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Drug interaction studies between paclitaxel (Taxol) and OC144-093 - A new modulator of MDR in cancer chemotherapy

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SUMMARY

The MDR modulator, OC144-093, is a potential candidate for use in cancer therapy and exhibits potent biological activity *in vitro* and *in vivo* when combined with anticancer agents such as paclitaxel [1]. Its inhibitory interaction with P-glycoprotein (Pgp), the mdr1 gene product and a mechanistic participant in multidrug resistance[2], underlies its activity as a modulator of MDR. Having previously shown that OC144-093 is not a substrate for CYP3A [4] we first examined the effects of OC144-093 on paclitaxel metabolism *in vitro*. Using human liver microsomes, we have demonstrated that OC144-093 inhibited the CYP3A mediated metabolism of paclitaxel at high concentrations only (Ki = 39.8 ± 5.1 μM, n=3). Pharmacokinetic results also show that an oral dose of OC144-093, co-administered with paclitaxel caused negligible disturbance of the pharmacokinetic profile for paclitaxel when injected intravenously. In contrast, AUC values were elevated approximately 1.5-fold in all groups treated orally with paclitaxel and OC144-093. Cmax was enhanced approximately 2-fold in the co-dosed group. These characteristics are consistent with Pgp blockade in the gut enhancing oral bioavailability. Elimination properties of paclitaxel were affected only upon multiple dosing of OC144-093. These results warrant the further clinical assessment of OC144-093 as an MDR reversing agent.

INTRODUCTION

Multidrug resistance (MDR) persists as a major problem in the chemotherapeutic treatment of a wide variety of human carcinomas. The mdr1 gene product, P-glycoprotein (Pgp), is the active component involved in the mechanism of many incidences of MDR and acts as an ATP dependent drug efflux pump. Chemotherapeutic agents are actively expelled from MDR tumor cells that over express Pgp. This phenomenon limits the effectiveness and inhibits the target cell toxicity of drugs such as doxorubicin, paclitaxel, etoposide and vincristine [2].

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Nonstandard abbreviations: PgP = P-glycoprotein, MDR = multidrug resistance, HPLC = high performance liquid chromatography, AUC = area under the curve

Studies have shown that P-glycoprotein is a 170 kDa membrane-associated transport protein, localized on the apical surface of cells and tissues and extensively expressed throughout the human body. Its function appears to be protective since it acts as a barrier to guard against the penetration of toxic compounds [6]. Pgp expression is predominant in tissues responsible for absorption and/or elimination processes e.g. small and large intestine, adrenal cortex, renal proximal tubule, pancreatic ductiles, biliary hepatocytes and to a lesser extent, the blood brain barrier, placenta, lungs and stomach [7, 8, 9]. Tumor expression of Pgp can be observed in cancer types exhibiting inherent resistance to chemotherapy or in tumors following treatment with antineoplastic agents known to be Pgp substrates such as doxorubicin, paclitaxel or vincristine [10]. Pgp's function in MDR, being one of effecting drug efflux from target cells, is similar to its endogenous role and appears to be an evolutionary protective mechanism exploited by tumor cells.

Consequently, several strategies have been taken to combat Pgp-mediated MDR including chemosensitization where Pgp inhibitors are used in combination with anticancer agents.

Numerous compounds have been screened/developed for the purpose of MDR modulation and belong to a number of chemical classes ranging from the calcium channel blockers and anti-malarials to anti-arrythmics and immunosuppressants. In general they have been classified as those belonging to the first, second or third generation of Pgp directed MDR modulators. First generation compounds, such as verapamil [11, 12, 13] and cyclosporin A (CsA; [14]) are those which also exhibit inherent pharmacological activity since their MDR reversal properties were observed secondary to their functional clinical use. These agents displayed significant toxicities at doses required to achieve Pgp inhibition. Second generation compounds were developed specifically for the purpose of Pgp inhibition and, in general, were relatively pharmacologically inert structural analogs of their first generation counterparts such as CsA derivative, PSC 833 [15]. Although inherently less toxic, many of these agents induced toxicity complications arising from pharmacokinetic (PK) interaction with co-administered anticancer drugs [16, 17, 18, 19]. OC144-093 is one of the latest third generation modulating compounds investigated for MDR reversal. These compounds are being designed with the intent of alleviating the drug-drug interaction experiences with first and second generation modulators.

