Activity of Oxaliplatin against Human Tumor Colony-forming Units

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ABSTRACT

This study was conducted to identify tumor types warranting Phase II clinical trials of oxaliplatin using the human tumor cloning assay. Oxaliplatin was tested at concentrations ranging from 0.5 to 50.0 μg/ml in 1-h and 14-day continuous exposures along with 1.4 μg/ml carboplatin and 0.2 μg/ml cisplatin for comparison. We defined in vitro response as tumor growth inhibition >50% of control. In the 1-h exposure schedule, in vitro responses were observed in 9 of 116 (8%), 18 of 115 (16%), 38 of 103 (37%), and 7 of 13 (54%) tumor specimens at concentrations of 0.5, 5.0, 10.0, and 50.0 μg/ml oxaliplatin, respectively. In the 14-day exposure schedule, in vitro responses were observed in 10 of 121 (8%), 37 of 121 (31%), 57 of 106 (54%), and 15 of 15 (100%) tumor specimens at concentrations of 0.5, 5.0, 10.0, and 50.0 μg/ml oxaliplatin, respectively. Activity was observed against colon cancer, non-small cell lung cancer, gastric cancer, and melanoma colony-forming units. In both cisplatin-resistant and cisplatin-sensitive tumors, the activity of oxaliplatin was concentration and time dependent. A 1-h exposure to 5.0 and 10.0 μg/ml oxaliplatin led to 7.4 and 23.4% in vitro responses, respectively, in specimens resistant to 1-h exposure of 0.2 μg/ml cisplatin. Moreover, 1-h exposures to 5.0 μg/ml and 10.0 μg/ml oxaliplatin showed in vitro antitumor responses in 10.2 and 24.3%, 17.2 and 34.5%, 10.0 and 20.0%, 6.7 and 16.7%, and 11.4 and 34.3% of specimens resistant to 1.4 μg/ml carboplatin, 6.0 μg/ml 5-fluorouracil, 3.0 μg/ml irinotecan, 10.0 μg/ml paclitaxel, and 0.04 μg/ml doxorubicin, respectively. The effect in those drug-resistant specimens was improved when oxaliplatin was used on the protracted exposure regimen. Our data indicate that oxaliplatin is an active drug in vitro against a large variety of human tumors. Both concentration and duration of exposure are important factors for oxaliplatin cytotoxicity. The broad spectrum of activity and the in vitro activity against some tumors primarily resistant to conventional anticancer drugs encourage further clinical investigations of oxaliplatin in patients with advanced cancer refractory to conventional chemotherapy.

INTRODUCTION

Of the new-generation platinum compounds that have been evaluated, those with the dach1 carrier ligand have received the most attention in recent years (1). Kidani et al. (2, 3) were the first to suggest that the spatial conformations of the dach carrier ligand might affect the interactions of dach-platinum compounds with DNA and, therefore, the cytotoxicity of these compounds (2). From these compounds, oxaliplatin [trans-1,2-diaminocyclohexane]oxalatoplatinum] was selected based on its preclinical activity in a variety of tumor xenografts (4–6), the absence of renal toxicity (7), and its mild hematological toxicity in Phase I clinical trials (8). Although oxaliplatin forms platinum-DNA adducts very slowly in vitro, the kinetics of intracellular platinum-DNA adduct formation (1, 9, 10), the percentages of the different intranuclestrand adducts, and the sites of DNA binding appear similar to those of cisplatin in many respects (1). However, in spite of those similarities, dach-platinum adducts are bulkier and more hydrophobic than cis-diammine-platinum adducts and may be more effective at inhibiting DNA synthesis (11) and cell growth than cisplatin adducts (12). In addition, monoadduct-to-diadduct conversion is slower for dach-platinum adducts (13), presumably because the N-Pt-N bond angle is more constrained for dach-platinum-DNA adducts than for cis-diammine-platinum-DNA adducts (14, 15). Finally, recent evidence suggests that the loss of mismatch repair that may occur early in the acquisition of cis-diammine-platinum resistance in several human cancers displays a strong carrier ligand specificity for cis-diammine versus dach-platinum adducts (16). Consistent with these molecular biology studies, the National Cancer Institute cytotoxic screening in vitro showed that the dach-platinum compounds, including oxaliplatin, can be identified as a separate family with a unique mechanism(s) of action and a lack of, or only partial, cross-resistance with cis-diammine compounds (12).

