ELSEVIER

Journal of Chromatography B, 663 (1995) 181–186

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Short communication

# Quantitative determination of unchanged cisplatin in rat kidney and liver by high-performance liquid chromatography

Kazuhiko Hanada, Naomi Nagai, Hiroyasu Ogata*

*Department of Biopharmaceutics, Meiji College of Pharmacy, Yato-cho 1-22-1, Tanashi-shi, Tokyo 188, Japan*

First received 23 June 1994; revised manuscript received 13 September 1994

**Abstract**

A quantitative analytical method for measuring unchanged cisplatin (CDDP) and high- and low-molecular-mass metabolites (fixed and mobile metabolites) in rat kidney and liver was developed. Unchanged CDDP, separated from fixed and mobile metabolites in tissue homogenates by consecutive procedures of fractionation and ultrafiltration, was determined by high-performance liquid chromatography (HPLC) with post-column derivatization. Although unchanged CDDP was found to be partly metabolized to fixed metabolites during the preparation of cytosolic ultrafiltrates, the recovery of unchanged CDDP gave a constant value (about 70%), which was independent of tissue type and CDDP concentration (from 1 to 10 $\mu$g/ml). The detection limit for unchanged CDDP in the cytosolic ultrafiltrate was 20 ng/ml, corresponding to a concentration detection limit of 65 ng Pt per g of tissue in the kidney and liver. The concentrations of fixed and mobile metabolites were determined as platinum concentrations in the tissue homogenate and in the cytosolic ultrafiltrate using atomic absorption spectrometry after correcting for transformation of unchanged CDDP to fixed metabolites. The distribution of unchanged CDDP, mobile metabolites and fixed metabolites in rat kidney and liver, after bolus injection of CDDP (5 mg/kg), was determined using this method.

## 1. Introduction

Cisplatin (CDDP) is an effective cancer chemotherapeutic agent used in the management of various tumors, however, its clinical use is often complicated by dose-related nephrotoxicity [1]. Although the mechanisms of CDDP nephrotoxicity remain unclear, kidney platinum levels have been shown to be several fold higher than plasma levels and above those found in other organs following administration of CDDP to animals [2,3]. Patients with evidence of nephrotoxicity have been shown to have higher kidney platinum concentrations than patients without kidney damage [4]. Although CDDP reacts extensively with proteins and various low-molecular-mass compounds in biological fluids, producing high- and low-molecular-mass platinum (fixed and mobile metabolites), the principal platinum species in the initial distribution phase after intravenous administration is unchanged CDDP [5]. Furthermore, unchanged CDDP and several low-molecular-mass metabolites are actively secreted and/or reabsorbed by the kidney during the early phase. These pharmacokinetic properties may be related to the observation that

* Corresponding author.

0378-4347/95/$09.50 
*SSDI* 0378-4347(94)00415-3

the critical effects of CDDP on the kidney occur within 0–4 h after its administration [5–7]. To understand the relationship between the toxic effect and CDDP disposition, it is essential to clarify the distribution and biotransformation of unchanged CDDP in the organs showing side effects as well as in plasma and urine. Although there are a lot of methods available for analyzing unchanged CDDP [8–15], most of them measure unchanged CDDP in plasma and/or urine.

In the present study, we have applied a previously published HPLC method [13] with partial modification for the quantitative determination of unchanged CDDP in rat kidney and liver, after correcting for the loss of unchanged CDDP in the preparation of kidney and liver cytosolic ultrafiltrates.

## 2. Experimental

### 2.1. Chemicals and reagents

CDDP (Randa®) was a gift from Nippon Kayaku (Tokyo, Japan). CDDP was dissolved in a 0.9% (w/v) sodium chloride solution (1 mg/ml), which was used as the stock solution for HPLC analysis, and as administration solution for animal studies. Each standard solution for HPLC analysis was prepared by diluting the stock solution consecutively with 0.9% (w/v) sodium chloride solution. All other chemicals and reagents were purchased from Wako Pure Chemical Industries (Tokyo, Japan) and were of analytical and reagent grade.

