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Cellular Distribution and Cellular Reactivity of Platinum(II) Complexes

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ABSTRACT. We have investigated whether or not the cellular content of reactive platinum, aside from total cellular and DNA-bound platinum, is a measure of the growth inhibitory potential of a given platinum complex. Human MCF-7 breast cancer cells, after treatment with cisplatin [*cis*-diamminedichloroplatinum(II)] and several 1,2-diphenylethylenediamineplatinum(II) complexes at a fixed dose of 3 μM, were analyzed for their contents of platinum in total cells, isolated nuclei, chromosomal DNA, and the cellular pool of reactive platinum, and compared with ED_{50}-values. Platinum was measured by atomic absorption. Reactive platinum was identified after its reaction with calf thymus DNA that had been added to the cells before their lysis. The amounts of platinum binding to chromosomal DNA were related to previously established ED_{50}-values, and such a correlation could not be found for platinum in total cells, nuclei, and, especially, reactive platinum. The observed differences in the platinum contents of DNA were referred to variations in the rate of adduct formation rather than repair because two representative platinum complexes were indistinguishable by their effects on the chloramphenicol acetyltransferase (EC 2.3.1.28) transfection system. One of the other platinum complexes accumulated, showing an increased growth inhibition in support of this interpretation with regard to the other platinum complexes. During prolonged treatment of MCF-7 cells with the platinum(II) complexes, pools of reactive platinum were found to persist even after drug depletion in the culture medium. This suggested a hitherto unrecognized cellular storage and availability of reactive platinum. BIOCHEM PHARMACOL 52;1:7–14, 1996.

KEY WORDS. cisplatin; prodrug activation; platinum metabolism; platinum DNA; reactive platinum; chemotherapy

Cisplatin† and numerous other platinum(II) complexes have been intensively studied with regard to their molecular mode of action, which ultimately results in the growth inhibition of tumor cells (for a recent review see [1]).

The success of a metal complex in inhibiting tumor growth has been attributed to a number of properties, including penetration of the cellular membrane, accumulation in the tumor cell and nucleus, and efficiency in coordinating with chromosomal DNA. This reaction with DNA is considered the key event that leads to growth inhibition. The degree of DNA platinum lesions in cell nuclei and, thus, the growth inhibitory potential of a given platinum(II) complex, is believed to depend, among other preconditions, both on the cellular level of chemically reactive platinum at the time of the treatment and on the persistency of reactive platinum in the cells. However, these two important properties have not been adequately investigated in the past, probably because their measurement is tedious and time-consuming.

We treated human mammary breast cancer MCF-7 cells with selected platinum complexes that differed in their already published ED_{50}-values (the concentration of a platinum complex effecting 50% growth inhibition) [2, 3]. We measured the platinum contents in total cells, nuclei, chromosomal DNA, and the pool of reactive cellular platinum, and compared the various contents with the magnitude of the ED_{50}-values. We also followed the platinum contents after depletion of the reactive platinum in the culture medium.

MATERIALS AND METHODS

Materials

The platinum complexes K104, r-4F-Pt, and m-4F-Pt (Fig. 1) were gifts of Dr. Schönenberger (Regensburg, Germany), and cisplatin was a gift of Degussa (Frankfurt/Main, Germany). Dulbecco's MEM Eagle's medium without Phenol Red and fetal calf serum were purchased from Gibco. Proteinase K® (EC 3.4.21.14), ribonuclease A (EC 3.1.27.5), ribonuclease T1 (EC 3.1.27.3), and trypsin (EC 3.4.21.4)-

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† *Abbreviations:* Cisplatin, *cis*-diamminedichloroplatinum(II); K104, diaqua[*meso*-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]platinum(II) sulfate; m-4F-Pt, diaqua[*meso*-1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) sulfate; r-4F-Pt, diaqua[*racem*-1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) sulfate; TE-buffer, buffer containing 10 mM Tris-HCl, 1 mM EDTA, pH 8.0; DDTC, diethyldithiocarbamate; Mes, 2-(N-morpholino)ethanesulfonic acid.