In clinical trials, pharmacokinetic data revealed that after a 2-h i.v. injection of 130 mg/m2 oxaliplatin, peak plasma levels of total and ultrafiltrable platinum were 5.1 ± 0.2 and 2.1 ± 0.2 μg/ml, respectively (7). In those studies, oxaliplatin showed a very large volume of distribution (330.1 ± 41.9 liters). Inter-

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The abbreviations used are: dach, 1,2-diaminocyclohexane; 5-FU, 5-fluorouracil; 95% CI, 95% confidence interval.
estingly, oxaliplatin accumulated rapidly in cells. Pharmacokinetic studies showed that 2 h after injection, only 50% of platinum remained in the plasma (about 67% bound to proteins and 33% ultrafiltrable), and the drug progressively accumulated in RBCs with a long terminal half-life close to that of erythrocytes (17). This partitioning of platinum in erythrocytes appears to be unique for oxaliplatin (18, 19). Although some in vitro studies have shown that most of the intraerythrocyte ultrafiltrable platinum remains nonexchangeable, the biotransformation of oxaliplatin in erythrocytes remains not clearly understood (1). As a result, oxaliplatin shall be considered as a prodrug that requires biotransformation in solution. However, clinical data were mainly reported as μg/ml platinum instead of μg/ml oxaliplatin. Therefore, this may generate a discrepancy between the concentrations of oxaliplatin required to achieve significant antiproliferative effects in vitro and the achievable in vivo concentrations. To date, clinical experiences were mainly focused on patients with advanced colorectal cancer using oxaliplatin either in short-term (20, 21) or continuous chronomodulated infusion (22). In patients with colorectal cancer, the overall response rates of oxaliplatin alone were 20% in first-line chemotherapy (23) and 10% in patients treated previously with 5-FU (24). Moreover, the antitumor activity was increased when oxaliplatin was combined with 5-FU with or without folinic acid (21, 25) in a small but constant proportion of patients with complete histological responses. Those data obtained in patients with colon cancer, which is usually considered very resistant to any conventional chemotherapy, including cisplatin, may warrant further clinical investigation of oxaliplatin in other tumor types.

On the basis of the preclinical and pharmacokinetic data available for the selection of relevant concentrations and schedules, we investigated the antiproliferative effects of oxaliplatin at concentrations ranging from 0.5 to 50.0 μg/ml against human tumor colony-forming units in vitro. The aims of this study were to identify tumor types with in vitro response for future Phase II clinical trials and to determine the relative antiproliferative effects of short-term versus prolonged exposure to oxaliplatin at clinically relevant concentrations.

MATERIALS AND METHODS

Collection of Tumor Cells. After written informed consent was obtained according to institutional guidelines, tumor specimens (biopsies, bone marrow, pleural effusions, and ascites) were collected by sterile standard techniques as part of routine clinical procedures. Samples were collected and delivered within an average time of 4 h and were processed immediately upon arrival in the laboratory. Internal controls indicated that the viability of cells was maintained when samples were processed within 3 days for solid specimens and 5 days for fluid ones. Biopsies of solid tumors were stored in McCoy’s 5A medium (Life Technologies, Inc., Grand Island, NY) containing 10% newborn calf serum, 10 mm HEPES, 100 μg/ml of penicillin, and 90 μg/ml streptomycin (all from Life Technologies, Inc.) for transport to the laboratory. Preservative-free heparin (10 units/ml; O’Neill, Johns, and Feldman, St. Louis, MO) was added immediately after collection of fluids to prevent coagulation. Solid specimens were minced and repeatedly passed through metal sieves with 100 μm mesh (EC Apparatus, St. Petersburg, FL) to obtain a single-cell suspension. When necessary, effusions were centrifuged at 150 × g for 5–7 min and passed through 25-gauge needles to obtain single-cell suspensions. All specimens were washed twice in McCoy’s 5A medium containing 5% horse serum (Sigma Chemical Co.), 10% heat-inactivated FCS (Hyclone, Logan, UT), 2 mm sodium pyruvate, 2 mm glutamine, 90 units/ml penicillin, 90 μg/ml streptomycin, and 35 μg/ml l-serine (all from Life Technologies, Inc.). The viability of cells (from 40 to 100%) was determined on a hemocytometer with trypan blue. Only viable cells determined the final concentration of plated cells.

