MICROSCALE SYNTHESIS OF CARBOPLATIN LABELED WITH THE AUGER EMITTER PLATINUM-193m: RADIOTOXICITY VERSUS CHEMOTOXICITY OF THE ANTITUMOR DRUG IN MAMMALIAN CELLS

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ABSTRACT

The 4.3 d Platinum-193m emits numerous low energy Auger electrons by virtue of its 3-step isomeric decay almost entirely by internal conversion. We have produced this radionuclide by the $^{192}Pt(n,\gamma)^{193m}Pt$ reaction, and synthesized ^{193m}Pt labeled carboplatin, and carboplatin with a stable Pt metal center using a microscale synthetic approach. The clonogenic survival of Chinese hamster V79 cells is investigated following 2-3 h exposure of the cells to various extracellular concentrations of the cold drug as well as the radiolabeled compound. At 37% survival, the extracellular concentration of nonradioactive carboplatin is about $140\,\mu M$ compared to $35\,\mu M$ with the radiolabeled analog. These results suggest the potential of ^{193m}Pt-carboplatin in chemo-Auger combination therapy.

INTRODUCTION

Platinum-coordinated compounds have been of much interest as potential chemotherapeutic agents since the serendipitous discovery of the effects of cis-diamminedichloroplatinum(II) (cisplatin) by Rosenberg *et al.* (1) in 1969. Other compounds with transition metals and main group elements have also been investigated (2), but they have not demonstrated the same therapeutic efficacy toward cancer as cisplatin. However, there are problems associated with cisplatin treatment despite its successes, the foremost being renal toxicity and physiological discomfort.

A large number of cisplatin analogs are being investigated at present for their viability as chemotherapeutic agents. One compound which has yielded promising results for a variety of carcinomas is cis-diammine(1,1-cyclobutanedicarboxylato)-platinum(II) or carboplatin (Fig. 1.) The renal toxicity and other associated deleterious physiological phenomena are strongly diminished and clearance of the drug from the body is much faster
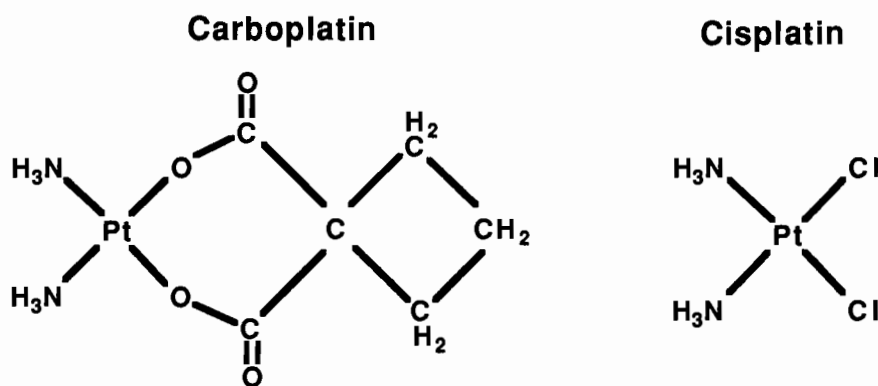
Carboplatin   **Cisplatin**

FIG. 1. Carboplatin, an analog of cisplatin. Both species have square planar molecular geometry, the leaving groups are the 1,1-cyclo-butanedicarboxylato in carboplatin and the chlorides in cisplatin.

than for cisplatin (3). The drug dose necessary to produce fifty percent survival in V79 cells is approximately seven times greater for carboplatin

than for cisplatin (4). Because platinum is a heavy metal, platinum-containing compounds are toxic to the necessary and beneficial body cells as well as to the malevolent ones. Such chemical toxicity may be strongly reduced, in principle, by administration of the drug at lower dosages without affecting the therapeutic efficacy, as indicated below.

It is well known that the antitumor action of these drugs (*e.g.* cisplatin, carboplatin) is intimately related to specific binding of the drug molecules to the DNA of the cells. It is also well established that the high radiotoxicity of Auger emitters is manifest upon their binding to the DNA (*e.g.* ^{125}IUdR). Studies *in vitro* and *in vivo* have repeatedly demonstrated the severe cytocidal effects of ^{125}IUdR, a thymidine analog, when incorporated into the DNA of proliferating cells (5,6). These considerations, when combined together, point to the potential of a new class of radiolabeled platinum coordination compounds as chemo-Auger therapeutic agents. The central goal of our research is to synthesize such compounds and investigate their effectiveness in model cell systems. By virtue of their highly internally-converted isomeric-decay modes, the radionuclides ^{193m}Pt and ^{195m}Pt are copious emitters of Auger electrons (7,8). Platinum-193m is of particular value as a metal center in the drug molecules because it can be produced at a high specific activity (see below). The present work is concerned with microscale synthesis of carboplatin labeled with ^{193m}Pt and study of the biological effects in a mammalian cell line in culture.