Recent evidence suggests that overlapping substrate specificities and tissue distribution exist between drug metabolizing enzymes and Pgp [3]. In particular, cytochrome P450 3A has been shown to be one of the major enzymes involved in the metabolism of several anticancer agents and modulators of MDR [5, 20, 21]. Saturation of its capacity to function as a metabolic catalyst in the liver and extrahepatic metabolic tissues is thought to result in drug-drug interactions [22]. Predetermination of resulting adverse drug reactions is therefore a crucial step in drug development. In addition, the fundamental mechanism of action of Pgp inhibitors may disrupt pharmacokinetic properties of drugs by inhibiting mechanisms of drug absorption, distribution and elimination [23] which must also be addressed.

OC144-093 has been shown to have potent biological MDR reversal activity both *in vitro* and *in vivo* [1]. Since its mechanism of action relies upon the blockade of Pgp activity and common substrates exist between this moiety and drug metabolizing enzymes questions are raised regarding drug interactions between OC144-093 and anticancer agents such as paclitaxel. This report describes an investigation into potential drug interactions between OC144-093 and paclitaxel. Human liver have been used to investigate the inhibition of paclitaxel metabolism by OC144-093. In addition, we report a pharmacokinetic study in CD-1 mice in which pharmacokinetic profiles for paclitaxel administered orally and intravenously are compared with and without three dosing regimes for oral dosing of OC144-093.

Materials and methods

Materials

Human Liver samples were obtained from The Department of Pathology following the removal of metastatic liver tumors at Vancouver Hospital, Vancouver, BC. Histologically normal tissue surrounding tumors was used. Upon surgical removal, liver samples were preserved on ice for up to 1h before flash freezing using liquid nitrogen for storage at -80°C. Paclitaxel (Taxol) was obtained from Bristol Myers Squibb Pharmaceutical Group in vials containing 5ml of a 6mg/ml solution in Cremophor EL: Ethanol (1:1; v:v). OC144-093 and OC144-092 (Internal Standard) were supplied by Ontogen Corp., Carlsbad, CA. Pierce BCA Protein Determination Kit, tolbutamide, sulfaphenazole, quinidine and NADPH (Sigma-Aldrich Canada Ltd., Oakville, Ontario). Magnesium chloride (BDH Chemicals, Toronto, Ontario). Furaphylline (Gentest Corporation, Woburn, MA). Ketoconazole (ICN Pharmaceuticals Inc., Costa Mesa, CA). Diethyldithiocarbamate (ddC) (Aldrich Chemical Company Inc., Milwaukee, Wisconsin). Other chemicals and solvents including acetic acid, acetonitrile, methanol and potassium phosphate were of analytical

grade and were purchased from Fisher Scientific (Fair Lawn, NJ, Canada)

Preparation of human liver microsomes

Washed microsomes were prepared by the classical differential sedimentation method [24]. Microsomes were diluted by suspension in 0.067 M phosphate buffer and the protein concentration was determined using the Pierce BCA Protein Determination Kit with bovine serum albumin as the protein standard. The microsomes were stored at -80°C until use.

Incubations

Human liver microsomal protein (1mg) was incubated at 37°C for 30min with 1-25μM paclitaxel, $MgCl_2$ (10mM), NADPH (5mM) and 0.067 M phosphate buffer (pH 7.4) in a final volume of 0.5ml. Incubations were terminated and paclitaxel and its metabolites were extracted using acetonitrile (2ml) containing 0.8μM internal standard (Baccatin III). The organic extracts were evaporated to dryness and the residues reconstituted in the HPLC mobile phase (120μl) for analysis of 100μl by HPLC. Recovery of paclitaxel was >90%. The limit of paclitaxel detection was 10 ng/incubation volume (0.5ml).

