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Oxaliplatin, Tetraplatin, Cisplatin, and Carboplatin: Spectrum of Activity in Drug-Resistant Cell Lines and in the Cell Lines of the National Cancer Institute's Anticancer Drug Screen Panel

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ABSTRACT. The present study was designed to explore the activity of platinum compounds in cisplatin-resistant cell lines, the unselected cell lines of the National Cancer Institute's Anticancer Drug Screen, and the potential for use in combination. The activities of four platinum compounds in cisplatin-resistant KB and A2780 cells were investigated. The cells were highly resistant to cisplatin and cross-resistant to carboplatin, but less than one-tenth as resistant to oxaliplatin and tetraplatin. Cellular accumulation of all platinum compounds was decreased in both resistant cell lines. When the activities of cisplatin and oxaliplatin were evaluated in the National Cancer Institute's Anticancer Drug Screen, marked differences were observed. Evaluation of the activity profile using the COMPARE program revealed a different pattern for both agents: the cisplatin activity profile was similar to those of other diamine-platinum compounds, alkylating agents including melphalan, and camptothecin analogs, whereas the activity profile of oxaliplatin resembled those of other "dach" (diaminocyclohexane) platinum compounds and of acridine derivatives. The sensitivity profiles are influenced by the target(s)/mechanism(s) of action and the mechanism(s) of resistance of a drug. The dissimilarity in profiles suggests that these two platinum compounds have a different target(s)/mechanism(s) of action, a different mechanism(s) of resistance, or most likely both. Studies evaluating combinations of cisplatin/oxaliplatin suggest that the activities of these two agents are at least additive and possibly synergistic. Oxaliplatin has a different spectrum of activity and low cross-resistance to cisplatin and should be valuable in cisplatin refractory patients or in combination with cisplatin. *Copyright © 1996 Elsevier Science Inc.* BIOCHEM PHARMACOL 52;12:1855–1865, 1996.

KEY WORDS. platinum; oxaliplatin; cisplatin; platinum resistance; drug screen; drug discovery

Accidentally discovered as an anticancer agent in 1968, cisplatin has been in clinical use since 1971 when studies first demonstrated its efficacy [1]. Cisplatin and more recently, cisplatin analogues have been used in the treatment of a wide range of malignancies, most importantly testicular and ovarian cancer [2, 3]. Recognition of the limitations of cisplatin therapy has served as a catalyst for both the development of more active and less toxic derivatives and for studies of the mechanisms of resistance.

As with all other chemotherapeutic agents, cisplatin efficacy has been limited by the existence or development of resistance [4, 5]. Although identification of the mechanism(s) of cisplatin resistance has often provided conflicting or incomplete explanations, an examination of the published literature suggests that cisplatin resistance is likely to be multifactorial and that additional mechanisms other than those heretofore described may help to complete our understanding [4, 5]. Clarification of the mechanism(s) of cisplatin resistance should provide greater insight into its mechanism of action, and a stimulus for rational attempts to overcome resistance. Although no single agent has been examined extensively, reversal of resistance has been attempted using a variety of non-chemotherapeutic agents, including cyclosporin A, several methylxanthines, trifluoperazine, forskolin, BSO,‡ and amphotericin [6–11]. In addition to these attempts, the use of novel platinum compounds offers an alternative approach, especially if lack of

* The order of the first two authors is to be considered arbitrary.

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‡ *Abbreviations:* BSO, buthionine sulfoximine; "dach," 1,2-diaminocyclohexane; RPMI, Roswell Park Memorial Institute; FBS, fetal bovine serum; and TCA, trichloroacetic acid.

cross-resistance can be demonstrated. Emerging *in vitro* and clinical evidence suggests that this latter goal may be achieved using platinum compounds from different classes.

Of special interest in overcoming resistance is the "dach" family of platinum compounds. Synthesized originally in 1972 by Connors [12], the substitution of the amine radicals in cisplatin by a "dach" radical resulted in a stable complex with good antitumor activity; however, the prototype complex was virtually insoluble in water. To improve solubility, several "dach" platinum derivatives were prepared by replacing the chloride leaving groups with a variety of anionic leaving groups. Interest in this class of compounds was increased further when Burchenal *et al.* [13] demonstrated activity against cisplatin-resistant L-1210 leukemia. Kidani *et al.* [14, 15] succeeded in separating the "dach" platinum derivatives into geometric isomers, *cis* and *trans*, and then separated the *trans* into two optical isomers: *trans-d* and *trans-l*. Oxaliplatin, the *trans-l* "dach" oxalato platinum compound, was shown to be soluble in aqueous solvents and active in the L1210 leukemia model [16]; it has undergone Phase I and Phase II evaluation in Europe [17–24]. These clinical studies together with *in vitro* results suggest that oxaliplatin and other "dach" platinum compounds possess a wide spectrum of activity and are active in cisplatin-resistant tumors [25–27].

In the present study, information obtained from the drug sensitivity profile of the cell lines comprising the NCI Drug Screen Panel and cisplatin-resistant cell lines is presented. The results demonstrate a different spectrum of activity for "dach" platinum compounds, including oxaliplatin, compared with cisplatin and carboplatin. The data suggest that oxaliplatin is active in cisplatin-resistant cells and lacks cross-resistance with melphalan and carboplatin. Potential clinical implications of these results are discussed.

MATERIALS AND METHODS

Materials

Cisplatin and carboplatin were products of the Bristol-Myers Co., Wallingford, CT. Oxaliplatin was obtained from Debiopharm, Lausanne, Switzerland. Tetraplatin was obtained from the Pharmaceutical Resources Branch of the Developmental Therapeutics Program of the National Cancer Institute.