PRODUCTION OF RADIONUCLIDES

The half-lives of ^{195m}Pt and ^{193m}Pt are 4.02 d and 4.33 d, respectively (9). Both radionuclides can be produced through bombardment with thermal neutrons in a reactor: ^{194}Pt(n,γ)^{195m}Pt, ^{192}Pt(n,γ)^{193m}Pt. The average Auger yields calculated through Monte Carlo methods for the two radionuclides are comparable; 26 and 33 Auger electrons per decay of ^{193m}Pt and ^{195m}Pt, respectively (7). The following considerations indicate the relative merits for the two radionuclides in biomedical applications.

1. Platinum-194 constitutes 32.9% of naturally occurring platinum whereas platinum-192 makes up only 0.78%. Highly enriched ^{192}Pt (57%) is available through Oak Ridge National Laboratory, albeit expensive.

2. The cross sections for the above nuclear reactions are 2 barns (9) for ^{193m}Pt and 0.09 barn for ^{195m}Pt (9). The attainable specific activity of ^{195m}Pt is therefore limited. In contrast, ^{193m}Pt can be produced at specific activities 10-40 times higher than ^{195m}Pt by the (n,γ) reaction.

3. ^{193m}Pt can also be produced through the following nuclear reactions: $^{193}Ir(d,2n)^{193m}Pt$ and $^{192}Os(\alpha,3n)^{193m}Pt$. Accordingly, it may be obtained at a very high specific activity following chemical separation of the target material, since the only platinum in the system is ^{193m}Pt. The cross sections for the iridium-193 and osmium-192 reactions are 0.07 barn (10) and 0.5 barn (11), respectively. The latter of the two reactions is being explored as a possible method of production.

4. ^{195m}Pt is easily produced at a modest cost. The X ray and γ ray yields for the two radionuclides differ greatly, with ^{195m}Pt having the edge in this comparison. ^{195m}Pt has a 0.17 yield (12) for the 98.9 keV γ ray (most pronounced). ^{193m}Pt has a 0.0011 yield (13) for its most pronounced γ ray of 135.5 keV. The Pt K X Ray yields for ^{195m}Pt and ^{193m}Pt are 0.77 and 0.15, respectively (7,8). The high photon yields for ^{195m}Pt make it an excellent candidate for tracer studies. For therapeutic applications, however, the high specific activity of ^{193m}Pt is essential.

MICROSCALE SYNTHESIS OF CARBOPLATIN(^{193m}Pt)

To minimize the chemotoxicity of platinum, it is important to use as small an amount of the metal as possible. We have developed a scheme of synthesis involving only micromolar quantities of platinum. Here we briefly describe the procedures and mechanism of the synthesis.
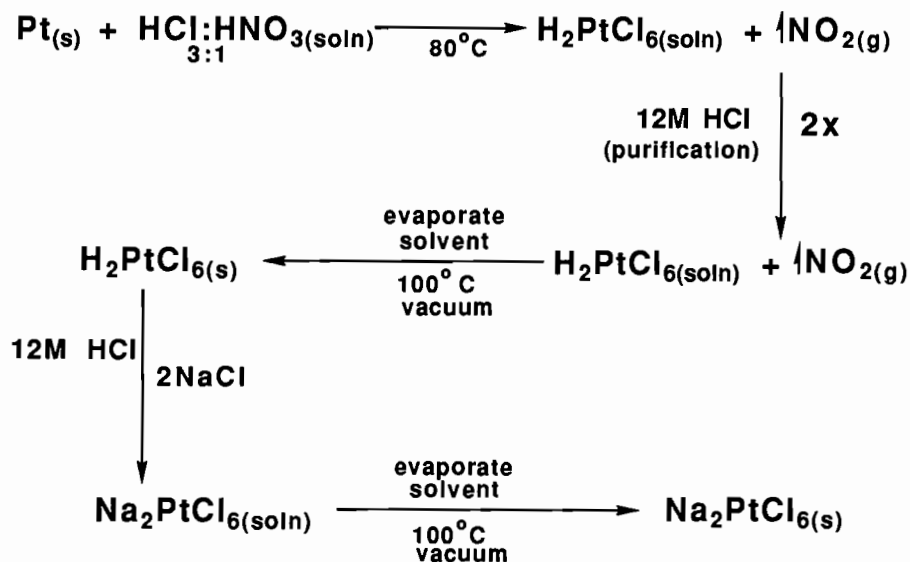
Five milligrams of ^{192}Pt enriched to approximately 57% were irradiated for one week with thermal neutrons at a flux of about 4×10^{14} neutrons/cm^2-s at the University of Missouri Reactor Facility. The sample was dissolved in aqua regia and shipped from Oak Ridge National Laboratory.