Kinetic and inhibitor studies

Experiments were carried out using human liver microsomes to determine apparent K_i values for OC144-093 inhibition of CYP3A4 mediated paclitaxel metabolism. Enzyme activity was measured by quantification of metabolite formation. It was pre-determined that M1 was a product of CYP3A4 metabolism using specific inhibitors of CYP3A4, ketoconazole (0-3μM) and troleandomycin (0-10 0μM). This product of CYP3A4 metabolism is reported by Desai et. al. [25] to be the 3'-p-hydroxylated metabolite.

To determine whether OC144-093 inhibits paclitaxel metabolism the K_i value for OC144-093 inhibition of paclitaxel metabolism was determined. Metabolite formation was measured at 1, 5, 10 and 25μM concentrations of paclitaxel in the presence of six concentrations of OC144-093 (1-50μM; n=3). Dixon plots were generated (1/V vs. [I]) using mean data from three incubations and Sigmaplot® 4.0 for Windows 95 was the data analysis software used for K_i determination. All kinetic and inhibition studies were carried out in duplicate under linear conditions and incubations contained 1mg of protein and were for 30min.

Pharmacokinetic study for paclitaxel treated alongside OC144-093 in CD-1 mice

Thirty four groups (three mice per group) of female CD-1 mice were administered paclitaxel (taxol) either intravenously or by oral gavage at 16mg/kg and 10mg/kg respectively in a 200μl volume (saline/Cremophor/ethanol; 8/1/1, v/v/v). For groups dosed intravenously with paclitaxel, OC144-093 was administered orally at a dose of 30mg/kg . For groups dosed orally with paclitaxel OC144-093 was co-administered by oral gavage at a dose of 20mg/kg either at the time as paclitaxel or 1 hour before paclitaxel administration. In addition OC144-093 was administered as a split dose, 10mg/kg 1 hour before and 10mg/kg co-administered alongside paclitaxel. OC144-093 was prepared for oral dosing in PEG400:Tween20; 9/1 v/v. Mice in the control group were administered 200μl of vehicle (p.o.). At timepoints 15min, 30min, 45min, 1h, 1.5h, 2h, 4h and 8h following oral paclitaxel administration, mice were anaesthetized with CO_2 and blood was collected by cardiac puncture into microtainer tubes containing EDTA prior to euthanization by cervical dislocation. For the groups dosed intravenously with paclitaxel blood was collected at timepoints described for oral dosing with the exception of the 45min and 1.5h timepoints. Plasma was obtained by centrifugation at 2500rpm for 10 min and 200μl samples were extracted using 1ml Acetonitrile spiked with internal standard, Baccatin III (internal standard, 0.16μM) before evaporation to dryness using a nitrogen stream at room temperature. Samples were reconstituted into 120μl mobile phase (ddH_2O:Acetonitrile, 9:1) and HPLC was carried out for sample analysis using paclitaxel standard curves for quantification. Winnonlin™ software (Pharsight Corporation) was used to determine pharmacokinetic parameters.

HPLC analysis

The HPLC system consisted of an integrated Waters 600E multisolvent delivery pump, 717plus autosampler and 996 photodiode array detector (Waters Corp., Milford, MA). Paclitaxel and the IS (Baccatin III) were resolved on a Nova-Pak C_{18} column (4μm, 150mm x 3.9mm inside diameter; Waters, Milford, Massachussets) with Mobile Phases A and B, ddH_2O and Acetonitrile respectively. The following gradient profile was used: t = 0min, 90% A, 10% B; t = 5 min, 90% A, 10% B; t = 30 min, 35% A, 65% B; t = 40 min, 35% A, 65% B; t = 45 min, 90% A, 10% B; t=50min, 90% A, 10% B. The gradient was formed using a high pressure mixer. The flow rate was 1.0 ml min^{-1}. A Waters 996 Photo Diode Array Detector was used to scan at multiple wavelengths and chromatograms were processed for traces obtained at 230nm.