Cell Lines

The cell lines comprising the National Cancer Institute's Anticancer Drug Screen Panel were obtained and processed as previously described [28, 29]. Briefly, following an initial acquisition, *in vitro* expansion was followed by cryopreservation of a large number of master stock samples for serial rethawing at 20 passage intervals. All cell lines have been analyzed for the presence of potential pathogens and have undergone karyotyping, isoenzyme analysis, and morphologic analysis at both the light and electron microscopic levels.

Cisplatin-resistant cell lines were derived from KB 3-1 cells (a single clone of KB cells, a HeLa subclone) and A2780 (1A9) cells (a single clone of A2780 ovarian carcinoma cells) by stepwise increases in the extracellular concentration of cisplatin. All cells were maintained in RPMI medium with 10% FBS, 2 mM glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin. Briefly, cells were exposed initially to 0.25 μM cisplatin, and a single clone was isolated from the few surviving cells. This clone was expanded and carried as a population during the subsequent selection process. The concentration of cisplatin was increased at intervals of several weeks to several months. The resistant cell lines used in these studies were propagated in medium containing 20 μM cisplatin [KB CP(20)] or 80 μM cisplatin [A2780-E(80)].

Cytotoxicity Assays

Cytotoxicity studies were performed in triplicate in the presence of increasing concentrations of drug in 96-well plates. Eight hundred to 1200 cells were seeded in each well and treated 24 hr after plating. The assay was terminated by fixing cells with 10% TCA 4 days after the addition of drug. Then cells were stained for determination of protein using 0.4% sulforhodamine B as previously described [29]. Unbound dye was washed from the plates, and the bound dye was extracted prior to measuring optical density at 565 nm using an LKB Ultraspec Plus. A separate plate that had been fixed at the time of drug addition was used to determine the zero value. Untreated control wells were assigned a value of 100%, and the IC_{50} was defined as the concentration of drug that reduced the optical density measured at 564 nm to 50% of the control value.

Platinum Accumulation

For these experiments, 5×10^6 cells were plated in 15-cm dishes. The following day, fresh 37° medium containing various concentrations of platinum was added. All experiments were performed in triplicate. After incubation for 2 hr at 37°, the medium was decanted, and the cells were washed three times with ice-cold PBS before scraping and collecting the cells by centrifugation. An aliquot was removed to determine cell counts, and the rest was processed for platinum accumulations. To measure intracellular platinum, cell pellets were prepared using the method of McGahan and Tyczkowska [30]. Concentrated nitric acid (0.5 mL) was added to the cells, and this was incubated overnight at room temperature. Then the sample was boiled for 5 min and cooled to room temperature; 0.5 mL of 30% H_2O_2 was added, and the solution was boiled again and then cooled at room temperature. A Perkin–Elmer model 3030 Atomic Absorption Spectrophotometer and Zeeman background correction was used to analyze the sample [31]. Platinum standards were prepared by serial aqueous dilution using SPEX™ Aqueous Standard Dilution Cisplatin $H_2PtCl_3H_2O$ (1000 g/mL). Aliquots (50 μL) were mea-

sured, and a standard curve was plotted using integrated absorbance-seconds.

COMPARE Analysis and Determination of Pearson Correlation Coefficients

The version of COMPARE used in this work differs from the original version of COMPARE that made comparisons based on calculated mean difference in "deltas" [32]. The current version of COMPARE is usually configured to calculate pairwise correlations with the $-\log_{10}$ (minus log 10) of one of the specific NCI cell line activity parameters: GI_{50}, TGI or LC_{50} [33]. For instance, the GI_{50} is the NCI designation for a time zero corrected LC_{50}, that is, the concentration of agent causing a 50% growth inhibition. Thus $-\log_{10}$ (GI_{50} values) for a seed or probe compound is correlated with the corresponding data from each compound in a database containing data for tens of thousands of screened compounds. In this study, the GI_{50} data were utilized in the COMPARE studies with the seed data provided by the platinum compounds of interest (cisplatin and oxaliplatin). The correlation coefficients reported are Pearson correlation coefficients that are output by the SAS^R procedure PROC CORR using the out = output option (SAS^R is a registered trademark of the SAS Institute Inc., SAS Circle, P.O. Box 8000 Cary, NC 27512-8000).

Determination of Combination Indices: Antagonism/Synergism/Additivity

Evaluation of the activity of cisplatin and oxaliplatin alone or in combination was performed according to the method described by Chou and Talalay [34, 35]. Mathematical evaluation of the data was carried out by computerized analysis of the median dose effect and the combination index. Cytotoxicity curves were performed using either agent alone or in combination at one of three cisplatin:oxaliplatin molar ratios: 1:1, 2:1, and 1:2. The dose ranges studied were chosen such that the IC_{50} values for the individual drugs fell in the middle of the concentration range examined (IC_{50} is the concentration giving 50% inhibition). A median effect plot was constructed and, using the least squares method of linear regression, the median effect parameters m and Dm were calculated. With these values, the fraction affected for each dose was then calculated. Once these values were available, the combination index was calculated, and plotted on the vertical axis versus the fraction affected on the horizontal axis. This plot describes the extent of synergism, additivity, or antagonism at each dose or fraction affected. The combination index was calculated using both the mutually exclusive assumption and the mutually non-exclusive assumption.

RESULTS

Single clone isolates from both KB 3-1 cells and the A2780 subclone, 1A9, were obtained by exposure to 0.25 μM cisplatin. These clones were expanded and exposed to gradually increasing concentrations of cisplatin, over a period of 2–3 years, before reaching the concentrations of 20 and 80 μM cisplatin for the KB and A2780 sublines, respectively.