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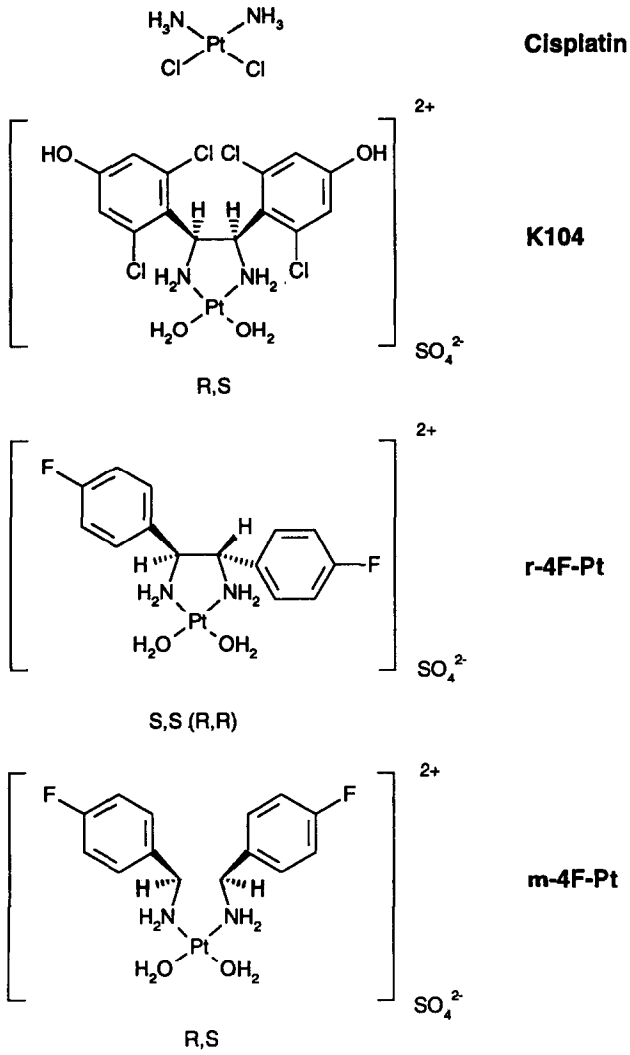

FIG. 1. Platinum(II) complexes used in the treatment of MCF-7 cells. Cisplatin, *cis*-diamminedichloroplatinum(II); K104, diaqua[*meso*-1,2-bis(2,6-dichloro-4hydroxyphenyl)-ethylenediamine]platinum(II) sulfate; m-4-F-Pt, diaqua-[*meso*-1,2-bis(4-fluorophenyl)ethylenediamine]platinum-(II) sulfate; r-4F-Pt, diaqua[*racem*-1,2-bis(4-fluorophenyl)-ethylenediamine]platinum(II) sulfate.

EDTA were purchased from Boehringer, Germany. L-Glutamine and a *Mycoplasma* test kit were purchased from KG Biochrom, Germany. All other chemicals were obtained from Merck, Germany, and were all of analytical grade.

Cell Line and Culture Conditions

The MCF-7 human breast cancer cell line was obtained from the American Type Culture Collection. Cells were propagated as described for the measurement of ED_{50}-values [2, 3]. They were maintained in Dulbecco's medium supplemented with 10% fetal calf serum and 2 mM L-glutamine using 75 cm^2 culture flasks (Greiner, Frickenhaus, Germany). They were grown at 37°C under 5% CO_2 in a humidified incubator and were serially passaged weekly following treatment with 0.05% trypsin/0.02% EDTA. Only *Mycoplasma*-negative cultures were used. Cells were counted as described [2].

Platinum Treatment of Cells

Cells were seeded in 58 cm^2 Petri dishes and grown for 72 h before one of the drugs was added to the culture medium at a final concentration of 3 μM. They were treated with the platinum complexes for variable lengths of time, at maximum 30 hr. The medium was then removed. The monolayer was washed, then suspended in ice-cold 0.9% NaCl, and pelleted at 200 × *g* for 10 min.

Isolation of Nuclei

The resulting cell pellet was resuspended in $CaCl_2$ buffer (10 mM Tris · HCl, pH 7.5; 3 mM $CaCl_2$). After 10 min on ice, the cells were lysed in a 5% (v/v) lysis solution [5% (v/v) benzalkoniumchloride alkyldimethylbenzylammonium chloride), 5% (v/v) acetic acid]. The lysate was layered on top of a 3-mL 0.25 M sucrose cushion in $CaCl_2$ buffer and centrifuged for 10 min at 200 × *g*. The resulting pellet was washed twice with $CaCl_2$ buffer to remove the sucrose. The isolated nuclei were stored at −20°C.

Isolation of Chromosomal DNA

Purification of chromosal DNA followed a modified method of Fichtinger-Schepman [4]. Briefly, cells were lysed with 10% SDS, and protein digested with Proteinase K® (250 μg/mL). The suspension was extracted with phenol and the aqueous phase precipitated with ethanol in the presence of 2.0 M ammonium acetate. The resuspended pellet was digested with ribonuclease A (75 μg/mL) and ribonuclease T1 (75 μ/mL) and extracted with chloroform/isoamylalcohol. After precipitation with ethanol, the isolated DNA was washed with 70% ethanol and dried. DNA was quantified by the fluorimetric method of Thomas and Farquhar [5].