Table 1: Summary of pharmacokinetic parameters determined for paclitaxel administered both intravenously and orally with and without OC144-093.

	C_{max} (ng/ml)	AUC (ng hr/ml)	$T_{1/2}$ (hr)
Paclitaxel 10mg/kg po	639.4	2187.9	8.0
Paclitaxel 10mg/kg po + 20mg/kg OC144-093 po	1258.9	3054.3	5.8
Paclitaxel 10mg/kg po + 20mg/kg OC144-093 po pre-dosed 1hr	486.7	3364.6	NA
Paclitaxel 10mg/kg po + 2x10mg/kg OC144-093 po pre-dosed 1hr and coadministered	740.8	3942.1	10.3
Paclitaxel 16g/kg iv	3034.7	4425.5	0.75
Paclitaxel 16g/kg iv 30mg/kg OC144-093 po	4795.3	5350.4	0.87

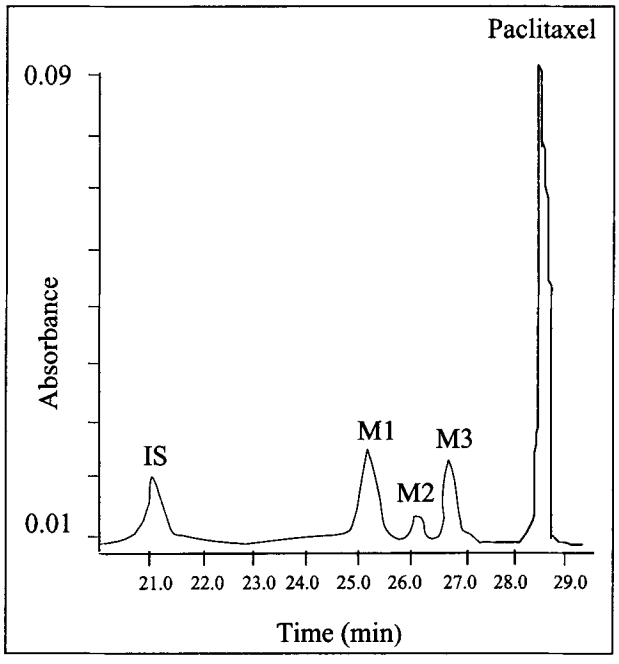

Fig. 1: HPLC trace for Paclitaxel metabolism (10μM) in human liver microsomes (UV detection at 230 nm)

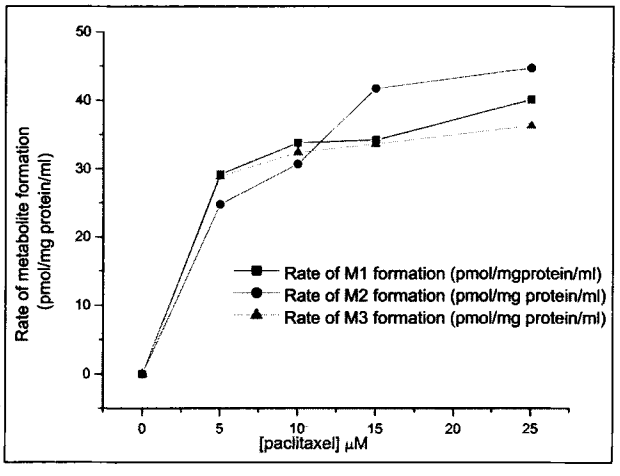

Fig. 2: Graph plotting the rate of formation of paclitaxel metabolites M1, M2 and M3 in human liver microsomes.

