243 POSTER

A liquid chromatography/tandem mass spectrometry assay for *cis*-amminedichloro(2-methylpyridine) platinum(II) (ZD0473) in human plasma ultrafiltrate

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The clinical use of platinum drugs as anticancer agents has encountered problems when attempting to relate pharmacokinetic profiles with efficacy and toxicity. This has been mainly due to the lack of specific and sensitive analytical methodology to examine concentrations of the unbound drug in plasma. The presence of a carbocyclic ring on the new anti-tumor reagent platinum drug, *cis*-amminedichloro(2-methylpyridine)platinum(II) (ZD0473) suggested that it would be possible to develop the first stable isotope dilution LC/MS assay for a platinum drug in human plasma samples. The dichloro form of the drug exists in equilibrium with various aquated forms in plasma. The molecular form of the drug therefore depends upon the length of time that the plasma sample is maintained at room temperature before freezing. Therefore, we have developed a method that quantitatively converts the various aquated species back to the dichloro form of the parent drug so that a single molecular species can be analyzed. Selected reaction monitoring was performed on the transition of *m/z* 393 $[M + NH_4]^+$ to *m/z* 304 $[M + NH_4\text{-}NH_3\text{-}2xH^{35}Cl]^+$ for ZD0473, and *m/z* 400 $[M + NH_4]^+$ to *m/z* 310 $[M + NH_4\text{-}NH_3\text{-}^2H^{35}Cl\text{-}H^{35}Cl]^+$ for $[^2H_7]$ZD0473. The standard curves were fitted to a quadratic regression over the range from 10 ng/mL to 5000 ng/mL in human plasma ultrafiltrate. The lower limit of quantitation for ZD0473 was 10 ng/mL for 100 μL of plasma ultrafiltrate (Table).

Table: Validation data for plasma ultrafiltrate QC samples

QC sample	LLQ	LQC	MQC	HQC	UQC
Theoretical (ng/mL)	10	40	400	4,000	25,000
Number of QCs	15	15	15	15	15
Determined (ng/mL)	11.1	43.1	419.9	4,057	25,272
Precision (%)	12.1	5.0	2.2	1.5	1.3
Accuracy (%)	110.9	107.7	105.0	101.4	101.1

This simple, rapid, reliable, and sensitive method of quantitation had excellent accuracy and precision. Inter-day variations (n = 3) for batches of QC samples (n = 5/day) are shown below. Plasma ultrafiltrate samples containing ZD0473 were shown to be stable at -80 °C for at least three months. The assay is being used to determine pharmacokinetic parameters for ZD0473 in Phase II/III studies.

244 POSTER

Influence of the cytoprotective agent amifostine on the pharmacokinetics of paclitaxel

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Background: Amifostine is a cytoprotectant agent that has shown to reduce paclitaxel toxicity in vitro and in clinical trials. Pharmacokinetic changes has been described for several compounds when these are combined with amifostine. The objective of present study is to evaluate the influence of amifostine on the pharmacokinetic parameters of paclitaxel.

Methods: Paclitaxel was administered weekly as 1-hour infusion at dose of 80 mg/m2, without amifostine or with amifostine 500 mg 15 min before paclitaxel. Blood samples were obtained at the end of the infusion (0 min) and 15, 30, 60, 120 and 240 min postinfusion. Paclitaxel plasma concentrations were measured by a high-performance liquid chromatography (HPLC) assay. Paclitaxel's pharmacokinetic characteristics were evaluated using a open two-compartment model. Area under the concentration-time curve (AUC), the peak plasma concentrations (Cmax), the duration of time that plasma paclitaxel concentration were >0.1 μM (TPP>0.1) and >0.05 μM (TPP>0.05), and clearance were studied. Statistical analysis was performed using paired t test.

Results: Twenty-six courses of paclitaxel were analysed, 13 courses without amifostine and 13 with amifostine. No statistically significant differences were observed between patients without or with amifostine in Cmax (2.94±1,32 vs 3.09±0.77 μM), AUC (4.2±1.9 vs 3.4±1.2 μM* h), TPP>0.1 (192±140 vs 191±182 min) and TPP>0.05 (572±844 vs 589±568 min). Previous administration of amifostine increased by 35% (497±222 vs 772±424 mL/min/m2) ($p<0.05$) paclitaxel clearance.

