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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.

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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.

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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

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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