ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
14 (1996) 367–372

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Short communication

Liquid chromatography–mass spectrometry for the detection of platinum antineoplastic complexes

Robbin B. Burns [a], Roland W. Burton [a], Simon P. Albon [a], Leanne Embree [a,b,*]

[a] *Division of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, University of British Columbia, 2146 East Mall, Vancouver, B.C. V6T 1Z3, Canada*

[b] *Division of Medical Oncology—Laboratory Operations, British Columbia Cancer Agency, 600 West 10th Avenue, Vancouver, B.C V5Z 4E6, Canada*

Received for review 12 January 1995

Keywords: Liquid chromatography–mass spectrometry; Carboplatin; Sensitive detection; Platinum antineoplastic complexes

1. Introduction

Platinum complexes represent a major class of antineoplastic drugs, with cisplatin (*cis*-diamminedichloroplatinum(II)) and carboplatin (*cis* - diammine(1,1 - cyclobutanedicarboxylato) - platinum(II), CBDCA) being important representatives approved for the treatment of patients with cancer. Both cisplatin and carboplatin have demonstrated cytotoxicity in a number of sarcoma, melanoma and leukaemia tumour models and have shown clinical efficacy, especially in bladder, lung, ovarian and testicular carcinomas [1,2].

Novel platinum complexes are under development to improve efficacy, decrease toxicity and alter the spectrum of activity of cisplatin and carboplatin [3,4]. Modifications at the ammine ligands of carboplatin have provided new agents for evaluation, including DWA2114R (Fig. 1) [3,5–14]. Complete evaluation of the clinical pharmacokinetic and pharmacodynamic behaviour of these and other new platinum antineoplastic complexes will play a significant role in optimizing their therapeutic use. Essential to these investigations are assay methods capable of sensitive and specific quantitation of the parent platinum complexes in the presence of their in vivo and in vitro degradation products.

Efforts to preclude interference from the degradation products of carboplatin while quantitating the parent complex have required the addition of liquid chromatographic (LC) separations prior to detection [15–22]. Off-line atomic absorption spectrometry following LC provides the desired sensitivity [23–25], but this method has not been automated and presents significant reproducibility problems. Methods using pre-column derivatization described for cisplatin are not methods of

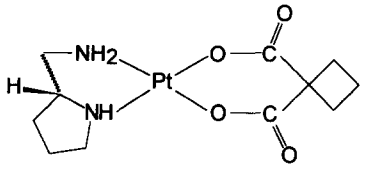

Fig. 1. Structure of DWA2114R.

* Corresponding author.

SSDI 0731-7085(95)01594-9

choice as they lack specificity for the parent complex [23,26–30]. Electrochemical (EC) detection following LC separation has been used for cisplatin analysis, but carboplatin and other platinum complexes lack sufficient electroactivity for sensitive LC–EC analysis owing to ligand effects on the central platinum metal [16,31–35]. Isotope dilution gas chromatography–MS [36,37] techniques offer sensitive detection of platinum species but lack specificity owing to the sample handling procedures used. The recently reported inductively coupled plasma (ICP)–MS [38] and LC–ICP–MS [39,40] methods are extremely sensitive but a chromatographic component is required for specificity.

This paper describes the utility of LC–MS using positive electrospray ionization for the sensitive and specific determination of the platinum antineoplastic complexes carboplatin and DWA2114R.

