A Microscale Synthesis of A Promising Radiolabelled Antitumor Drug: cis-1,1-cyclobutanedicarboxylato (2R)-2-methyl-1,4-butanediamine platinum(II), NK121

Masato SUWA [1], Osamu KOGAWA [1], Hiroyoshi NOWATARI [2],
Yuko MURASE [3], Yoshio HOMMA [3] and Yutaka HASHIMOTO [1]

Research Laboratories of Pharmaceuticals Group [1], Nippon Kayaku Co., Ltd., 3-31-12, Shimo, Kita-ku, Tokyo 115, Japan, Takasaki Research Laboratories [2], Nippon Kayaku Co., Ltd., 219, Iwahana-machi, Takasaki, Gumma 370-12, Japan, Kyoritsu College of Pharmacy [3], 1-5-30, Shibakoen, minato-ku, Tokyo 105, Japan.

SUMMARY

A promising antitumor drug, cis-1,1-cyclobutane-dicarboxylato (2R)-2-methyl-1,4-butanediamine platinum(II), NK121, was synthesized from radionuclides of platinum such as ^{193m}Pt, ^{195m}Pt and ^{191}Pt which were produced by neutron irradiation of enriched ^{192}Pt. The overall yield was 38.6% in a synthesis time of 10 hours. The radioactivities present in 8.39 mg of NK121 were 115.3 μCi as ^{193m}Pt, 29.9 μCi as ^{197}Pt, 22.0 μCi as ^{195m}Pt, and 4.8 μCi as ^{191}Pt at the end of synthesis. The specific activity of the NK121 was 13.7 μCi (^{193m}Pt)/mg NK121 at the end of synthesis. The radiochemical purity of NK121 was typically 99 %. HPLC analyses confirmed that NK121 was in an adequate chemical purity and suitable for animal experimentation.

Key Words: ^{193m}Pt-labelled antitumor drug; microscale synthesis; NK121

INTRODUCTION

Since Rosenberg and the co-workers(1) reported the antitumor activity of cis-dichlorodiammineplatinum(II), cis-DDP, a number of investigations on the drug have been made in recent times to clarify the mechanism of antitumor action(2), apply pharmacokinetics to the

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optimization of drug therapy(3), confirm metabolic products(4), and deal with the distribution of the drug in the body(5). A detailed investigation of the use of the drug, however, showed that the principal difficulty with the drug centers around its dose-limitting renal toxicity(6).

In our efforts to improve the therapeutic properties of platinum-based antitumor agents, a number of platinum complexes have been prepared and evaluated in an animal tumor screen in our laboratories. In a preceding paper(7), we reported that cis-1,1-cyclobutanedicarboxylato (2R)-2-methyl-1,4-butanediamine Pt(II) (hereafter abbreviated NK121) is a promising antitumor drug with low nephrotoxicity and myelosuppression compared with the antitumor drugs cis-DDP and carboplatin. It is therefore of importance to synthesize radiolabelled NK121, which allows measurement of local tissue concentrations, metabolic rate and the utilization of NK121 in laboratory animals. The present paper reports a highly refined procedure for the microscale synthesis of radiolabelled NK121, which was evaluated for authenticity and purity by HPLC. The results of animal studies will be reported separately.

MATERIALS AND METHODS

Target and irradiation. The radionuclides of platinum used in this study were ^{191}Pt, ^{193m}Pt, ^{195m}Pt and ^{197}Pt, which were produced by thermal neutron irradiation of 12.75 mg of enriched ^{192}Pt (purchased from Isotopic Sales at Oak Ridge National Laboratory Tenn., U.S.A.) in the nuclear reactor JRR-4 (Tokai, Ibaraki, Japan) for 3 days (4-6 hours each day) leaving 16 hours in between. The total duration of bombardment was 16 hours. The thermal neutron flux was approx. 5 x 10^{13}n cm^{-2} s^{-1}. The isotopic enrichments were: ^{190}Pt(<0.05%), ^{192}Pt(56.97%), ^{194}Pt(26.16%), ^{195}Pt(11.23%), ^{196}Pt(4.74%) and ^{198}Pt(0.90%).