Drugs. Purified oxaliplatin provided by Debiopharm S.A. (Lausanne, Switzerland) was diluted in water to create concentrated stocks. Aliquots of each stock solution were stored at −70°C and were thawed immediately before the experiment. Four final concentrations of 0.5, 5, 10, and 50 μg/ml were tested at both 1-h and continuous exposures. Because of the reproducible high cytotoxic activity, testing at 50.0 μg/ml was discontinued after 7 and 15 samples at 1-h and 14-day continuous exposure, respectively.

Oxaliplatin activity was also evaluated in tumor specimens that showed resistance to a panel of clinically relevant cytotoxic drugs. Drug concentrations were selected based on our previous experience with these anticancer drugs in the cloning assay (26, 27). These drug concentrations were previously associated with in vitro responses and, for some drugs, were predictive of clinical responses in previously published studies (26, 27). For comparison, specimens were exposed, along with oxaliplatin, to 1.4 μg/ml carboplatin and 0.2 μg/ml cisplatin at both 1-h and 14-day exposures. Furthermore, because of the potential clinical applications, the cytotoxicity of 1-h and 14-day oxaliplatin exposures were evaluated in specimens resistant to the following concentrations and schedules: 1-h exposure to 6.0 μg/ml 5-FU; 1-h exposure to 0.3, 1.5, and 3.0 μg/ml irinotecan (CPT-11; 7-ethyl-10-[4-(piperidyl)-1-piperidyl]carbonyloxy-camptothecin); 1-h exposure to 0.25, 2.5, and 10.0 μg/ml paclitaxel; 1-h exposure to 0.04 μg/ml doxorubicin; and 1-h exposure to 3.0 μg/ml cyclophosphamide. The in vitro concentrations used in this study correlate with achievable in vivo concentrations in humans that were previously shown to be effective in animals and/or in clinical trials against a large variety of human tumor types, including those used in this study (26, 27).

Human Tumor Cloning Assay. The human tumor cloning assay was performed using the two-layer system described by Hamburger and Salmon with several modifications (28). Briefly, cells were suspended in 0.3% agar in enriched Connaught Medical Research Laboratories medium 1066 supplemented with 15% heat-inactivated horse serum, penicillin (100 units/ml), streptomycin (2 mg/ml), glutamine (2 mm), insulin (3 units/ml), asparagine (0.6 mg/ml), and HEPES buffer (2 mm). For the continuous exposure, compounds were added directly to the above mixture. Cells were plated in 35-mm Petri dishes in a top layer of agar over an underlayer of agar to prevent growth of fibroblasts. Three plates were prepared for each data point. The plates were incubated at 37°C and were removed on day 14 for counting of the colonies. For the 1-h exposure studies, the cells were incubated with the compound for 1 h and then washed and plated in the top layer of agar, just as was done for the contin-
uous-exposure studies. The number of colonies (defined as >50 cells) formed in the three compound-treated plates were compared to the number of colonies formed in the three control plates, and the percentage of colonies surviving at that concentration of compound was calculated.

