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DETERMINATION OF ETHYLENEDIAMINEPLATINUM(II) MALONATE IN INFUSION FLUIDS, HUMAN PLASMA AND URINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A selective and convenient high-performance liquid chromatographic assay was developed for ethylenediamineplatinum(II) malonate (JM-40) in plasma ultrafiltrate and urine. A μPorasil silica column (30 cm) was used with acetonitrile—water (90:10, v/v) as the mobile phase and the elution of compounds was monitored by ultraviolet absorbance at 214 nm. A linear dynamic range of at least three decades (1—1000 μg/ml) was achieved. The detection limit in plasma ultrafiltrate was 0.35 μg/ml. The stability of JM-40 was determined in 0.9% sodium chloride, 5% glucose, plasma ultrafiltrate and urine. More stable drug solutions were obtained with 5% glucose than with 0.9% sodium chloride. JM-40 was also determined in plasma ultrafiltrate and urine samples of one patient receiving short-term infusions of the drug. In plasma ultrafiltrate unmetabolized JM-40 was detected during the first 5 h after administration and had a half-life of 21.3 ± 1.6 min. The parent drug was excreted in the urine in rapidly decreasing amounts. Eighteen per cent of the dose was recovered as unmetabolized drug during the first 6 h.

INTRODUCTION

Since the discovery of the antineoplastic activity of cisplatin [1, 2], many analogues of this platinum complex have been synthesized in the search for a

compound with a better therapeutic index [3]. The second-generation platinum compound ethylenediamineplatinum(II) malonate (JM-40) (see Fig. 1) was selected by the EORTC New Drug Development Committee for phase I studies on account of its antitumour activity and lack of emetic effect in the ferret [4, 5]. The drug is presently under clinical investigation.

In general, pharmacokinetic studies of cisplatin and its analogues have been performed using total platinum levels in clinical samples determined by flameless atomic absorption spectrophotometry (AAS) [6—8]. This method is very sensitive, but cannot differentiate between the original platinum complex and its degradation products. Moreover, it cannot be used on-line with a high-performance liquid chromatographic (HPLC) system.

The purpose of our study was to develop a selective HPLC procedure using ultraviolet (UV) absorbance detection for the quantification of JM-40. The applicability of this method is demonstrated by the analysis of JM-40 in infusion solutions and body fluids.

EXPERIMENTAL

Materials
The chemicals used were of analytical grade. The mobile phase consisted of acetonitrile (LiChrosorb®, Merck, Darmstadt, F.R.G.) and double-distilled water (90:10, v/v). Glucose, used for the stability experiments, was of Ph. Eur. quality (Brocacef, Amsterdam, The Netherlands). Chemically pure JM-40 (99.8% by elemental analysis) which was used for in vitro experiments was kindly provided by Johnson Mathey (Reading, Berkshire, U.K.). JM-40 ampoules (50 mg of JM-40 per 10 ml of water), used for intravenous administration to patients, were supplied by Bristol Myers (Brussels, Belgium).

An ultrafiltrate of pooled plasma samples from healthy individuals was prepared with Amicon Centriflo® ultrafiltration membrane cones, CF 50A. The model MPS-1 micropartition system provided with YMT filters (both from Amicon, Oosterhout, The Netherlands) was used for the filtration of plasma samples from patients.

Instrumentation
The chromatographic system consisted of a Model 6000A solvent delivery system, a 30 × 0.39 cm column packed with μPorasil, 10 μm (both from Waters, Etten-Leur, The Netherlands) and an LDC UV III monitor Model 1203 with a fixed wavelength of 214 nm (Charles Goffin, De Bilt, The Netherlands). For the stability experiments, 10-μl samples were injected with a WISP 710B autoinjector equipped with a datamodule and a system controller (all from Waters). Plasma ultrafiltrates and urine samples of patients were injected with a Valco Universal Inlet equipped with a 10-μl loop (Chrompack, Middelburg, The Netherlands). The chromatograms were recorded with a Model PM 8251 strip-chart recorder (Philips, Eindhoven, The Netherlands). UV spectra were determined with a Model 25 spectrophotometer (Beckman Instruments, Mijdrecht, The Netherlands).

Total platinum concentrations in plasma, plasma ultrafiltrate and urine samples were determined with a Model 5000A atomic absorption spectro-

photometer at 265.9 nm. The atomic absorption instrumentation included a HGA 500 graphite furnace, an AS 40 autosampler and a PRS printer-sequencer (all from Perkin-Elmer, Gouda, The Netherlands).