Sieving Methods

Method 1: Typically, 5 × 10^7 cells in 2 mL TE-buffer (consisting of 10 mM Tris · HCl, 1 mM EDTA, pH 8.0) were lysed by sonification. After centrifugation for 15 min at 20,000 × *g*, 0.5 mL of supernatant was loaded on a NAP-5 column (Sephadex G25, Pharmacia) and eluted with 2.5 mL TE-buffer. Method 2: Cells were ruptured by sonication and pelleted as described. The supernatant was passed through a Centricon C10 and a Centricon C3 ultrafiltration system (Amicon, Beverly, MA).

Derivatization with DDTC

Derivatization followed the method described by Bannister *et al.* [6]. Cells were lysed by sonification and incubated for 30 min at 37°C with 0.1 vol DDTC solution consisting of

10% DDTC (w/v) (Sigma)/0.1 N NaOH. The suspension was cooled on ice and extracted twice with 0.5 vol. chloroform. The organic phase was dried under vacuo.

Derivatization with Calf Thymus DNA

Cells (typically 5×10^7) were suspended in 2 mL of a solution containing 3.1 mg/mL calf thymus DNA (Sigma) in 10 mM Mes [2-(N-morpholino)ethanesulfonic acid] buffer, pH 5.5. After sonification (6 pulses of 10 sec), the lysate was allowed to react for 30 min at 37°C. The reaction was stopped on ice by the addition of 0.1 M ammonium carbonate. DNA was quantitatively isolated and the amount of totally bound platinum measured by atomic absorption. This method was the same as that for the measurement of DNA-bound platinum in the absence of added calf thymus DNA. By calibrating the yield of DNA during sample preparation, it was possible to refer the measured DNA-bound platinum, either in the absence or in the presence of the added calf thymus DNA, to a given number of cells (usually 10^6) in the sample. For this number of cells, chemically reactive platinum is calculated (and thus defined) as the total amount of DNA-bound platinum in the presence of calf thymus DNA minus the amount of platinum in the absence of calf thymus DNA (corresponding to platinum bound to chromosomal DNA only). The relative standard error for the chemically reactive platinum was ±15%.

Measurement of Platinum

Platinum-containing material was dried and mineralized by heating for 5 min in 65% HNO_3 (w/v) at 100°C. The product was dried under vacuo and analyzed by atomic absorption. A Varian atomic absorption AA 30 spectrometer equipped with a GTA 96 graphite atomizer was used. The dried samples were dissolved in 0.5% HNO_3, and each sample was measured in 40 μL quantities at 4 different dilutions. To optimize the experimental accuracy, the measured absorbance values were treated by linear progression analysis plotting absorbance vs the reciprocal of the dilution factor. The platinum content was calculated from the slope with a relatively high precision (standard errors of ±10 pg). Background absorbances, which were the same within a series of dilutions, appeared in the abscissa and were, thus, eliminated. By this optimization, the detection limit of the method was 50 pg of platinum.

If the platinum content of a particular cell compartment and the ED_{50} value are strictly correlated, and the degree of growth inhibition varies hyperbolically with the dose of the drug, the following relation is considered:

$$[Pt] = \phi \cdot [3 \cdot 10^{-6}]/(ED_{50} + 3 \cdot 10^{-6}],$$

where [Pt] is the concentration of platinum in the compartment, $[3 \cdot 10^{-6}]$ the dose of the platinum complex (in mol/L), and ϕ a not further characterized cell culture type and compartment-specific parameter. In the simplest case, ϕ is the same for all investigated platinum complexes. The qualitative prediction of platinum contents in Table 1 is calculated in the light of this assumption.

Repair of Platinum Adduct-Containing Plasmid DNA

Plasmid pA2(-331/-289)tk-CAT 8+ (a gift from Dr. Klein-Hitpaß/Essen, Germany) was allowed to react with the desired platinum complex, yielding an average incorporation of 1 mol platinum per mol reporter gene of chloramphenicol acetyl transferase (EC 2.3.1.28) (CAT). This amount of platinum inhibited the expression of the reporter enzyme by 75%. After transfection into MCF-7 cells (in analogy to the method of Meyer *et al.* [7]; in the presence of 0.01 μM estradiol) the expression of the enzyme activity was followed with reference to the absence of platinum. The recovery of CAT activity was interpreted as corresponding to DNA repair.

RESULTS

Experimental Strategy

Our goal was to relate the differences in growth-inhibition potential of selected platinum complexes to the platinum content in whole cells, isolated cell nuclei, the fraction of platinum binding to chromosomal DNA, and the fraction of chemically reactive platinum. Growth inhibitor potentials in terms of ED_{50}-values together with qualitative estimates of platinum are given in Table 1. These ED_{50} values have been previously measured under conditions [2, 3] that were very similar to those used in our present experiments.

