NOTE

SYNTHESIS OF [^{13}N]CISPLATIN

Martin T. Haber*, Arthur J. L. Cooper*†, Karen C. Rosenspire††, James Z. Ginos† and David A. Rottenberg†

*Departments of Neurology and Biochemistry, Cornell University Medical College, New York, NY 10021; †Department of Neurology and ††Biophysics Laboratory, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

SUMMARY

A method for the "carrier-added" synthesis of [^{13}N]cisplatin is described. Yields were ~1-4 mCi from 20-40 mCi of [^{13}N]ammonia with a total synthesis time of 19-28 minutes. The product was ~96% radiochemically pure as judged by HPLC analysis and had a specific activity of ~100 mCi/mmole in 1.0 ml of saline. [^{13}N]Cisplatin was administered intraperitoneally to mice. Of the tissues investigated, concentration of label was highest in kidneys. At 10 min, considerable label in the blood, liver, and kidney was in a form other than cisplatin. However, no evidence was obtained that [^{13}N]ammonia was released from [^{13}N]cisplatin *in vivo*. [^{13}N]Cisplatin may be used to assess drug delivery to primary and metastatic brain tumors in patients receiving intravenous or intraarterial cisplatin chemotherapy.

Key Words: [^{13}N]ammonia, [^{13}N]cisplatin, brain

INTRODUCTION

A number of biologically important compounds containing positron-emitting radionuclides have been synthesized within the last few years for *in vivo* positron emission tomographic (PET) studies, particularly of the central nervous system (see Phelps *et al* (1) for a recent review). PET measurements of tissue radioactivity concentration may be useful in determining the uptake of systemically administered drugs by a target organ or tumor. Cisplatin (cis-diamminedichloroplatinum) is an effective anticancer drug that is being evaluated for the treatment of malignant brain tumors (2, 3). It is well known that very little platinum enters normal brain tissue following cisplatin administration (4, 5, 6). However, Stewart *et al* (7) analyzed surgical and post-mortem specimens of brain and intracerebral tumor tissue from patients who received i.v. or intracarotid cisplatin and concluded that potentially therapeutic concentrations of platinum are obtained in tumor and adjacent

0362-4803/85/050509–08$01.00
© 1985 by John Wiley & Sons, Ltd.
Received August 27, 1984
Revised October 17, 1984

brain tissue but not in remote normal brain regions. Thus, it appears that cisplatin (or platinum-containing breakdown products) penetrate, or are actively taken up by some human brain tumors. In order to develop a non-invasive PET method for measuring uptake of cisplatin (or breakdown products) in vivo, we have developed a rapid synthesis of [^{13}N]cisplatin.

METHODS

Synthesis of [^{13}N]cisplatin. Theoretically, one could react ammonia directly with K_2PtCl_4, but this was not attempted because of reported side reactions, low yields, and the necessity for a high molar ammonia ratio (8). On the other hand, the method of Hoeschele et al (8) gives excellent yields of pure cisplatin, uses essentially stoichiometric amounts of ammonia and is designed for small-scale synthesis. Therefore, we simplified this method to give maximum yield of [^{13}N]cisplatin within the time constraints imposed by the short half-life of ^{13}N ($t_{½}$=9.96min). The method is as follows: [^{13}N]Ammonia was produced in the Cyclotron Facility at Memorial Sloan-Kettering Cancer Center (MSKCC) as described (9) and bubbled into 1.0-2.0 ml of water. K_2PtI_4 (0.29 M) was prepared in water in a Microfuge tube by reacting 177 µl of 0.4 M K_2PtCl_4 with 68 µl of 5.8 M KI for 10 min at 25°C. To this solution was added 750 µl of aqueous [^{13}N]ammonia containing 20-40 mCi of activity (specific activity 50-200 mCi/µmole) and 25 µl of 5.2 M ammonium hydroxide. The mixture was heated in a 95°C water bath for 5 min, cooled on ice for 2 min and centrifuged for 30 sec in a Beckman Microfuge (9,000 g). The supernatant was discarded. Radiochemical yields at this first step were 70-80%. To the precipitate was added 340 µl of 0.4 M silver nitrate, the mixture was agitated and heated at 95°C for 3 min, cooled on ice for 1 min, and centrifuged for 30 sec. To the tube was added 63 µl of 1 M HCl and the mixture was heated for 1 min, cooled and centrifuged. The supernatant was transferred to another tube. The radiochemical yield for this second step was 80-85%. To the supernatant was added 70 µl of conc HCl, the mixture was heated for 4 min, cooled on ice for 2 min, centrifuged, and the supernatant was discarded. Radiochemical yields for this step were 70-80%. A white precipitate formed first (AgCl), followed by a yellow precipitate (cisplatin).

