In Vitro Cytotoxicity, Protein Binding, Red Blood Cell Partitioning, and Biotransformation of Oxaliplatin

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ABSTRACT

The in vitro cytotoxicity, protein binding, partitioning of platinum from whole blood into erythrocytes, its exchange back into plasma, and the in vitro biotransformation in plasma were studied for the new nonnephrotoxic platinum analogue oxaliplatin. The cytotoxicity studies were carried out against a panel of human tumor cell lines derived from carcinomas of the ovary (A2780, A2780/cp), bladder (TCCSUP, RT4), colon (HT-29), melanoma (SKMEL-2, HTB144), and glioma (U373MG and U87MG). The relative potency of the five platinum complexes was oxaliplatin = tetraplatin > cisplatin > iproplatin > carboplatin. Oxaliplatin was active against HT-29 and only minimally cross-resistant with cisplatin against A2780/cp. Both bladder carcinoma cell lines, both melanoma cell lines, and one of the two glioblastoma cell lines were resistant to both oxaliplatin and tetraplatin. The cytotoxicity profiles of the drug pairs oxaliplatin-tetraplatin and cisplatin-carboplatin showed statistically significant correlation by the Spearman rank correlation test. Oxaliplatin was similar to cisplatin and tetraplatin in protein binding; 85–88% of all platinum from oxaliplatin (5, 10, or 20 µg/ml) was bound to plasma proteins within the first 5 h with an average half-life of 1.71 ± 0.06 h. When oxaliplatin was incubated in whole blood (5, 10, and 20 µg/ml), the erythrocytes took up 37.1 ± 2.1% of the total platinum in 2 h (maximum uptake) which was not exchangeable into plasma. Thus the erythrocyte-bound fraction does not serve as a reservoir of drug. In plasma, oxaliplatin was unchanged at 0.5 h, but at 1 h, 30% of the total platinum in plasma was in a peak which had identical retention to that of (trans-1,2-diaminocyclobexane)dichloroplatinum(II), the major biotransformation product of tetraplatin. At 2 h, (trans-1,2-diaminocyclobexane)dichloroplatinum(II) and three other platinum-containing peaks were detected but no unchanged oxaliplatin. All the platinum eluted in a single peak near the solvent front at 4 h. The marked similarity in cytotoxicity between oxaliplatin and tetraplatin may be due to the formation of (trans-1,2-diaminocyclobexane)dichloroplatinum(II) in tissue culture media.

INTRODUCTION

Oxaliplatin is a platinum complex undergoing Phase II clinical evaluation in Europe which will shortly be introduced into clinical trial in the United States. It differs from the currently commercially available platinum complexes, cisplatin and carboplatin, in its toxicity profile and in its spectrum of antitumor activity in early clinical trials (1–5). The drug has neither marked renal nor myelotoxicity; its dose-limiting toxicity is peripheral neuropathy (1). In preliminary clinical studies, it has shown antitumor activity against colorectal carcinoma, melanoma, and glioma, as well as against the tumors traditionally treated with platinum complexes (2–5). Pharmacological information on the drug is limited (2). Oxaliplatin exhibited no cross-resistance with cisplatin when tested in cisplatin-resistant L1210 tumors both in vivo and in vitro (6). In order to gain more information about the differences between oxaliplatin and other platinum complexes and to prepare for clinical trials of the drug in our institute, we have carried out studies in vitro of the pattern of cytotoxicity of oxaliplatin against a series of human tumor cell lines, its plasma protein binding, erythrocyte uptake, retention, and biotransformation in plasma. In these studies, we used cell lines derived from tumors normally sensitive (ovary, bladder) and normally resistant to platinum complexes (melanoma, glioma, and colorectal carcinoma). For comparison, we have studied four platinum complexes (Fig. 1), the two commercially available divalent platinum complexes cisplatin and carboplatin, and two quadrivalent platinum complexes iproplatin and tetraplatin. Iproplatin has undergone Phase I and II clinical evaluation with demonstrated clinical activity (7); tetraplatin has also undergone clinical trial (8), and like oxaliplatin, is a dach platinum complex. In this study, we demonstrate that oxaliplatin is converted to PtCl2(dach) in plasma, and suggest that formation of this species in the normally high chloride-containing media may be the reason for similar cytotoxicity profiles observed in this study for oxaliplatin and tetraplatin. This study also reveals that a significant portion of platinum is lost irreversibly to the erythrocytes when incubated in whole blood.