**Quality Control.** A test was defined as an experiment performed on a unique tumor tissue sample that contained untreated control, positive control, and three specified compound concentration levels. A test having an average of 20 or more colonies present on day 14 in the untreated control plates and <30% survival in the positive control was considered evaluable. To ensure the presence of an excellent single-cell suspension, a positive control consisting of the cell toxin orthosodium vanadate at a concentration of 200 μg/ml was used. For an experiment to be considered assesable, the orthosodium vanadate had to produce less than 30% survival of colony-forming units. Three untreated control plates also were set up on day 0. On the day of culture reading there should have been <30% survival of tumor colony-forming units in the positive control plates. If there was no effect on colony formation, then the single-cell suspension on day 0 was poor (because orthosodium vanadate does not affect clumps), and the tumor sample test was considered nonevaluable. The use of a positive control has been shown to greatly increase the reproducibility of the human tumor cloning assay (28). The results were expressed as the percentage of survival of tumor colony-forming units for a particular drug relative to its control. This quantity was calculated as the ratio between the mean number of colonies surviving in the drug-treated plates and the mean number of colonies growing in control plates. Survival rates were expressed as means and SD. A response was defined as growth of tumor colony-forming units in treated plates that was ≤50% of that in control plates (considered as a relevant antiproliferative effect). The in vitro response rate was the ratio of the number of in vitro responses among evaluable specimens and was expressed as mean and 95% CI. A specimen was considered resistant to a drug having an average of 20 or <30% survival of colony-forming units. Three untreated control plates also were set up on day 0. On the day of culture reading there should have been <30% survival of tumor colony-forming units in the positive control was considered nonevaluable. To ensure the presence of an excellent single-cell suspension, a positive control consisting of the cell toxin orthosodium vanadate at a concentration of 200 μg/ml was used. For an experiment to be considered assesable, the orthosodium vanadate had to produce less than 30% survival of colony-forming units. Three untreated control plates also were set up on day 0. On the day of culture reading there should have been <30% survival of tumor colony-forming units in the positive control plates. If there was no effect on colony formation, then the single-cell suspension on day 0 was poor (because orthosodium vanadate does not affect clumps), and the tumor sample test was considered nonevaluable. The use of a positive control has been shown to greatly increase the reproducibility of the human tumor cloning assay (28). The results were expressed as the percentage of survival of tumor colony-forming units for a particular drug relative to its control. This quantity was calculated as the ratio between the mean number of colonies surviving in the drug-treated plates and the mean number of colonies growing in control plates. Survival rates were expressed as means and SD. A response was defined as growth of tumor colony-forming units in treated plates that was ≤50% of that in control plates (considered as a relevant antiproliferative effect). The in vitro response rate was the ratio of the number of in vitro responses among evaluable specimens and was expressed as mean and 95% CI. A specimen was considered resistant to a cytotoxic drug when the growth of tumor colony-forming units in plates treated with the higher concentration of this drug was ≥50% of that in control plates.

To date, most authors agree that a 30% in vitro response rate may translate into responses in clinical trials. However, for some tumor types that are typically very resistant to classical antitumor agents, a 20% in vitro response rate may also be expected to translate into clinical benefit in clinical trials.

**Statistics.** Head-to-head comparisons of duration of exposure were made using Wilcoxon's signed rank test. Comparisons of response rates between drugs were made with Fisher's exact test or the χ² test as appropriate. A two-sided P < 0.05 was considered to indicate statistical significance.

**RESULTS**

**Antiproliferative Effect against Human Tumor Colony-forming Units.** As can be seen in Table 1, a total of 224 and 243 freshly explanted tumor specimens were exposed to oxaliplatin for 1 h and 14 days, respectively. Of these specimens, 116 (52%) and 121 (50%) specimens exposed to oxaliplatin for 1 h and 14 days, respectively, were considered evaluable for in vitro response. The major subgroups of tumors tested (and the number of colonies forming units) were colon cancer (51%), melanoma (10%), neuroblastoma (9%), gastric cancer (8%), sarcoma (8%), ovarian cancer (8%), breast (6%), and urothelial bladder (6%).