### 2.2. HPLC apparatus and analytical conditions for unchanged CDDP determination

The HPLC system consisted of a Shimadzu HPLC apparatus (Kyoto, Japan), a LC-6A HPLC pump, a SPD-6A spectrometric detector operated at a wavelength of 290 nm with a range of 0.01 AUFS and a C-R6A Chromatopac integrator. Samples were introduced into a 100-$\mu$l loop (Rheodyne, Cotati, CA, USA). Separation of unchanged CDDP from other metabolites was performed at 40°C on an analytical column (150 × 4.6 mm I.D.) packed with Hitachi No. 3013-N, 5 $\mu$m particle size (Chromato Research, Tokyo, Japan). The mobile phase was 10 m*M* sodium chloride–acetonitrile (85:15, v/v) at a constant flow-rate of 0.9 ml/min. A guard column (50 × 4.6 mm I.D.) was packed with the same resin as the analytical column. Unchanged CDDP was detected after post-column derivatization with both 26 $\mu M$ potassium dichromate and 6.6 m*M* sodium hydrogensulfite, pumped at 0.6 and 0.3 ml/min, respectively. The post-column derivatization system consisted of two HPLC pumps (Shimadzu LC-9A, Kyoto, Japan) and two reaction columns (PTFE tubes, 7 m × 0.5 mm I.D. and 30 m × 0.25 mm I.D.).

### 2.3. Apparatus and analytical conditions for platinum determination

The platinum concentration was determined with a Hitachi Model Z-9000 atomic absorption spectrometer (Hitachi Co., Tokyo, Japan). After a 20-$\mu$l sample had been applied to the tube cuvette, platinum was monitored at 265.9 nm using a four-stage temperature program consisting of a 30-s dry stage from 80°C to 120°C, a 17-s ash stage at 1300°C, a 15-s atomize stage at 2800°C and a 5-s clean stage at 3000°C, respectively. Argon gas was used as a carrier gas at a flow-rate of 200 ml/min.

### 2.4. Recovery of unchanged CDDP from kidney and liver tissue

Wistar male rats (200–280 g body weight) anesthetized with diethyl ether were sacrificed, and the kidney and liver were removed after intra-arterial perfusion of ice-cold 0.9% (w/v) sodium chloride solution. After immersion and decapsulation in ice-cold 0.9% (w/v) sodium chloride solution, each organ was blotted on filter paper and weighed. The losses of unchanged CDDP during homogenization and ultracentrifugation were estimated according to the following separately performed experiments. To determine the loss of unchanged CDDP during homogenization, each organ was homogenized (1:4, w/v) in an ice-cold CDDP

standard solution (5 or 10 μg/ml of CDDP in 0.9% (w/v) sodium chloride solution for the kidney, 2 or 5 μg/ml for the liver). An aliquot of the homogenate was taken for total platinum analysis. The rest of the homogenate was ultracentrifuged (100 000 *g*) at 4°C for 60 min [5,16]. A 450-μl sample of the supernatant was subjected to ultrafiltration (4000 *g*) at 4°C for 30 min using an UFC 3GC membrane with 10 000 molecular-mass cut-off (Japan Millipore, Tokyo, Japan). All samples (tissue homogenate, cytosol and cytosolic ultrafiltrate) were frozen and stored below −20°C until analysis. For HPLC analysis of unchanged CDDP, a sample of cytosolic ultrafiltrate was immediately thawed in hot water and an aliquot (50–100 μl) was loaded directly onto the analytical column. A 20-μl aliquot of each sample (tissue homogenate, cytosol and cytosolic ultrafiltrate) was loaded onto the tube cuvette for platinum analysis. The recovery of unchanged CDDP was calculated as the percentage ratio of unchanged CDDP (platinum equivalent) to the added CDDP (platinum equivalent). To determine the loss of unchanged CDDP during ultracentrifugation, each organ was homogenized (1:4, w/v) in an ice-cold 0.9% (w/v) sodium chloride solution. CDDP was added to the kidney homogenate at a concentration of 1, 2, 5, or 10 μg/ml and to the liver homogenate at a concentration of 1, 4, or 10 μg/ml. A 500-μl aliquot of the homogenate was used for total platinum analysis. Cytosolic ultrafiltrates were prepared according to the procedure described above.

