP2C8. Interestingly, the rates of rosiglitazone metabolism and paclitaxel hydroxylation have been shown to correlate in human liver microsomes expressing CYP2C8 [4]. In healthy subjects, a 2 mg oral dose of rosiglitazone is safe, >99% bioavailable and the plasma concentration at 3 h is highly predictive of AUC(0,∞) (r^2 = 0.98) (Clarke, S, GlaxoSmithKline, personal communication), making rosiglitazone an attractive probe candidate.

Despite the known *in vitro* correlation, rosiglitazone has not been previously used as a probe for CYP2C8 to predict paclitaxel exposure in patients. In this study we used rosiglitazone and ERMBT as surrogates of CYP2C8 and CYP3A4 activity, respectively, and hypothesized that the combination of these probes would partially explain the variability in paclitaxel exposure in cancer patients.

Methods

This study was conducted at the General Clinical Research Center (GCRC) and approved by the Institutional Review Board at UNC Chapel Hill. Eligible patients were >18 years old with solid tumour malignancies, were scheduled to receive weekly paclitaxel and provided written informed consent.

On study day 1, the erythromycin breath test (ERMBT) was carried out as previously described [5]. The percentage of the dose exhaled as ^{14}C over 1 h (AUC(0,1 h)) was $\log_e$ transformed and used as a measure of CYP3A4 activity. Next, each subject (all of whom fasted for >6 h) was administered a 2 mg oral dose of rosiglitazone. A blood sample was collected 3 h after dosing and plasma concentration analysis was performed as previously described [6] with an assay lower limit of quantification of 3 ng ml^{-1} and a coefficient of variation <15%.

Approximately 6 h after the rosiglitazone dose, each subject was administered a 1 h infusion of paclitaxel (75 mg m^{-2} to 90 mg m^{-2}) as per their standard treatment. Blood samples (7 ml) were collected prior to infusion and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, and 18–24 h after the start of infusion. Limited sampling, shown to reasonably estimate paclitaxel exposure [7], was employed for patient convenience. The 18–24 h sample was intended for estimation of AUC(0,∞), which was subsequently deemed unreliable due to inadequate estimation of the terminal phase elimination rates. Plasma was separated and stored at −80°C before undergoing analysis via LC/MS/MS as previously described with an assay lower limit of quantification of 10 nM and a coefficient of variation < 15%, to measure total paclitaxel concentration [8].

Paclitaxel pharmacokinetic analysis was performed in WinNonlin Pro Version 5.2.1 (Pharsight Corp., Mountain View, CA) using non-compartmental methods. AUC(0,6 h) was calculated using the linear trapezoidal method and $\log_e$ transformed for use in the primary analysis. Prior to statistical analysis, paclitaxel and rosiglitazone concentration values were adjusted to correspond to standardized doses (81.4 and 1.11 mg, respectively) based on the study mean patient BSA (1.81 m^2), to minimize variability due to differences in doses received and patient size, and were $\log_e$ transformed. BSA was selected as the metric for body composition based on the practice of dosing paclitaxel by BSA. All later discussion refers to dose-adjusted and $\log_e$-transformed values, except where indicated.

Statistical analysis was conducted in SAS 9.2 (SAS Institute Inc, Cary, NC) using a multiple linear regression model with rosiglitazone and ERMBT AUC(0,1 h) the independent variables and paclitaxel AUC(0,6 h) the dependent variable. Descriptive statistics are reported as mean (±SD or min, max), as appropriate.

Results

Twenty patients with planned or ongoing weekly paclitaxel treatment were enrolled. Demographic data for the 14 subjects who had evaluable samples for paclitaxel pharmacokinetic analysis are displayed in Table 1. The dose-adjusted mean paclitaxel AUC(0,6 h) was 6787 (±2506) ng ml^{-1} h, rosiglitazone concentration was 82.3 (±26.0) ng ml^{-1} and ERMBT AUC(0,1 h) was 2.76% h^{-1} (±1.17). In the two-variable regression model rosiglitazone was a statistically significant predictor of paclitaxel AUC(0,6 h) (P = 0.019). However, ERMBT was not (P = 0.47). After exclusion of ERMBT, rosiglitazone alone explained about 38% of the variability in paclitaxel AUC(0,6 h) (r^2 = 0.38, P = 0.018, Figure 1). In follow-up exploratory analyses no other relevant covariates (e.g., age, albumin, cancer type and

Table 1

Subject demographic and clinical characteristics

	Study population (n = 14)
Gender	
Female	11 (79%)
Male	3 (21%)
Ethnicity	
Caucasian	12 (86%)
African American	1 (7%)
Other	1 (7%)
Age (years): Mean (min, max)	45.7 (25, 64)
BSA: Mean (min, max)	1.81 m^2 (1.51, 2.33)
Cancer type	
Breast	4 (29%)
Lung	6 (43%)
Melanoma	2 (14%)
Ovarian	1 (7%)
Other	1 (7%)
Paclitaxel dose: Mean (min, max)	81.37 mg m^{-2} (75, 90)
Concomitant chemotherapy*	
Herceptin	4 (29%)
Carboplatin	6 (43%)
None	4 (29%)

*Neither agent is known to modulate CYP2C8 or CYP3A4 activity.