The ED_{50} values in Table 1 were measured after at least 4 days of incubation without renewal of the medium. For cisplatin and K104, shorter time periods were examined [2]. Growth inhibition was not manifested before 15 hr after the addition of the platinum complexes, following a first-order time dependence [2]. The dose-dependences appear

TABLE 1. Growth inhibition of human mammary MCF-7 tumor cell cultures by platinum(II) complexes

Platinum(II) complex*	ED_{50} (μM)†	Predicted platinum content‡
Cisplatin	0.5§	high
K104	20§	low
m-4F-Pt	3‖	low-medium
r-4F-Pt	0.3‖	high

* For the definition and structures of complexes, see Figure 1.

† Taken from the literature. The dose for 50% growth inhibition after 4 days [3] or 5 days [2] of treatment with the platinum complexes. The number of surviving cells (drug present) relative to control cells (drug absent) were measured by the crystal violet method described in ref. [3]. Standard deviations are within 20% of the given values.

‡ Relative platinum content estimated under the assumption of a direct correlation between content and ED_{50} value as described in the text.

§ Reference [2].

‖ Reference [3].

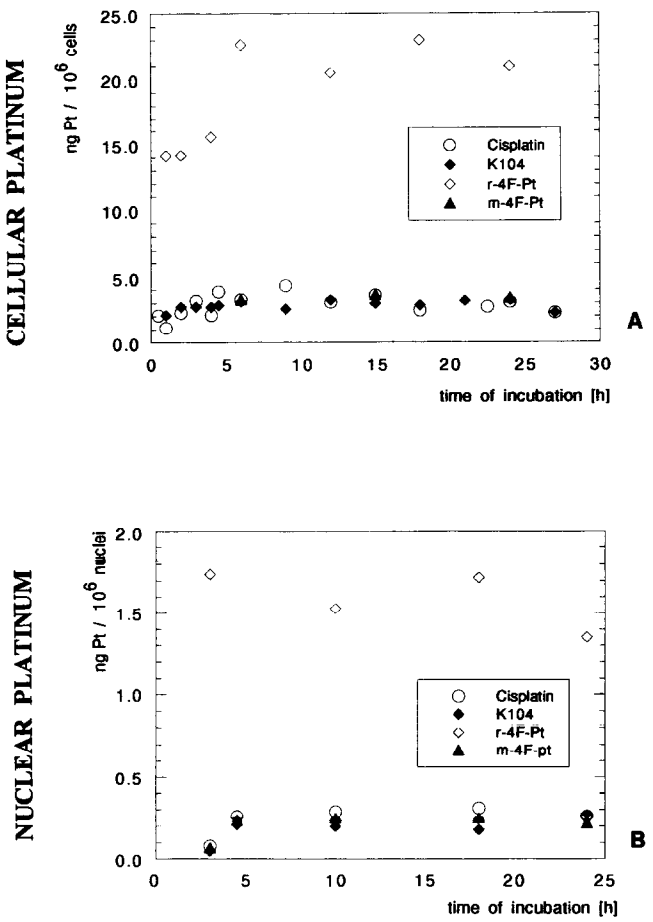

FIG. 2. Total cellular and nuclear platinum of MCF-7 cells treated with platinum complexes at a dose of 3 μM for varying lengths of time. Panel A, total cellular platinum; Panel B, nuclear platinum.

to be hyperbolical. From the data for r-4F-Pt and m-4F-Pt in ref. [3], it would seem that these properties were of the same kind. To reveal the features that led to the different inhibition efficiencies of the complexes, we then measured the platinum contents of the treated cells during the first 30 hr of incubation.

Platinum Uptake by Whole Cells and Nuclei

The uptake of platinum by whole cells and nuclei followed dependences that were similar for cisplatin, K104, and m-4F-Pt. However, amounts were exceptionally high for r-4F-Pt (Fig. 2A, B). The platinum content did not show the correlation considered in Table 1. The high uptake for r-4F-Pt suggested that this platinum complex was accumulated.

Platinum Bound to Chromosomal DNA

The platinum content in chromosomal DNA followed the order r-4F-Pt > cisplatin >> K104 (Fig. 3). The amount for m-4F-Pt and K104 was similar (only 3 data points, not shown). Thus, the level of DNA-bound platinum correlated with the predictions in Table 1.