### 2.5. Tissue sample preparation

Male Wistar rats (200–280 g body weight) were anesthetized with sodium pentobarbital (40 mg/kg i.p.). The rats were fasted for 16 h prior to the experiment, but had free access to water. The animals were administered CDDP (5 mg/kg) as a bolus injection and subsequently sacrificed at 1, 3, 5, 15, 30, 60 or 120 min. After intra-arterial perfusion with ice-cold 0.9% (w/v) sodium chloride solution, the kidney and liver were immediately removed. After immersion and decapsulation in an ice-cold 0.9% (w/v) sodium chloride solution, each organ was blotted on filter paper, weighed and homogenized in ice-cold 0.9% (w/v) sodium chloride solution (1:4, w/v) at 4°C. The preparation of cytosolic ultrafiltrates and analysis of unchanged CDDP and platinum were performed according to the procedures described above. The concentrations of unchanged CDDP, mobile metabolites and fixed metabolites in the kidney and liver were calculated according to the following equations:

- UPt = unchanged CDDP in cytosolic ultrafiltrate as determined by the HPLC method (platinum equivalent)/0.7.
- UPtloss = UPt − unchanged CDDP in cytosolic ultrafiltrate as determined by the HPLC method (platinum equivalent).
- MPt = platinum in cytosolic ultrafiltrate − unchanged CDDP in cytosolic ultrafiltrate as determined by the HPLC method (platinum equivalent).
- FPt = (platinum in tissue homogenate − platinum in cytosolic ultrafiltrate) − UPtloss

where UPt, MPt and FPt are the concentrations of unchanged CDDP, mobile metabolites and fixed metabolites after correcting for loss of unchanged CDDP in the sample preparation, UPtloss is the concentration of decomposed CDDP which is transformed to fixed metabolites during sample preparation, and 0.7 is the correction factor, representing the recovery of unchanged CDDP from the tissue homogenates.

## 3. Results and discussion

We have applied the previously published HPLC method [13] to determine unchanged CDDP in the kidney and liver. Unchanged CDDP is known to be so reactive with proteins and some biologically relevant low-molecular-mass compounds [5,14,17] that it can be transformed to either fixed metabolites or mobile metabolites during sample preparation. Thus, the stability of unchanged CDDP during the preparation of tissue samples was investigated. Secondly, it was necessary to determine the optimal HPLC conditions under which HPLC

peaks from tissue ultrafiltrates did not interfere with the chromatogram of unchanged CDDP.

The cytosolic fraction was prepared by consecutive homogenization and ultracentrifugation according to published methods [5,16]. The loss of unchanged CDDP was estimated separately under the following two conditions: (1) kidney and liver were homogenized with CDDP solution, (2) CDDP was added to each tissue homogenate prior to ultracentrifugation. Table 1 shows the recovery of unchanged CDDP from rat kidney and liver. The recovery of unchanged CDDP was estimated to be approximately 70% in both kidney and liver, and this was independent of the CDDP concentration over the range 1–10 μg/ml. Platinum levels in the cytosol and cytosolic ultrafiltrate did not change; however, the platinum level equivalent to the fixed metabolites increased after preparing the cytosolic fraction. CDDP was added to a tissue blank homogenate, and the time course of the level of unchanged CDDP was monitored. Decomposition of unchanged CDDP occurred shortly after the addition of CDDP. Therefore, the loss of unchanged CDDP during sample preparation was attributed to the rapid transformation of unchanged CDDP to fixed metabolites, although this could be corrected for by using the correction factor (0.7). The kinetics of CDDP in the kidney and liver after CDDP administration can be evaluated as unchanged CDDP, mobile and fixed metabolites according to the equations described in the experimental section.

