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Journal of Chromatography B, 675 (1996) 107–111

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Determination of the two diastereoisomers of lobaplatin (D-19466) in plasma ultrafiltrate of cancer patients with a normal or an impaired kidney or liver function by high-performance liquid chromatography with ultraviolet detection

J. Welink[a], B. Pechstein[b], W.J.F. van der Vijgh[a,*]

[a] *Department of Medical Oncology, Academisch Ziekenhuis Vrije Universiteit, De Boelelaan 1117, 1081 HV Amsterdam, Netherlands*

[b] *Department of Clinical Biochemistry, Arzneimittelwerk Dresden, Dresden, Germany*

First received 23 May 1995; revised manuscript received 23 August 1995; accepted 23 August 1995

Abstract

Lobaplatin consists of two diastereoisomers, LP-D1 and LP-D2. Being a new cytostatic agent it represents platinum compounds of the third generation and is active in several in vitro tumor models of murine and human origin. To determine the pharmacokinetics of LP-D1 and LP-D2 in cancer patients with and without a normal kidney and liver function, an HPLC procedure was developed and validated. Plasma ultrafiltrate samples were injected into the HPLC system after solid-phase extraction. The standard curves of LP-D1 and LP-D2 in plasma ultrafiltrate were linear over the range 0.071–9.100 and 0.067–8.639 μM, respectively. The recovery from plasma ultrafiltrate was 84% for both diastereoisomers. The within-day accuracy ranged from 98.1 to 100.3% for LP-D1 and from 96.5 to 106% for LP-D2. The between-day accuracy ranged from 99.2 to 101.5% for LP-D1 and 97.7 to 101.2% for LP-D2. The within-day and the between-day precision were $\leq$6.0% and $\leq$6.1% for LP-D1 and $\leq$3.8% and $\leq$6.5% for LP-D2, respectively. For pharmacokinetic purposes the method proved to be sufficiently sensitive, specific and accurate for analysing clinical samples.

Keywords: Lobaplatin

1. Introduction

Lobaplatin (D-19466; 1,2-diamminomethylcyclobutaneplatinum(II)lactate) is a water-soluble platinum compound which consists of a nearly 1:1 mixture of two diastereoisomers (LP-D1 = *RRS* configuration and LP-D2 = *SSS* configuration). Their structural formulas are shown in Fig. 1. Lobaplatin is under investigation in the treatment of cancer. In vitro the compound is most sensitive in xenografts of non-small cell lung, ovarian, stomach and breast cancer [1]. Preclinical data suggest a lack of cross-resistance of lobaplatin in a number of platinum-resistant tumor models [1,2]. An objective response was

* Corresponding author.

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SSDI 0378-4347(95)00353-3

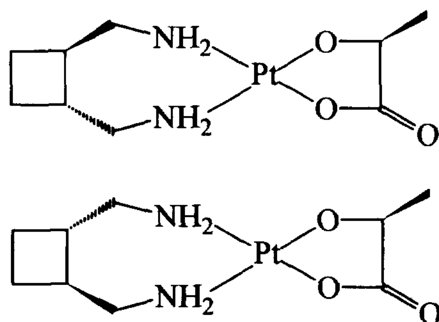

Fig. 1. Structural formulas of the two diastereoisomers of lobaplatin.

found in 8 out of 31 (26%) patients with ovarian cancer in phase I and II trials [1,3].

Lobaplatin was chosen for a further phase I trial, in which patients participated who had an impaired kidney or liver function. To study the pharmacokinetics in these patients, an accurate and specific assay for both diastereoisomers of lobaplatin in plasma ultrafiltrate must be available. Lobaplatin has previously been analysed in infusion solutions [4] and in plasma ultrafiltrate [5] without separation of the two diastereoisomers. We have developed a selective HPLC assay preceded by a solid-phase extraction procedure to analyse the two diastereoisomers of lobaplatin separately in plasma ultrafiltrate of cancer patients with a normal or an impaired kidney or liver function.

2. Experimental

2.1. Materials

Lobaplatin trihydrate ($M_r = 451$), consisting of 51.3% LP-D1 and 48.7% LP-D2, was provided by Asta Medica (Frankfurt, Germany). Acetonitrile (gradient grade) was obtained from Merck (Amsterdam, Netherlands), methanol (HPLC grade), KH_2PO_4 and KOH from Baker (Deventer, Netherlands) and triethylamine from Pierce (Rockford, IL, USA). C_{18} solid-phase cartridges (BondElut C_{18}, 100 mg, 1 ml, metal frits) were obtained from Varian (Harbor City, CA, USA).