Figure 1 presents cytotoxicity curves using four different platinum compounds in the two sets of parental and cisplatin-resistant cell lines. Both resistant cell lines demonstrated high levels of resistance to the selecting agent, cis-

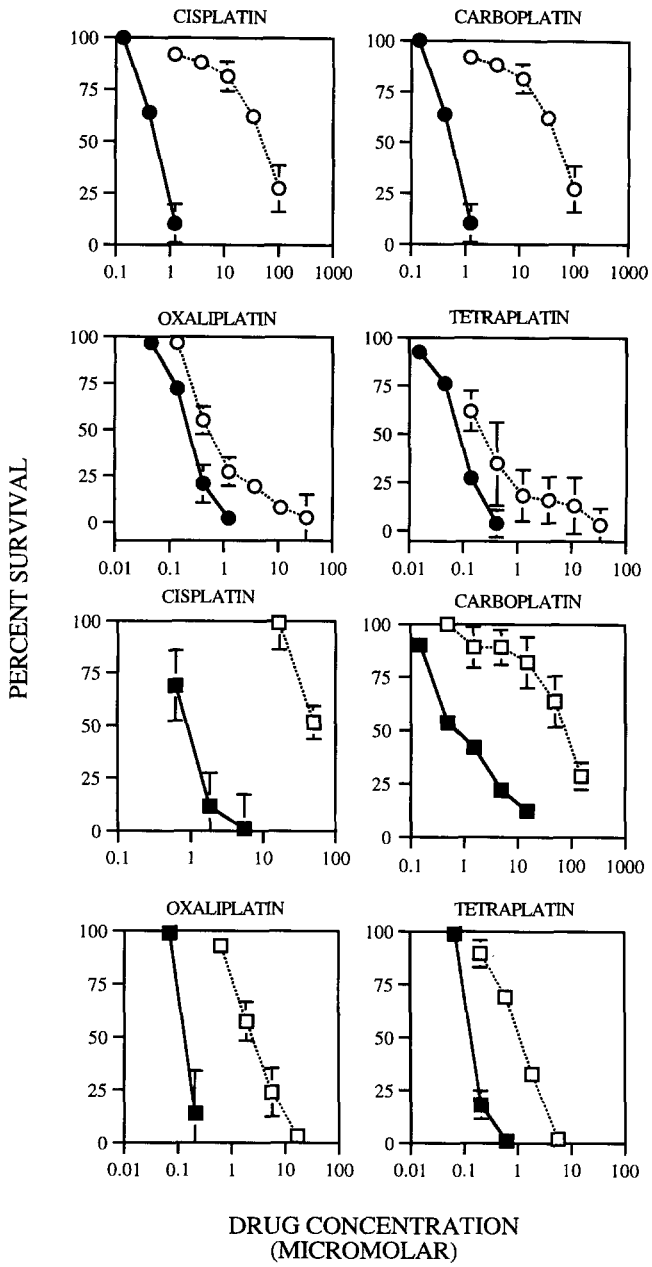

FIG. 1. Cytotoxicity curves. Relative resistance to four platinum compounds was determined in cisplatin-resistant cell lines. Filled symbols: parental cells; open symbols: resistant sublines. Upper four panels show results with cisplatin-resistant KB cells [KB CP(20)]. Lower four panels present results with cisplatin-resistant A2780 cells [A2780-E(80)]. Where standard deviations were greater than the symbols, these are shown. N = 4 independent experiments.

platin, and also to carboplatin, with less cross-resistance to the two "dach" platinum compounds, oxaliplatin and tetraplatin. These results as well as the resistance to two other agents are summarized in Table 1. As can be seen, compared with the resistance observed for cisplatin and carboplatin, that to oxaliplatin and tetraplatin was less than one-tenth as great. In addition, one can see that both cell lines were also cross-resistant to the alkylating agent, melphalan, an observation that is addressed in greater detail below.

Reduced accumulation of cisplatin is the most common observation in models of cisplatin resistance, and in some can fully account for the increase in tolerance [36]. To evaluate the possibility that the low level of cross-resistance observed for oxaliplatin and tetraplatin could potentially be explained by a reduction in platinum accumulation, we investigated the accumulation of the four platinum compounds shown in Fig. 1, following a 2-hr incubation. These results are shown in Fig. 2, which depicts one experiment performed in triplicate, where accumulation of all drugs was examined. Similar results were observed in independent experiments. For all drugs, at least two experiments and as many as five were performed. As shown in Fig. 2, over a broad concentration range, in both drug-resistant cell lines, the accumulation of all four platinum compounds was reduced. The magnitude of this decrease varied with the concentration of extracellular platinum and was greatest at the lower concentrations, where platinum levels in resistant cells were only a few percent that of parental cells. At the higher concentrations, significant reductions were still observed with values of 15–38% that of parental observed for all four platinum compounds. In cisplatin-resistant A2780 cells, for example, at the highest concentration (33 μM extracellular platinum), cisplatin and carboplatin accumulation were 19 and 15% that of parental cells, while oxaliplatin and tetraplatin levels were 38 and 33% of parental values. Similar reductions were observed as well for iproplatin (at 33 μM: A2780-E(80), 14% of parental; KB CP(20), 22% of parental). This decrease in accumulation may explain the low levels of cross-resistance observed for oxaliplatin and tetraplatin.