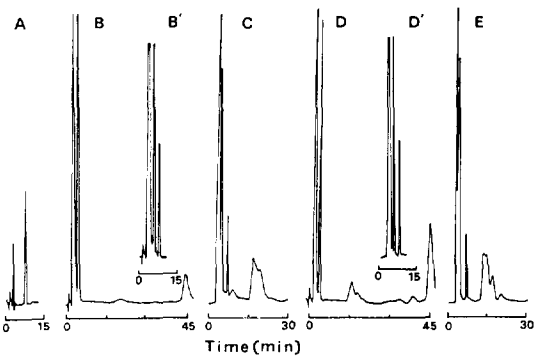


Fig. 1. Chromatograms of unchanged CDDP in 0.9% (w/v) sodium chloride solution (1 μg/ml) (A) and in rat-kidney cytosolic ultrafiltrate taken at 30 min (C) and in rat-liver cytosolic ultrafiltrate taken at 5 min (E) following bolus injection of CDDP (5 mg/kg). (B) and (D) represent chromatograms of blank kidney and liver cytosolic ultrafiltrates obtained from non-treated rats, and chromatograms of unchanged CDDP (1 μg/ml) spiked in the same blank cytosolic ultrafiltrates (B' and D').

Fig. 1 shows chromatograms of unchanged CDDP in the kidney and liver cytosolic ultrafiltrates. There are several peaks from cytosolic ultrafiltrates, but no interfering peak was detected at the retention time for unchanged

Table 1
Recovery of unchanged CDDP from rat kidney and liver

| Kidney | | Liver | |
|---|---|---|---|
| CDDP added (μm/ml) | Recovery (%) | CDDP added (μg/ml) | Recovery (%) |
| *Ultracentrifugation* | | | |
| 1 | 68.7 ± 2.7 | 1 | 70.2 ± 6.2 |
| 2 | 68.8 ± 7.5 | 4 | 73.7 ± 7.2 |
| 5 | 70.7 ± 6.4 | 10 | 70.6 ± 7.7 |
| 10 | 71.2 ± 4.6 | | |
| *Homogenization* | | | |
| 5 | 74.5 ± 8.3 | 2 | 70.6 ± 5.7 |
| 10 | 69.8 ± 5.2 | 5 | 69.9 ± 8.9 |

Experimental conditions: see text.
Values are expressed as mean ± S.D. ($n = 4$).

CDDP (9 min, Fig. 1B and D). The peak of unchanged CDDP was identical to the chromatograms of the blank cytosolic ultrafiltrates spiked with unchanged CDDP (Fig. 1B′ and D′). The detection limit with a 100-μl injection was 20 ng/ml at a signal-to-noise ratio of 2 in the cytosolic ultrafiltrate, corresponding to a detection limit of 65 ng Pt per g tissue in both kidney and liver.

The concentrations of unchanged CDDP, mobile and fixed metabolites in the rat kidney and liver after bolus injection of CDDP (5 mg/kg) were determined using this HPLC method and atomic absorption spectrometry. Fig. 1C and E show typical chromatograms of kidney and liver cytosolic ultrafiltrates after bolus injection of CDDP (5 mg/kg) to rats. Fig. 2 shows the time courses of total platinum, fixed and mobile metabolites, and unchanged CDDP levels in rat kidney (left) and liver (right). Unchanged CDDP rapidly reached its maximum level immediately after bolus injection of CDDP. The concentration of unchanged CDDP was lower than the detection limit and the fixed metabolite was a major species at 120 min. The distribution and biotransformation of CDDP were considered to be organ specific. These results are the same as previously reported data [5,18]. In the liver, the total platinum level was far less than that in the kidney, and the mobile and fixed metabolites might be produced at almost the same rate during the first 15 min. However, the fixed metabolites were selectively and extensively produced in the kidney.

The intracellular CDDP metabolites in the kidney have been investigated using size exclusion and ion-exchange chromatography [18], or HPLC fractionation and atomic absorption spectrometry [16]. Although unchanged CDDP was labile in biological fluids, the stability of unchanged CDDP during preparation of tissue samples was not examined in the previous studies. In the present study, we have found loss of unchanged CDDP during sample preparation, and proposed a correction factor. Furthermore, we have applied HPLC separation and on-line post-column derivatization. Thus the analytical time was shortened and the time courses of unchanged CDDP could be easily monitored. This method will be suitable for the quantitative determination of unchanged CDDP in organs showing side effects and will be useful for toxicokinetic studies.