The yellow product was dissolved in hot saline (1 ml), and AgCl was removed by centrifugation. The supernatant was used for HPLC analysis or for tissue distribution studies. All reagents were made up on the day of the experiment.

HPLC analysis of [^{13}N]cisplatin. A solution (20 µl) of the final product in saline was injected onto a Whatman Partisil PXS-10/25 SCX cation exchange column and eluted with 5 mM potassium phosphate-HCl, pH 2.55, at a flow rate of 1.0 ml/min. At 8 minutes the eluting phase was switched to 20 mM potassium phosphate-HCl, pH 3.50. Under these conditions ammonia was eluted with the second buffer. Fractions were collected every 30 sec and counted with a gamma counter up to 10 minutes; thereafter, fractions were collected every minute. The HPLC analysis is similar to that reported by Scanlon (10).

Tissue distribution studies. An aliquot (150 µl) of the final product was injected i.p. (10-60 mg/kg), and groups of mice were killed by cervical dislocation at 1, 5 and 10 minutes after injection. Whole organs, pooled chest blood, and a sample of thigh muscle were removed, counted for radioactivity and weighed. Data are expressed in % dose/organ and as relative concentration (fraction of dose found in the organ or specimen/fraction of body weight contained in that specimen or organ; ref. 11).

Analysis of radioactivity in kidney, liver and blood after an i.p. injection of [^{13}N]cisplatin. Each mouse was injected i.p. with [^{13}N]cisplatin in 1 ml of saline (20-560 mg/kg). Ater 10 min the liver, kidneys or blood were homogenized in a four to six-fold excess of ice-cold 1% picric acid and 800 µl was centrifuged for 30 sec in a microfuge. An aliquot of the clear yellow supernatant was analyzed by HPLC as described above. In a separate experiment the picrate suspension was centrifuged and the precipitate was further washed (x3) with an equal volume of picric acid. The combined supernatants and final precipitate were separately counted in order to compare radioactivity bound to protein (and other high-molecular-weight components) to that in low molecular-weight soluble components.

Determination of label in glutamine, urea and free ammonia. Animals were injected with 0.5 ml of the [^{13}N]cisplatin solution (20-150 mg/kg). The liver, kidneys and blood were separately homogenized and centrifuged as described above. A portion of the supernatant (600 µl) was added to a Dowex 1 acetate column (0.5 x 0.5 cm) to remove picric acid. The pass-through and a 400 µl wash with 100 mM L-aspartate (to remove any residually bound amino acids) were combined. To this solution was added 125 µg of jack bean urease (80 U) in 200 µl of 1 M potassium phosphate, pH 6.0. After incubation at 37°C for 5 min, 600 µl was removed, placed in the chamber of a Conway micro-diffusion apparatus, and [^{13}N]ammonia was collected over a period of 15 min by diffusion into 50 µl of 1 M H_2SO_4 (11). In order to determine the label in glutamine (amide), the experiment was repeated with urease replaced by glutaminase (250 µg, 8 U) in 200 µl of water and 100 µl of 0.1 M sodium acetate, pH 4.9. After 20 min of incubation at 37°C, [^{13}N]ammonia was determined following Conway microdiffusion. Analysis by HPLC using o-phthalaldehyde (13) showed no detectable glutamine in the glutaminase-treated samples. Further treatment of the urease-treated samples (200 µl) with excess urease (0.1 mg) yielded no detectable increase in ammonia under conditions in which added urea (0.1 µmole) was rapidly converted to ammonia.

RESULTS

Synthesis of [^{13}N]cisplatin. Typically, final radiochemical yields were 2-4 mCi (30-50% theoretical yield) with a total synthesis time of 19-28 minutes, and final specific activity of 50-200 mCi/mmole, calculated from the amount of carrier added and the radiochemical yield. The radiochemical purity was consistently 96% as judged by HPLC analysis. A standard prepared according to the method of Hoeschele et al (8) had an identical retention time (3.5 min, UV detection) to that of the major radioactive peak obtained from analysis of [^{13}N]cisplatin.

Tissue distribution of label in mice. A number of organ and tissue distribution studies of radioactive and non-radioactive platinum derived from cisplatin have been carried out in various animal species (4, 5, 6, 14) and in a few patients (15, 16). Since the Pt-N bond is thought to be stable (17), it was assumed that the short-term distribution of label derived from [^{13}N]cisplatin would be the same as that of Pt derived from cisplatin. This hypothesis was verified. As was previously shown for Pt uptake following administration of cisplatin (4 , 5), the present studies demonstrate a high uptake of [^{13}N]cisplatin-derived label in kidney (Table 1). As was noted by others for Pt (e.g. 4, 5), very little label derived from [^{13}N]cisplatin accumulated in brain, and some of this brain label was undoubtedly due to radioactivity in residual blood.

