On-Line Differential Pulse Polarographic Detection of Carboplatin in Biological Samples after Chromatographic Separation

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A method for the determination of the antitumor platinum(II) complex carboplatin has been developed that uses high-performance liquid chromatography with differential pulse polarographic (DPP) detection. Carboplatin is reduced at -1.77 V vs. Ag/AgCl. DPP detection was used because of its sensitivity and selectivity. The influence of differential pulse polarographic parameters on detector performance was investigated. By use of a pulse amplitude of -250 mV at a potential of -1.6 V, with a 0.5 -s drop time and a 50 -ms memory time constant, detection limits of 0.1 μ M carboplatin in plasma ultrafiltrate and 1 μ M in urine were achieved. With an injection volume of 50 μ L precision was 1.5% and 4.7% , respectively. As an example, the pharmacokinetic profile of carboplatin has been determined in a patient after an intravenous bolus injection.

Carboplatin (diammine(1,1-cyclobutanedicarboxylato)-platinum(II), CBDCA, Figure 1) is a promising analogue of the antitumor platinum complex cisplatin (*cis*-diamminedichloroplatinum(II)) (1). It is in an advanced stage of clinical investigation and for this reason its pharmacokinetic profile needs to be elucidated in detail. Therefore, a sensitive assay of carboplatin in body fluids is required. Since pharmacokinetics of platinum compounds is usually compared with pharmacokinetics of platinum determined by atomic absorption spectrophotometry (AAS), the method to be developed for carboplatin should be at least as sensitive as AAS.

Assays of carboplatin have been described using normal-phase (2, 3) as well as reversed-phase (4-6) HPLC systems. Selective and sensitive on-line detection of the eluted carboplatin appeared to be the critical step in determining low concentrations. The only method used for pharmacokinetic investigations was based on normal-phase HPLC and UV detection at 225 nm (3). Because of the low molar absorptivity of carboplatin at this wavelength ($\lambda_{\max}$ 210 nm, $\epsilon = 4300$ L/(mol cm)) the detection limit in plasma ultrafiltrate was 10 μ M, which limits pharmacokinetic and metabolic studies of carboplatin in patients. Other spectrophotometric techniques used to detect carboplatin are quenched phosphorescence detection (4) and postcolumn reaction detection (5). However, these methods did not provide the desired sensitivity either.

Platinum compounds can be determined by electrochemical reduction or oxidation. Although the polarographic reduction of platinum(II) complexes has been the subject of investigation for years, its electrode reaction mechanism is still not completely understood (7-10). Recently, differential pulse polarography (DPP) has been used for the determination of total platinum in inorganic and biological media (11-13). Liquid chromatography with electrochemical detection (LC-EC) has been proven to provide a sensitive and selective determination of cisplatin (14-16), iproplatin (*cis*-dichloro-*trans*-dihydroxo-*cis*-bis(isopropylamine)platinum(IV)) (14, 15) and

spiroplatin (aqua(1,1-bis(aminomethyl)cyclohexane)[sulfato]platinum(II)) (17, 18). Until now, electrochemical detection of carboplatin was only explored in the oxidative mode with a relatively poor detection limit of 14 μ M in aqueous solution (14), while in the reductive mode no signal was observed at polarographic (15) as well as gold/mercury (14) electrodes. The present paper describes a sensitive detection of carboplatin by means of reductive DPP.

EXPERIMENTAL SECTION

Instrumentation. The chromatographic system consisted of a Waters Model 590 pump (Etten-Leur, The Netherlands), a Valco air-actuated injection valve provided with a 50 - μ L injection loop (Chrompack, Middelburg, The Netherlands), and a Phase Sep Spherisorb S5 ODS2 column (4.6×150 mm, particle size 5 μ m, ATS Chromatography, Waddinxveen, The Netherlands) combined with an Alltech direct-connect guard column (2.1×30 mm, Amstelveen, The Netherlands) filled with Serva octadecyl = Si 100 Polyol 0.03 mm (Brunswick, Amsterdam, The Netherlands). The HPLC pump was provided with an extra pulse dampner (Waters) to eliminate flow noise.