MATERIALS AND METHODS

Drugs. Oxaliplatin was obtained from Debiopharm, Lausanne, Switzerland. Cisplatin was purchased from Sigma Chemical Co. Tetraplatin was a gift from Upjohn Co. Carboplatin and iproplatin were gifts from Bristol-Myers Co. [3H]PtCl2(dach) was kindly provided by Dr. R. D. Haugwitz of National Cancer Institute.

Cell Lines. All cell lines used in the study are of human origin. These include two bladder carcinoma cell lines (RT4 and TCCSUP), one ovarian carcinoma cell line (A2780), its cisplatin-resistant subline (A2780/cp) (7), one colon carcinoma cell line (HT-29), two glioblastoma cell lines (U-373MG and U-87MG), and two melanoma cell lines (SK-MEL-2 and HT-144). All cell lines except the ovarian carcinoma lines were obtained from the American Type Culture Collection (Rockville, MD). The A2780 cell line and its cisplatin-resistant subline (A2780/cp) were kindly provided by Dr. R. Oszos of Fox Chase Cancer Center, Philadelphia, PA.

Cytotoxicity Studies. The cytotoxicity studies were carried out with the sulforhodamine-B microculture colorimetric assay as described previously (10, 11). Typically, cells were plated into 96-well plates on day 0 and exposed to the drug on day 1; the sulforhodamine-B assay was carried out 48 h after drug exposure. The plates were incubated at 37°C in 5% CO2 and 100% relative humidity at all times except when adding the drug and during the final assay period. The initial number of cells plated for the assay ranged from 2,000 to 20,000 cells/50 µl/well. The numbers of cells for plating and the drug exposure time were based on pilot studies using the criteria that (a) the cells in control media are still in the log phase of growth on the day of the assay; (b) the maximum absorbance for the untreated controls on the day of the assay is in the range of 1.0 to 1.5; and (c) cells go through >2 doublings during the drug exposure (10, 11). Typically, 9 drug concentrations, ranging from 0.1 to 100 µM for cisplatin and oxaliplatin, 1 to 1000 µM for carboplatin, 0.1 to 1000 µM for iproplatin, and 0.01 to 100 µM for tetraplatin were used. Eight wells were used per concentration. The plates were read at 570 and/or 540 nm using a Biotek Instruments model EL3109 microplate reader interfaced with an IBM PC.
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Cytotoxicity Data Analysis. Cytotoxicity data were analyzed using computer programs EPI-STAT and EPI-TUS 1-2-3 format by the computer program DATALOG, and survival fractions were calculated by comparing the drug treated with controls.

Cytotoxicity Data Analysis. Dose response data were fit with the Sigmoid-Emax concentration-effect model (12) with nonlinear regression and weighted by the reciprocal of the square of the predicted response. The fitting software G-PLATE was developed in-house by Dr. W. Greco, Roswell Park Cancer Institute, with Microsoft FORTRAN and uses the Marquardt (13) algorithm as adapted by Nash (14) for the nonlinear regression. Best fit parameter estimates and slopes are provided along with SE and 95% confidence limits.

Spearman's rank correlation test was used for evaluating the similarity of the cytotoxicity profiles of drug pairs (15, 16) using the computer program EPI-STAT. The IC50 values for each of the drugs against each of the cell lines was numerically ranked, and correlations were evaluated.

Protein Binding Experiments. Platinum complexes were incubated in human plasma at 37°C at 5, 10, and 20 μg/ml for 48 h. At intervals, two 1-ml aliquots were subjected to centrifugal ultrafiltration using Amicon centrifree micropartition systems at 1500 x g for 30 min. Platinum in the PUF was measured by FAAS by injection of 50-μl aliquots. Controls containing the platinum complex in water showed no binding to the membrane of the Centrifree tubes. Protein binding of oxaliplatin in vitro in plasma was compared with that of cisplatin and tetraplatin at equimolar concentrations (50 μM).

Partitioning of Oxaliplatin into Erythrocytes. Several concentrations of the drug were incubated in whole blood at 37°C. Aliquots were centrifuged at 1500 x g for 20 min to obtain plasma and erythrocytes. PUF was obtained as described above. Plasma and erythrocytes (200 μl) were digested at 90°C with 1 and 2 volumes of 70% nitric acid, respectively, and the residue redissolved in 0.1 N HCl prior to FAAS. Standards were prepared identical to the samples in the same biological matrix.