2. Materials and methods

2.1. LC–MS

The LC–MS system was composed of a Hewlett-Packard Model 1090 Series II liquid chromatograph (Hewlett Packard, Avondale, PA, USA) with a diode-array detector, coupled to a VG Quattro Quadropole MS (Fisons, Altrincham, UK) operated in the positive electrospray ionization mode. The UV detector was set at 210 nm. During the scan mode, the MS conditions included a source temperature of 100°C, a cone voltage of 30 V and relative high-mass/low-mass resolution values of 12/12, whereas during the selected-ion recording (SIR) mode the source temperature, cone voltage and relative mass resolution values were 80°C, 24 V and 9/9, respectively. Chromatographic analysis was performed using a Waters μBondapak ODS column (3.9 mm i.d. × 150 mm, 10 μm) at ambient temperature with an injection volume of 20 μl and a mobile phase flow rate of 0.5 ml min^{-1}. Following chromatography the flow was split, with one-tenth of the chromatographic eluent entering the MS. For compound separation, gradient elution was used with water as component A and methanol as component B. The gradient consisted of 100% A for the first 6 min, followed by 100% A to 75% A–25% B over 1 min, with an additional 8 min at 75% A–25% B. Subsequently, water was replaced with aqueous formic acid (0.02%, v/v) in the mobile phase. Mass spectra were obtained by scanning from m/z 200 to 1000 at 200 amu s^{-1}. Limits of detection (LOD) for carboplatin and DWA2114R were determined independently using mobile phases of aqueous formic acid (0.02%)–methanol (95:5 and 75:25) and SIR at m/z 371.3 and 437.3, respectively.

2.2. LC–UV

UV LODs were determined using a Hewlett-Packard Model 1050 liquid chromatograph with a variable-wavelength detector, a Rheodyne 7125 injector (Alltech Associates, Deerfield, IL, USA) with a 20 μl sample loop and a Hewlett-Packard Model 3396A integrator. The mobile phase was water–acetonitrile (98:2 for carboplatin and 90:10 for DWA2114R). The chromatographic column, mobile phase flow rate and analytical wavelength were as described for the LC–MS analysis.

2.3. Materials

Methanol and acetonitrile (both of HPLC grade) were obtained from Fisher Scientific (Fairlawn, NJ, USA). Formic acid (AnalaR grade) was purchased from BDH (Toronto, Ontario, Canada). Carboplatin Injection (10 mg ml^{-1}) was obtained from David Bull Laboratories (Vaudreuil, Quebec, Canada). A gift of DWA2114R was provided by Chugai Pharmaceutical (Shizuoka, Japan). HPLC-grade water (Milli-Q; Millipore, Bedford, MA, USA) was prepared on-site.

2.4. Preparation of standards and reagents

Aqueous stock solutions of 100 μg ml^{-1} carboplatin and 110 μg ml^{-1} DWA2114R were made from Carboplatin Injection and DWA2114R powder, respectively. An aqueous mixture containing both carboplatin and DWA2114R at 50.0 μg ml^{-1} was prepared from these stock solutions for mass

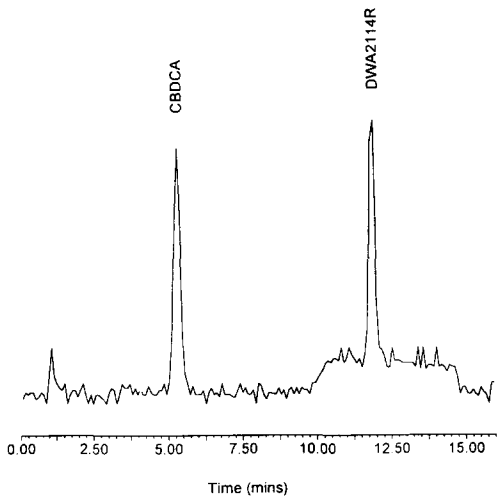

Fig. 2. Chromatogram of carboplatin and DWA2114R at 50 μg ml^{-1} with detection at 210 nm and gradient elution. The injection volume was 20 μl.

spectra determination. For UV LOD determinations, 2.00 μg ml^{-1} carboplatin and DWA2114R solutions in water were prepared separately and further diluted to 600, 400, 200 and 100 ng ml^{-1}. For MS LOD determinations, solutions of carboplatin and DWA2114R at 200 ng ml^{-1} were further diluted to 50.0, 35.0, 20.0, 10.0 and 5.00 ng ml^{-1}.