Electrochemical detection and polarographic measurements were performed with a PAR Model 310/303 static mercury drop electrode (SMDE) (EG&G Instruments, Nieuwegein, The Netherlands) provided with a saturated Ag/AgCl reference electrode and a platinum counter electrode. Type G171 capillaries (PAR) were used to prevent mercury drop fall at potentials beyond -1.2 V. The medium mercury drop size (1.57 mm²) was used. Potential control and current sampling of the SMDE were made by a PAR Model 174A polarographic analyzer. The electronic circuit of the DPP mode was modified as follows: (i) the range of pulse amplitudes was extended with values of 250 and 500 mV by changing the resistors R43-R47 to 800 , 100 , 60 , 20 , and 20 k Ω , respectively (19), (ii) the sample width was changed from 16.5 to 20 ms to suppress noise from the 50 -Hz line frequency by changing resistor R271 from 165 to 200 k Ω (19), and (iii) the memory time constant and (iv) the pulse width were made adjustable both according to ref 20. The actual pulse width was measured by a Hewlett-Packard Universal Counter HP 5325A (Amsterdam, The Netherlands). UV absorption detection was performed with an LDC/Milton Roy UV-III Model 1203 detector with a fixed wavelength of 214 nm (Charles Goffin, De Bilt, The Netherlands). Signals were recorded by a BD 100 strip chart or BD 91 XYY' recorder (Kipp & Zonen, Delft, The Netherlands). Platinum concentrations in eluent fractions, collected by an LKB 2112 RediRac fraction collector (Zoetermeer, The Netherlands), were determined by a Perkin-Elmer Model 5000 atomic absorption spectrophotometer (Gouda, The Netherlands) as described previously (21).

Chemicals. Carboplatin was from Johnson Matthey (Reading, Berkshire, U.K.) and cisplatin was provided by Bristol Meyers (Brussels, Belgium). Distilled demineralized water was used throughout. Mercury of polarographic quality was obtained from Merck (Amsterdam, The Netherlands). All other chemicals were of analytical grade. The mobile phase consisted of 0.05 M NaClO₄ and was deoxygenized as described previously (17). Blank urine and plasma samples were pooled from healthy volunteers.

Procedures. The plasma ultrafiltrate pool for in vitro experiments was prepared with Amicon Centriflow CF 50A ultrafiltration cones (Oosterhout, The Netherlands). Patient plasma samples (1 mL) were ultrafiltered by the Amicon MPS-1 system

Figure 1. Structural formula of carboplatin.

provided with YMT filters (22). Recovery of carboplatin after this ultrafiltration step was tested in duplicate by spiking plasma to obtain concentrations of 5, 50, and 100 μM . Each solution was ultrafiltered immediately after constitution. Peak heights in the first 200 μL of ultrafiltrate were compared with those from the same concentrations in spiked plasma ultrafiltrate. Plasma ultrafiltrate and urine samples were immediately frozen, stored at -25°C , and thawed just before injection of 50 μL into the HPLC system without any further pretreatment. Separation and detection were performed at ambient temperature. Calibration curves of carboplatin in spiked plasma ultrafiltrate and urine samples were prepared daily. The guard column was repacked after every 100 samples. Peak heights were recorded rather than peak areas.

RESULTS AND DISCUSSION

Separation. Carboplatin was retained on a C-18 reversed-phase column with a mobile phase of water containing 0.05 M sodium perchlorate as supporting electrolyte. Sodium perchlorate was chosen because it does not form complex bonds with Pt(II) (23) and therefore it will not cause degradation of platinum compounds by ligand exchange during separation or detection. The chromatographic separation step could be simple, because of the high selectivity of the applied detection method. A capacity factor (k') of 1.8 appeared to be sufficient to separate carboplatin from detectable endogenous compounds.

Recovery of carboplatin from freshly spiked plasma samples was 97% and independent of the concentration (5–100 μM). Carboplatin injected in aqueous solutions, freshly spiked plasma ultrafiltrate, or urine was totally recovered from the column in one peak as determined by AAS analysis of the fractionated eluent. Possible metabolites or degradation products of carboplatin like *cis*-diamminediaquaplatinum(II), *cis*-diammineaquachloroplatinum(II), and *cis*-diamminedichloroplatinum(II) (cisplatin), which may be formed by exchange of the 1,1-cyclobutanedicarboxylate ligand, eluted at or close to the solvent front. This was expected on account of the high polarity of these molecules. Sodium perchlorate had no influence on retention of carboplatin, as measured by UV detection at 214 nm using the same column and only water as mobile phase. It seems plausible that the hydrophobic cyclobutane group of carboplatin is responsible for its retention on RP-18 columns with purely aqueous mobile phases.