The amount of radioactivity produced ranges between 35-100 mCi, depending upon reactor flux and quantity of target material. Since this is a significant amount of radioactivity, proper safety precautions must be taken. In addition to lead shielding, mirrors are strategically placed to observe the

system. Manipulation of the materials is done through automation, vacuum line or applied mechanics, as necessary.

The first step in the radiosynthesis of carboplatin is the isolation of contaminants ([199]Au and ^{192}Ir) from nuclear side reactions. Solvent extraction with methylethylketone and precipitation are used, respectively, for removal of these radionuclides. The synthetic scheme most often used by our group is presented in Fig. 2. There are other synthetic avenues being investigated at present, however none have yielded the same quality of the product. In order to increase the yield of the cis isomer over the trans isomer, most synthetic chemists convert the tetrachloroplatinate salt to the tetraiodo complex because of the enhanced trans-directing properties of iodide. However, we do not do this because, during the conversion from the halide complex to the aquo complex through the addition of silver nitrate, the silver halide precipitate occludes much of the platinum. The microscale quantity of platinum requires a minimization of precipitation reactions and transfers.

Purification of the carboplatin is achieved through column separation and ion exchange resins. Analyses are performed with UV-visible spectroscopy and thin layer chromatography.

$$Pt_{(s)} + HCl{:}HNO_3 \underset{3:1}{}_{(soln)} \xrightarrow[80°C]{} H_2PtCl_{6(soln)} + \mathord{\uparrow}NO_{2(g)}$$

12M HCl (purification) | 2x

$$H_2PtCl_{6(s)} \xleftarrow[\substack{100° C \\ vacuum}]{\text{evaporate solvent}} H_2PtCl_{6(soln)} + \mathord{\uparrow}NO_{2(g)}$$

12M HCl | 2NaCl

$$Na_2PtCl_{6(soln)} \xrightarrow[\substack{100°C \\ vacuum}]{\text{evaporate solvent}} Na_2PtCl_{6(s)}$$

$$Na_2PtCl_{6(s)} \ + \ 0.1M \ HCl \xrightarrow[\substack{5 \ min}]{0°C} Na_2PtCl_{6(soln)}$$

$$\underset{\substack{25°, \ 40°, \ 80°, \ 25°C \\ 2 \ min \ intervals}}{\overset{\textbf{Variable}}{\textbf{Temperature}}} \quad \overset{\substack{\textbf{Argon} \\ \textbf{Atmosphere}}}{0.5N_2H_4 \cdot 2HCl}$$

$$Na_2PtCl_{4(s)} \xleftarrow[\Delta]{vacuum} Na_2PtCl_{4(soln)} \ + \ \downarrow N_{2(g)} \ + \ \downarrow HCl_{(g)}$$

$$\underset{\substack{\textbf{Solvent}}}{\overset{\textbf{H}_2\textbf{O}}{}} \ \Bigg| \ \underset{\substack{\Delta \ 80°C \\ 2Min}}{\overset{\textbf{2NH}_3}{}}$$

$$\underset{85\%}{\text{cis-Pt(NH}_3)_2Cl_{2(aq)}} \ + \ \underset{15\%}{\text{trans-Pt(NH}_3)_2Cl_{2(aq)}}$$