With the goal of further understanding the differences among the various platinum compounds, we examined the sensitivity patterns of cisplatin and oxaliplatin in the cell lines of the NCI Anticancer Drug Screen. It has been established previously that the sensitivity profile or mean graph of a given drug can predict its target(s)/mechanism(s) of action, utilizing the COMPARE program [32, 37]. In addition, the sensitivity profile is also influenced by the existence of mechanisms of resistance [38]. Thus, both the target of a drug as well as its mechanisms of resistance are responsible for the profile. Drugs that share these properties have similar profiles, whereas drugs that differ have dissimilar profiles. Figure 3 depicts the sensitivity pattern or mean graph of 47 cell lines for cisplatin, carboplatin, oxaliplatin, and tetraplatin. The data presented are the GI_{50} values, which represent the concentration of drug that reduced cell growth by 50%. The vertical line represents the mean value of GI_{50} for all cell lines, while the horizontal bars indicate the extent to which individual GI_{50} values differ from this mean value. Cell lines with bars pointing to the left have a GI_{50} that is greater than the mean value, and are less sensitive, whereas those with bars pointing to the right have GI_{50} values that are lower than the mean and are more sensitive. Visual inspection demonstrates differences between the two compounds. For example, in the colon cancer cell lines, the GI_{50} values of all colon cancer cell lines were higher than the mean for cisplatin, whereas a majority were more sensitive than the mean for oxaliplatin. In contrast, a majority of the cell lines established from CNS cancers were more sensitive to cisplatin than the mean value, but more resistant to oxaliplatin. It must be emphasized that the vertical line represents a mean value and this was lower for oxaliplatin compared with cisplatin. Consequently, although in the oxaliplatin mean graph the ovarian cancer cell lines were more resistant than the mean, their GI_{50} values on average were comparable to those of cisplatin.

Similar data are available for nearly 40,000 different compounds, and the relative similarity of these patterns can be determined utilizing the COMPARE program. In this analysis, the compound of interest is used as the "seed," and the degree of similarity between the mean graph of the "seed" compound and all other compounds is determined. These results are then ranked according to Pearson correlation coefficients. Compounds with a mean graph similar to that of the "seed" compound have high Pearson correlation coefficients, whereas those with dissimilar graphs

TABLE 1. Cytotoxicity profile of A2780(1A9) and KB 3-1 cells, and resistance profile of A2780 E(80) and KB CP(20) cells

Drug	A2780(1A9) IC_{50} (μM)	A2780-(E(80) fold resistance	KB 3-1 IC_{50} (μM)	KB CP(20) fold resistance
Cisplatin	0.21 ± 0.05*	92 ± 11	0.75 ± 0.38	78 ± 15
Carboplatin	0.35 ± 0.13	64 ± 12	1.65 ± 0.88	57 ± 13
Tetraplatin	0.15 ± 0.07	4.6 ± 1.4	0.11 ± 0.02	4.1 ± 2.1
Oxaliplatin	0.12 ± 0.07	4.7 ± 0.9	0.39 ± 0.22	2.7 ± 1.2
Iproplatin†	0.31	28	1.1	9
Melphalan†	0.33	51	0.25	26

* Values are means ± SD, N = 4.

† Average of two experiments.

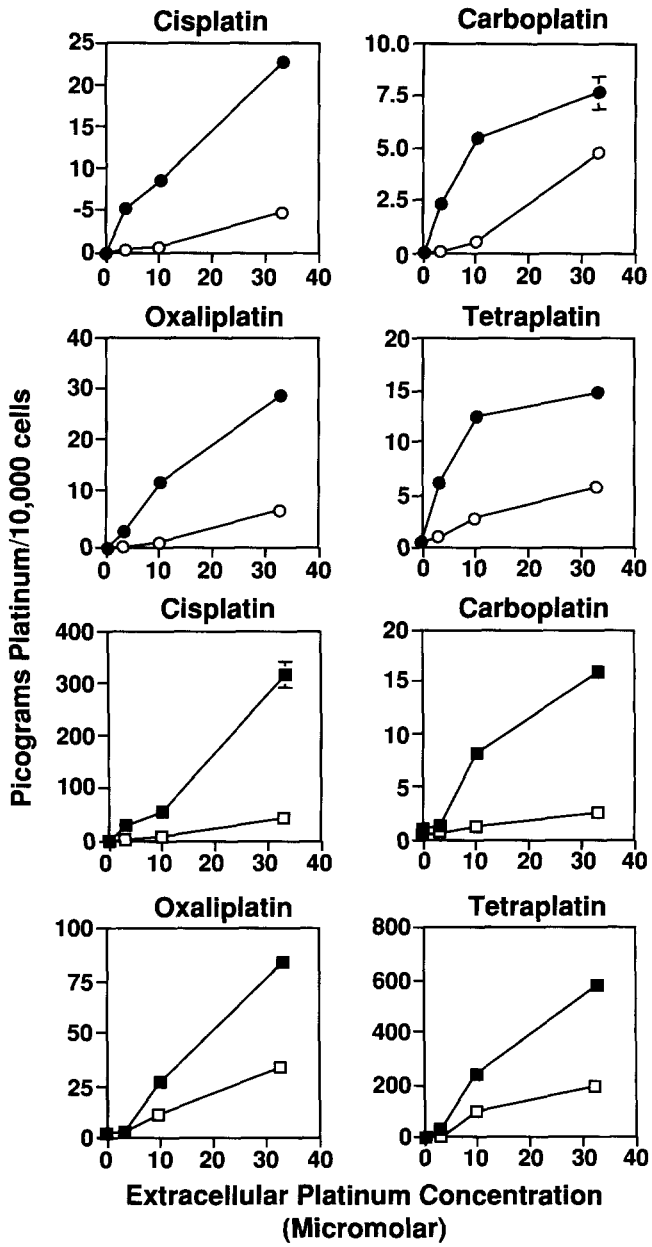

FIG. 2. Platinum accumulations. Absolute levels of platinum accumulation in the two cisplatin-resistant cell lines were determined for all four platinum compounds investigated in Fig. 1. Filled symbols: parental cells; open symbols: resistant sublines. Upper four panels show results with cisplatin-resistant KB cells [KB CP(20)]. Lower four panels present results with cisplatin-resistant A2780 cells [A2780-E(80)]. One experiment performed in triplicate is depicted. Similar results were observed in independent experiments. Where standard deviations were greater than the symbols, these are shown.

have low correlation coefficients. When the COMPARE analysis was carried out using cisplatin and oxaliplatin as the "seed" compounds, two different profiles were obtained, as shown in Tables 2 and 3 and Fig. 4. In the case of cisplatin, the 21 compounds with the highest correlation coefficients included carboplatin, other *cis*-diamine platinums, several alkylating agents, and camptothecin analogues. Cisplatin appears on the list because it has been screened multiple times, and the data bank contains additional mean graphs other than the one used as the "seed." The high correlation coefficients obtained with different cisplatin data entries were expected, and confirm the validity of the analysis. A high correlation coefficient was observed for carboplatin. In contrast, the correlation coefficients for oxaliplatin and tetraplatin were markedly lower.