2.2. Sample pretreatment

A stock solution of 1.774 mM racemic lobaplatin was prepared in water. Fresh drug-free heparinized blood was centrifuged at room temperature for 15 min at 1400 g. The plasma obtained was filtered through CF 25 ultrafiltration membrane cones (Amicon, Beverly, MA, USA) by centrifuging for 45 min at 1000 g. A freshly spiked plasma ultrafiltrate stock solution (17.738 μM) was prepared by a 100-fold dilution of the lobaplatin stock solution with plasma ultrafiltrate. Plasma ultrafiltrate standard solutions were prepared before each run by serial dilutions (v/v) from the lobaplatin stock solution in plasma ultrafiltrate. Nominal lobaplatin standard concentrations were 17.738, 8.869, 4.435, 2.217, 0.554, 0.227, 0.139 and 0 μM. Plasma ultrafiltrates of patients were prepared by filtering 4 ml plasma through CF 25 ultrafiltration membrane cones. If necessary (when the expected sample concentrations are above 17.738 μM lobaplatin) patient samples were diluted with blank plasma ultrafiltrate. Lobaplatin was extracted from plasma ultrafiltrate samples by using C_{18} solid-phase cartridges placed in a 21-port vacuum manifold (Baker, Deventer, Netherlands). The extraction procedure is described in Table 1. Methanol eluent obtained was evaporated under a stream of nitrogen at room temperature. The residue was dissolved in 0.3 ml water, transferred to autosampler vials and 100 μl of it was injected into the HPLC system.

Unknown concentrations in plasma ultrafil-

Table 1
Solid-phase extraction procedure

1. Conditioning of C_{18} Sep Pak Cartridge (Varian, Bond Elut):
   Prime each cartridge with 5.0 ml methanol, 1.0 ml acetonitrile followed by 5.0 ml water.
2. Application of sample:
   Dilute 0.5 ml plasma ultrafiltrate with 0.5 ml water and load this onto the cartridge.
3. Cartridge wash:
   Wash the cartridge with 1.0 ml water.
4. Cartridge elution:
   Elute lobaplatin with 1.0 ml methanol.

trate samples were calculated by interpolation on the calibration curves of lobaplatin in plasma ultrafiltrate, covering the concentration range of 0.071–9.100 μM for LP-D1 and 0.067–8.639 μM for LP-D2.

2.3. Chromatography

The HPLC system consisted of a Marathon autosampler with a cooled tray (4°C), a Spectroflow 480 solvent delivery system and a degasser, Model GT 103 (all from Separations, H.I. Ambacht, Netherlands). A computer (Olivetti M290S) provided with Axxiom Chromatography Software (Model 727, version 3.92, Axxiom Chromatography, CA, USA) was used for the acquisition and processing of data. The system was equipped with a Lichrocart guard column (4×4 mm I.D., Merck, Amsterdam, Netherlands), which was packed with Lichrosorb RP-18, 5 μm and a 5-μm Hypersil ODS column, 250×4 mm I.D. (Knauer, Berlin, Germany). The mobile phase —consisting of 1.36 g KH_2PO_4 which was dissolved in 1 l acetonitrile–water–triethylamine (26:973.7:0.3, v/v/v) and adjusted to a pH of 6.4 with 5 M KOH— was used at a flow-rate of 1.0 ml/min. After the analyte was eluted from the column, the analytical column was washed for 2.0 min with a mobile phase consisting of 1.36 g KH_2PO_4 which was dissolved in 1 l acetonitrile–water–triethylamine (200:799.7:0.3, v/v/v) and adjusted to a pH of 6.4 with 5 M KOH, at a flow-rate of 1.0 ml/min. An ultraviolet detector (210 nm) from Applied Biosystems (Separations, H.I. Ambacht, Netherlands) was used.

2.4. Validation of the assay

The procedure was validated on six days by the duplicate analysis of the quality control samples (spiked plasma ultrafiltrate with concentrations of 0.341, 1.706 and 6.823 μM for