$$\Big| \ 10\% \ xs \ AgNO_3$$

$$\text{cis-[Pt(NH}_3)_2(H_2O)_2](NO_3)_{2 \ (aq)} \ + \ 4AgCl_{(s)}$$

$$\Big| \ Na_2(CBDC)*$$

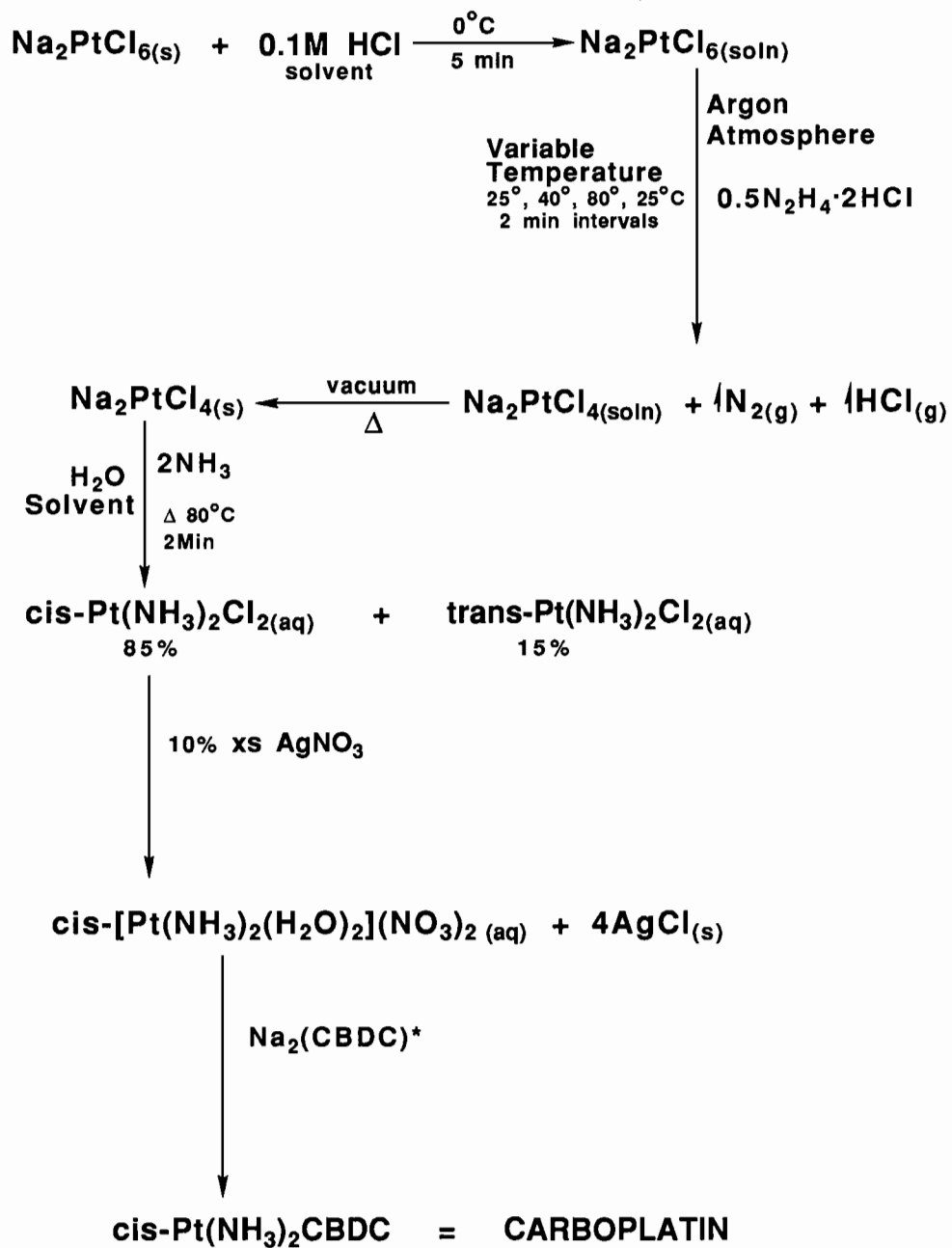$$\text{cis-Pt(NH}_3)_2CBDC \ = \ \textbf{CARBOPLATIN}$$

FIG. 2. Schematic of the synthetic mechanism of carboplatin.
*Na₂(CBDC) = sodium cyclocbutanedicarboxylate.

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After the carboplatin has been synthesized, the dried product is dissolved in sterile water. The drug concentration and activity are then assessed. Spectrophotometric methods and thin layer chromatography are, once again, used for the chemical analysis and NaI(Tl) and Ge(Li) detectors for the radioanalysis. Thin layer chromatography with photographic paper affixed to the silica gel plates works very well for purity analysis and precludes the use of an indicator.