The assay of the radionuclides was made using an intrinsic Ge detector (Princeton Gamma-Tech) coupled to a 4096-channel analyzer (Camberra series 35) and an automatic gamma counter (Packard, Model 5650). The intrinsic Ge-multichannel analyzer system had a 1.98 keV resolution (FWHM) at 1.33 MeV and a peak-to-compton ratio of 32:1. The detector was absolutely calibrated with a set of LMRI gamma-ray standard sources. The dead time losses were always less than 10 %. The energy and the intensity of the photopeaks used were: ^{191}Pt (409.44 keV, 8%), ^{193m}Pt (135.5 keV, 0.111%), ^{195m}Pt (98.66 keV, 11.3%), ^{197}Pt (77.35 keV, 17.0%). The automatic gamma counter was also used in the radiometric determination of the yield of the synthesis.

The radioactivities measured 40 hours after the end of irradiation were: ^{191}Pt (12.9 μCi), ^{193m}Pt (306.0 μCi), ^{195m}Pt (57.3 μCi), ^{197}Pt (88.5 μCi). Thus, the labelled compound described below is a mixture of ^{191}Pt-NK121, ^{193m}Pt-NK121, ^{195m}Pt-NK121 and ^{197}Pt-NK121. Instead of these nomenclatures, however, *Pt-NK121 will hereinafter be employed. The synthetic route adopted for the preparation of *Pt-NK121 is shown in Fig. 1. K_2*$PtCl_4$ was prepared from the irradiated platinum by a method analogous to that described by Kauffman and Cowan (8) and *Pt-NK121 was synthesized from K_2*$PtCl_4$ according to the modified method of Nowatari et al.(7).

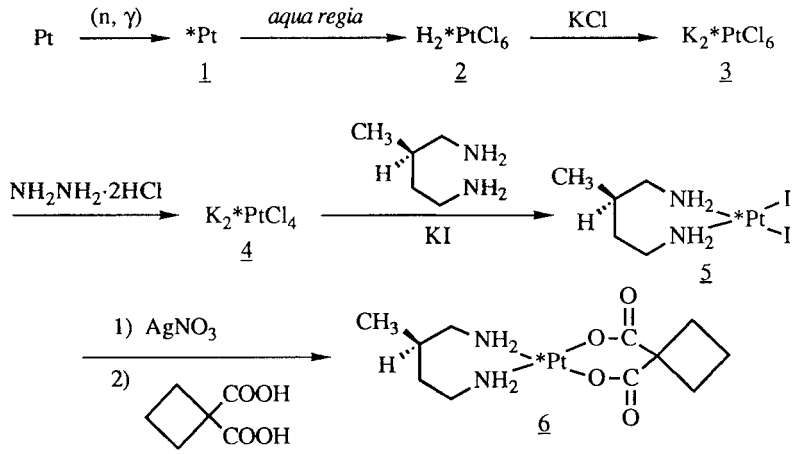

Fig. 1. Synthesis of *Pt-NK121

H_2*$PtCl_6$(2). The irradiated platinum (1) was dissolved in 1 ml of *aqua regia* in a 10 ml Pyrex conical centrifuge tube upon gentle heating. The platinum *aqua regia* solution was repeatedly evaporated to near dryness with a few ml of concentrated HCl in order to destroy any nitro complexes.

K_2*$PtCl_6$(3). To the residue (H_2*$PtCl_6$)(2) was added 9.75 mg KCl in 0.5 ml of water while the mixture was stirred for 5 min at about 35-40 °C, and then allowed to remain at room temperature for additional 5 min. A few ml of 80% aq. ethanol was added and the reaction mixture was cooled in an ice bath for 5 min, centrifuged at 2000 rpm for 5 min. The precipitated (K_2*$PtCl_6$) (3) was washed with a small amount of ice-cold 80% aq. ethanol, ethanol, ether and finally air-dried.

K_2*$PtCl_4$(4). To the precipitate(3) was added 3.43 mg of N_2H_4•2HCl in 0.1 ml of water. The mixture was stirred mechanically and kept at 65°C for 30 min. The temperature of the mixture was then raised to 80 to 90°C, to ensure completion of the reaction, for periods up to 10 min. In order to remove excess N_2H_4•2HCl, the final residue, K_2*$PtCl_4$(4), was recrystallized from ethanol-acetone-ether and dissolved in 3 ml of water. Ten µl aliquots were sampled for the identification and assay of gamma-emittering nuclides.