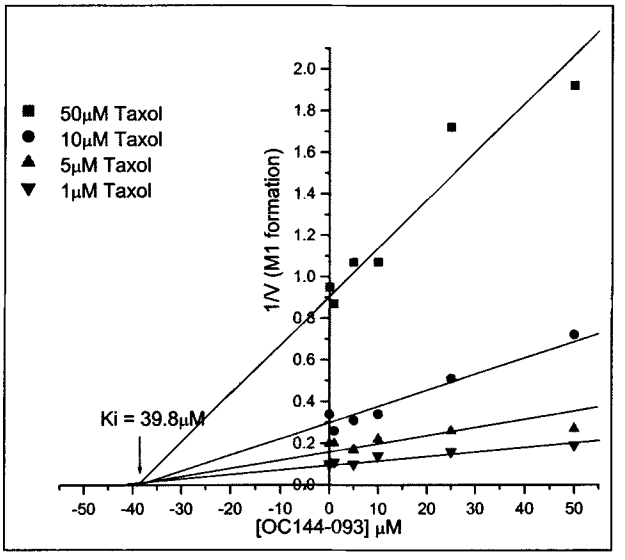

Fig. 3: Graph representing a Dixon plot for determining the K_i of OC144-093 for inhibition of Taxol metabolite M1 formation.

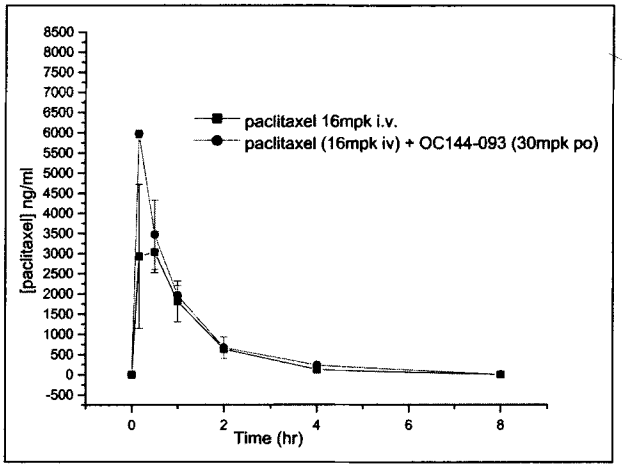

Fig. 4: Pharmacokinetic profiles for paclitaxel coadministered i.v. with orally administered OC144-093.

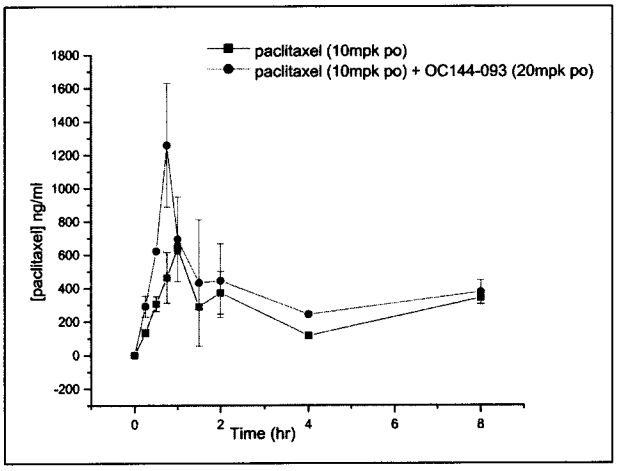

Fig. 5a: Pharmacokinetic profiles for paclitaxel coadministered orally with OC144-093 administered at a dose of 20mpk (po).

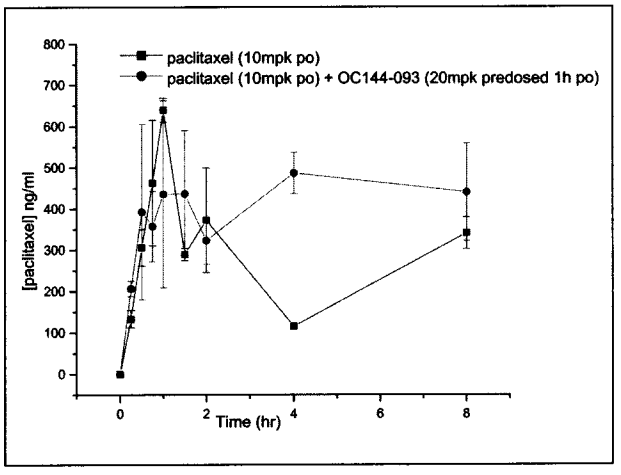

Fig. 5b: Pharmacokinetic profiles for paclitaxel administered orally following a predose (1hr) of OC144-093 administered at a dose of 20mpk (po).