In order to project the electron spectrum from the nuclear decay, Monte Carlo calculation methods were utilized. Computer simulations of the nuclear and atomic transitions were performed on the Physics VAX at the University of Massachusetts. The average electron spectrum from ^{193m}Pt decay in Fig. 3 includes electrons from internal conversion, Auger, Coster-Kronig and super Coster-Kronig processes (7).
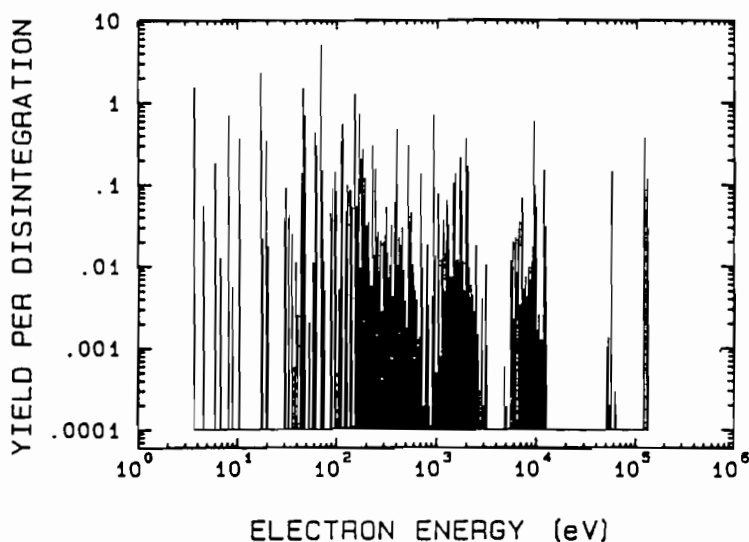

FIG. 3. Monte Carlo simulation of the average electron spectrum in decay of ^{193m}Pt.

BIOLOGICAL STUDIES WITH CARBOPLATIN

Chinese hamster lung fibroblasts (V79 cells) were used in these studies. The advantages of this mammalian cell line as a model for *in vitro* studies have been noted by Kassis *et al.* (14). Clonogenic survival of the cells exposed to the radiolabeled carboplatin or the unlabeled drug was the biological end

point. The kinetics of uptake, post-incubation retention and subcellular distribution of the radiolabeled compound were also studied.

The experimental methods and protocols were essentially the same as developed by Kassis *et al.* (15) The details are also given by Howell (7). The cells were cultured in Gibco (Grand Island, NY) minimum essential medium (MEM) supplemented with 2 mM L-glutamine, 10 mM Hepes buffer, 10% fetal calf serum, 50 units/ml penicillin, 50 µg/ml streptomycin, and 0.1 mM non-essential amino acids. The pH was adjusted to 7.0 with $NaHCO_3$. Medium with calcium (MEMA) was used for survival studies; and MEMB, free of calcium, for uptake and retention studies which require the cells to be mobile. Use of MEMB reduced the probability of cell clumping, and lack of calcium had no effect on cell doubling time or survival (15). Cells in suspension were counted using a Coulter counter and verified with a hemocytometer.

Kinetics of Cellular Uptake

Exponentially growing V79 cells were trypsinized, suspended in MEMB, and conditioned for 4 h at 37°C in an atmosphere of air with 5% CO_2, with the incubation tubes gently shaken on a rocker-roller. The cells (2×10^5 cells/ml) were then treated with radiolabeled carboplatin of various concentrations, and incubated further under the same conditions. Aliquots of the suspension were taken at different times, the cells were washed four times and counted. The microfuge method of Kassis and Adelstein (16) was used to determine the whole cell uptake, the radioactivity being assayed with a Ge(Li) well-detector. The fraction of the total cell uptake when plotted as a function of incubation time (Fig. 4) shows saturation within 2-3 h. Accordingly, cells were incubated with the drug for 2.5 h for all the rest of the experiments.

Cellular Retention

Following 2.5 h of incubation with the radiolabeled carboplatin, the cells were washed four times with MEMB, resuspended in the calcium-free medium and incubated further. At various later times, aliquots of the suspension were taken and the cellular content of radioactivity determined.
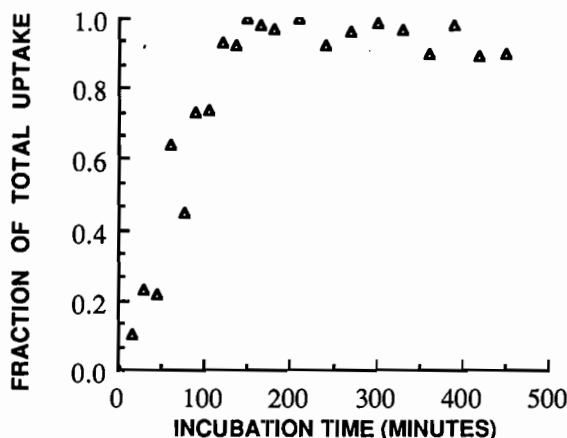
FIG. 4. Kinetics of cellular uptake of radiolabeled carboplatin by V79 cells as a function of time.