Chemically Reactive Platinum

It was necessary to establish a method, and thereby define *reactive* platinum. We found that the identification of reactive platinum with low-molecular-weight platinum was inadequate: sieving on NAP-5 columns indicated 50% of the total cellular platinum in the form of low-molecular-

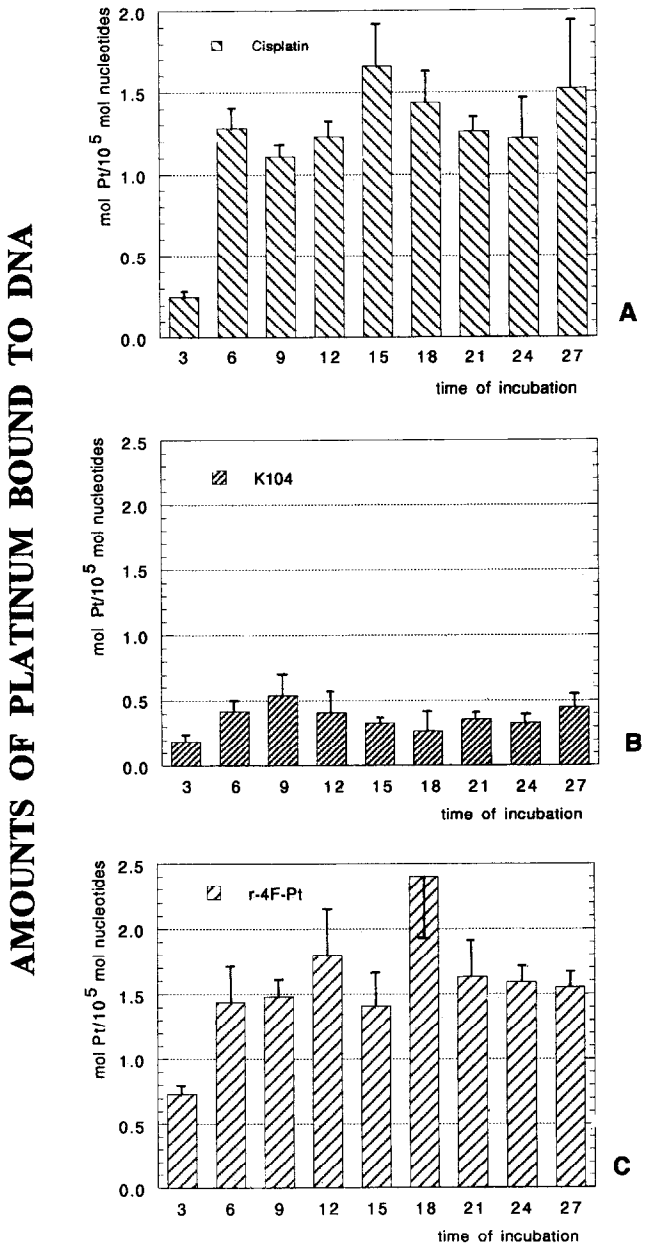

FIG. 3. Quantities of platinum bound to chromosomal DNA of MCF-7 cells after treatment with 3 μM platinum complexes for various lengths of time. Error bars are shown with respect to the average value from 2 measurements.

weight platinum; in contrast, ultrafiltration through Centricon C3-filters indicated 3% as low-molecular-weight platinum. Sieving appeared to be conceptually inappropriate. For instance, low molecular weight cellular constituents, such as glutathion [8] or GTP, were to inactivate platinum (for a recent review, see ref. [1]). Because the method of derivatization seemed to be more appropriate, cellular platinum was allowed to react with DDTC. By this method, 50% of the total cellular platinum was "reactive." Because DDTC can expel ligands in the *trans*-position, a dissociation of platinum from biological ligands and, thus, an overestimation of reactive platinum seemed likely. Finally, derivatization with calf thymus DNA, added to cells before lysis, was used as the method of choice, especially because DNA was the supposed target of the platinum complexes. The conditions of the derivatization reaction, in particular the incubation time of 30 min, were established on the basis of previous results [9], and were such that the reactions of the monoaqua or diaqua forms of the platinum(II) complexes were complete. In cases where the reaction time was prolonged by another 60 min, the observed additional increase in DNA-bound platinum was less than 5% of the amount for the standard incubation time.

The results in Fig. 4 suggest for K104 and, especially, for cisplatin an initial increase and then an approach to plateau levels of 0.2–0.4 ng reactive Pt/10^6 cells. For m-4F-Pt, data points were collected between 15–30 hr with an average of 0.3 ng ± 0.02 ng reactive Pt/10^6 cells (3 time points, not shown). The levels for r-4F-Pt were higher at short incubation times, supporting the idea that at these times a higher amount of platinum was in a reactive form for this complex than for the other platinum complexes.