The pattern obtained when oxaliplatin was used as the seed was completely different. In addition to tetraplatin, a large number of additional "dach" platinum compounds, and three acridine derivatives with alkylating properties were found to have high correlation coefficients. Cisplatin and carboplatin, in contrast, had very low correlation coefficients. Figure 4 presents the structures of the platinum compounds identified by the COMPARE analysis. Panel A shows the structures of those with a high correlation coefficient when cisplatin was the "seed" compound, and panel B, those identified when oxaliplatin was the "seed." Those with high correlation coefficients with cisplatin are diamino platinum compounds like cisplatin, whereas those with high correlation coefficients with oxaliplatin are "dach" platinum compounds. The structural similarities emphasize the difference between the two classes, and also provide evidence of the validity of the analysis. These results, together with those in the cisplatin-selected cell lines, suggest that these agents belong to two different classes of platinum compounds. These COMPARE differences may reflect differences in target(s)/mechanism(s) of action and mechanism(s) of resistance.

Finally, to begin to explore the possibility of using platinums from two different classes in combination, we performed initial experiments using combinations of cisplatin and oxaliplatin at three different molar ratios. The activities of cisplatin:oxaliplatin molar ratios of 1:1, 2:1, and 1:2 were compared with that of cisplatin or oxaliplatin alone in parental KB cell lines and A2780 (1A9) cells. Mathematical evaluation of the data was carried out using a computerized analysis of the median dose effect and the combination index developed by Chou and Talalay [34, 35]. The results (means ± SD) of four independent experiments are shown in Fig. 5. Plots of fraction affected on the horizontal axis versus the combination index on the vertical axis are shown. The combination index was calculated using both the mutually exclusive assumption (similar mechanisms of action and/or resistance) and the mutually non-exclusive assumption (quite dissimilar mechanisms of action and/or resistance). Theoretically, a combination index (CI) value of 1 indicates additivity; a value greater than 1, antagonism; and a value less than 1, synergism. Because this theoretical ideal is difficult to achieve practically, the shaded area indicates the range of additivity. This area was determined as the experimental variability observed when both platinum compounds were the same, and can be considered the area of additivity. Specifically, experiments were conducted in