3. Results and discussion

3.1. Compound separation and determination of spectra

Fig. 2 shows the chromatogram obtained from the LC–MS system for an aqueous mixture of carboplatin and DWA2114R. The compounds were well separated on the LC column, eluting at 5.3 and 11.9 min, respectively. The void time for the system was 2.9 min, generating capacity factors (k') of 0.83 for carboplatin and 3.1 for DWA2114R. Mass spectra from m/z 325 to 925 (Fig. 3A and 3B) for the peaks due to carboplatin (371.3 g mol^{-1}) and DWA2114R (437.4 g mol^{-1}) indicate that, in addition to molecular ion (MH^+) formation, significant dimerization and sodium adduct formation occurred. MH^+ ion peaks were observed at m/z 372 for carboplatin and m/z 438 for DWA2114R, while the corresponding dimers were found at m/z 743 and 875, respectively. Monomeric and dimeric sodium adducts appeared at m/z 394 and 765 for carboplatin and m/z 460 and 897 for DWA2114R. Furthermore, each m/z group was comprised of several mass values corresponding to the major platinum isotopes ^{194}Pt, ^{195}Pt, ^{196}Pt and ^{198}Pt (natural abundances 32.9, 33.8, 25.3 and 7.2%, respectively) [37]. Platinum-containing ions in the spectra are distinguishable by the m/z values resulting from the presence of these isotopes, as demonstrated by spectra of selected m/z regions (Fig. 4A and 4B). For carboplatin, additional peaks due to an $[MH - NH_3]^+$ ion and its sodium adduct are evident in Fig. 4A at m/z 354–358 and 376–380, respectively.

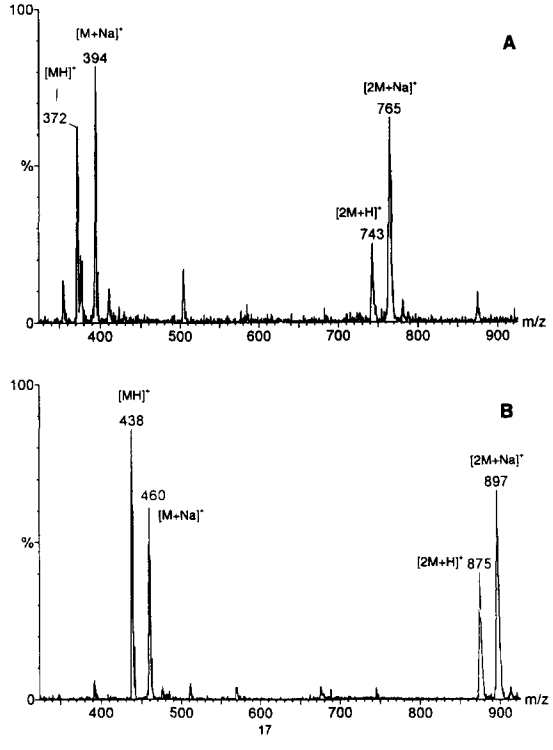

Fig. 3. Mass spectra for (A) carboplatin and (B) DWA2114R showing tentative peak assignments.

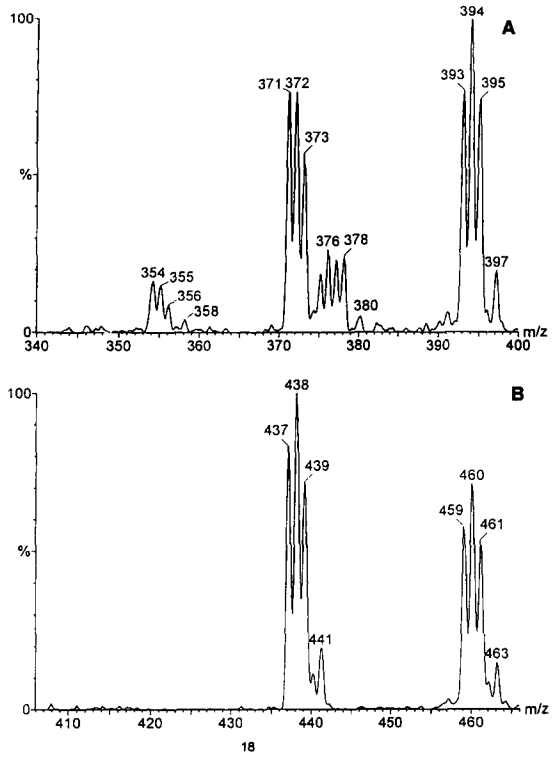

Fig. 4. Isotope clusters for (A) carboplatin and (B) DWA2114R.