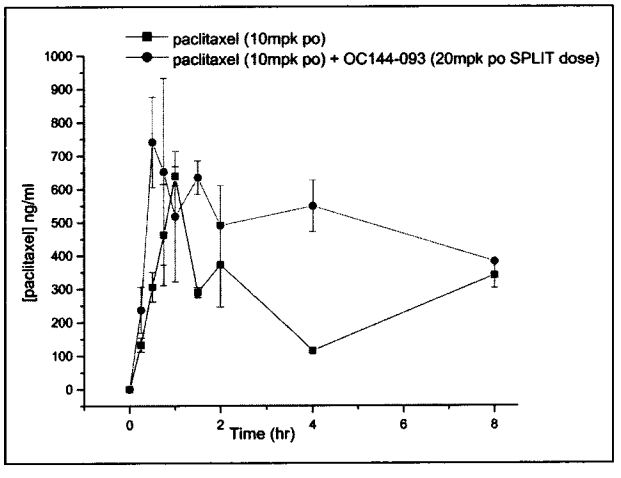

Fig. 5c: Pharmacokinetic profiles for paclitaxel administered orally following a predose (1hr) of OC144-093 administered as a split dose (2 x 10mpk po).

Results

To investigate the potential of *in vivo* drug interactions between paclitaxel and the MDR modulator OC144-093, the metabolic profiling of paclitaxel was carried out *in vitro*. Initial studies were carried out with human liver microsomes to identify the CYP2C8 derived, 6α-hydroxylated metabolite as well as the product of CYP3A mediated metabolism (3'-p-hydroxypaclitaxel). As shown in Figure 1, paclitaxel, and three metabolites M1, M2 and M3 were detected following extraction of the microsomal incubation mixture. Retention times were 28.3min, 21min, 25.7min, 26.1min and 26.5min for paclitaxel, IS, M1, M2 and M3 respectively (Fig. 1). Paclitaxel metabolite M3 co-eluted with a 6α-hydroxypaclitaxel standard. Inhibition studies carried out to determine which of the metabolites of paclitaxel was generated by the activity CYP3A using low concentrations of ketoconazole (<5μM) and troleandomycin, definitively showed that only M1 formation is inhibited by these two specific inhibitors of CYP3A4 and is therefore likely to be the 3'-p-hydroxylated metabolite [25]. M2 and M3 were not affected by either of the inhibitors.

Velocity vs [S] plots are shown in Figure 2 to illustrate the formation of paclitaxel metabolites in human liver microsomes at the four concentrations examined (5, 10, 15 and 25μM). The graph illustrated in Figure 2 demonstrates that at 25μM paclitaxel enzyme activity is either saturated or rapidly approaching saturation (Vmax) for all three metabolites respectively. Experiments were then carried out to obtain a K_i value for inhibition of CYP3A4 mediated paclitaxel metabolism by OC144-093 using 2, 5, 10 and 25μM paclitaxel with 0, 1, 5, 10, 25 and 50μM concentrations of OC144-093. Sigmaplot® version 4.0 data analysis software program was utilized giving a K_i value of 39.8 ± 5.1μM (determined using mean data from three incubations). A representative Dixon plot of the data from one of these incubations (1/V vs. [I]) is shown in Figure 3. 1. 5, 10 and 50μM concentrations of paclitaxel were used to determine the Ki of OC144-093 for the inhibition of CYP3A4 mediated metabolite formation (M1, 3'-p-hydroxypaclitaxel). Six concentrations of OC144-093 are included in the plot: 0, 1, 5, 10, 25 and 50μM. K_i is illustrated here at the point where 'lines of best fit' for the four inhibition curves converge.