## References

[1] N.E. Madias and J.T. Harrington, *Am. J. Med.*, 65 (1978) 307.
[2] C.L. Litterst, A.F. LeRoy and A.M. Guarino, *Cancer Treat. Rep.*, 63 (1979) 1485.
[3] C.L. Litterst, I.J. Torres and A.M. Guarino, *J. Clin. Hematol. Oncol.*, 7 (1976) 169.
[4] D.H. Stewart, N.Z. Mikhael, A.A. Nanji, R.C. Noir, S. Kacew, K. Howard, W. Hirte and J.A. Maroun, *J. Clin. Oncol.*, 3 (1985) 1251.
[5] P. Mistry, C. Lee and D.C.H. McBrien, *Cancer Chemother. Pharmacol.*, 24 (1989) 73.
[6] P.T. Daley-Yates and D.C.H. McBrien, *Biochem. Pharmacol.*, 34 (1985) 1423.

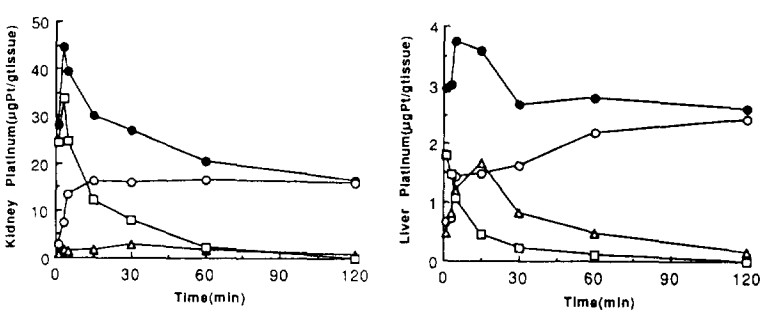


Fig. 2. Concentration–time curves of total platinum (●), fixed metabolites (○), mobile metabolites (△) and unchanged CDDP (□) in the kidney and liver following bolus injection of CDDP (5 mg/kg) to rats. Values are expressed as mean ($n = 2$).

[7] P.A. Reece, I. Stafford, M. Davy and S. Freeman, *Clin. Pharmacol. Ther.*, 42 (1987) 320.
[8] S.J. Bannister, L.A. Sternson and A.J. Repta, *J. Chromatogr.*, 273 (1983) 301.
[9] R. Safirstein, M. Daye and J.B. Guttenplan, *Cancer Lett.*, 18 (1983) 329.
[10] P.T. Daley-Yates and D.C.H. McBrien, *Biochem. Pharmacol.*, 33 (1984) 3063.
[11] S.K. Mauldin, F.A. Fichard, M. Plescia, S.D. Wynck, A. Saucer and S.G. Chaney, *Anal. Biochem.*, 157 (1986) 129.
[12] R. Kizu, K. Hayakawa and M. Miyazaki, *Biomed. Chromatogr.*, 3 (1989) 14.
[13] M. Kinoshita, N. Yoshimura, H. Ogata, D. Tsujino, T. Takahashi, S. Takahashi, Y. Wada, K. Someya, T. Ohno, K. Masuhara and Y. Tanaka, *J. Chromatogr.*, 529 (1990) 462.
[14] Z. Zhao, K. Tepperman, J.G. Dorsey and R.C. Elder, *J. Chromatogr.*, 615 (1993) 83.
[15] A. Andersson and H. Ehrsson, *J. Chromatogr.* B, 652 (1994) 203.
[16] R.P. Sharma and I.R. Edwards, *Biochem. Pharmacol.*, 32 (1983) 2665.
[17] A.J. Repta, D.F. Long, in A.W. Prestakyo, S.T. Crooke and S.K. Carter (Editors), *Cisplatin – Current Status and New Developments*, Academic Press, New York, NY, 1980, p. 285.
[18] R.W. Mason, S.T. Hogg and I.R. Edwards, *Toxicology*, 38 (1986) 219.