Conclusion: The majority of Paclitaxel's phamacokinetic parameters (Cmax, AUC, TPP>0.1 and TPP>0.05) are comparable in the presence or absence of amifostine. Further trials evaluating the role of the increase of paclitaxel clearance in toxicity or efficacy are warranted.

245 POSTER

Analysis of *cis*-amminedichloro(2-methylpyridine) platinum(II) (ZD0473) in human urine

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There is accumulating evidence that platinum drugs with carbocyclic rings such as carboplatin and oxaliplatin can undergo biotransformations *in vivo*. The analysis of platinum drugs in urine has typically relied upon atomic absorption spectrophotometry or more sensitive inductively-coupled plasma-mass spectrometry (ICP-MS) methodology. As urine may contain inactive low molecular weight platinum bound adducts and possibly biotransformation products, urinary platinum concentrations may not reflect concentrations of the excreted drug. The use of liquid chromatography (LC) in combination with ICP-MS improves specificity considerably. However, this method has not yet been applied to the analysis of platinum drugs in urine. Furthermore, ICP-MS requires conversion of molecules to atomic platinum, so that the molecular species being analyzed cannot be definitively characterized. We anticipated that the use of LC/tandem mass spectrometry (MS/MS) would overcome these problems. This turned out to pose a significant analytical challenge for *cis*-amminedichloro(2-methylpyridine)platinum(II) (ZD0473) because there are three major isotopes of platinum and two of chlorine. Molecular species ate complex and sensitivity is limited by segregation of the MS signal into a number of different isotopes. However, by careful choice of LC/MS conditions, it was possible to analyze ZD0473 with acceptable detection limits. Dichloro-platinum drugs such as cisplatin and ZD0473 are unstable in solution because they readily equilibrate with water to form at least two aquated forms. Therefore, assay methodology has been developed that avoids extraction and purification of the sample. Urine samples were simply diluted with isotonic saline, and a heavy isotope internal standard ($[^2H_7]$ZD0473) was then added. Quantitation was conducted using LC/selected reaction monitoring (SRM)/MS under electrospray ionization conditions. SRM was performed on the transition of *m/z* 393 $[M + NH_4]^+$ to *m/z* 304 $[M + NH_4\text{-}NH_3\text{-}2xH^{35}Cl]^+$ for ZD0473, and *m/z* 400 $[M + NH_4]^+$ to *m/z* 310 $[M + NH_4\text{-}NH_3\text{-}^2H^{35}Cl\text{-}H^{35}Cl]^+$ for $[^2H_7]$ZD0473. The standard curves were fitted to a quadratic regression over the range from 0.15 μg/mL to 500 μg/mL in human urine. The lower limit of quantitation for ZD0473 was 0.20 μg/mL for 100 μL of urine. This simple and rapid stable isotope dilution assay will be used for the quantitation of ZD0473 in the urine of patients dosed with ZD0473.

Phase I studies

246 POSTER

Changes in the methodology of Phase I clinical trials of anticancer agents and their impact in a single centre between 1983 and 1999

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We have analysed all phase I trials performed at our centre over the last 15 years to assess the impact and practical implications of changes in Phase I trial design.

Trial Design: In all, 68 studies were performed using 75 treatment schedules. Forty studies were of single agents (SA), of which 29 were classic cytotoxics. The remaining 28 were studies of novel combinations (CS), the numbers of which increased over this period (2 during 1983-89, 9 during 1990-94 and 17 during 1995-99). Starting dose (SD) was determined from clinical +/- animal data in 49 studies. Of the 24 with SD based on animal data alone, 13 used 0.1 LD10 whereas the remaining 11 were more conservative. Traditional "modified Fibonacci" dose escalation schemes were used in 18 SA studies with more aggressive strategies preferred in the remaining 22 SA studies; escalations that were empirical were used more frequently in the 28 CS. Forty-six studies included pharmacokinetic analyses which often aided dose escalation. Only 4 incorporated pharmacokinetically guided dose escalation and had limited success; other mathematical escalation models

were not attempted. Intra-patient escalation was permitted in 18 studies. Definitions of dose limiting toxicity (DLT) varied widely, with no clear pattern over time or between SA and CS; only 3 studies incorporated prophylactic use of colony-stimulating factors.