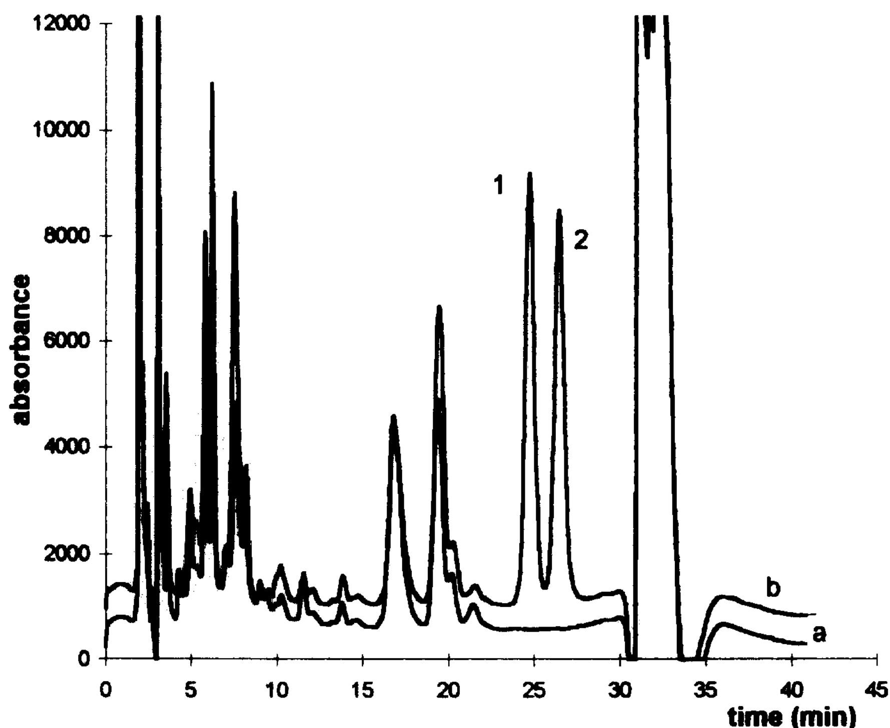

Fig. 2. Chromatograms of extracted plasma ultrafiltrate from a representative patient with an impaired kidney function: (a) just before and (b) 30 min after a bolus injection of 50 mg/m^2 lobaplatin. Peak identity: 1 = LP-D1 (ca. 6 μM), 2 = LP-D2 (ca. 5.6 μM).

Table 2
Recoveries of the diastereoisomers LP-D1 and LP-D2 at various concentrations in plasma ultrafiltrate after solid-phase extraction

Sample (μM)	Recovery (%)
LP-D1	
0.454	85.3
0.908	84.7
2.275	84.1
9.100	82.0
LP-D2	
0.433	81.0
0.866	86.0
2.160	85.9
8.639	83.3

LP-D1 and 0.324, 1.620 and 6.479 μM for LP-D2) to determine the between-day precision and accuracy and by the 6-fold analysis of these samples on one day to determine the within-day precision and accuracy. The recovery of both diastereoisomers of lobaplatin after extraction from plasma ultrafiltrate was calculated from the ratio of an injected solution of lobaplatin in water and the extracted sample. The specificity of the assay was determined by the analysis of blank plasma ultrafiltrate from cancer patients with a normal or an impaired kidney or liver function.

3. Results and discussion

Fig. 2 shows the chromatograms of lobaplatin in plasma ultrafiltrate from a representative patient with an impaired kidney function after bolus injection of 50 mg/m^2 lobaplatin. No interfering peaks were detected at the retention times of the diastereoisomers (LP-D1: 24.0 ± 1 min and LP-D2: 25.5 ± 1 min) in the plasma ultrafiltrate of cancer patients with a normal or an impaired kidney or liver function. The LLQ (lowest concentration of analyte in a sample which can be determined with a precision and accuracy within 20%) was 0.071 μM for LP-D1 and 0.067 μM for LP-D2. Calibration curves in plasma ultrafiltrate (0.071–9.100 μM for LP-D1 and 0.067–8.639 μM for LP-D2) were calculated by linear regression with a weight-factor of $1/y$. This procedure resulted in correlation coefficients of ≥0.999 over the entire concentration range. The regression data of the calibration curves of LP-D1 and LP-D2 were 626.1 ± 13.7 AU/min and 650.0 ± 16.7 AU/min for the slope and −5.8 ± 6.7 AU and −4.2 ± 5.3 AU for the intercept, respectively. The detection limit of the

Table 3
Within-day and between-day precision and accuracy for the determination of the diastereoisomers LP-D1 and LP-D2 in plasma ultrafiltrate

Quality control sample (μM)	Within-day (n = 6)			Between-day (n = 6)		
	Determined concentration (μM)	C.V. (%)	Accuracy (%)	Determined concentration (μM)	C.V. (%)	Accuracy (%)
LP-D1						
0.341	0.342	6.0	100.3	0.346	6.1	101.5
1.706	1.674	2.9	98.1	1.693	3.6	99.2
6.823	6.739	3.8	98.8	6.915	3.4	101.3
LP-D2						
0.324	0.326	3.7	100.6	0.327	6.5	100.9
1.620	1.564	3.7	96.5	1.582	4.1	97.7
6.479	6.405	3.8	98.9	6.556	3.1	101.2