Table 1. Distribution of ^{13}N Following Intraperitoneal Injection of [^{13}N]Cisplatin Into Mice*

Tissue	Relative Concentration			% Dose/Organ		
	1 min	5 min	10 min	1 min	5 min	10 min
Blood	1.90±0.04	2.10±0.22	3.28±1.09			
Liver	0.73±0.37	1.42±0.25	1.95±0.16	3.17±1.59	6.50±1.01	8.23±1.34
Kidneys	0.62±0.21	4.93±0.91	5.44±0.89	1.28±0.04	6.09±0.79	6.73±1.19
Muscle	0.23±0.21	0.59±0.07†	0.67±0.20			
Brain	0.03±0.01	0.10±0.01	0.12±0.02	0.07±0.02	0.22±0.03	0.21±0.03

% dose/organ was measured directly in the dissected organ. For muscle, a small piece (20-80 mg) was removed from the thigh muscle and for blood a small amount was removed from pooled chest blood following severence of the heart vessels. Average body weights for the mice were 16.5 ± 0.6, 17.0 ± 1.0, and 20.0 ± 1.0 g for the 1, 5, and 10 min time points, respectively. The values shown are the mean ± S.E.M., n = 4 at 1 and 5 min; n = 6 at 10 min. The mice were females of the B6D2F$_1$ strain.
† n = 3.

Radioactivity bound to acid-precipitable material. Following administration of cisplatin to experimental animals, platinum is rapidly bound (within minutes) to high molecular weight components in tissues (e.g. 18, 19) and in plasma (4). Binding to human plasma proteins has also been well documented (20). These studies have employed either ultrafiltration or acid precipitation to separate high-molecular-weight from low-molecular-weight components. We find 26%, 26%, and 11% of the total tissue nitrogen label associated with acid-precipitable material in blood, liver and kidney, re-

spectively, 10 min following i.p. injection of [^{13}N]cisplatin in mice (n = 2).

Analysis of 1% picric acid-soluble material. In order to ascertain the amount of label still present as [^{13}N]cisplatin at 10 min after injection, an HPLC analysis was carried out on the deproteinized tissue extract (see Methods). We found that in blood, liver and kidney only 48%, 58% and 82%, respectively, of the radioactivity was still associated with the cisplatin peak. The remainder of the activity in each case was spread among many components. It was noted that small amounts of radioactivity in the HPLC profiles eluted in the regions assigned to ammonia (8 min), glutamine (5 min) and glutamate (4.5 min); cisplatin co-elutes with urea (3.5 min). When tissue samples were analyzed for free [^{13}N]ammonia plus urea-derived [^{13}N]ammonia (see Methods), < 0,01%, < 0.01% and < 0.07% of the total counts were found in this fraction in blood, liver and kidney, respectively (n = 2). Similarly, no detectable (< 1.0%) free [^{13}N]ammonia was found in glutaminase-treated tissue extracts (n = 2).

DISCUSSION

The present studies were carried out to test the feasibility of using [^{13}N]cisplatin to assess drug uptake in patients with brain tumors. It was important to determine whether some [^{13}N]ammonia is liberated from [^{13}N]cisplatin *in vivo*, because ammonia will be rapidly taken up by brain and incorporated into glutamine (21). Uptake of such secondary labeled products would complicate the interpretation of brain and tumor ^{13}N concentrations as determined by PET. Although it is generally agreed that the Pt-N bond of cisplatin is strong (17), NH_3 can be displaced from cisplatin in reactions with biomolecules under forcing conditions. For example, in the reaction with methionine, the S and N of one methionine displace two chlorides, but on heating with excess methionine, a second methionine S displaces one ammonia ligand trans to the first sulfur (22). Also, one cannot rule out *a priori* the possibility of enzymatic attack of the Pt-N bond with release of [^{13}N]ammonia. However, the present studies show that very little, if any, [^{13}N]ammonia is

released from [^{13}N]cisplatin within 10 min in mice tissues. Moreover, double-label (^{195m}Pt, ^{14}C) experiments in rats with the cisplatin analog, ethylenediaminodichloroplatinum (II), have shown that the label ratio remains constant in vivo over a period of days (23).