3.2. Effect of formic acid

Substitution of aqueous formic acid (0.02%) for water in the gradient system did not significantly affect the retention times of carboplatin and DWA2114R observed with UV detection (5.4 and 12.0 min, respectively). Mass chromatograms of m/z 372.1 and 438.2, corresponding to the MH^+ ions of carboplatin and DWA2114R, are shown in Fig. 5A and 5B. Mass spectra of these complexes (Fig. 6A and 6B) show a significant reduction in both adduct and dimer formation. Addition of formic acid to the mobile phase increased the sensitivity (based on peak areas for mass chromatograms of the MH^+ ions) 2–3-fold for each compound. The ability of formic acid to suppress adduct formation can be attributed to the decrease in pH of the mobile phase, making formation of the MH^+ ion more favourable relative to other processes observed (adduct and dimer formation).

3.3. MS LOD determinations

Some optimization of the mass spectrometer conditions was attempted. The mass resolution was lowered during SIR, resulting in improved signal-to-noise ratios due to both increased peak areas and reduced baseline noise. Changing the mass resolution also caused a slight shift in the m/z patterns observed, with maximal MH^+ ion formation of carboplatin and DWA2114R occurring at m/z values of 371.3 and 437.3 for lower (9/9) resolution compared with 372.1 and 438.3 for higher (12/12) resolution. The former set of m/z and mass resolution values was used for subsequent LOD determinations. Isocratic mobile phase conditions were selected such that the retention times for carboplatin (4.5 min; $k' = 0.55$) and DWA2114R (5.1 min; $k' = 0.76$) were similar. For areas taken from the corre-

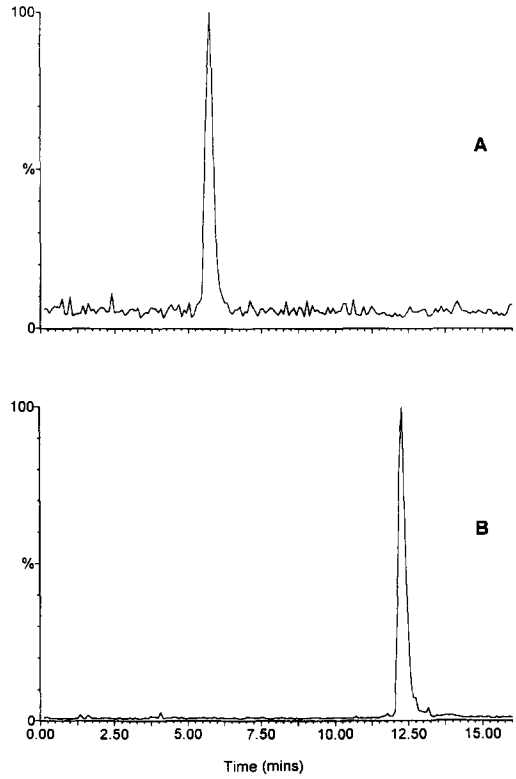

Fig. 5. Mass chromatograms of 50 μg ml^{-1} (A) carboplatin at m/z 372.1 and (B) DWA2114R at m/z 438.2 in the presence of formic acid (0.02%) using gradient elution.

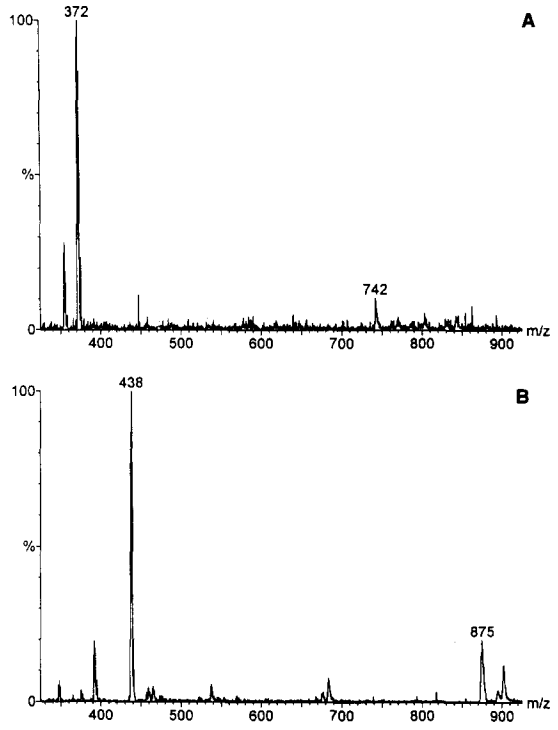

Fig. 6. Mass spectra of (A) carboplatin and (B) DWA2114R in the presence of formic acid (0.02%).