To determine the exchangeability with plasma of the erythrocyte-bound platinum, oxaliplatin was incubated in blood at 37°C for 2 h. After centrifugation, the erythrocyte fraction was washed twice with 0.9% NaCl and resuspended in drug-free plasma to a volume equivalent to the original and incubated for 4 h. Aliquots were removed at intervals, plasma and erythrocyte fractions were collected and digested with nitric acid, and platinum was determined by FAAS.

Biotransformation of Oxaliplatin. These studies were carried out with 50 μg/ml of oxaliplatin because the platinum is progressively lost to the plasma proteins. Oxaliplatin was added to plasma and incubated at 37°C; 1-ml aliquots were subjected to centrifugal ultrafiltration, and the ultrafiltrate (100 μl) was injected into HPLC. For the 2- and 4-h samples, the ultrafiltrate was concentrated twice by lyophilization.

HPLC System. A Waters Associates HPLC system, consisting of two M6000A pumps with a system controller for making gradients and a U6K injector, was used. Fractions were collected at 0.2-min intervals using a Gilson fraction collector, and platinum was determined by FAAS.

HPLC Procedure. A reverse phase ion pair HPLC procedure was used to separate oxaliplatin from the other biotransformation products (17). The column was an Alttech C4 with a mobile phase gradient of 5 min heptane sulfonic acid to 90% methanol at 1 ml/min flow rate. The elution profile consisted of 10 min at initial conditions (100% heptane sulfonic acid), a 30-min gradient from heptane sulfonic acid to 90% methanol, and 10 min at the final conditions (90% methanol).

Atomic Absorption Spectrophotometry. A Perkin Elmer Model 403 atomic absorption spectrophotometer with a Model 2100 graphite furnace was used. A 3-stage temperature program with 50-s drying, 50-s charring, and 20-s atomization was used. A 50-μl sample was injected into the furnace for platinum measurement.

RESULTS

Cytotoxicity Studies. The dose response curves for the five platinum complexes (Fig. 1) in eight human tumor cell lines are shown in Fig. 2. The IC50 values are presented in Table 1. The ovarian carcinoma cell line A2780 is the most sensitive to all the platinum complexes tested. The data reveal the following general characteristics of the five platinum complexes: (a) the potency is in the order of oxaliplatin = tetraplatin > cisplatin > iproplatin > carboplatin; (b) the two dach-Pt complexes show similar profiles and in many instances the two curves overlap, yielding similar IC50 values; and (c) the slopes of the dose response curves of the two dach-Pt complexes differ substantially from those of the other platinum complexes.

In relation to A2780, the other cell lines exhibit different degrees of resistance to each of the five platinum complexes. This relative resistance expressed as "fold resistance" relative to A2780 is shown in Fig. 3. The data indicate that (a) 5 of 7 cell lines used in this comparison are relatively more resistant to the two dach-Pt complexes than the other platinum agents. These include the two melanoma cell lines (SKMEL-2 and HT-144), one of the glioblastoma cell lines (U-87MG), and both bladder cell lines (RT-4 and TCCSUP); (b) a general collateral sensitivity (or resistance) exists between cisplatin and carboplatin and between oxaliplatin and tetraplatin; (c) 4 of 7 cell lines (HT-29, RT4, U-87MG, and U373MG) exhibit less resistance to iproplatin than to the other platinum complexes. The one cell line (other than A2780) that shows the most sensitivity to oxaliplatin is the colon carcinoma cell line HT-29 (Fig. 3; Table 1). The glioblastoma cell line U373MG shows approximately the same relative resistance to all the platinum complexes. The bladder cell line TCCSUP is relatively sensitive to cisplatin and carboplatin but is resistant to the two dach-Pt complexes.

The five platinum complexes were also tested against A2780/cp, a variant of A2780 ovarian carcinoma made resistant to cisplatin in vitro (Fig. 4). This cell line is 18- and 15-fold resistant to cisplatin and carboplatin, respectively, but relatively sensitive to oxaliplatin. The degree of resistance seen in this cell line for oxaliplatin, tetraplatin, and iproplatin are 2.4, 3.7, and 3.9, respectively.

The results of the Spearman rank correlation analysis for the pair wise comparison of the five platinum complexes used in this study are presented in Table 2. The only two drug pairs that show a correlation in their activity are oxaliplatin-tetraplatin and cisplatin-carboplatin. These correlations are highly significant.