Methods

The stability of JM-40 was determined at ambient temperature. A freshly prepared solution of JM-40 in water was used as the external standard. Plasma ultrafiltrate and urine samples from healthy volunteers and solutions of 0.9% sodium chloride and 5% glucose were spiked with a concentrated stock solution of JM-40 to obtain a final drug concentration of 100 μg/ml. After preparing the solutions, 10-μl aliquots were periodically introduced into the chromatographic system without prior purification.

JM-40 was administered to the patients immediately after dilution of the pharmaceutic preparation in 300 ml of 5% glucose. Doses of 300 and 400 mg/m^2 were given as intravenous infusions of 12 and 25 min duration, respectively. The interval between subsequent drug administrations was one month. Blood samples were drawn at the end of infusion and 10, 20, 30, 60, 90, 120, 150, 210, 240 and 360 min thereafter. Urine was obtained from one patient every 2 h. All samples were immediately processed and analysed within 30 min after collection.

RESULTS AND DISCUSSION

Chromatography

Several stationary phases, including silica and silica-bonded phase packings ($-$CN, $-$NH$_2$, $-$NO$_2$) were examined. Elution was carried out using various combinations of acetonitrile and water. Silica columns offered a wide range of retention times for JM-40 allowing a choice of various eluents. An optimal separation of JM-40 from endogeneous constituents present in plasma ultrafiltrate and urine was obtained with a 30-cm μPorasil column and a mobile phase consisting of acetonitrile$-$water (90:10, v/v). Fig. 1 shows representative chromatograms of plasma ultrafiltrate and urine from a patient before and after administration of JM-40. The chromatographic conditions for the separation of JM-40 from endogeneous compounds are comparable to those used for CBDCA [*cis*-diamine-1,1-cyclobutanedicarboxylatoplatinum(II)] [9]. Although JM-40 lacks a cyclobutane group, its polarity may be similar to that of CBDCA due to the ethylene group at the amine site. Higher proportions of water in the eluent resulted in decreased resolution of JM-40 from plasma ultrafiltrate and urine constituents. The retention volume of 25 ml required a flow-rate of 2 ml/min. The use of a 10-μl injection volume was a compromise between detection limit and resolution; a larger injection volume caused a reduction in the number of theoretical plates from 4000 with 10 μl to 3000 with 20 μl.

The UV absorption spectrum (500$-$190 nm) of JM-40 in eluent showed a maximum at 200 nm with a molar absorptivity of $5.35 \cdot 10^3$ l mol^{-1} cm^{-1}. A comparable value ($5.05 \cdot 10^3$ l mol^{-1} cm^{-1}) was obtained at 214 nm corresponding to the fixed wavelength of the UV detector.