Trial Results: Sixty-seven studies are completed with data available from 63, which recruited 1939 patients (pts). The median (M) number of dose levels (DL) was 4 (range 2-14); significantly more DL were explored for SA than CS (M 6.5 vs 3; P<0.001), and for conventional compared to more aggressive dose escalation regimens (4 vs 7; P=0.01). Fewer pts per study were treated below the RD in combination than in SA trials (7 vs 16 patients, P=0.13), and in trials using more aggressive dose escalation strategies (12 vs 15; P= 0.15). The first dose level at which DLT ocurred was close to the final MTD (M ratio 1.0). There were 16 toxic deaths (8 in SA and 8 in CS). Although patients appeared at greater risk in CS and more aggressive SA studies, this mirrors the greater proportion of pts treated at or above the RD.

Conclusion: The number of Phase I trials of anti-cancer drugs, in particular combination trials, has increased. More aggressive study designs can reduce the size of Phase I trials, limit the number of patients treated at doses below the RD and appear to have an appropriate safety profile.

247 POSTER

Phase I study of intermittent (weekly) topotecan in non small cell lung cancer (NSCLC)

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Introduction: Topotecan (T) is a water soluble, semisynthetic analog of the alkaloid camptothecin, which is a specific inhibitor of topoisomerase I. Clinically, it has antitumor efficacy in small cell lung cancer and ovarian cancer when administered as a 30-minute intravenous infusion given daily for 5 consecutive days, repeated every 3 weeks. In preclinical studies, the tumor growth inhibition rates were similar between 5 consecutive days and intermittent administration at the same total dose.

A Phase I study was undertaken to determine the MTD and recommended dose of T when administered intermittently to patients (pts) with NSCLC, previously treated and untreated, and to study the pharmacokinetics.

Method: T was administered once a week (Days 1, 8 and 15), by 30-minute intravenous infusion; the cycle was repeated every 4 weeks. The dose was escalated from the starting dose of 4 mg/m2 in 2 mg/m2 increments. At least three pts were treated at each escalated dose level.

Results: 12 pts were given 20 cycles of treatment (median 2, range 1-2). No dose limiting toxicities (DLTs) were observed at 4 mg/m2 in previously treated pts. At 6 mg/m2, DLTs were observed in all three previously treated pts (1 patient with of Grade 4 febrile neutropenia, and 2 pts with Grade 3 infection without neutropenia). Therefore, the MTD and recommended dose for the intermittent dose schedule in previously treated pts were 6 and 4 mg/m2, respectively.

In previously untreated pts, no DLT was observed at 6 mg/m2, and only one patient showed DLT (Grade 4 neutropenia persisting for at least 3 days) at 8 mg/m2. However, 2 pts including the one with DLT could not receive the treatment on day 15 because their hematological parameters failed to show recovery to the predetermined level. On the basis of these results, MTD was estimated at 8 mg/m2 and the recommended dose at 6 mg/m2 in previously untreated pts.

No differences were noted in pharmacokinetic parameters between previously treated and untreated pts at 6 mg/m2. Urinary excretions of T (lactone plus carboxylate form) were similar at two dose levels in both subgroups of pts, and no altered elimination was observed.

Regression of the primary lung tumor and/or lymph node metastases was observed in some of the previously treated and untreated pts, but none of them achieved PR or CR.

Conclusions: Further investigation is necessary to determine the efficacy and dosing schedule of T for NSCLC.