In vivo data for paclitaxel dosed in combination with OC144-093 was generated and pharmacokinetic profiles were plotted for paclitaxel following HPLC analysis of mouse plasma extracts. These profiles are shown in Figure 4, Figure 5a, Figure 5b and Figure 5c which were obtained for paclitaxel administered alone by both intravenous or oral administration and in combination with an oral dose of OC144-093. Co-administration of OC144-093 with

paclitaxel had varied effects on its pharmacokinetic profile which appears to depend upon the route of administration of paclitaxel and the time of administration in relation to that of paclitaxel. Pharmacokinetic parameters for each profile are summarized in Table I.

The pharmacokinetic profiles obtained for paclitaxel administered intravenously either alone (16mg/kg) or co-administered with OC144-093 (30mg/kg po) are illustrated in Figure 4. There is a considerable difference between the 10min timepoints for each group and C_{max} is 2-fold higher in the group co-administered OC144-093 with paclitaxel (see Table I). After this time, however, the traces follow a super-imposable profile suggesting that the rate of elimination of paclitaxel in both groups is the same. Co-administration of OC144-093 orally appears to enhance the AUC by a factor of 1.25 (see Table I). The values measured for $t_{1/2}$ were 0.75h and 0.87h for the groups dosed with paclitaxel alone and co-administered OC144-093, respectively.

Figure 5a illustrates that co-administration of OC144-093 with paclitaxel dosed orally produced an elevated C_{max} value of 1258.9ng/ml at 45min which returned to control levels (639.4ng/ml) after 1hour. The area under the curve (AUC) for this group was 3054.3 ng hr/ml, a 1.5-fold enhancement of the control value (2187.9ng hr/ml). Plasma half-life ($t_{1/2}$) was reduced to 5.8h compared with 8.0h for the control.

There was no effect of pre-dosing with OC144-093 (20mg/kg po) on C_{max} (486.7ng/ml) compared with the control (639.4ng/ml; Figure 5b) although AUC was enhanced 1.5-fold to 3364.6ng hr/ml compared with the control value of 2187.9ng hr/ml. Elevated levels of paclitaxel were maintained, however up until the 8h timepoint such that a value for $t_{1/2}$ could not be determined within the timeframe examined.

Upon administration of a split dose of OC144-093, 10mg/kg one hour before and 10mg/kg at the same time as dosing orally with paclitaxel, elevated plasma paclitaxel levels were observed after 30min which were greater than 2-fold those observed for the control (Figure 5c). This is comparable with the effect of OC144-093 in the group pre-dosed (20mg/kg po) group although in the split-dose group a $t_{1/2}$ value was measurable at 10.3h, 2 hours longer than the control (8.0h). The distribution and rate of paclitaxel elimination in the split dosed group appears to be comparable with that of the pre-dosed group (20mg/kg) in which the 4 hour time point showed a two-fold elevation in plasma paclitaxel concentration compared with the control (Figures 5b and 5c).

Discussion

First and second generation MDR modulators have been limited in their therapeutic applicability because of inherent toxicity and adverse drug-drug interactions. This has been particularly evident when these compounds were presented at physiological concentrations required to overcome MDR [16, 17, 18, 19]. Drug-interactions between anticancer agents and first/second generation modulators have been manifested by pharmacokinetic alterations that occur when both anticancer agents and modulators are co-administered. These are likely due to overlapping substrate specificities and tissue distribution of Pgp and the enzymes involved in the metabolism of several anticancer agents and modulators of MDR [3].

Investigations suggest that interactions between MDR modulators and anticancer agents may result from saturation of the capacity of drug metabolizing enzymes that function as a metabolic catalysts in the liver and extrahepatic metabolic tissues [22]. This results in the formation of potentially toxic, alternative waste products, which may be generated via interaction with enzymes involved in otherwise minor routes of metabolism, or the accumulation of toxic levels of parent drug compound. In addition, pharmacokinetic profiles of Pgp substrates may become altered by the presence of other substrates for Pgp which compete for or inhibit distribution and/or elimination processes.