Survival Study

Survival studies were performed using carboplatin with radiolabeled and non-radiolabeled platinum metal centers in the same time frame under the same conditions. The cells were trypsinized and conditioned for four hours at 37°C (5% CO_2 atmosphere) in MEMB on a roller at a concentration of 400,000 cells/ml. The cells were diluted to a cell concentration of 200,000/ml with MEMB, treated with radiolabeled or non-radiolabeled carboplatin of various concentrations and incubated for the 2.5 h uptake period under the same incubation conditions. The extracellular concentrations for the radiolabeled carboplatin ranged from 5 to 55 µM. The concentrations for the non-radiolabeled carboplatin varied from 15 to 200 µM. The cells were removed and immediately placed on ice, and were maintained in that condition when not being manipulated. A 0.8 ml aliquot was extracted from each tube containing the radioactive drug, and put aside for the whole cell uptake study. The remaining cell suspension was washed four times with MEMA, and resuspended in MEMA. Serial dilutions were performed on each tube so that final cell concentrations of 20,000/ml; 2000/ml; and 200/ml were obtained. Incubation flasks (25 cm²) were seeded with 1.0 ml of each of the dilutions and nutrified with 4.0 ml of MEMA. The cells were then incubated for one week under standard conditions to allow for colony formation. The

resulting colonies were washed three times with normal saline, fixed with methanol and stained with crystal violet. The colonies were then counted. The criterion for cell survival was taken to be its ability to form a colony with 50 or more cells (7). The survival fractions were determined in comparison with colonies formed by cells unexposed to the drug. The data thus accrued in the radiolabeled and non-radiolabeled studies are plotted in Fig. 5.
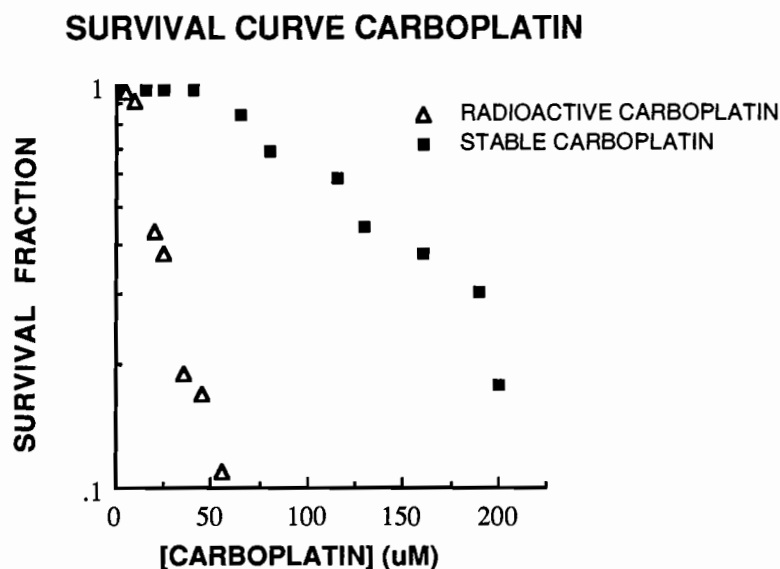
SURVIVAL CURVE CARBOPLATIN

FIG. 5. Survival of V79 cells. The survival fraction is plotted against the extracellular concentration of carboplatin in the incubation medium in studies with the "cold" and radiolabeled compounds.

Whole Cell Uptake

The whole cell uptake studies were carried out using the 0.8 ml aliquot taken from each of the tubes from the survival study. The cell suspension was centrifuged for 5 min at 2000 rpm and 4°C. After centrifugation, 100 µl of the supernatant was removed and layered onto 300 µl of fetal bovine serum (FBS) in a 450 µl centrifuge tube and centrifuged at 15,000 rpm for one minute, frozen in liquid nitrogen, and then the tip was cut off and placed in a borosilicate gamma tube for radioassay. This supernatant fraction was used to correct for the actual activity in the cell pellet. Another 10 µl of the

supernatant from the 2000 rpm centrifugation was spotted onto absorbent paper and placed in a gamma tube to determine the concentration of radioactivity in the medium. The supernatant was decanted (the 2000 rpm sample), the cell pellet was broken up by vortex mixing and then resuspended in MEMA. An 100 µl aliquot of the cell suspension of known cell concentration (measured via Coulter counter) was layered onto 300 µl of fetal bovine serum in a 450 µl centrifuge tube and centrifuged at 15,000 rpm. The same tube-cutting and radioassay procedure was used. The measured radioactivity in the cell pellet was corrected using the values obtained for the supernatant (with no cells). All trials were done in triplicate.