sponding SIR chromatograms, injection of 1 ng (20 μl of 50.0 ng ml^{-1} solution) of carboplatin and DWA2114R gave an area ratio of 0.52, suggesting the potential for greater sensitivity for DWA2114R relative to carboplatin. In addition, baseline noise was greater at m/z 371.3 than m/z 437.3. The LODs were 35.0 ng ml^{-1} (700 pg per injection) for carboplatin and 10 ng ml^{-1} (200 pg per injection) for DWA2114R, with signal-to-noise ratios of 3.0 and 4.0, respectively.

3.4. UV LOD determinations

Acetonitrile was substituted for methanol in the mobile phase in order to decrease the background absorbance and reduce baseline noise. Retention times were 4.9 min (void time of 2.6 min; $k' = 0.88$) for carboplatin and 5.9 min ($k' = 1.3$) for DWA2114R. The LODs were 200 ng ml^{-1} for both carboplatin and DWA2114R, with signal-to-noise ratios of 5.5 and 4.5, respectively.

4. Conclusions

This study examined the utility of LC–MS with positive electrospray ionization for the detection of carboplatin and DWA2114R. For both these complexes, detection limits were significantly better for LC–MS than for LC–UV under similar chromatographic conditions. The LC–MS procedure described here did not provide the sensitivity reported for ICP–MS [36,39]; however, there is greater potential for specificity with the current conditions as derivatization and/or degradation of the parent platinum complex are not required. The effect of biological fluids on the observed LODs is under investigation. Further optimization of both the chromatographic and detection portions of this assay method may further increase its sensitivity for platinum complexes.

Acknowledgements

The authors gratefully acknowledge Dr. Frank S. Abbott for time on the LC systems and thank the Chugai Pharmaceutical for their generous gift of DWA2114R. Financial support was provided by the British Columbia Cancer Agency, the British Columbia Cancer Foundation and the University of British Columbia (University Graduate Fellowship for Robbin B. Burns).

References

[1] M.K. Wolpert-DeFilippes, in A.W. Prestayko, S.T. Crooke and S.K. Carter (Eds.), Cisplatin: Current Status and New Developments, Academic Press, New York, 1980, pp. 183–191.
[2] E. Reed, K.W. Kohn, in B.A. Chabner and J.M. Collins (Eds.), Cancer Chemotherapy: Principles and Practice, Lippincott, Philadelphia, 1990, pp. 465–490.
[3] M.C. Christian, Semin. Oncol., 19 (1992) 720–733.
[4] N. Farrell, Cancer Invest., 11 (1993) 578–589.
[5] K. Akamatsu, K. Endo, T. Matsumoto, K. Morikawa, M. Koizumi, K. Koizumi and H. Mitsui, Jpn. J. Cancer Res., 82 (1991) 724–731.
[6] T. Matsumoto, K. Endoh, K. Akamatsu, K. Kamisango, H. Mitsui, K. Koizumi, K. Morikawa, M. Koizumi and T. Matsuno, Br. J. Cancer, 64 (1991) 41–46.