Repair of Plasmid DNA

To clarify whether or not the relatively low content of platinum in chromosomal DNA after treatment with K104 was the result of a more active repair, the expression of chloramphenicol acetyl transferase from the corresponding platinum-bearing plasmids was measured after their transfection into MCF-7 cells. Expression was blocked by these lesions. The results in Fig. 5 indicate a time-dependent recovery that was similar for K104 and cisplatin lesions. The results were interpreted by assuming that DNA repair was similar for both platinum complexes. Because of the similarity, DNA repair was unlikely to account for the different platinum contents in chromosomal DNA.

Persistence of Chemically Reactive Platinum in MCF-7 Cells

The time-dependences for total cellular platinum (Fig. 2A), nuclear platinum (Fig. 2B), DNA-bound platinum (Fig. 3), and reactive platinum (Fig. 4) show plateaus towards prolonged incubation times. The plateaus did not parallel the levels of the reactive platinum contained in the culture

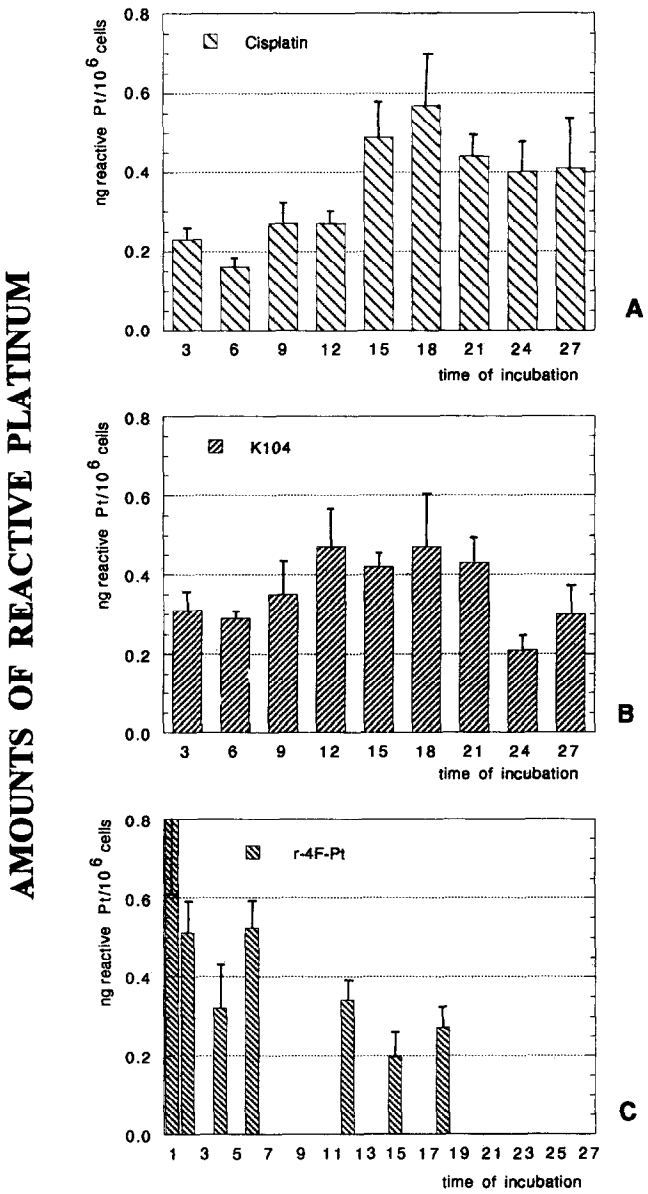

FIG. 4. Quantities of reactive platinum in MCF-7 cells after cell treatment with 3 μM platinum complexes for various length of time. *Reactive* platinum refers to platinum after derivatization with calf thymus DNA as described in Methods. Error bars are shown with respect to the average value from 2 measurements.

medium (Fig. 6). This decreased by 80% within 3 hr and almost disappeared over 21 hr in the case of K104, m-4F-Pt, and r-4F-Pt. In the case of cisplatin, the level declined continuously and corresponded to only 30% residual reactive platinum after 21 hr. The extracellular platinum pool apparently ceased to have an effect on the intracellular platinum content after a prolonged period of incubation. To test this assumption, cells were treated for 18 hr with cisplatin and then transferred to fresh culture medium that did not contain the drug. During the following 15 hr, a plateau level of 0.51 ng Pt/10^6 cells ±20% persisted, in agreement with the above assumption.