Protein Binding. When oxaliplatin was incubated with plasma, the percentage of free (nonprotein bound) platinum recovered in PUF fell rapidly to 12–15% in about 5 h. The extent of protein binding was independent of oxaliplatin concentrations at 5, 10, and 20 μg/ml (Fig. 5). The half-lives for the disappearance of free platinum as determined by log-linear regression analysis using the program LAGRAN (18)
were 1.65, 1.77, and 1.72 h, respectively, for 20, 10, and 5 µg/ml initial concentrations. From 6 to 48 h the fraction of platinum recovered was constant.

A comparison of oxaliplatin (20 µg/ml; 50 µM) with equimolar concentrations of cisplatin and tetraplatin indicated that all three behaved similarly in the extent and time course of protein binding in plasma (Table 3). Half-lives for the binding appeared to be indistinguishable, and recovery of platinum in PUF between 6 and 48 h were similar.

**Partitioning of Platinum in Whole Blood.** When oxaliplatin was incubated in whole blood (5, 10, and 20 µg/ml), platinum uptake into the erythrocytes was rapid in the first 2 h and then leveled off. The

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**Table 1** Cytotoxicity of the five platinum agents against the eight human tumor cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cisplatin IC₅₀ (µM)</th>
<th>Carboplatin</th>
<th>Iproplatin</th>
<th>Tetraplatin</th>
<th>Oxaliplatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2780</td>
<td>0.76 ± 0.02*</td>
<td>19.80 ± 0.77</td>
<td>6.68 ± 0.19</td>
<td>0.17 ± 0.007</td>
<td>0.17 ± 0.006</td>
</tr>
<tr>
<td>HT-29</td>
<td>3.65 ± 1.68</td>
<td>339.74 ± 26.8</td>
<td>28.74 ± 3.86</td>
<td>2.05 ± 0.54</td>
<td>0.97 ± 0.09</td>
</tr>
<tr>
<td>TCC SUP</td>
<td>6.35 ± 0.12</td>
<td>64.80 ± 2.66</td>
<td>77.77 ± 3.51</td>
<td>10.64 ± 0.92</td>
<td>7.03 ± 0.48</td>
</tr>
<tr>
<td>RT-4</td>
<td>12.30 ± 0.50</td>
<td>216.38 ± 13.29</td>
<td>21.94 ± 2.15</td>
<td>1.80 ± 0.25</td>
<td>11.10 ± 0.54</td>
</tr>
<tr>
<td>U-87MG</td>
<td>14.38 ± 0.27</td>
<td>201.60 ± 3.86</td>
<td>48.12 ± 2.5</td>
<td>6.49 ± 0.25</td>
<td>17.6 ± 0.84</td>
</tr>
<tr>
<td>U-373MG</td>
<td>11.36 ± 0.59</td>
<td>253.90 ± 12.0</td>
<td>84.99 ± 3.70</td>
<td>4.03 ± 0.7</td>
<td>2.95 ± 0.69</td>
</tr>
<tr>
<td>SK-MEL2</td>
<td>39.77 ± 2.45</td>
<td>343.42 ± 28.27</td>
<td>230.02 ± 3.35</td>
<td>24.87 ± 2.28</td>
<td>30.94 ± 1.53</td>
</tr>
<tr>
<td>HT-144</td>
<td>7.74 ± 0.30</td>
<td>110.88 ± 7.55</td>
<td>99.88 ± 12.22</td>
<td>4.34 ± 0.34</td>
<td>7.85 ± 0.23</td>
</tr>
</tbody>
</table>

*Mean ± SE derived by "G"-plate; n = 3 for all.
uptake profile and the extent of erythrocyte partitioning were very similar at the different concentrations. The mean percentage recoveries of the total platinum in blood into erythrocytes, plasma, and PUF is shown in Fig. 6. The average amount of platinum partitioned into the erythrocytes at the three concentrations tested amounted to 37.1 ± 2.1% of the total. In the whole blood, platinum binding to plasma proteins was complete by 3 h as shown by the recovery of platinum in PUF (Fig. 6).

When erythrocytes incubated in whole blood containing 10 and 20 μg/ml of oxaliplatin were washed and resuspended in drug-free plasma, no significant efflux of platinum back into plasma was observed (data not shown).

Biotransformation of Oxaliplatin in Plasma. The retention time for oxaliplatin was approximately 19 min (Fig. 7). At 1 h of incubation in plasma at 37°C, the first biotransformation product of oxaliplatin was seen, which eluted at 12 to 13 min. By 2 h, oxaliplatin was no longer detectable in the PUF while four other platinum-containing peaks including the one at 12 min were observed. By 4 h of incubation, there was essentially only one platinum-containing peak in PUF which eluted near the solvent front. This may represent negatively charged biotransformation products as all the neutral and positively charged species are retained on the column under our chromatography conditions. Under identical HPLC conditions [3H] PtCl2(dach) standard chromatographed with a retention of 12 to 13 min.