Because of the very low uptake of label into normal brain and the increased permeability of the tumor-brain barrier to small polar molecules, [^{13}N]cisplatin may afford a reasonable contrast between tumor and healthy brain. Indeed, Stewart et al showed a marked difference (1:2) between platinum uptake in normal brain and brain tumor in biopsy and post-mortem specimens from patients receiving cisplatin (7). Experiments designed to test the usefulness of [^{13}N]cisplatin to monitor drug uptake during the chemotherapy of human brain tumors are under development in our laboratory. By starting with ten times the activity of [^{13}N]ammonia (within the capability of the MSKCC cyclotron) it should be possible to increase both the yield and specific activity of [^{13}N]cisplatin into the range required for PET imaging of human brain tumors.

Acknowledgements - This work was supported by NINCDS Grant NS 15665, NIH Grant CA-33732, and United States Department of Energy Contract EE-77-S-02-4668.

REFERENCES

1. Phelps, M.E., Mazziota, J.C. and Huang, S-C. - J. Cereb. Blood Flow Metabol. 2: 113-162 (1982).
2. Stewart, D.J., O'Bryan, R.M., Al-Sarraf, M.,Costanzi, J.J. and Dishi, N. - J. Neuro-Oncol. 1: 145-147 (1983).
3. Yung, W-K.A., Wallace, S., Feun, L., Leavans, M. and Fields, W.S. - Neurology 33: 66 (1983).
4. LeRoy, A.F, Lutz, R.J., Dedrick, R.L., Litterst, C.L. and Guarino, A.M. - Cancer Treat. Rep. 63: 59-71 (1979).
5. Litterst, C.L., LeRoy, A.F., and Guarino, A.M. - Cancer Treat. Rep. 63: 1485-1492 (1979).
6. Taylor, D.M. - Biochimie 60: 949-956 (1978).
7. Stewart, D.J., Leavens, M., Luna, M., Seifert, W., Loo, T.L. and Benjamin, R.S. - ASCO Abs. 359 (1981). Stewart, D.J., Leavens, M., Maor, M., Feun, L., Luna, M., Bonura, J., Caprioli, R., Loo, T.L., and Benjamin, R.S. - Cancer Res. 42: 2474-2479 (1982).
8. Hoeschele, J. D., Butler, T.A., Roberts, J.A. and Guyer, C.E. - Radiochimica Acta 31: 27-36 (1982).
9. Gelbard, A.S., Clarke, L.P., McDonald, J.M., Monahan, W.G., Tilbury, R.S., Kuo, T.Y.T. and Laughlin, J.S. - Radiology 116: 127-132 (1975).
10. Scanlon, K.J., Safirstein, R.L., Thies, H., Gross, R.B., Waxman, S. and

Guttenplan, J.B. - Cancer Res. 43: 4211-4215 (1983).
11. Woodard, H.Q., Bigler, R.E., Freed, B.R. and Russ, G.A. - J. Nucl. Med. 16: 958 (1975).
12. Conway, E.J. - Microdiffusion Analysis and Volumetric Errors (First American Edition), p. 90, Crosby, Lockwood and Sons, London (1963).
13. Jones, B.N., Pääbo, S. and Stein, S. - J. Liquid. Chromat. 4: 565-586 (1981).
14. Hoeschele, J.D., VanCamp, L. - In: Advances in Antimicrobial and Antineoplastic Chemotherapy (vol. II, Hejlzar, M., Semonský, M., and Masá, S., eds.), pp. 241-242, University Park Press, Baltimore (1972).
15. Lange, R.C., Spencer, R.P. and Harder, H.C. - J. Nucl. Med. 13: 328-330 (1972).
16. Smith, P.H.S. and Taylor, D.M. - J. Nucl. Med. 15: 349-351 (1974).
17. Cleare, M.J. - J. Clin. Hemat. Oncol. 7: 1-21 (1977).
18. Sharma, R.P. and Edwards, I.R. - Biochem. Pharmacol. 32: 2665-2669 (1983).
19. Choie, D.D., Del Campo, A.A. and Guarino, A.M. - Toxicol. Applied Pharmacol. 55: 245-252 (1980).
20. Himmelstein, K.J., Patton, T.F., Belt, R.J., Taylor, S., Repta, A.J. and Sternson, L.A. - Clin. Pharmacol. Ther. 29: 658-664 (1981).
21. Cooper, A.J.L., McDonald, J.M., Gelbard, A.S., Gledhill, R.F. and Duffy, T.E. - J. Biol. Chem. 254: 4982-4992 (1979).
22. Thomson, A.J., Williams, R.J.P. and Reslova, S. - Struct. Bonding 11: 1-46 (1972).
23. Taylor, D.M., Jones, J.D. and Robins, A.B. - Biochem. Pharmacol. 22: 833-839 (1973).