OC144-093 was shown in this report to be generally non-inhibitory with regard to paclitaxel metabolism. It was observed that at low concentrations, where CYP3A4 is known to be specifically inhibited (typically <3μM; [26]), ketoconazole inhibited the formation of the metabolite designated here as M1. Troleandomycin, a mechanism based inhibitor of CYP3A4 also inhibited M1 formation. Based upon these results we have assigned paclitaxel metabolite M1 formation as one mediated by CYP3A4. M1 is likely, therefore to be the 3'-p-hydroxylated metabolite of paclitaxel [25]. M3 co-eluted with a 6α-hydroxylated standard of paclitaxel. 6α-hydroxypaclitaxel has been reported previously in the literature as being formed as the result of a CYP2C8 dependent biotransformation [5] which is in agreement with our results which show that it was not affected by specific inhibitors of CYP3A4.

The metabolic profile of OC144-093 has been studied and the major metabolic pathway characterized as O-deethylation [4]. It has been determined that cytochromes P450 CYP2C9, CYP2B6, CYP2E1 and CYP3A4 are not involved in the formation of this metabolite, however, evidence suggests that this pathway is cytochrome P450 mediated [4]. OC144-093 was inhibitory towards CYP3A mediated paclitaxel metabolism only at high concentrations (39.8 ± 5.1μM) and those significantly higher than plasma concentrations required to achieve effective Pgp blockage (1μM, [1]). This is consistent with the finding that metabolism of OC144-093 is not mediated

primarily by CYP3A4 [4]. The formation of neither M2 or M3 was inhibited by OC144-093. We can therefore conclude, based upon these results, that OC144-093 does not interfere with the metabolism of paclitaxel.

As is the case for most Pgp substrates, OC144-093 has the potential to disrupt the pharmacokinetic profile of drugs whose therapeutic dose delivery is regulated by the Pgp efflux pump, and interfere with mechanisms involving tissue distribution and elimination processes. It has been suggested that enhancement of oral bioavailability may be achieved by co-administering a substrate for Pgp and that this phenomenon occurs irrespective of whether or not a compound is a substrate for CYP3A which is co-expressed in the gut [27]. The effects of dosing OC144-093 with paclitaxel appear mainly to be those involved in absorption processes. OC144-093 enhanced the oral absorption of paclitaxel only when co-administered orally. This effect was manifested by way of an increase in both C_{max} and AUC compared with the control (Table I) and is likely to be due to interactions with Pgp in the gut where absorption events may be disrupted. Pre-treatment with OC144-093 caused an extension in $t_{1/2}$ compared with the control and the group treated simultaneously with paclitaxel and OC144-093. This could be due to an effect on Pgp expressed in the kidney and other tissues involved in distribution and elimination processes which occurs only following either long-term or multiple treatment with OC144-093. From the profiles generated after dosing groups with paclitaxel intravenously either alone or with OC144-093 (orally administered) we can conclude that OC144-093 may interfere with the pharmacokinetics of paclitaxel only in the initial distribution phase and does not significantly affect elimination processes. Compared with results obtained with other modulators of MDR, such as PSC833, which caused a 10-fold increase in Cmax following oral co-administration with paclitaxel [23], OC144-093 has a relatively minor effect on the pharmacokinetics of paclitaxel. Judging from the metabolism studies carried out in human liver fractions *in vitro*, it is also unlikely that metabolic interactions with CYP3A are involved here.

In conclusion, this study has provided important information concerning co-administration of paclitaxel, a clinically relevant anticancer agent, with OC144-093, a promising new modulator of MDR. Compared with other modulators of MDR, such as PSC833 which may affect the metabolism of anticancer agents such as paclitaxel, vinblastine and etoposide [28], OC144-093 does not appear to affect the metabolism or drastically alter the pharmacokinetics of paclitaxel. This compound therefore exhibits many desirable characteristics that may influence its utility as a modulator of MDR in a clinical setting.

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