Intracellular Activity Distribution

The intracellular activity distribution study was conducted in a manner similar to the survival study. The cells were processed the same way up to the wash. The contents of the ten culture tubes were combined. The cell concentration was determined using a Coulter counter. The cell suspension was washed two times with cold calcium-free salt solution composed of the following: 0.4 mM KH_2PO_4, 0.4 mM $Na_2HPO_4 \cdot 7H_2O$, 0.74 mM $MgSO_4 \cdot 7H_2O$, 5 mM KCl, and 0.12 M NaCl. The cells were then suspended in 2 ml of cold hypotonic sucrose buffer (0.25 M sucrose, 3 mM $CaCl_2$, 50 mM Tris, pH 7.0) and then placed on ice for five minutes and aliquots were taken for cell counting and radioassay. Disruption of the cell membrane was done by adding 2 ml of sucrose buffer containing 2% Triton X-100 and vortex mixing (17). The cells were placed on ice for an additional five minutes and then vigorously vortexed for thirty seconds. At this point a sample for radioassay was collected. This sample contained cytoplasmic components and whole nuclei. The nuclei were centrifuged at 2000 rpm for 15 min at 4°C. The nuclei formed a pellet at the bottom of the centrifuge tube. The supernatant contained the cytoplasmic fraction. The cytoplasmic fraction was decanted and put on ice for further radioanalysis. The nuclei were washed once with cold sucrose buffer (40 ml) and suspended in 2 ml of the same.

Aliquots of the the suspended cell nuclei were assayed for the content of radioactivity in the whole nucleus. Guanidine-HCl precipitable activity analysis (indicative of the drug bound to the DNA) was done by taking a 5 ml aliquot of the nuclei suspension and adding 10 ml of cold guanidine-HCl (6 M) to it and gently mixing with a glass rod. 15 ml of cold ethanol was added followed by further gentle mixing. The contents of the Erlenmyer flask were transferred to a Gelman Type A-E filter and then the flask, stirring rod and

filter were washed three times with 10 ml cold guanidine-HCl: ethanol solution with each wash being filtered to ensure isolation of all DNA remnants. The filter paper was transferred to a gamma tube for radioassay.

Aliquots of cytoplasmic fractions were radioassayed. The remainder of the fractions were centrifuged at 15,000 rpm for 20 minutes. The supernatant was decanted and the pellet containing the mitochondria was washed once, resuspended in sucrose buffer, and assayed for radioactivity.

RESULTS AND DISCUSSION

In this paper, we have presented the initial results of our studies with carboplatin, an anti-tumor drug, labeled with ^{193m}Pt, a prolific emitter of Auger electrons. Rather than a 50 mg Pt target used traditionally in the ORNL procedures for cisplatin synthesis (^{195m}Pt metal center), we have used only a 5 mg Pt target with 57% enrichment in ^{192}Pt. Our approach to microscale synthesis of the carboplatin complex labeled with ^{193m}Pt, as well as the synthesis of the compound with the stable metal center, gave about a 50% yield. Three alternative mechanisms are also explored, but the same yield has not been achieved. Two of the alternative mechanisms also utilize silver halide precipitation reactions, Ag_2O to separate thiocyanate and Ag_2SO_4 to separate halide components. In microscale synthesis, precipitation reactions create avenues by which significant amounts of the radionuclide can coprecipitate by being occluded in the precipitating moiety, the silver halides in this case. Therefore precipitations are minimized, and the yields are not as high as reported for larger scale operations. The third synthetic scheme uses dimethyl formamide (DMF) as the solvent and removes undesired species through filtration of precipitates, albeit in lower quantity than the other three syntheses. However, DMF is quite toxic and removal from the system creates some problems due to the microscale quantity being carried in the vapor to the upper regions of the reaction glassware. The analyses indicate that the purification processes are satisfactory for the method outlined in this paper.

The biological applications of the drug to the V79 cells yielded some promising results. The uptake kinetics data indicate a saturation after 2-3 hours of the incubation. Howell *et al.* have found similar kinetics of uptake by V79 cells (7,8) for trans-diamminedichloroplatinum(II) or transplatin, radiolabeled with ^{195m}Pt. Preliminary results of the retention study show that

the bulk of the intracellular activity is eliminated in 2-3 days post-incubation. This is comparable to the results of Howell *et al.* with [195mPt]-transplatin (7,8).