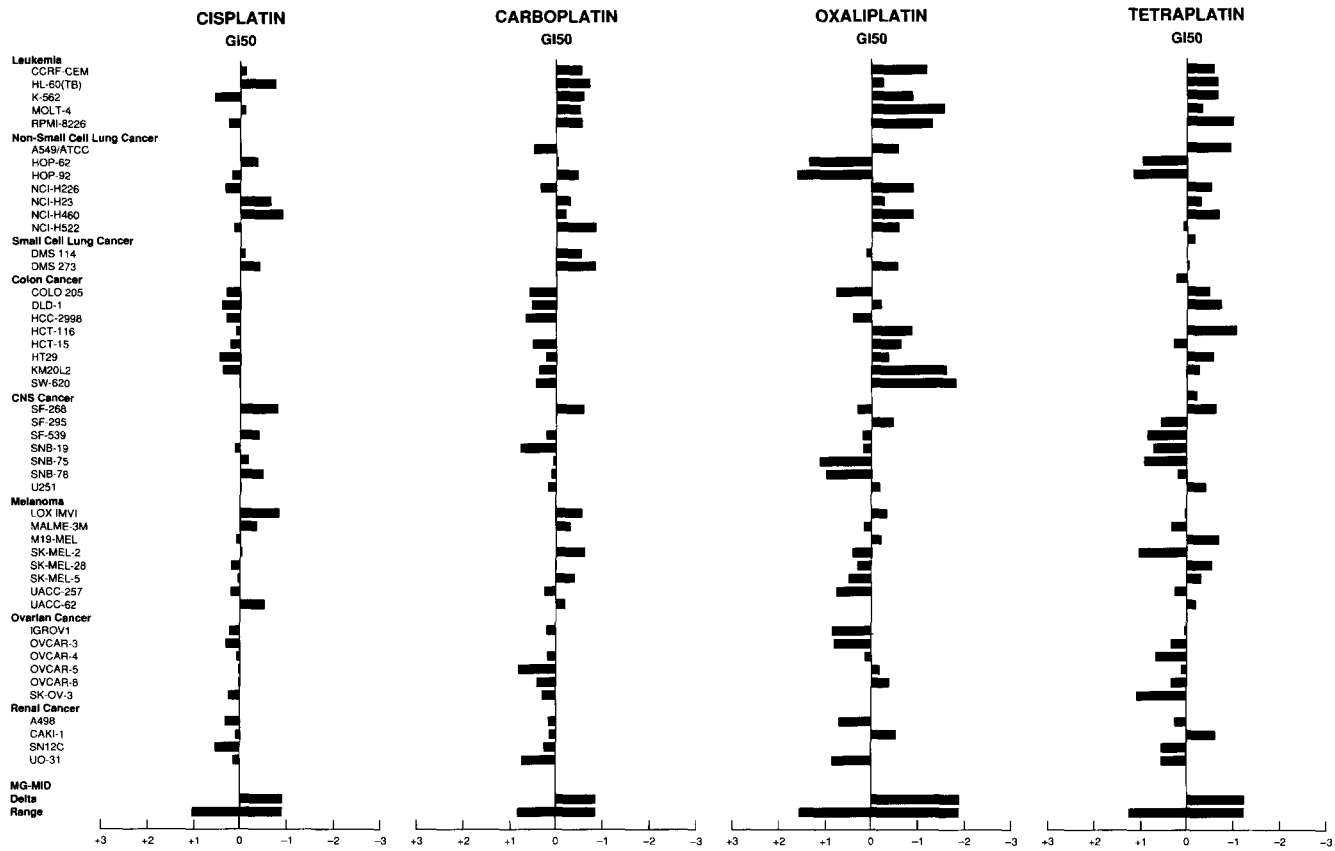

FIG. 3. Mean graph for cisplatin, carboplatin, oxaliplatin, and tetraplatin. The sensitivities of the cell lines in the National Cancer Institute's Anticancer Drug Screen Program for cisplatin, carboplatin, oxaliplatin, and tetraplatin are depicted. The vertical line represents the mean GI_{50} (concentration required to inhibit 50% growth) for all the cell lines. The horizontal bars represent the difference in the GI_{50} of a particular cell line relative to the mean. A log scale is utilized. Cell lines with a horizontal bar pointing to the left have a GI_{50} that is greater than the mean and are more resistant than the mean, whereas cell lines with a horizontal bar pointing to the right have a GI_{50} that is less than the mean and are more sensitive.

which the activities of different molar ratios of cisplatin: cisplatin and oxaliplatin:oxaliplatin were determined. Because the same platinum compound was used as both platinum compounds, a combination index of 1 would have been expected, theoretically, since combining the same platinum compound should be simply additive, not synergistic or antagonistic. However, experimental variation around the theoretical value of 1 was obtained, and it is this range of variability that is depicted as the shaded area. Additivity (and possibly some synergism) was observed throughout the range of concentrations tested; some experiments demonstrated marked synergism. Taken together, the results suggest that in combination the activities of these two platinum compounds are at least additive and possibly synergistic.

DISCUSSION

In the present study, we report our findings on the activities of four different platinum compounds in both drug-selected and unselected cell lines. Beginning with cisplatin-selected A2780 ovarian and KB 3-1 epidermoid cells, we found low levels of cross-resistance to both oxaliplatin and tetraplatin, although high levels of resistance to both cisplatin and carboplatin were demonstrable. Data in the NCI Anticancer Drug Screen demonstrated different patterns in the COMPARE analysis for cisplatin and oxaliplatin. The latter provides evidence that the compounds may have dissimilar targets, mechanisms of resistance, or both. Studies to determine the effectiveness of cisplatin and oxaliplatin in combination suggest that these drugs are at least additive and possibly synergistic. These results confirm and extend previous observations documenting differences in the resistance profile for different platinum compounds [27, 39–44].

Selection of A2780 and KB 3-1 cells with cisplatin was successful in establishing sublines with high levels of cisplatin resistance after prolonged incubation in medium containing gradually increasing concentrations of cisplatin. High levels of cross-resistance to carboplatin were observed. In contrast, lower levels of cross-resistance were found to both oxaliplatin and tetraplatin. These results confirm and extend previous observations in cisplatin-resistant cells [25–27, 41–43]. Cross-resistance to oxaliplatin was somewhat higher in the cisplatin-resistant A2780 E(80) cells, probably a result of the higher level of cisplatin resistance in this subline; but in both cisplatin-resistant sublines, rela-

TABLE 2. Results obtained using cisplatin(II) as the "seed" compound to probe the NCI Anticancer Drug Screen database*

Cisplatin(II) COMPARE Analysis			
Rank	**PCC**	**NSC No.**	**Name or description**
1	0.998	119875	Cisplatin(II)
2	0.849	146067	Cis-Diamminemalonato platinum(II)
3	0.843	632608	Diammino diazacyclooctadecane platinum(II)
4	0.807	119875	Cisplatin(II)
5	0.798	241240	Carboplatin(II)
6	0.794	634047	Bis(*cis*-diammine) platinum
7	0.783	348948	Cyclodisone
8	0.781	34462	Uracil nitrogen mustard
9	0.758	48034	Bis-aziridine
10	0.757	132313	Dianhydrogalactitol
11	0.752	629	Butane diepoxide
12	0.751	119875	Cisplatin(II)
13	0.747	Discreet	Cisplatin derivative
14	0.746	135758	Piperazinedione
15	0.744	Discreet	Camptothecin analog
16	0.735	344007	Piperazine alkylator
17	0.732	25154	Pipobroman
18	0.729	Discreet	Disulfur alkylating agent
19	0.728	166199	Adamantyl-bis-aziridine phosphoramide
20	0.728	Discreet	Camptothecin analog
21	0.726	8806	Melphalan
	0.340	363812	Tetraplatin
	0.302	266046	Oxaliplatin

* The 21 compounds with the highest Pearson correlation coefficients (PCC), tetraplatin, and oxaliplatin, are shown.

