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Note

High-performance liquid chromatographic analysis of unchanged *cis*-diamminedichloroplatinum (cisplatin) in plasma and urine with post-column derivatization

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cis-Diamminedichloroplatinum(II), cisplatin (CDDP), is one of the most potent anticancer drugs in current use [1]. However, its severe nephrotoxicity limits its clinical application [2]. After administration, CDDP undergoes a ligand-exchange reaction producing a mixture of protein-bound platinum (fixed metabolite), ultrafiltrable platinum complexed with several nucleophilic functional groups (mobile metabolites) and unchanged CDDP [3–5]. Although the biologically active (anticancer and nephrotoxic) species is unchanged CDDP [3,4], the pharmacokinetics of unchanged CDDP has not yet been widely applied for clinical therapy owing to the lack of a specific analytical method.

High-performance liquid chromatography (HPLC) has been adopted for de-

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termination of unchanged CDDP in biological fluids. Several investigators have used initial HPLC separation of platinum species followed by quantitation with off-line [6,7] and on-line [8] atomic absorption spectrometry, UV detection after post-column derivatization [9,10], reductive amperometric detection [11,12], direct UV detection [13], UV detection after column switching [14], electrochemical detection [15] or on-line radioactivity detection [16]. However, none of these methods has been widely accepted for clinical pharmacokinetics.

This paper describes the use of a Hitachi No. 3013-N analytical column and the post-column derivatization for the analysis of unchanged CDDP in clinical pharmacokinetics.

EXPERIMENTAL

Materials

CDDP (Landa) was a gift from Nihon Kayaku (Tokyo, Japan). All other chemicals and reagents were of analytical grade.

Apparatus and analytical conditions

We used a Shimadzu HPLC system (Kyoto, Japan) consisting of an LC-3A pump, an SPD-2A spectrometric detector operated at 0.02 a.u.f.s and a wavelength of 290 nm, a CTO-2A column oven maintained at 40°C and a C-R1A Chromatopac. The sample injector was a syringe-loading injector with a 100-μl loop (Rheodyne Model 7125). A Guard-Pak module with cyano (CN) insert (Waters Assoc., Milford, MA, U.S.A.) was used as a precolumn. The analytical column (15 cm$\times$4.6 mm I.D.) was packed with an anionic exchange resin, Hitachi No. 3013-N, 5 μm particle size (Chromato Research, Tokyo, Japan). The mobile phase, acetonitrile–10 m*M* sodium chloride (85:15, v/v), was pumped at a constant flow-rate of 0.7 ml/min. The platinum levels were determined with a Hitachi Model Z-8000 atomic absorption spectrometer (Hitachi, Tokyo, Japan). After 10 μl of sample were injected into the tube cuvette, a four-stage temperature programme was used for each determination. It consisted of a 30-s dry stage from 80 to 120°C, a 17-s ash stage at 1300°C, a 10-s atomization stage at 2700°C and a 3-s cleaning stage at 2800°C. Argon gas was used as the carrier gas. The platinum 265.9-nm line was monitored and lamp current was 12.5 mA.

Post-column derivatization system

The post-column derivatization system was composed of two minichemical pumps (SP-T-2501U; Nihon Seimitsu, Tokyo, Japan) and two different sizes of PTFE tubes (7 m$\times$0.5 mm I.D. and 30 m$\times$0.25 mm I.D.) as the reaction columns. These tubes were kept cylindrically wound. The reaction solutions

were $2.6 \cdot 10^{-5}$ *M* potassium dichromate and $6.6 \cdot 10^{-3}$ *M* sodium hydrogensulphite, which were pumped at 0.14 and 0.07 ml/min, respectively.

Standard solutions

A stock solution (1 mg/ml), prepared by dissolving CDDP in 0.9% sodium chloride solution, was stable (less than 5% loss) for at least one month at 4°C. Standard solutions were prepared by diluting the stock solution with 0.9% sodium chloride solution.

Sample preparation

Blood (5 ml) was collected in a heparinized dry test-tube. The sample was immediately centrifuged (1000 *g*) for 10 min at 4°C. The plasma obtained was subjected to ultrafiltration (4000 *g*) for 30 min at 4°C with a UFC 3GC membrane, which has a 10 000 molecular weight cut-off (Japan Millipore, Tokyo, Japan). Adsorption of CDDP on the membrane was negligible. Urine was collected periodically and its volume was measured. Each sample (plasma, ultrafiltered plasma and urine) was stored at −20°C until analysis. An ultrafiltered plasma sample was immediately (within 30 s) thawed in hot water (80°C), and a 100-μl aliquot was injected directly on to the column. Each urine sample was thawed in the same way as the ultrafiltered plasma sample and centrifuged at 1000 *g* for 1 min. A 50-μl aliquot of the upper phase was diluted (1:10) with distilled water, and 100 μl of this diluted fluid were injected directly on to the column. The stock periods for ultrafiltered plasma and urine samples were restricted to five days after sampling because of the instability of CDDP in biological fluids.

RESULTS AND DISCUSSION

The following points describe several modifications and further investigations of the system reported by Marsh and co-workers [9,10].

Unchanged CDDP in biological fluid was separated by an analytical column (Hitachi No. 3013-N), then determined at 290 nm after on-line post-column derivatization with potassium dichromate (activating agent) and sodium hydrogensulphite (derivatizing agent). Marsh et al. [10] separated the platinum species with a solvent-generated anion-exchange column [ODS Hypersil column coated with hexadecyltrimethylammonium bromide (HTAB) before analysis], which was time-consuming and showed poor reproducibility. The Hitachi No. 3013-N column overcame these deficiencies. The extensive length of tube was employed for a sufficient reaction delay time in the post-column derivatization. Although band-broadening was previously observed in tubes of large I.D. (0.79 mm) [10], both the lengths of tubes and the I.D. used in this study (7 m×0.5 mm I.D. and 30 m×0.25 mm I.D.) resulted in sufficient retention for clinical application without band-broadening.