Modification of the Polarographic Analyzer. The polarographic analyzer has been provided with a limited maximum pulse amplitude (–100 mV) and a rather long memory time constant (MTC) of the DPP mode (83 ms). The observed time constants were 2.1 s and 4.2 s with a drop time of 0.5 s and 1 s, respectively (24). These long time constants have been reason to reject differential pulse polarographic detection (15, 25). Therefore, a circuit with an adjustable MTC was added, as described by Jackson et al. (20). The effect of the MTC on peak height, plate number, and noise is shown in Table I. It can be decided from the ratio between plate number and noise level as well as from the ratio between peak height and noise level that an MTC of 50 ms gives the best results. This effect is even more pronounced with a drop time of 1 s. An MTC of 50 ms and a drop time of 0.5 s were chosen as optimum values to ensure sharp peaks with a reproducible peak height.

The pulse width was also made variable (20) to increase sensitivity (26). The measured relationship between pulse

Table I. Influence of the Memory Time Constant (MTC) on Some Chromatographic Parameters after Injection of 50 μL of 10 μM Carboplatin

MTC, ms	plate no.	peak height, nA	noise level, nA
83	2080	214	0.4
50	3390	255	0.5
20	4230	289	0.7
10	4270	298	1.2

Figure 2. Relationship between the pulse width of DPP detection and the chromatographic peak height of a 50- μL injection of carboplatin 10 μM .

width and sensitivity (peak height) is shown in Figure 2. An increase in sensitivity of 57% was observed when the pulse width was shortened from the original 40.9 ms to 3.4 ms. The noise level did not change, but the height of the base line was doubled, eliminating the sensitivity advantage in the lower concentration ranges. Therefore, the original pulse width was used for the analysis of carboplatin. Optimization of the pulse amplitude is discussed below.

Polarographic Detection. Figure 3a shows a sampled direct current polarogram (SDCP) and background subtracted SDCP and DPP polarograms of carboplatin in 0.05 M sodium perchlorate. The reduction wave has a half-wave potential ($E_{1/2}$) of -1.77 V vs. Ag/AgCl. It appeared irreversible according to the Tomeš criterion ($|E_{3/4} - E_{1/4}| = 51.7/n\alpha$) (27): from the SDCP curve of Figure 3a it can be calculated that the transfer coefficient $\alpha = 0.32$ when the number of electrons $n = 2$. The limiting current at -1.85 V showed a linear relationship with the carboplatin concentration over the range of 1.0×10^{-6} to $1.7 \times 10^{-4}\text{ M}$ suggesting a diffusion-controlled process. Reduction waves of Pt(II) complexes at potentials more negative than -1.3 V have been attributed to catalytic hydrogen evolution (9–11). This is in accordance with our observation that the limiting current of the SDCP curve (Figure 3a) is 3 times higher than the value calculated from the Cottrell equation (27), which affirms that the observed process is not merely a reduction of $\text{Pt}^{2+} \rightarrow \text{Pt}^0$. The highly negative reduction potential makes high demands upon the dropping mercury electrode. Nevertheless, the wave was considered to be suited to detect carboplatin in HPLC effluents. Detection at the hanging mercury drop electrode (HMDE) as used for spiropalladium (18) was not feasible because of electrode surface contamination by adsorbed species from injected biological samples. Therefore, the static mercury drop electrode (SMDE) was used throughout.

Figure 3. Voltammograms of carboplatin 10^{-4} M: (a) (---) sampled direct current polarogram of carboplatin and electrolyte (0.05 M sodium perchlorate), (—) background subtracted SDCP (X) and differential pulse polarograms; (b) hydrodynamic SDC (X) and DP voltammograms. Pulse amplitudes of -100 mV (●), -250 mV (■), and -500 mV (▲), medium drop size, drop time of 0.5 s, and scan rate of 5 mV/s were used.

In order to determine optimum detection conditions with the described HPLC system, hydrodynamic voltammograms of carboplatin were recorded with the polarographic detector in the SDCP and DPP mode and with several pulse amplitudes (Figure 3b). The hydrodynamic voltammograms corresponded with the background-subtracted polarograms of Figure 3a. Background subtraction of the batch polarograms was employed to allow comparison with the hydrodynamic voltammograms, which are composed of chromatographic peak heights measured from the base line. Shape, peak potential, and $|E_{3/4} - E_{1/4}|$ of hydrodynamic and batch voltammograms are essentially the same. This means that at the flow rate used, the electrode process has not become rate determining, despite the increased mass transport to the mercury drop.