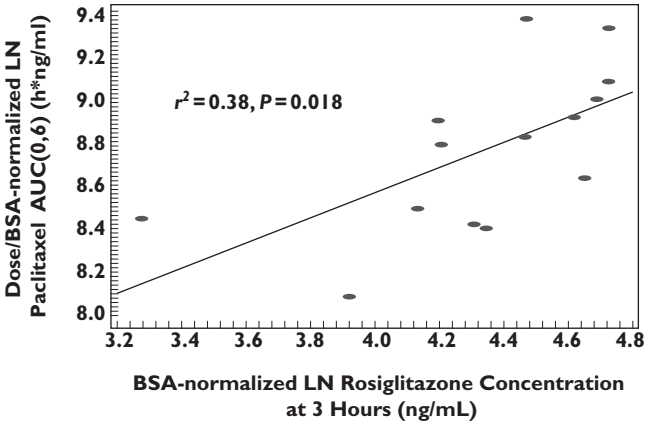

Figure 1

Relationship between $\log_e$ rosiglitazone 3 h concentration (x-axis) and $\log_e$ paclitaxel AUC(0,6 h) (y-axis) (n = 14). Concentrations of both drugs were adjusted to a standard (mean) dose per BSA

smoking status) significantly contributed to the model (data not shown).

Discussion

In this pilot study, a 3 h rosiglitazone plasma concentration after a 2 mg oral dose explained 38% of the variability in paclitaxel exposure. As expected, higher rosiglitazone concentrations were associated with increased paclitaxel exposure. For a variety of reasons, including drug transporters, absorption and compensatory metabolic pathways, rosiglitazone may not be the optimal CYP2C8 probe. Other potential probes, such as pioglitazone which may be more sensitive to changes in CYP2C8 activity, should also be considered for further clinical CYP2C8 probe research [9].

Because this was a small pilot study, supportive analyses of the data were conducted to investigate the robustness of our findings; with results consistent with the primary analysis. The correlation between paclitaxel AUC(0,6 h) and rosiglitazone without dose adjustment was statistically significant (r^2 = 0.33, P = 0.029) while adjusting for mean weight in kg instead of BSA strengthened the findings modestly (r^2 = 0.50, P = 0.004). The correlation between paclitaxel AUC(0,∞) and AUC(0,6 h) was strong (r^2 = 0.92) and use of AUC(0,∞) instead of AUC(0,6 h) did not meaningfully change the results (r^2 = 0.32, P = 0.034). Finally, an even better relationship was found (r^2 = 0.51, P = 0.004) when paclitaxel AUC(0,6 h) and rosiglitazone were rank transformed prior to analysis, suggesting that the findings did not result from the impact of a particularly influential value.

The highest rosiglitazone concentration seen was 120 ng ml^{-1} while the reported K_i of rosiglitazone is 1998 ng ml^{-1} [10]. Thus, same day administration of rosiglitazone and paclitaxel is unlikely to have resulted in competitive enzyme inhibition. In a similar study ERMBT explained 67% of the variability in clearance of docetaxel [3], which is metabolized exclusively by CYP3A4. Our finding that ERMBT was not predictive of paclitaxel clearance was disappointing, but consistent with other evidence that CYP2C8 is the primary enzyme responsible for paclitaxel metabolism. It is also likely that a portion of our unexplained variability can be attributed to the role of drug transporters in the pharmacokinetics of ERMBT and paclitaxel [11]. It should also be noted that both rosiglitazone and paclitaxel are substrates for P-glycoprotein [12, 13] and this may contribute to the correlation we observed.

In conclusion, this report supports further study of rosiglitazone or similar agents as *in vivo* probes of CYP2C8 activity in humans. This is the first report of an *in vivo* association between rosiglitazone and paclitaxel exposure and supports the hypothesis that rosiglitazone may be a reasonable probe for *in vivo* exposure to CYP2C8 substrates.

Competing Interests

UNC has received research funding for various clinical trials from GlaxoSmithKline and Bristol-Myers Squibb, the makers of rosiglitazone and paclitaxel.

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