TABLE 3. Results obtained using oxaliplatin(II) as the "seed" compound to probe the NCI Anticancer Drug Screen database*

Oxaliplatin(II) COMPARE Analysis			
Rank	**PCC**	**NSC No.**	**Name or description**
1	1.000	266046	Oxaliplatin
2	0.870	266047	A cyclohexyldiammino platinum(II)
3	0.821	614887	A cyclohexyldiammino platinum(II)
4	0.778	638728	A cyclohexyldiammino platinum(II)
5	0.766	271674	A cyclohexyldiammino platinum(II)
6	0.756	363812	Tetraplatin
7	0.742	658360	Pyrazolo acridine
8	0.735	649900	Pyridobenzimidole
9	0.733	631197	9-Amino acridine with aklylating side chain
10	0.731	Discreet	Pyridole indole derivative
11	0.729	628115	9-Amino acridine with alkylating side chain
12	0.728	268242	Dibenzyldaunorubicin
13	0.726	658777	A thio-pyrimidone glucoside
14	0.722	281279	A cyclohexyldiammino platinum(II)
15	0.716	146268	2-Thio-6-azapyrimidone riboside
16	0.715	265459	A cyclohexyldiammino platinum(II)
17	0.712	633685	9-Amino acridine with alkylating side chain
18	0.711	363812	Tetraplatin
19	0.710	626541	9-Amino acridine with alkylating side chain
20	0.705	628114	9-Amino acridine with alkylating side chain
	0.302	119875	Cisplatin
	0.058	241240	Carboplatin

* The 20 compounds with the highest Pearson correlation coefficients (PCC), cisplatin, and carboplatin are shown.

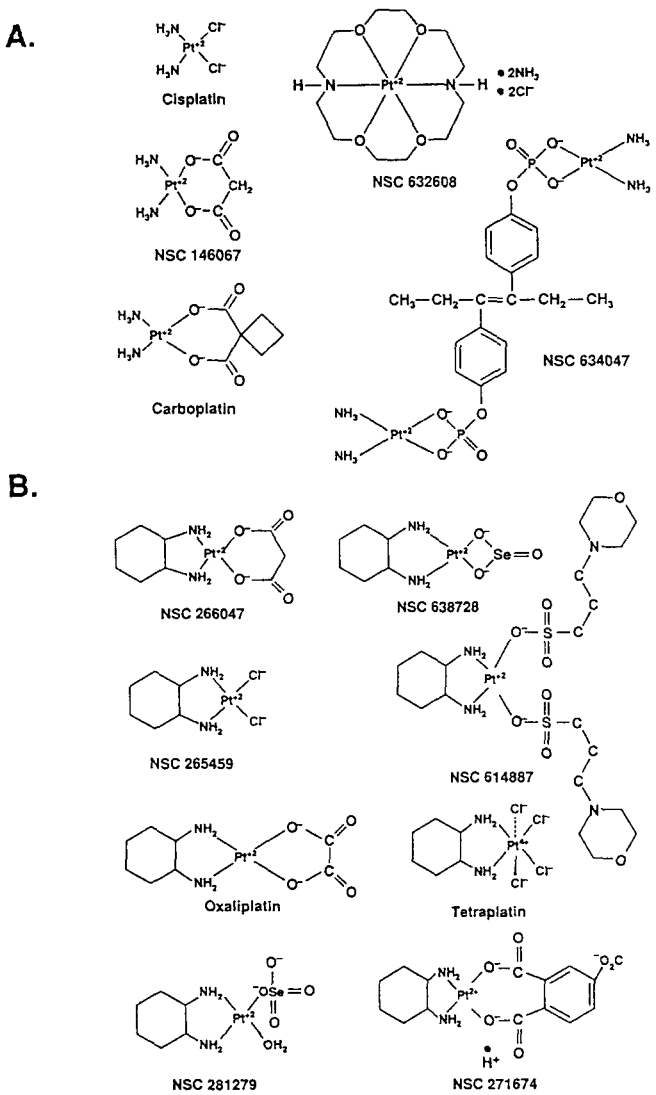

FIG. 4. Chemical structures of platinum compounds. Platinum compounds identified by the COMPARE analysis as having a mean graph or drug sensitivity profile similar to cisplatin are shown in panel A, whereas those whose mean graph resembles that of oxaliplatin are shown in panel B.

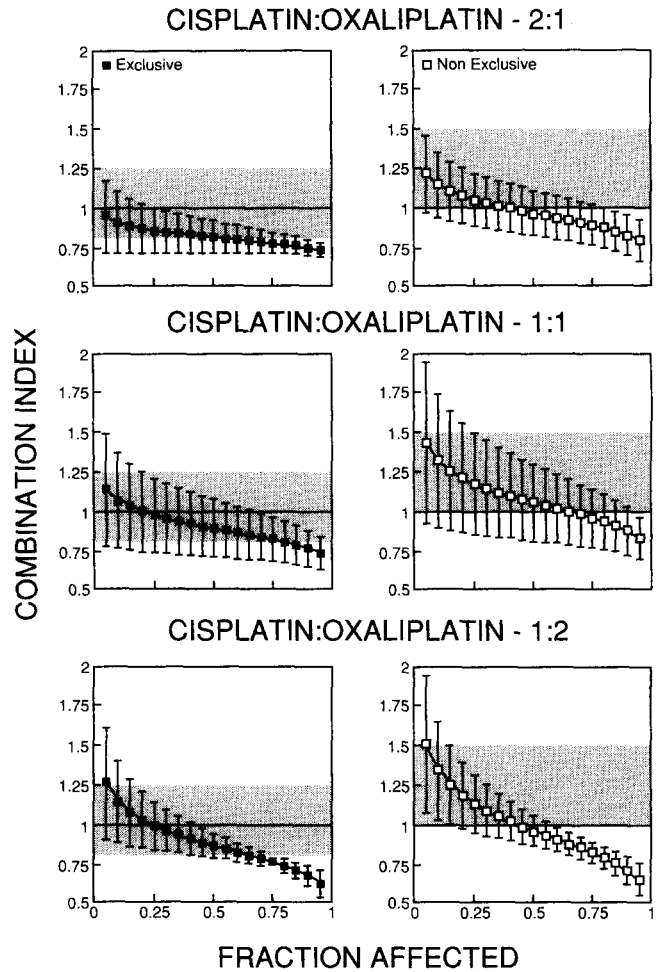

FIG. 5. Analysis of synergy/antagonism/additivity for the combination of cisplatin and oxaliplatin. The results of studies determining the activity of cisplatin/oxaliplatin combinations are shown. Theoretically, a value of 1 indicates additivity with values greater than 1 indicative of antagonism and those less than 1 consistent with synergy. Practically, the shaded areas represent the areas of additivity as determined in control experiments, where the experiments were performed with the same platinum compound added as if two different compounds were being used. The molar ratios of cisplatin to oxaliplatin as shown in the figure are from top to bottom: 2:1, 1:1, and 1:2. The range of standard deviations are shown. N = 4 independent experiments.

tive resistance to oxaliplatin was at least 10-fold less than cisplatin and carboplatin resistance. A similar low level of cross-resistance to tetraplatin was also observed.