![Table 1](http://clincancerres.aacrjournals.org)
number of evaluable specimens exposed for 1 h and 14 days) were ovarian (19 and 22), colon (15 and 12), breast (14 and 18), non-small cell lung (14 and 13), and renal cell (7 and 11) carcinomas. As shown in Table 1, both short-term and prolonged exposure of oxaliplatin had a concentration-dependent effect on growth inhibition of human tumors.

For the 1-h exposure schedule, in vitro responses were observed in 9 of 116 (8%; 95% CI, 4–14), 18 of 115 (16%; 95% CI, 10–24), 38 of 103 (37%; 95% CI, 28–47), and 7 of 13 (54%; 95% CI, 25–81) evaluable specimens at concentrations 0.5, 5.0, 10.0, and 50.0 µg/ml, respectively (Table 1). Head-to-head comparisons of survival showed clear concentration-response effects in the 1-h exposures (P < 0.01). For this schedule, relevant antiproliferative effects were observed at the relatively high concentration of 10.0 µg/ml in breast (43%; 95% CI, 18–71), colon (33%; 95% CI, 12–62), and non-small cell lung (31%; 95% CI, 9–61) cancers. Although the number of samples is limited, in vitro responses were also observed in four gastric carcinomas (100%; 95% CI, 40–100), four sarcomas (67%; 95% CI, 22–96), and three melanomas (43%; 95% CI, 10–82). No relevant antiproliferative effects were observed against ovarian cancer for concentrations below 50.0 µg/ml in the 1-h exposure schedule.

For the 14-day continuous-exposure schedule, in vitro responses were observed in 10 of 121 (8%; 95% CI, 4–15), 37 of 121 (31%; 95% CI, 23–40), 57 of 106 (54%; 95% CI, 44–64), and 15 of 15 (100%; 95% CI, 78–100) specimens at concentrations of 0.5, 5.0, 10.0, and 50.0 µg/ml, respectively (Table 1). As previously observed in the 1-h exposure schedule, head-to-head comparison of percentage survival demonstrated concentration-response effects in the continuous exposures (P < 0.01). In continuous exposure, relevant antiproliferative effects were observed at concentrations starting at 5.0 µg/ml in renal (45%; 95% CI, 17–77), colon (42%; 95% CI, 15–72), ovarian (27%; 95% CI, 11–50), and non-small cell lung (23%; 95% CI, 5–54) carcinomas. In vitro responses were also observed in four melanomas (80%; 95% CI, 28–99), three sarcomas (50%; 95% CI, 12–88), and two gastric carcinomas (50%; 95% CI, 7–93).

Effects of Short-Term Versus Prolonged Exposures. Interestingly, whereas increased concentrations showed a limited increase in cytotoxicity, the longer duration of exposure was effective in maximizing the antiproliferative effects of oxaliplatin. Data for direct head-to-head comparisons of the antiproliferative effects of 1-h versus 14-day continuous exposure were available in 84, 83, 73, and 11 specimens at concentrations of 0.5, 5.0, 10.0, and 50.0 µg/ml, respectively. In this study, the in vitro response rates were significantly higher with 14-day exposure to oxaliplatin as compared to 1-h exposure (P < 0.01). At all concentrations tested, there were significantly higher antiproliferative effects with continuous-exposure oxaliplatin.
Table 2  *In vitro* antitumor effects of oxaliplatin in specimens showing primary resistance to classical anticancer drugs