DISCUSSION

This study was initiated because of the limited pharmacological data available on oxaliplatin and because the preliminary reports indicate that oxaliplatin is showing some initial clinical activity in tumors that are not normally highly sensitive to cisplatin (2-5). The results of the in vitro cytotoxicity comparisons carried out in this study indicate that oxaliplatin is indeed active against the one colon carcinoma cell line (HT-29) tested. The molar potency of oxaliplatin was considerably greater than that of cisplatin against the three cell lines A2780, HT-29, and U373-MG. The potency of the two drugs was roughly similar against four additional cell lines (RT-4, U87MG, SKMEL-2, and HT-144) and cisplatin was considerably more potent than oxaliplatin in one cell line (TCCSUP).

Both melanoma cell lines, both bladder cell lines, and one of the two glioblastoma cell lines were more resistant to oxaliplatin than to cisplatin. These cell lines were also more resistant to tetraplatin than to cisplatin. This differential sensitivity to platinum agents showing more often higher resistance to tetraplatin than cisplatin has been reported by other investigators in panels of ovarian carcinoma cell lines.
have been predicted from these potency data (1). The relative molar potency (oxaliplatin = tetraplatin> cisplatin> iproplatin> carboplatin) of the five platinum agents noted in this study was consistent with the observations of others (9, 15, 16) and in the case of cisplatin, iproplatin and carboplatin reflects the trend in the maximum tolerated doses of these drugs in humans. However, the maximum tolerated dose of oxaliplatin in humans is higher than would have been predicted from this potency data (1).

A comparison of oxaliplatin with other platinum agents used in the study showed that oxaliplatin is similar to tetraplatin both in potency and in the slope of the dose response curves but differ markedly from the other complexes. The pair-wise comparison of the platinum agents showed significant correlation for only the oxaliplatin-tetraplatin and cisplatin-carboplatin pairs. It should be noted that the statistical power of this analysis is somewhat limited by the small number of cell lines. However, statistically significant correlations for oxaliplatin-tetraplatin and cisplatin-carboplatin do indicate that the sensitivity pattern of these drug pairs is very similar. The remaining drug pairs would not appear to have similar activity pattern based on the values of Spearman’s correlation coefficient obtained. The correlations obtained for pairs including cisplatin, carboplatin, iproplatin, and tetraplatin are entirely consistent with those obtained by other investigators in different model systems (9, 15, 16).

Based on the structural similarities of the carrier ligands, one would indeed expect a similarity in the activity of oxaliplatin and tetraplatin but not necessarily in their potency. For example, sulfosalicylato-dach Pt, a dach Pt complex we evaluated earlier, shows the same general activity profile as tetraplatin, but its potency is approximately one log lower than that of tetraplatin (22). The observed close similarity (with overlapping dose response curves) between oxaliplatin and tetraplatin in the present study leads to the conclusion that these two platinum complexes not only share the same active moiety but the kinetics of the reactions involving their corresponding leaving ligands are similar. This conclusion is substantiated by the in vitro protein binding and the biotransformation studies, which indicate similar reactivities and at least one common biotransformation product.

Limited pharmacokinetic studies carried out during the European clinical trials of oxaliplatin showed a biphasic plasma decay of total platinum with a prolonged terminal phase half-life (70 h), implying platinum binding to the plasma proteins similar to that for other platinum complexes (2). However, not all platinum complexes bind to plasma proteins directly; the clinical pharmacokinetics of iproplatin showed extensive platinum binding to plasma proteins (23), but the in vitro studies indicated that iproplatin itself does not bind to the plasma proteins (24–25). It is the divalent metabolite(s) of iproplatin generated in vivo which appears to be the source for this platinum binding to proteins (24). Although in vitro studies show protein binding of tetraplatin, it appears likely that it is the divalent metabolite(s) of tetraplatin that bind to the proteins, as tetraplatin is reduced rapidly (estimated t1/2 of 3 s) in rat plasma (26). Although oxaliplatin is a
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Fig. 7. Biotransformation of L-OHP in in vitro incubation of the drug in plasma. A, L-OHP in water; B-F, L-OHP in plasma (B, 0 time; C, 0.5 h; D, 1.0 h; E, 2.0 h; F, 4.0 h). Recovery of injected platinum is >90% at all time points.

divalent platinum complex and quite likely would bind to the proteins either directly or through aquation reactions, the kinetics of its binding to the plasma proteins have not been determined until now. This study of plasma protein binding, RBC cell sequestering of platinum, and in vitro biotransformation of oxaliplatin is expected to provide a basis for the interpretation of the forthcoming clinical pharmacokinetic data for this compound.