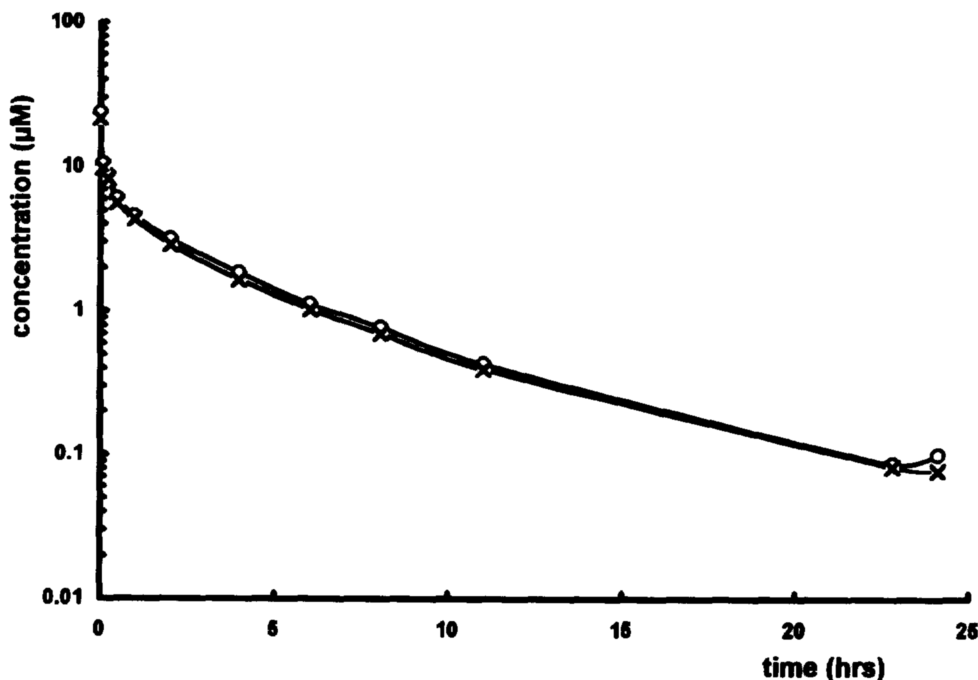

Fig. 3. Semilogarithmic plasma concentration–time curves in a representative patient with an impaired kidney function after receiving 50 mg/m^2 lobaplatin as an i.v. bolus injection (× = LP-D1 and ○ = LP-D2).

assay (3 × noise at the highest sensitivity) was 0.067 μM for LP-D1 and 0.062 μM for LP-D2. The overall recovery was about 84% for both diastereoisomers and independent of the concentration (Table 2).

The coefficient of variation (C.V.) of the within-day repeatability ($n = 6$) was ≤6.0% and ≤3.8% for LP-D1 and LP-D2, respectively (Table 3). The C.V. of the between-day repeatability ($n = 6$) was ≤6.1% for LP-D1 and ≤6.5% for LP-D2. The within-day accuracies for LP-D1 and LP-D2 ranged from 98.1–100.3% and 96.5–100.6%, respectively. The between-day accuracies ranged from 99.2–101.5% for LP-D1 and 97.7–101.2% for LP-D2.

Fig. 3 is an example from our study. It shows a semilogarithmic plot of the plasma concentration versus time curve of LP-D1 and LP-D2 in a representative patient who received 50 mg/m^2 of lobaplatin as an i.v. bolus injection. This figure clearly shows that our assay allows us to monitor the pharmacokinetics of lobaplatin for at least 24 hours, which is much longer than in another study using an HPLC procedure even without the separation of the two diastereoisomers [5].

We conclude that our assay of lobaplatin proved to be simple, accurate and precise. Its sensitivity was low enough to determine the pharmacokinetics of the intact drug over at least 24 h.

References

[1] R. Voegeli, W. Schumacher, J. Engel, J. Respondek and P. Hilgard, J. Cancer Res. Clin. Oncol., 116 (1990) 439.

[2] C. Meijer, N.H. Mulder, H. Timmer-Bosscha, G.J. Meersma and E.G.E. de Vries, Proc. Am. Ass. Cancer Res., 32 (1991) 408.

[3] J.A. Gietema, H.J. Guchelaar, E.G.E. de Vries, P. Aulenbacher, D.T. Sleijfer and N.H. Mulder, Anti-Cancer Drugs, 4 (1993) 51.

[4] H.J. Guchelaar, D.R.A. Uges, P. Aulenbacher, E.G.E. de Vries and N.H. Mulder, Pharm. Res., 9 (1992) 808.

[5] J.A. Gietema, G.J. Veldhuis, H.J. Guchelaar, P.H.B. Willemse, D.R.A. Uges, A. Cats, H. Boonstra, W.T.A. van der Graaf, D.T. Sleijfer, E.G.E. de Vries and N.H. Mulder, Br. J. Cancer, 71 (1995) 1302.