The relationship between peak height and concentration of aqueous JM-40 solutions was linear over a range of 1$-$1000 μg/ml on a log$-$log scale (r =
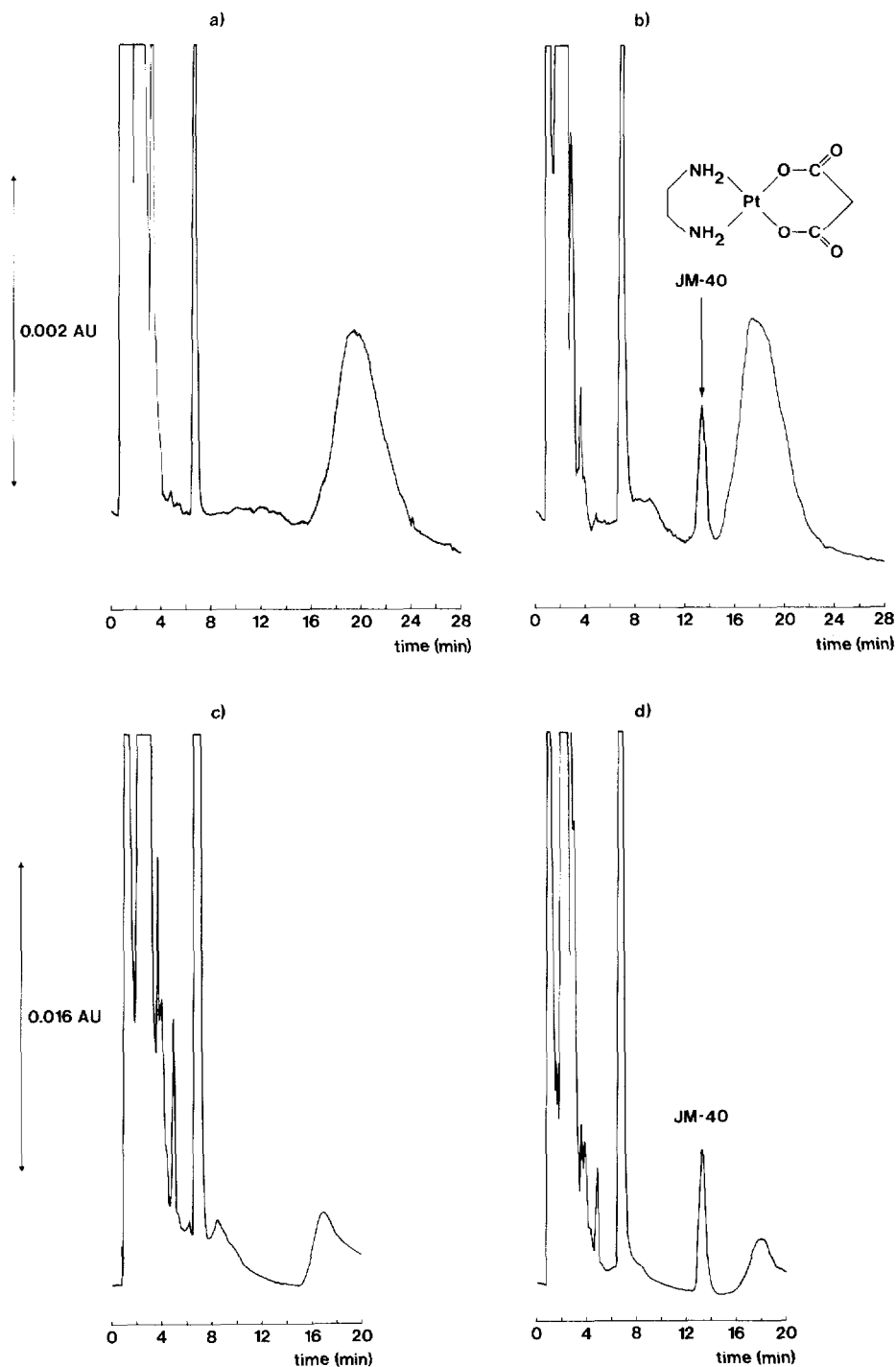
Fig. 1. Chromatograms of plasma ultrafiltrate and urine from a patient. (a) Ultrafiltrate before and (b) 90 min after administration of 300 mg/m² JM-40 (concentration 8.5 μg/ml); (c) urine before and (d) 2—4 h after administration of 400 mg/m² JM-40 (concentration 54.4 μg/ml). Column: μPorasil, 10 μm, 30 × 0.39 cm. Mobile phase: acetonitrile—water (90:10, v/v). Flow-rate: 2 ml/min. UV detection at 214 nm.

0.999, n = 6). Concentrations of JM-40 in biological fluids were calculated by linear interpolation of daily prepared standard curves for aqueous drug solutions. The sensitivity was $13.6 \cdot 10^{-3}$ absorbance units per μg. A practical detection limit of 0.35 μg/ml in plasma ultrafiltrate was achieved at a signal-to-noise ratio of 3:1. The recovery of JM-40 from the HPLC system was determined with AAS by the ratio of the platinum content of the JM-40 peak and the amount of platinum originally present in the sample injected. Using 10-μl samples containing 90.5 μg/ml JM-40, drug recovery from plasma ultra-filtrate, urine and aqueous solutions was 91.3 ± 3.5% (n = 6), 91.3 ± 2.7% (n = 3) and 90.9 ± 3.9% (n = 4), respectively. The overall precision of the assay, determined with an aqueous solution of 103 μg/ml JM-40 over three subsequent days, was 1.6% (n = 7). Repeated analysis of 50 μg/ml JM-40 in plasma ultrafiltrate, freshly prepared before each injection, revealed a mean within-day precision of 1.8% (three days, n = 6 per day) and an overall precision of 3.5% (n = 18).