Maximum sensitivity for carboplatin was reached in the DPP mode with a pulse amplitude of -250 mV. An initial potential of -1.60 V, instead of -1.65 V, was chosen to reduce the contribution from the cathodic background current (Figure 3). The maximum pulse height of -100 mV originally present in the polarographic analyzer produced a lower peak current due to the irreversibility of the reduction wave. A -500 mV pulse did not substantially increase sensitivity; a possible decrease of selectivity led to the choice of -250 mV as the optimal pulse amplitude. The pulse amplitude had no influence on noise levels. Compared to SDCP, DPP detection offered not only an increased sensitivity but also a reduced flow noise. DPP detection is also less susceptible to mercury

Figure 4. Chromatograms of plasma ultrafiltrate (a) and urine (b) of a patient. Blank samples and samples which contained 10.8 and 60.0 μ M carboplatin, respectively. Column, Spherisorb S5 ODS2, 4.6×150 mm; mobile phase, 0.05 M NaClO_4 , 1 mL/min. DPP detection with an initial potential of -1.60 V and a pulse amplitude of -250 mV.

drop anomalies (e.g., Barker effect) because less negative potentials are applied than with SDCP detection during the main part of drop life. These advantages are of special significance at the applied highly negative potentials.

Determination of Carboplatin in Biological Samples.

Carboplatin was determined in human body fluids under the optimized detection conditions. Plasma ultrafiltrate and urine samples were injected without any prepurification or degassing. It can be seen from the chromatograms in Figure 4 that, due to the selectivity of the detection mode, a sufficient separation can be obtained from detectable endogenous compounds in a simple and short chromatographic run. Detection limits were 0.1 and 1 μ M in plasma ultrafiltrate and urine, respectively. This is higher than the detection limit of carboplatin in water (0.05 μ M), owing to base line irregularities. Nevertheless, the reached value of 1 μ M in urine is low enough (because, e.g., 200 mL of urine containing 1 μ M of carboplatin represents only 0.013% of a dose of 350 mg/ m^2). Linear calibration lines could be obtained up to 150 μ M ($r > 0.999$) in both body fluids. A small increase of electronic

noise due to a shorter MTC (Table I) is not significant because of the base line irregularities and, therefore, the increased sensitivity is pure benefit.

The within-day coefficient of variation (CV) of five measurements of carboplatin in plasma ultrafiltrate (0.9 μM) and in urine (9 μM) was 1.5% and 4.7%, respectively. The between-day CV in water was 2.5% ($n = 4$). The short retention time of carboplatin and the absence of detectable peaks with longer retention times allowed the analysis of 10–12 samples per hour.

The developed procedure was used to determine the stability of carboplatin in plasma ultrafiltrate and urine at ambient temperature. Degradation half-lives of 20 and 7 days were found in plasma ultrafiltrate and urine, respectively. After an intravenous bolus injection of 350 mg/m² to one patient, a peak plasma concentration of 126 μM carboplatin was reached. A biphasic decay in plasma was observed with half-lives of 19 and 132 min. Cumulative urine excretion of carboplatin reached 40% of the administered dose within 6 h. Due to the low detection limit of 0.1 μM , carboplatin could be determined in plasma up to 24 h after administration. Therefore, our procedure is very well suited for pharmacokinetic and metabolism studies. In summary, our developed detection procedure for the determination of carboplatin in body fluids has a comparable or higher sensitivity than that with AAS, can be performed on-line, and is specific for the original compound.

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Convolution: A General Electrochemical Procedure Implemented by a Universal Algorithm

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The convolution of the faradaic current with an appropriate "convolution function" can generate surface concentration data in a variety of electrochemical situations. Eight circumstances are discussed, differing in geometry and with or without homogeneous reaction complications, and the corresponding convolution functions are presented. A convolution algorithm is derived that applies equally to all situations.

During the last 15 years, electroanalytical chemists have made increasing use of convolution techniques to process voltammetric current data (1). Though these techniques may be applied in investigations having analytical, kinetic, ther-

modynamic, or mechanistic goals, their fundamental purpose is always the determination of the instantaneous concentration of an electroactive species at the surface of an electrode from the faradaic current.

Consider an electrode reaction that generates a species into a phase initially devoid of that species. Diffusion then occurs away from the electrode surface with diffusion coefficient D . If the current density is $i(t)/A$ and the electrode reaction involves n electrons, then the surface concentration of the electrogenerated species has frequently been shown to be of the form

$$c^s(t) = \frac{i(t) * g(t)}{nAFD^{1/2}} \quad (1)$$