Reduced cisplatin accumulation is the most common observation in cisplatin-resistant cells, and in some models can fully explain resistance [36]. Thus, we sought initially to measure the level of platinum accumulation in parental and cisplatin-resistant cell lines, to determine the potential contribution of this as a mechanism of platinum resistance. Accumulation of cisplatin and carboplatin was reduced in both cell lines, with levels that were only 15–20% that of parental cells at high extracellular concentrations of drug; lower percentages were found at lower extracellular platinum concentrations. In addition, the accumulation of both oxaliplatin and tetraplatin was also reduced, although to a lesser extent than cisplatin and carboplatin in KB CP(20) cells. This reduction in oxaliplatin and tetraplatin accumulation provides a potential explanation for the low levels of cross-resistance to these compounds. Although reduced oxaliplatin accumulation was not observed in a previous report, the levels of cisplatin resistance in the subline utilized in that study were not as high, and cross-resistance to oxaliplatin could not be measured [26].

Additional insight is provided by the results obtained when the sensitivity patterns of the cell lines in the NCI Anticancer Drug Screen were examined. Previous analyses have utilized the mean graphs and the COMPARE algorithm to identify patterns common to drugs that share a common target or mechanisms of action [32, 37]. The mean graphs of drug sensitivity or "fingerprints" have also been

used to identify putative targets of new or unknown compounds. The high correlations observed in these analyses have suggested that the target(s) or the mechanism(s) of action is an important determinant of drug sensitivity as determined in the Drug Screen assays. In addition, recent evidence using a drug-resistance measurement (*mdr*-1 expression) as the "seed" suggests that the mechanism(s) of resistance also influences the drug sensitivity profile [38]. Thus, the sensitivity pattern of a drug appears to depend on both its target or mechanisms(s) of action as well as its mechanism(s) of resistance. The dissimilar patterns obtained for cisplatin and oxaliplatin, as evidenced by the low Pearson correlation coefficients, suggest but do not prove that both are different. Differences in the mechanism(s) of resistance were suggested by the cross-resistance data in the cisplatin-tolerant cells, whereas differences in the target(s)/mechanism(s) or action are inferred from the COMPARE analysis. Clarification of the latter would be important in furthering our understanding of the mechanism(s) of action of platinum compounds. Biophysical analysis of DNA modified by "dach" platinum complexes indicates that although adducts similar to those of cisplatin are formed by the "dach" platinums, different effects on DNA appear to result from steric crowding of the axially oriented cyclohexane ring [45, 46].

Visual inspection of the mean graphs shows differences in the sensitivity patterns for both the colon cancer and CNS cancer subpanels. Oxaliplatin was more toxic to the colon cancer cell lines than to the panel as a whole, whereas it appeared less active against the cell lines derived from CNS tumors. These results extend previous observations in a smaller number of cell lines [25]. Further studies will be required to pursue these observations, although determination of clinical activity will be most important. Preliminary results suggest that oxaliplatin may have some activity against colon carcinomas [18, 47].

Examination of the COMPARE analysis provides further information of the differences between different platinum compounds. For oxaliplatin, high Pearson correlation coefficients were observed principally with other "dach" platinum compounds and several acridine derivatives. In contrast, for cisplatin, high correlation coefficients were found with platinum agents containing diaminoplatinum, and also with camptothecin analogs and alkylating agents including uracil nitrogen mustard and melphalan. The latter result supports the observation of cross-resistance to melphalan in the cisplatin-selected cell lines. Although cyclophosphamide cannot be tested *in vitro*, one prediction of these results is that cross-tolerance to cyclophosphamide may be observed when this drug is used in combination with cisplatin. Combinations of either cisplatin and oxaliplatin or cyclophosphamide and oxaliplatin may be less cross-resistant.

To address this latter issue, and to obtain preliminary *in vitro* data on the use of platinum compounds from different classes in combination, we have performed preliminary experiments using combinations of cisplatin and oxaliplatin in several molar ratios. Previous experiments have shown some synergy using similar analyses with cisplatin and carboplatin. We conclude that the activity of these agents in combination is principally additive and possibly synergistic. Such additivity may indicate some similarity in the mechanism(s) of action of these two drugs. Although a previous abstract has suggested that these two platinums in combination could be potentially antagonistic, the results described in the present models and in limited preclinical studies, as well as preliminary clinical observations indicate that antagonism does not occur [21–24, 48].

In summary, the present study provides evidence that development of resistance to cisplatin (and carboplatin) need not confer cross-resistance to oxaliplatin (and tetraplatin). The results in the resistant sublines and in the COMPARE analysis suggest that these different platinum compounds have a dissimilar mechanism(s) of resistance and probably a different target(s)/mechanism(s) of action as well. Oxaliplatin may be effective against cisplatin-resistant tumors, and may be valuable in combination with other platinum compounds or alkylating agents. Future clinical trials should provide some of these answers and guide *in vitro* investigations.

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