Stability

The stability of JM-40 was determined in acetonitrile—water (9:1, v/v), 0.9% sodium chloride, 5% glucose, plasma ultrafiltrate and urine at ambient temperature. Parent JM-40 was determined periodically after preparation of the solutions. The results of these experiments are shown in Fig. 2.
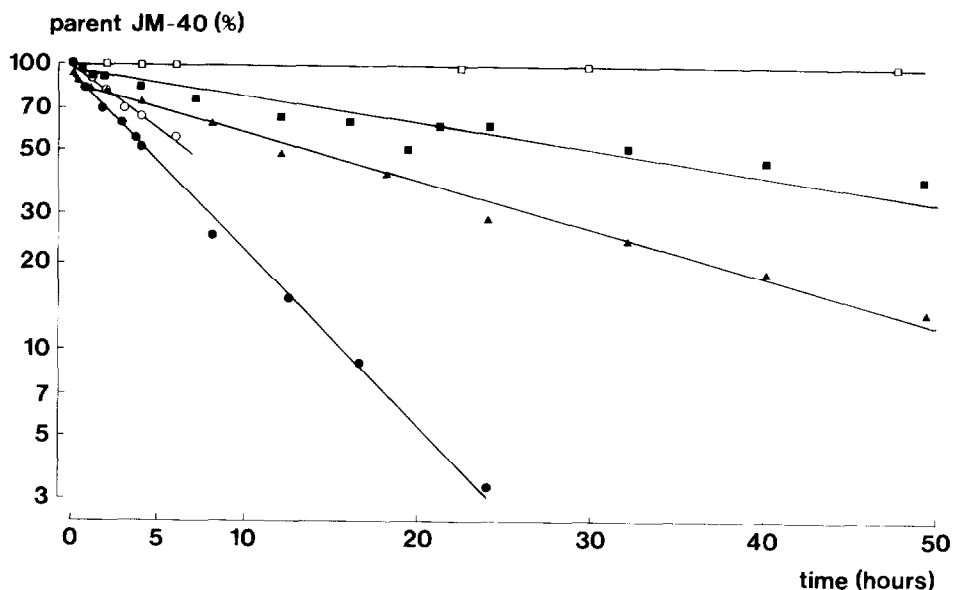

Fig. 2. Degradation curves for JM-40 in 5% glucose (□), 0.9% sodium chloride (■), plasma ultrafiltrate (▲), urine (●) and mobile phase (○).

In the mobile phase JM-40 had a half-life of 6.9 h (r = 0.994). This indicates that 2.1% of the injected drug may be lost during its 12.4-min residence in the column. JM-40 appeared to be stable in 5% glucose, confirming that 5% glucose is an appropriate vehicle for the administration of JM-40 to patients. Conversely, the drug appeared the be much less stable in saline, possibly due to

an exchange between the malonate group and the chloride ion [10]. The overall half-life in saline was 36.3 h (r = 0.95), although a half-life of 16.2 h (r = 0.97) was obtained during the first 4 h.

The reliability of the quantitation of JM-40 in body fluids is determined by the stability of JM-40 in these fluids. Fig. 2 shows that JM-40 disappears rapidly in plasma ultrafiltrate and urine, indicating the need for immediate analysis of patient samples. Half-lives of JM-40 in plasma ultrafiltrate and urine were 17.7 h (r = 0.994) and 4.9 h (r = 0.998), respectively. However, during the first 2 h, shorter half-lives of 10 h (r = 0.92) in plasma ultrafiltrate and 3.2 h (r = 0.97) in urine were obtained.

JM-40 concentrations in plasma ultrafiltrate could be determined within 30 min, at which point decomposition was limited to about 3%. Instability of JM-40 in urine was more severe. Therefore, decomposition during the stay of the drug in the bladder and during sample handling should be considered.

Pharmacokinetics

The assay was applied in the analysis of parent JM-40 in plasma ultrafiltrate and urine samples of a patient who received JM-40 in doses of 300 mg/m^2 and 400 mg/m^2. Fig. 3 shows the concentration—time curves for parent JM-40 in plasma ultrafiltrate compared to total platinum in plasma and plasma ultrafiltrate during the first 8 h after both infusions. The concentrations in plasma ultrafiltrate represent the amounts of non-protein bound forms of the
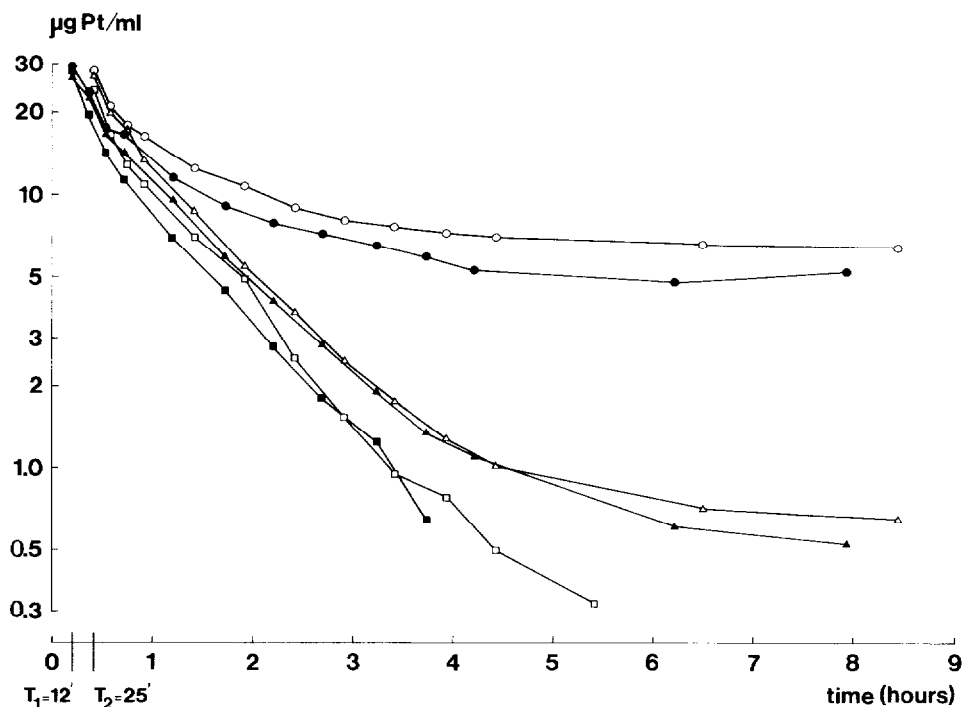