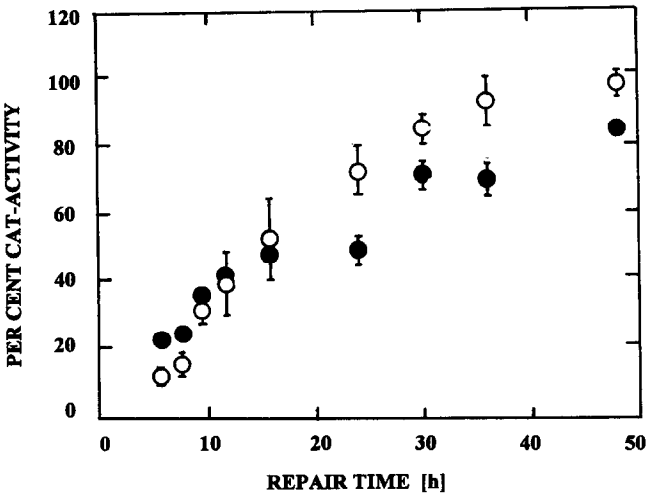

FIG. 5. Repair of a transient DNA plasmid containing a single adduct of cisplatin (open circles) or K104 (filled circles) per reporter gene in transfected MCF-7 cells. The expression of the reporter chloramphenicol acetyltransferase gene was followed. The activities are indicated with reference to expression in the absence of the platinum adduct. Error bars refer to deviations from the average value for 2 measurements.

DISCUSSION

Our goal was to establish a correlation between the cellular distribution of platinum and the ED_{50} values for the growth inhibition of MCF-7 cells by selected platinum(II) complexes. These compounds and their ED_{50} values are presented in Table 1. It can be argued that inhibition could be very sensitive to variations in experimental conditions, and a comparison of data such as in the table may be questionable. Because conditions in ref. [2] and [3] were quite comparable, we do not agree with this argument. Moreover, ED_{50} values of comparable sizes have been reported for other cell lines, such as human MDA-MB cells (cisplatin 0.7 μM; K104 40 μM) [2] or mouse P388 D_1 leukemia cells (cisplatin 0.5 μM; m-4F-Pt 3 μM; r-4F-Pt 0.5 μM) [10].

In our experiments, the platinum content in total cells, nuclei, and chromosomal DNA could be followed by conventional methods. However, a suitable technique had to be developed for reactive platinum. This was accomplished by derivatization of reactive platinum with calf thymus DNA. We are aware that the amount of reactive platinum measured by this technique does not necessarily reflect only *aquated* forms of the platinum(II) complexes employed. It is likely that other aquated adducts had been formed by the reaction of the drugs with biomolecules of the treated cells. They could bind to the added DNA and were also counted as reactive platinum. Clearly, a variety of reactive types of platinum complexes are defined by the condition of the derivatization reaction. The reactivities of these platinum complexes, however, were such that the derivatization was complete after 30 min. We believe that it is appropriate to call such platinum complexes "reactive" in a physiological sense. Other methods, such as molecular sieving or derivatization with DDTC, seemed to give unrealistic results and were not employed in our experiments, although such methods have been used in previously reported investigations [11, 12].

The amount of platinum binding to chromosomal DNA followed the growth inhibitory potential of the platinum complexes qualitatively, as predicted in Table 1. This finding confirms previous observations [13, 14], and is in agreement with the generally accepted mode of action of platinum(II) complexes on chromosomal DNA as the cellular target of these antitumor agents. In contrast to the 40-fold difference between the ED_{50} values for cisplatin and K104 in Table 1, the level of platinum-bound DNA varied only 4-fold. In modification of the above equation for the prediction of platinum contents, a strictly quantitative correlation of this kind is apparently not to be expected. Indeed, a few added lesions in appropriate positions of the genome may cause growth inhibition.

In contrast with the results for chromosomal DNA, the platinum content of total cells, of cell nuclei, and of the cellular reactive pool did not show a correlation with the ED_{50} values. For total cells and for nuclei, this finding is in agreement with the known reactivity of the contained biomolecules, such as proteins, certain amino acids, peptides, nucleotides, etc. (see the recent review [1]), and with the assumption that a large fraction of platinum could be bound to these biomolecules. Consequently, the small variation in the fraction of platinum bound to DNA could not be resolved.

If the level of reactive platinum was invariant, how was it possible that the platinum contents of chromosomal DNA varied? Assuming that the amount of platinum adducts of DNA reflected a steady-state equilibrium, mainly between the formation and repair of the lesions, the different levels could be the result of an effect on only one or on