When incubated in plasma, 85–88% of all platinum from oxaliplatin was bound to plasma proteins within the first 5 h with a concentration-independent half-life of approximately 1.5 h. Binding characteristics of oxaliplatin were found to be similar to cisplatin and tetraplatin. When incubated in whole blood, a significant portion of the total platinum was sequestered into the erythrocytes within the first 2 h, and this fraction was not exchangeable into plasma to any significant degree indicating that the erythrocyte partitioned platinum does not serve as a reservoir of drug. This is of interest since not all platinum complexes appear to be taken up by RBC. A comparative pharmacokinetic study carried out for five platinum agents (cisplatin, carboplatin, TNO-6, JM-40, and iproplatin) in dogs indicate that only iproplatin was taken up by RBC (27). None of the drugs used in that study have the dach moiety as the carrier ligand, and it is not known whether tetraplatin is taken up by the erythrocytes. In contrast to incubation in plasma where protein binding for oxaliplatin took 5 h to go to completion, whole blood took only 3 h at all the concentrations tested. The reason for this is unclear at the present time.

Since the protein binding is complete in a relatively short period (3–5 h), the biotransformation was explored in a 0- to 4-h incubation in plasma. In an ion pair HPLC system such as the one used in the present study, species with a net positive charge, such as the monoaquo and diaquo derivatives, are expected to elute after oxaliplatin. The retention of the metabolite peak detected at 1 h of incubation of oxaliplatin in plasma corresponds to $[^3$H]PtCl$_2$(dach). Since plasma contains a high chloride concentration (100 mM), it is not surprising that under these conditions the oxalato group may have been exchanged for chlorides, giving rise to PtCl$_2$(dach). PtCl$_2$(dach) is the major metabolite formed after quick reduction of tetraplatin in plasma (both in vitro and in vivo), giving rise to other chloride substituents more slowly (28). Tissue culture media also contain 100 to 120 mM concentrations of chloride, and perhaps under these conditions the oxalato group is displaced by chloride. Mauldin et al. (29) have demonstrated that in RPMI 1640 the malonato ligand of Pt(mal)(dach) is displaced by chloride, among others. They referred to this chloride displacement reaction as the “activation” pathway since PtCl$_2$(dach) was taken up by L1210 cells 8 times faster than the Pt(mal)(dach) (30). The same group has also demonstrated the reduction of tetraplatin to PtCl$_2$(dach) in culture media (31). These observations suggest that the close similarity in activity in vitro between oxaliplatin and tetraplatin may be due to the formation of PtCl$_2$(dach) in the culture media. Although we see a close similarity between the two compounds in vitro, the in vivo disposition of these drugs may entirely be different. Chaney et al. (32) have recently demonstrated that under conditions where tetraplatin is not reduced in the outside medium to PtCl$_2$(dach), the cellular biotransformation results in at least two new products. Our in vitro incubation of oxaliplatin in plasma indicates
that conversion to PtCl₂(dach) is not a rapid reaction. Thus oxaliplatin in vivo may enter the cells in an unchanged form.

There is a remarkable difference in the degree of relative resistance exhibited by the cell lines used in this study to the platinum agents with the three different carrier ligands. The underlying mechanisms responsible for these differences in resistance are not known at this time and, as has been demonstrated in many model systems, may involve elevated glutathione levels, decreased drug accumulation, and increased repair of DNA damage. Studies are currently under way in our laboratory to examine the role of some of these mechanisms.

Two of the findings made in this study may have a bearing on the clinical pharmacokinetics of oxaliplatin. First, a significant portion of oxaliplatin in the whole blood is sequestered into the erythrocytes. What this means in terms of oxaliplatin disposition in vivo will depend on the clearance of the drug from the blood. Second, a major biotransformation product of oxaliplatin in vitro is identical to the reduced form of tetraplatin. However, the extent of formation of this metabolite in vivo again depends on the metabolic versus nonmetabolic clearance of the drug from blood. We plan to confirm and extend these observations in vivo during the phase I clinical trial of oxaliplatin.

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