Fig. 3. Concentration—time curves of total platinum in plasma (●, ○), total platinum in plasma ultrafiltrate (▲, △) and parent JM-40 in plasma ultrafiltrate (■, □) in one patient after infusion of 300 mg/m^2 in T_1 = 12 min (closed symbols) and 400 mg/m^2 in T_2 = 25 min (open symbols).

metabolized and the parent drug. These species are thought to be responsible for the therapeutic effect, since protein-binding of platinum compounds is irreversible [11] and generates inactive adducts [12]. The concentration—time curves show three exponential terms for total platinum in plasma and plasma ultrafiltrate and two exponential terms for parent JM-40, due to distribution and elimination. The occurrence of more than two terms may indicate that metabolites of JM-40 are formed, which is supported by the presence of a third phase in the curve of total platinum in plasma ultrafiltrate in contrast to that of parent JM-40. Initial half-lives of distribution of parent JM-40 in plasma ultrafiltrate and of total platinum in plasma and plasma ultrafiltrate were 21.3, 28.3 and 28.6 min, respectively, when measured over a time interval of 0—20 min and without correction for subsequent phases.

Table I shows parent JM-40 and total platinum in plasma ultrafiltrate as a percentage of total platinum in plasma. These data indicate that in contrast to cisplatin [7, 13] non-protein-bound platinum can still be detected up to 24 h after administration of JM-40. Even 4 h after administration, about 50% of total platinum in plasma ultrafiltrate represents parent drug.

The results of the determination of JM-40 in patients' urine during the second cycle are given in Table II. The analysis was limited to urine samples collected during short time intervals because of possible drug degradation in the bladder. Most of the platinum excreted in the urine during the first 2 h was

TABLE I

PARENT JM-40 AND TOTAL PLATINUM IN PLASMA ULTRAFILTRATE AS PERCENTAGE OF TOTAL PLATINUM IN PLASMA

Results represent the mean of two cycles in one patient.

Time after administration (h)	Total platinum in plasma ultrafiltrate (μg/ml)	Parent JM-40 in plasma ultrafiltrate (μg/ml)
0	93.4	90.4
0.5	82.8	67.0
1	75.8	57.9
2	46.4	31.6
3	26.5	15.8
4	17.6	7.1*
8	10.2	
24	9.3	

*Second cycle only.

TABLE II

PARENT JM-40 EXCRETED IN URINE AFTER A DOSE OF 400 mg/m²

Time interval (h)	JM-40 (μg/ml)	Volume (ml)	Percentage of dose	Percentage of total platinum
0—2	88.8	1000	11.7	73.9
2—4	54.4	620	4.5	53.0
4—6	48.8	270	1.7	39.4

parent JM-40 (73.9%). After 6 h 18% of the dose was excreted as parent JM-40, while total platinum accounted for 28.6% of the dose. At 24 h, 39.0% of the dose was excreted. The cumulative platinum excretion in urine after administration of cisplatin was 24.4% and 28.4% of the dose at 6 and 24 h, respectively [13]. The excretion of a higher percentage of the dose over 24 h after JM-40 may be explained by the prolonged presence of non-protein-bound platinum species in plasma after administration of this drug.

ACKNOWLEDGEMENTS

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