Sodium thiosulphate (STS) has been applied clinically as a neutralizer of CDDP. It contains sulphur, which suggests inhibition of the post-column derivatization of CDDP. CDDP standard solutions (5 μg/ml) containing STS (2, 5 and 20 mg/ml) were determined by both direct UV detection at 210 nm and post-column derivatization at 290 nm. The fact that the same results for CDDP concentration were determined by both these detection methods suggested that STS did not inhibit the post-column derivatization.

CDDP is known to be very unstable in both aqueous and biological samples [14], so its stability during both sample treatment and storage was investigated. CDDP was found to be stable when biological samples (ultrafiltered plasma and urine) were immediately (within 30 s) thawed in hot water (80°C), but showed a 5% reduction in amount when samples were thawed at room temperature for 20 min. When samples were stored at −20°C, CDDP was stable without any loss for at least five days.

Fig. 1 shows chromatograms of unchanged CDDP in ultrafiltered plasma and diluted urine. Unchanged CDDP was detected at 11 min, where no interfering peak was observed in either fluids. The detection limit with a 100-μl injection was 80 ng/ml at a signal-to-noise ratio of 2 for unchanged CDDP in ultrafiltered plasma and diluted urine. Calibration curves for unchanged CDDP showed high correlation ($r=0.999$) and good linearity to 30 μg/ml. Good accuracy and reproducibility were obtained (Table I).

This HPLC analysis method was applied to the determination of unchanged CDDP in biological fluids after 2-h infusion of CDDP (130 mg). Fig. 2 shows the time course of the plasma concentration (left) and urinary excretion rate (right) of total and ultrafiltrable platinum, and unchanged CDDP as platinum equivalent. The total platinum concentration in plasma hardly changed, as shown previously by Gormley et al. [4]. The ultrafiltrable platinum was eliminated from plasma after administration of CDDP with a half-life of 50 min.

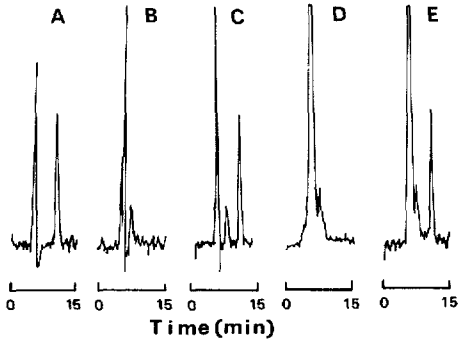

Fig. 1. Chromatograms for CDDP in saline (1 μg/ml, A), in ultrafiltered plasma (1 μg/ml, C) and in diluted (1·10) urine (10 μg/ml, E). B and D represent chromatograms for blank ultrafiltered plasma and blank diluted (1:10) urine, respectively.

TABLE I

WITHIN-DAY ACCURACY AND REPRODUCIBILITY OF THE PRESENT HPLC ANALYSIS FOR UNCHANGED CDDP IN ULTRAFILTERED PLASMA AND URINE

The observed values are expressed as mean ± S.D.; $n = 5$.

CDDP added (μg/ml)	CDDP found (μg/ml)	C.V. (%)	Recovery (%)
Ultrafiltered plasma			
0.5	0.48 ± 0.04	7.9	95.0
1.0	1.04 ± 0.05	5.3	104.0
2.0	2.05 ± 0.10	4.9	102.5
Urine			
5.0	4.96 ± 0.15	3.0	99.3
10.0	10.38 ± 0.57	5.5	103.8
20.0	19.60 ± 0.68	3.5	98.0

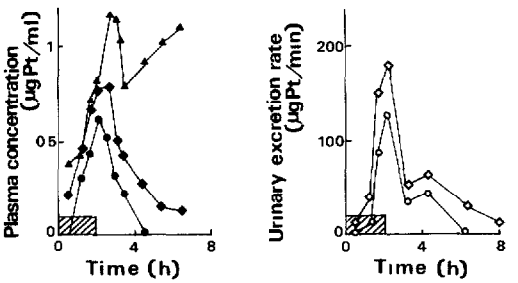

Fig. 2. Plasma concentration-time curves (left) and urinary excretion rate-time curves (right) for total platinum (▲), ultrafiltrable platinum (◆,◇) and unchanged CDDP (●,○) as platinum equivalent in a cancer patient. Patient characteristics: age, 67 years; sex, male; tumour location, lung; 24-h creatinine clearance, 97.4 ml/min. Administration protocol: after hydration (Solita T_3, 500 ml for 2 h), CDDP (80 mg/m^2) in saline (500 ml) was infused over 2 h (▨), followed by hydration for 4 h (Solita T_3, 500 ml, ×2).

On the other hand, unchanged CDDP was rapidly eliminated ($t_{1/2} = 25$ min) in comparison with ultrafiltrable platinum, as previously reported by Reece and Stafford [3]. The urinary excretion data also showed a difference in behaviour between unchanged CDDP and ultrafiltrable platinum. These results suggested that analysis of biologically active unchanged CDDP should be more clinically useful in CDDP chemotherapy than that of ultrafiltrable platinum.

This method, including a separation procedure using a Hitachi No. 3013-N column and quantitation by post-column derivatization, is applicable to clinical pharmacokinetic studies of unchanged CDDP.

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