248 POSTER

Phase I-II and pharmacokinetic study combining gemcitabine (GEM) with oxaliplatin (OX) in patients (pts) with advanced non-small-cell lung (NSCLC) and ovarian carcinoma (OC)

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Background: GEM and OX combinations have in vitro synergy, with a schedule dependency favoring GEM given prior OX (Faivre, 1999).

Aim: considering the non-overlapping toxicity for both drugs, and activity of GEM and OX as single agent in NSCLC and OC, we designed a phase I-II outpatient combination schedule to establish the maximal tolerated dose (MTD), the dose limiting toxicity (DLT), the recommended dose (RD), the pharmacokinetics profile, and to evaluate the antitumor activity of GEMOX in pts with potentially sensitive tumor i.e. advanced NSCLC and OC.

Methods: GEM was administered as a 30-minutes infusion followed by OX infused over 2 hours on D1 and D15 every 4 week (wk)-cycle (cy). Doses of GEM/OX were 800-1500/70-100 mg/m*, respectively. Results: 44 pts (M/F: 26/18, median age 55, 61% PS 0) received a total of 180 cycles. There were 35 NSCLC (5 platinum-pretreated, 2 resistant) and 9 OC pts (all platinum-pretreated, 2 resistant).

Toxicity: 44 pts were evaluable for acute and 32 pts for chronic toxicity. No acute DLT occurred at cy 1. Hematologic toxicity was < Gr3 except for 9 episodes of Gr3-4 non febrile neutropenia in 7 pts, and 3 episodes of Gr3-4 thrombocytopenia in 2 pts. Other toxicities were mild to moderate. Transient Gr3 asthenia occurred in 2% of cy. Eight pts (3 pretreated, 5 chemonaive) experienced cumulative Gr3 OX-related neurotoxicity requiring treatment discontinuation for 4 pts at cy 5.

Activity: Among 37 evaluable pts, 13/28 NSCLC and 3/9 OC pts showed an objective responses (1 CR, 15 PR including 3 PR in platinum-resistant pts). Pharmacokinetics suggested no drug interaction.

Conclusion: GEM/OX combination could be safely administered on a D1 and D15 schedule without acute toxicity, at the RD of 1500/85 mg/m2 q2 weeks. Promising antitumor activity is reported in pts with NSCLC and platinum-pretreated OC, deserving further evaluation of GEMOX.

249 POSTER

A phase IB study evaluating the scheduling and pharmacokinetic interaction between alimta and gemcitabine in patients with advanced cancer

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ALIMTA (pemetrexed disodium) is a novel multi-targeted antifolate agent, which inhibits thymidylate synthase (TS), dihydrofolate reductase (DHFR) and the purine biosynthetic enzyme, glycinamide ribonucleotide formyl transferase (GARFT). ALIMTA is active in breast, lung, bladder and GI malignancies in early clinical trials. We have completed a phase I study in which ALIMTA was administered 90 minutes after gemcitabine (GEM), based on a demonstration of sequence-dependent in vitro cytotoxic synergy. In the present trial, we have investigated the simultaneous administration of ALIMTA and GEM. 14 patients with solid tumors (9 male, 5 female; median age 60 (36-74); median ECOG PS 1) have received 70 courses of treatment at the MTD of our initial phase I study (GEM 1250 mg/m2 days 1 and 8 and ALIMTA 500 mg/m2 on d8). Cycles are repeated every 3 weeks. ALIMTA alone was given in cycle 1 to allow for pharmacokinetics (PK) sampling to evaluate the effect of GEM on the disposition of ALIMTA and vice versa. Folic acid/vitamin B12 supplementation was not included in this study. Neutropenia was the most common toxicity (grades 3 and 4 in 28 and 15% of patients in cycle 1, respectively). Non-hematologic toxicities were mild to moderate and included anorexia, nausea, fatigue, fever, rash, and pulmonary toxicity. 1 PR was noted in a patient with NSCLC. 8 patients had disease stabilization for 5 or more cycles (4 patients with 8 or more cycles). The change in schedule of drug administration does not appear to have any significant effect on the toxicity or efficacy of this combination. Results of PK studies will be discussed. Supported by grants from NCI (CA77112) and Eli Lilly and Company.