nm/ CONCISE COMMUNICATION

SYNTHESIS AND DISTRIBUTION OF A RADIOLABELED ANTITUMOR AGENT: cis-DIAMMINEDICHLOROPLATINUM (II)

Robert C. Lange, Richard P. Spencer, and Harold C. Harder

Yale University School of Medicine, New Haven, Connecticut

There are few opportunities to trace the distribution of antitumor agents during life since these compounds usually do not contain atoms which can be replaced by gamma-emitting analogs. In one compound that can be used, ^{131}I-iodoacetic acid, the radiolabel is apparently cleaved from the molecule as tissue binding occurs (*1*). It was therefore of interest to pursue studies of the radioactive synthesis and distribution of an antitumor agent that contains a metal, cis-diamminedichloroplatinum. The compound causes regression of several animal tumors (*2*) and is currently undergoing clinical trials at this center. There is evidence that cis-diamminedichloroplatinum interferes with nucleic acid synthesis (*3*), and Harder (*4*) has postulated that it creates an intrastrand DNA crosslink.

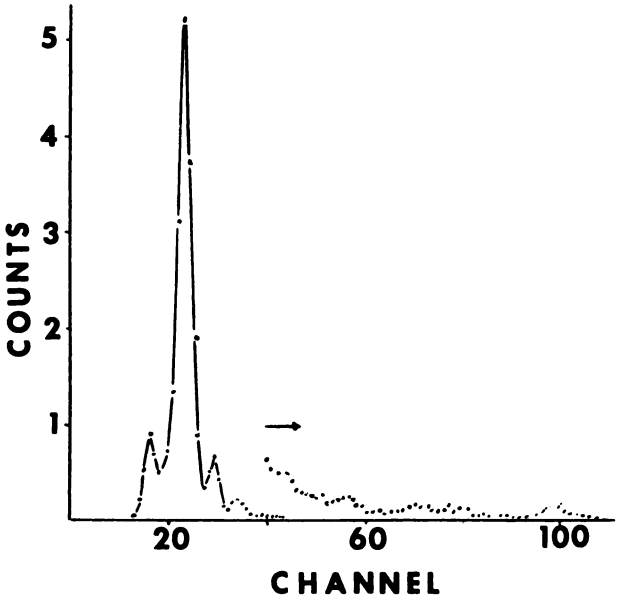
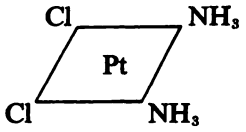


FIG. 1. Gamma-ray spectrum of ^{193m}Pt in final compound. Arrow indicates that latter portion of spectrum was enlarged ten times. Major peak corresponds to 67-keV K x-ray from ^{193m}Pt. Two small peaks (near 100 and 130 keV) are probably x-rays from ^{195m}Pt. Axis corresponding to counts is in arbitrary units.

SYNTHESIS

The compound can be represented as follows:



We began with ^{193m}Pt produced from 8.3 mg of 11.5%-enriched ^{192}Pt by the reaction ^{192}Pt (n,γ) ^{193m}Pt. This decays with a half-life of approximately 4 days by a highly converted isomeric transition. The external radiation from ^{193m}Pt consists almost entirely of K-characteristic x-rays. A gamma-ray spectrum of the material after purification is shown in Fig. 1. Irradiation was at a flux of 2.5×10^{14} neutrons/cm^2/sec for 12 days. The estimated specific activity upon removal from the reactor was 4.05 mCi/mg Pt. Radiochemically, 5 days after completion of irradiation the only major detectable impurity in the synthesized compound was ^{195m}Pt. Based on reported methods (*5,6*) a technique was evolved for maximizing the yield of cis-$Pt(NH_3)_2Cl_2$ from platinum. Basically the synthesis involved first converting the platinum metal to K_2PtCl_2. This was reduced to K_2PtCl_4 and then converted to cis-$Pt(NH_2)_2Cl_2$. The resulting compound had a specific activity of 192 mCi/mmole.

Verification of the structure of the compound was made in three different ways:

1. When chromatographed in 0.2 *M* glycine (Whatman No. 1 paper, ascending) there was a single radioactive peak with an R_f of 0.76. This was the same as that of authentic unlabeled cis-$Pt(NH_3)_2Cl_2$. Chromatography in

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For reprints contact: Richard P. Spencer, Nuclear Medicine Section, Yale University School of Medicine, 333 Cedar St., New Haven, Conn. 06510.

FIG. 2. Posterior gamma-camera scan of rabbit 40 min after intravenous injection of cis-$^{193m}Pt(NH_3)_2Cl_2$. Activity can be seen in liver, kidneys, and bladder (at bottom). Midline activity may be due both to blood pool and bone.

9:1 (V:V) acetone-water revealed activity at $R_f = 0.75$ with essentially no other peaks. The transcompound has a lower R_f in this solvent according to James D. Hoeschele of Michigan State University.