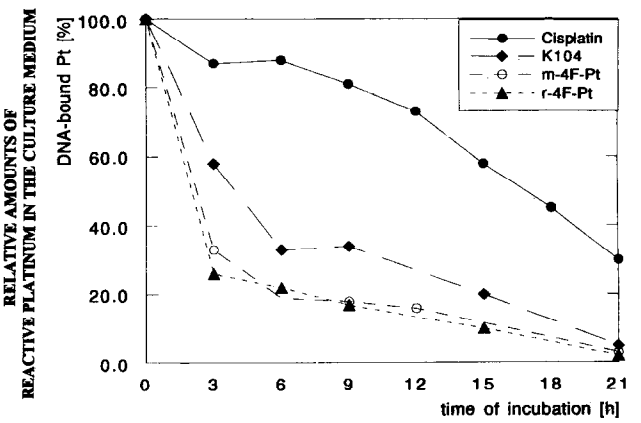

FIG. 6. Reactive platinum in the culture medium after various periods of incubation with 3 μM (corresponding to 100%) platinum(II) complexes. The experiments were carried out in the absence of cells. The binding of reactive platinum to calf thymus DNA was measured as described in *Materials and Methods*.

both of these reactions. The differential repair of DNA-platinum was measured for cisplatin and K104 as representative platinum complexes. The repair rates were found to be similar for both (Fig. 5). It was concluded that the rate of adduct formation was slower for K104 than for cisplatin; thus, accounting for the lower steady-state level of adducts for K104 in comparison with cisplatin. The same kind of phenomenon was assumed for m-4F-Pt and r-4F-Pt. As the concentrations of reactive platinum were nearly the same for cisplatin, K104, and m-4F-Pt, the values of rate constants for K104 and meso-4F-Pt had to be inferior to the rate constant for cisplatin. The kinetics for the reactions of cisplatin, K104, m-4F-Pt, and r-4F-Pt with purified DNA have been investigated [9], and it has been concluded that the reaction of nuclear DNA was probably faster for cisplatin than for the other platinum complexes at equimolar concentrations [9]. For r-4F-Pt, the reaction was faster and, thus, the growth inhibition stronger than for K104 or m-4F-Pt because of its high cellular concentration during the first half of the treatment (Fig. 4). The conclusion that different levels of DNA-bound platinum and, thus, antitumor efficiencies could be the results of variations in the chemical reactivity of platinum compounds is also shared by Yoshida *et al.* [14]. This does not rule out other contributions such as may originate from varying properties of DNA-platinum adducts.

The content of reactive platinum is similar for all the investigated platinum complexes except for r-4F-Pt. An average value of 0.4 ng for reactive platinum per 10^6 cells (plateau values in Fig. 4) was used to calculate the cellular concentration of reactive platinum as 1 μM (assuming an average cell volume of 2 picoliter [3]). This value is compared with the 3 μM concentration of the platinum complexes in the culture medium, indicating that reactive platinum in the case of these complexes is not accumulated relative to the platinum concentration outside the cells. In contrast, the concentration of reactive platinum for r-4F-Pt is high at short times of incubation, then declines, and becomes comparable with the concentrations for the other complexes (Fig. 4). The distribution of platinum in whole cells and in nuclei suggests a much larger uptake of r-4F-Pt than of the other complexes (Fig. 2). The stereoisomer m-4F-Pt does not show this high degree of uptake. It seems likely that both the high degree of uptake and the high concentrations of reactive platinum reflect the stereospecific transport of r-4F-Pt by an active pumping system. Before this conclusion is acceptable, however, dependence on consumption of ATP has to be demonstrated. The decline in cellular reactive r-4F-Pt seen in Fig. 4 may be due to a variety of reasons, such as the exhaustion of the free complex in the culture medium, the inactivation of the transport system, and/or an inactivation due to the reactions with intracellular bionucleophiles.

The time-dependence of reactive platinum in MCF-7 cells (Fig. 4) revealed an interesting phenomenon: a plateau level that persisted although reactive platinum in the culture medium became depleted, as indicated in Fig. 6 for K104, m-4F-Pt, r-4F-Pt (half-lives of 3 hr–4 hr), and cisplatin (half-life of 16 hr). A complete inactivation of platinum complexes in the culture medium has been reported to occur in less than 24 hr [3]. The finding of the plateau suggests that platinum can be stored in MCF-7 cells and that reactive platinum can be recruited from the storage. The assumption of a persistent level of reactive platinum offers an explanation as to why considerable levels of DNA-bound platinum are maintained during prolonged incubation times (Fig. 3). If reactive platinum did not persist, depletion of DNA-bound platinum could occur by excision repair, by DNA synthesis due to cell proliferation, and by the limitation of reactive platinum in the culture medium, as indicated in Fig. 6.

A storage and recruitment of reactive platinum may involve a new feature of platinum complexes that should be investigated in detail. The persistence phenomenon is probably more general, and may occur with other platinum complexes and cell types. For instance, it has been reported that cells show the same degree of growth inhibition after 6–12 hr of treatment with platinum complexes as after treatment for 5 or even 10 days [3]. Whether platinum-resistant cells can be distinguished by their inability to maintain sufficient levels of persisting reactive platinum has to be answered as does the question about the mechanism of the storage and activation of the stored metal.

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