Subcellular distribution studies show that about 70% of the activity that penetrated the cell is in the nucleus, and 60% of the latter bound to the DNA. In their studies with [195mPt]-transplatin, Howell *et al.* found that 57% of the intracellular activity was in the cell nucleus, 42% of which was bound to the DNA (7,8).

The survival curves for V79 cells (Fig. 5) exposed to radiolabeled versus non-radiolabeled carboplatin are strikingly different. For the non-radiolabeled carboplatin, the survival curve has a broad shoulder, and the extracellular concentration of the drug in the incubation medium is about 140 µM at 37% cell survival. These results are in excellent agreement with the work of Roberts and Fraval (4) on the chemical toxicity of non-radioactive carboplatin to V79 cells. In contrast, no shoulder is evident with the radiolabeled compound. The mean lethal drug concentration is about 35 µM at which no cell killing occurs with "cold" carboplatin alone. The issue of synergistic effects with ionizing radiation and platinum metal complexes is long standing. While our study does not address this question, we note that Douple and Richmond (18) reported no radiosensitization of V79 cells under oxic conditions for cisplatin and transplatin. Perhaps, the same may be true for carboplatin as well (19).

Although the Pt target for reactor irradiation is enriched in [192Pt] to the maximum extent available (57%), production of [195mPt] is inevitable. The average uptake/cell of the radiolabeled compound is approximately the same as for [195mPt]-transplatin (7). According to ORNL, the initial proportion of the activities of [193mPt] to [195mPt] was about 8:1. Quantitative estimates of the activities of the two radionuclides in the cell are essential for cellular dosimetry. No doubt, [193mPt] was predominant in the cell. Its quantification, however, presented an unanticipated difficulty. Although use of the Ge(Li) well-detector facilitated efficient detection of photons, this geometry also resulted in accidental sum peaks of the intense Pt K X rays emitted in the isomeric decays of the radionuclides. The characteristic γ photons, emitted in low yield, were masked by the sum peaks as a consequence. This technical problem is easily resolved by moving the source out of the well, and further studies are planned accordingly.

In spite of the above limitation, the present work is a significant new contribution. Carboplatin is an antineoplastic drug; when radiolabeled with the ^{193m}Pt moiety, its efficacy for cell killing is very high compared to the cold drug. This suggests that ^{193m}Pt labeled carboplatin may prove to be a viable chemo-Auger therapeutic agent that may achieve the same efficiency as the non-radioactive drug but at much lower dosages. Conceptually at least, a similar approach with cisplatin should be possible.

ACKNOWLEDGMENTS

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DISCUSSION

De Sombre, E.R. Do you have an explanation for the biology of the early uptake plateau? Have you saturated all the available sites?

Azure, M.T. Uptake curves with an early plateau as in this work (Fig. 4) are also observed in other cases (*e.g.* ^{195m}Pt-labeled transplatin (7)) for the same cell line. At the present time we do not fully understand the biological aspects of all possible binding sites. The uptake is concentration dependent, the tighter sites being filled preferentially at lower concentrations of the drug in the incubation medium. The plateau implies saturation of all the relevant sites.

De Sombre, E.R. What was the reason for the choice of carboplatin over cisplatin?

Azure, M.T. Convenience. Both are antineoplastic drugs. Cisplatin is effective at much lower concentrations than carboplatin. The specific activity of ^{193m}Pt available at present is not sufficiently high to delineate the effects of the Auger emitter from the chemical effects of cisplatin alone. Our goal is to examine the efficacy of ^{193m}Pt metal center in platinum antitumor drugs in general. These initial experiments with carboplatin demonstrate the idea. We plan to work with cisplatin with the radiolabel in the future as higher specific activities of the Auger emitter become available.

Kassis, A.I. What is the D_0 in decays/cell?

Howell, R.W. Approximately the same as in the case of ^{195m}Pt-transplatin (7). We encountered some systematic difficulty in quantification.

Sastry, K.S.R. Although ^{193m}Pt is the dominant one, there is also a significant amount of ^{195m}Pt. Use of a well-type Ge(Li) detector resulted in accidental sum peaks of the intense Pt K X rays. This effect masked the weak characteristic γ rays. This problem is easily rectified by placing the source outside the well. We will pursue these aspects further.

Kassis, A.I. Why carboplatin instead of transplatin?

Azure, M.T. Both of these are of interest. Carboplatin has tumorcidal efficacy while transplatin is merely chemotoxic. In these initial studies, we have preferred to work with carboplatin labeled with the Auger emitter.