cis-Diiodo (2R)-2-methyl-1,4-butanediamine platinum(II) (5) and *Pt-NK121(6). A solution of 39.965 mg of KI in 1 ml water was added to a solution of 5.270 mg of (2R)-2-methyl-1,4-butanediamine dihydrochloride in 1 ml of 0.1 N NaOH solution in an ice bath, and the resultant solution was added to the solution of K_2*$PtCl_4$ at 0°C. The reaction mixture was stirred mechanically while the temperature was slowly raised from 0 to 60°C over a period of 1 hour, and kept at 60°C for an additional 1 hour. The reaction mixture was centrifuged at 2000 rpm for 5 min, and the resultant precipitate(5) washed twice with a total of 4 ml water and allowed to react for 20 min with 18.0 mg of $AgNO_3$ in 1.8 ml of water at 60°C. The reaction mixture was then centrifuged and the supernatant was transferred to another conical centrifuge tube. The precipitate was washed with a total of 2 ml of water, centrifuged, and the aqueous washings were combined with the supernatant described above. To the solution was added 16.97 mg of 1,1-cyclobutanedicarboxylic acid in 2.35 ml of 0.1 N NaOH solution. The reaction mixture was maintained at 60°C for 1 hour, with stirring, and then filtered through 0.45µm membrane filter. The *Pt-NK121(6) was obtained by concentrating the filtrate to about 2.5 ml under reduced pressure at 40°C.

Purification of *Pt-NK121 was carried out using a Shimadzu Corp. LC-4A high-performance liquid choromatograph equipped with a sample injector (Model SSC-E1E005, Senshu Scientific Co. Ltd., Tokyo, Japan), a 1 ml-sample loop (Model E1E-2098, Senshu Scientific Co. Ltd., Tokyo, Japan), a Nucleosil $5C_8$ column (20 x 1.0 cm I.D.; Macherey Nagel Duren, West Germany), and a UV-spectrophotometric detector (Model SPD-2A, Shimadzu Corp., Kyoto, Japan). The mobile phase was methanol-water (1:5, v/v) at flow rate of 3 ml/min. The elution was carried out at ambient temperature (25 ± 2°C) and the column effluent was monitored at 215 nm. The methanol used for the mobile phase was of HPLC-grade (Junsei Chemical Co., Ltd., Tokyo, Japan). Approx. 0.8 ml aliquots of the resulting solution were injected into the chromatograph. The total sample solution (6) was purified in three injections

RESULTS AND DISCUSSION

The UV absorption spectrum of a typical *Pt-NK121 sample is illustrated in Fig. 2-A. The retention time for the *Pt-NK121 is 14.9 min. Radioactivity of the purified *Pt-NK121, which was collected from HPLC, showed that essentially 99 % of the total activity of (6) to be congruent with the peak corresponding to the radiochromatogram of *Pt-NK121 (Fig. 2-B). Unidentified minor components - an approx. 1 % of the total activity- could be eliminated on rechromatography of the 14.9-min fraction (Fig. 2). Thus, 8.39 mg of compound (6) was obtained in an adequate chemical purity by concentrating the14.9-min fractions to dryness under reduced pressure.

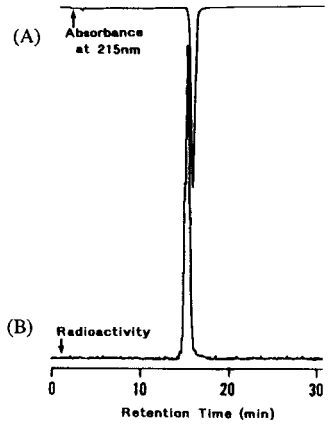

Fig. 2. HPLC chromatograms of purified *Pt-NK121

The yield, which was determined by comparing the total activity of K_2*$PtCl_4$ at the end of synthesis with that of the purified *Pt-NK121, at the end of synthesis, was 38.6 %. The radiochemical purity of the product (6) was typically 99 %. The radioactivities present in the final 8.39 mg of the purified *Pt-NK121 were 115.3 μ Ci as ^{193m}Pt, 29.9 μCi as ^{197}Pt, 22.0 μCi as ^{195m}Pt, and 4.8 μCi as ^{191}Pt at the end of synthesis. The specific activity of the final *Pt-NK121 was 13.7 μCi (^{193m}Pt)/mg *Pt-NK121 at the end of synthesis. A detailed investigation to measure the local tissue concentrations, metabolic rate and utilization of NK121 in laboratory animals, which will be published separately, showed that conversion electrons and gamma-rays from the radionuclides of platinum used in this study are especially useful for the animal and autoradiographic studies.

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