2. The cis- and trans-isomers of compounds of the type PtA_2X_2 can be distinguished by the Kurnakow test (*7*). The radiolabeled compound reacted correctly with thiourea in this assay.
3. The most sensitive test, a bioassay, was performed by comparing the effects of the radiolabeled compound with that of authentic cis-$Pt(NH_3)_2Cl_2$ on filament formation in E coli B (*8*). The radiolabeled compound exactly paralleled the unlabeled material in the rate and extent of filament formation. Both yielded filaments 50–100 times normal length in 90–95% of the cells in 4 hr at a concentration of 50 μM. The presence of other products such as K_2 $^{193m}PtCl_6$ would either inhibit growth and hence filament formation or else have no effect at all.

TABLE 1. QUANTITY OF ^{193m}Pt IN TISSUES 18 HR AFTER INTRAVENOUS INJECTION OF CIS-$^{193}Pt(NH_3)_2Cl_2$ INTO MALE RABBIT*

Organ	Percent injected dose $\times 10^{-5}$/gm tissue
Kidneys	5.64
Liver	2.52
Skull bone	1.81
Urine	1.73
Spleen	1.22
Lungs	1.18
Rib cage	1.03
Heart	0.76
Small intestine (+ contents)	0.71
Blood	0.45
Fat	0.14
Brain	0.04

* Other rabbits sacrificed at 18–24 hr gave essentially identical results.

Based on all the above criteria, it was concluded that the structure of the radiolabeled compound was verified and its purity was established.

METHODS

The compound was dissolved in physiological saline and used immediately in albino rabbits or Charles River strain white mice. The rabbits were anesthetized with sodium pentobarbital and placed under a gamma camera (70-keV peak, 25% window) with a coupled Nuclear Data 50/50 data acquisition system prior to intravenous injection (approximately 1 mCi each). The rabbits were sacrificed at 18–24 hr. Pairs of mice were injected intraperitoneally (0.05 ml of solution, containing about 20 μCi of ^{193m}Pt compound). Animals were sacrificed at 1, 2, 3, 24, and 48 hr. Tissues were removed and counted, and the activity/gram was calculated by comparison with a standard. Other mice were counted above a well scintillation counter and compared with a standard to determine the biological turnover of the radioactivity.

RESULTS

Following intravenous administration of cis-$^{193m}Pt(NH_3)_2Cl_2$ to rabbits, gamma-camera studies revealed generalized distribution and then accumulation in the liver, kidneys, and bladder (Fig. 2). Even after 18–24 hr, kidneys and liver retained most activity in the rabbits, and a typical assay is shown in Table 1. Of interest was activity in the skull bone (a bone without major hematopoietic function).

Intraperitoneal injection of cis-$^{193m}Pt(NH_3)_2Cl_2$ into mice followed by whole-body counting of four mice revealed rapid excretion. After the first day 79% of radioactivity had been eliminated (corrected

TABLE 2. TISSUE ACTIVITY OF ^{193m}Pt 2 HR AFTER INTRAPERITONEAL INJECTION OF CIS-$^{193m}Pt(NH_3)_2Cl_2$ IN MICE*

Tissue	Percent of dose/gm tissue
Kidneys	1.77
Liver	1.66
Small intestine (+ contents)	0.88
Fat	0.46
Skull bone	0.44
Rib cage	0.43
Spleen	0.42
Blood	0.35
Lungs	0.30
Heart	0.28
Brain	0.03

* Mean of two animals. Activity in brain might be due entirely to retained blood. Mice sacrificed 1 and 3 hr revealed similar values.

for physical decay). After 2 days 87% had been eliminated. Tissue assay in the mice at 2 hr after injection showed highest activity in the kidneys and liver (Table 2). At 24 and 48 hr the specific activity (counts/gm tissue) in the liver was about equal to that in the kidneys.

DISCUSSION

The cis-diamminedichloroplatinum was widely distributed after intravenous injection. Most activity was in the kidneys, urine, and the liver. Blood activity remained high. By contrast radioactivity in the brain was quite low. Indeed, brain radioactivity might be due almost entirely to retained blood. The brain content of radioactivity was about 7–10% of that in the blood; the blood is known to make up on the order of 7–10% of the brain weight.

Loss of activity from the animals, principally by the urine, was rapid over the first day (with about four-fifths lost from the mice). This rapid excretion, if true in man as well, might make for difficulties in maintaining adequate therapeutic levels. Studies are presently underway as to the chemical nature of the excreted and bound ^{193m}Pt, and the rate and extent of binding of the compound to various tumors.

SUMMARY

The antitumor compound cis-$Pt(NH_3)_2Cl_2$ was synthesized from ^{193m}Pt, and its structure confirmed by three criteria. Following intravenous injection into rabbits, most activity occurred in the kidneys, urine, and liver. In mice a similar distribution was shown with rapid excretion of the radiolabel (79% eliminated by 24 hr). There was little, if any, activity in the brain. The radiolabeled compound presents an almost unique opportunity to trace the distribution of an antineoplastic substance during life.

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