[7] M. Koizumi, M. Honda and K. Morikawa, Dev. Oncol., 54 (1988) 695–699.
[8] Y. Nio, S. Imai and T. Shiraishi, Anticancer Res., 11 (1991) 761–767.
[9] K. Tamura, S. Makino and Y. Araki, Cancer, 66 (1990) 2059–2063.
[10] H. Majima, in S.B. Howell (Ed.), Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy, Plenum Press, New York, 1991, pp. 345–355.
[11] A.J. Kraker, C.W. Moore and B.J. Roberts, Invest. New Drugs, 9 (1991) 1–7.
[12] H. Kobayashi, Y. Takemura and H. Miyachi, Proc. AACR, 31 (1990) 336.
[13] R.P. Perez, P.J. O'Dwyer and L.M. Hardel, Int. J. Cancer, 48 (1991) 265–269.
[14] K.-I. Akamatsu, H. Saito, T. Tsunenari, T. Matsumoto, K. Morikawa, M. Koizumi, H. Mitsui and K. Koizumi, Anticancer Res., 13 (1993) 2261–2266.
[15] P.J. Parsons, P.F. Morrison and A.F. LeRoy, J. Chromatogr., 385 (1987) 323–335.
[16] X.D. Ding and I.S. Krull, J. Liq. Chromatogr., 6 (1983) 2173–2194.
[17] L.A. Sternson, K.C. Marsh, S.J. Bannister and A.J. Repta, Anal. Proc., 20 (1983) 366–368.
[18] K.C. Marsh, L.A. Sternson and A.J. Repta, Anal. Chem., 56 (1984) 491–497.
[19] F. Elferink, W.J.F. van der Vijgh and H.M. Pinedo, Anal. Chem., 58 (1986) 2293–2296.
[20] R.C. Gaver and G. Deeb, Cancer Chemother. Pharmacol., 16 (1986) 201–206.
[21] Y.W. Cheung, J.C. Cradock, B.R. Vishnuvajjala and K.P. Flora, Am. J. Hosp. Pharm., 44 (1987) 124–130.
[22] S. Oguri, T. Sakakibara, H. Mase, T. Shimizu, K. Ishikawa, K. Kimura and R.B. Smyth, J. Clin. Pharmacol., 28 (1988) 208–215.
[23] P.A. Andrews, W.E. Wung and S.B. Howell, Anal. Biochem., 143 (1984) 46–56.
[24] C.M. Riley, L.A. Sternson, A.J. Repta and R.W. Siegler, J. Chromatogr., 229 (1982) 373–386.
[25] Y. Chang, L.A. Sternson and A.J. Repta, Anal. Lett., B11 (1978) 449–459.
[26] S.J. Bannister, L.A. Sternson and A.J. Repta, J. Chromatogr., 173 (1979) 333–342.
[27] P.A. Reece, J.T. McCall, G. Powis and R.L. Richardson, J. Chromatogr., 306 (1984) 417–423.
[28] R.F. Borch, J. H. Markovitz and M.E. Pleasants, Anal. Lett., 12 (1979) 917–926.
[29] J.D. Woollins, A. Woolins and B. Rosenberg, Polyhedron, 2 (1983) 175–178.
[30] H. Hasson and A. Warshawsky, J. Chromatogr., 530 (1990) 219–221.
[31] W.N. Richmond and R.P. Baldwin, Anal. Chim. Acta, 154 (1983) 133–142.
[32] I.S. Krull, X.D. Ding, S. Braverman, C. Selavka, F. Hochberg and L.A. Sternson, J. Chromatogr. Sci., 21 (1983) 166–173.
[33] F. Elferink, W.J.F. van der Vijgh, I. Klein, J.B. Vermorken, H.E. Gall and H.M. Pinedo, Cancer Treat. Rep., 71 (1987) 1231– 1237.
[34] P.J. Parsons and A.F. LeRoy, J. Chromatogr., 378 (1986) 395–408.
[35] F. Elferink, W.J.F. van der Vijgh and H.M. Pinedo, J. Chromatogr., 320 (1985) 379–392.
[36] S.K. Aggarwal, M. Kinter and D.A. Herold, J. Am. Soc. Mass Spectrom., 2 (1991) 85–90.
[37] S.K. Aggarwal, N.W. Gemma, M. Kinter, J. Nicholson, J.R. Shipe, Jr., and D.A. Herold, Anal. Biochem., 210 (1983) 113–118.
[38] K. McKay, Cancer Surv., 17 (1993) 407–414.
[39] Z. Zhao, K. Tepperman, J.G. Dorsey and R.C. Elder, J. Chromatogr. Biomed. Appl., 615 (1993) 83–89.
[40] W.R.L. Cairns, L. Ebdon and S.J. Hill, Anal. Proc., 31 (1994) 295–297.