<table>
<thead>
<tr>
<th>Concentrations and times of exposure to the parent drugs</th>
<th>No. of responses to oxaliplatin: No. resistant specimens (% <em>in vitro</em> responses)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 µg/ml cisplatin-resistant specimens</td>
<td></td>
</tr>
<tr>
<td>1-h exposure</td>
<td>1/81 (1.2%) 6/81 (7.4%) 19/81 (23.4%) 6/89 (6.7%) 28/89 (31.5%) 46/89 (51.7%)</td>
</tr>
<tr>
<td>14-day exposure</td>
<td>3/74 (4.0%) 9/74 (12.3%) 21/74 (28.4%) 5/109 (4.6%) 30/109 (27.5%) 57/109 (52.3%)</td>
</tr>
<tr>
<td>1.4 µg/ml carboplatin-resistant specimens</td>
<td></td>
</tr>
<tr>
<td>1-h exposure</td>
<td>3/78 (3.8%) 8/78 (10.2%) 19/78 (24.3%) 5/76 (6.6%) 25/76 (32.0%) 42/76 (55.3%)</td>
</tr>
<tr>
<td>14-day exposure</td>
<td>2/72 (2.8%) 6/72 (8.3%) 18/72 (25.0%) 3/109 (2.7%) 30/109 (27.5%) 56/109 (51.4%)</td>
</tr>
<tr>
<td>6.0 µg/ml 5-FU-resistant specimens</td>
<td></td>
</tr>
<tr>
<td>1-h exposure</td>
<td>3/29 (10.3%) 5/29 (17.2%) 10/29 (34.5%) 4/38 (10.5%) 8/38 (21.0%) 21/38 (55.3%)</td>
</tr>
<tr>
<td>14-day exposure</td>
<td></td>
</tr>
<tr>
<td>10.0 µg/ml paclitaxel-resistant specimens</td>
<td></td>
</tr>
<tr>
<td>1-h exposure</td>
<td>0/10 (0.0%) 1/10 (10.0%) 2/10 (20.0%) 1/17 (5.9%) 2/17 (11.8%) 8/17 (47.0%)</td>
</tr>
<tr>
<td>0.04 µg/ml doxorubicin-resistant specimens</td>
<td></td>
</tr>
<tr>
<td>1-h exposure</td>
<td>0/30 (0.0%) 2/30 (6.7%) 5/30 (16.7%) 1/35 (2.8%) 8/35 (22.8%) 15/35 (42.8%)</td>
</tr>
<tr>
<td>3.0 µg/ml cyclophosphamide-resistant specimens</td>
<td></td>
</tr>
<tr>
<td>1-h exposure</td>
<td>3/35 (8.6%) 4/35 (11.4%) 12/35 (34.3%) 4/43 (9.3%) 11/43 (25.6%) 23/43 (53.5%)</td>
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</table>

*Specimens were tested along with conventional agents. Inhibition is given as the ratio of the number of specimens inhibited [colony formation 0.5 or less times that of the control:number of assessable specimens (%)].

(Fig. 1). To evaluate the relative effects of the duration of exposure versus the concentration, we performed a scattergram analysis that compares the relative cytotoxic effect of short versus continuous exposures for each concentration of oxaliplatin (Fig. 2, *Upper panel*). As can be seen in Fig. 2, there was a clear time-related cytotoxic effect with oxaliplatin, whereas no time-related effects were observed with cisplatin and carboplatin (Fig. 2, *Lower panel*). As shown in Tables 1 and 2, protracted exposure induced higher cytotoxic effects in all of the tumor types and in the specimens resistant to a variety of classical anticancer drugs, respectively.

**Effects in Tumors Resistant to Classical Cytotoxic Agents.** Cisplatin and carboplatin were tested along with oxaliplatin to detect tumors primarily resistant to cis-diammineplatinum compounds. In cisplatin and carboplatin-resistant tumors, the activity of oxaliplatin was concentration and time dependent (Table 2). Head-to-head comparisons in tumor specimens showed that a 1-h exposure to 5.0 and 10.0 µg/ml oxaliplatin led to 7.4 and 23.4% *in vitro* responses, respectively; in specimens resistant to 0.2 µg/ml cisplatin at 1-h exposure, the same concentrations of oxaliplatin given as a 14-day exposure schedule led to 31.5 and 51.7% *in vitro* responses. Similarly, head-to-head comparisons showed that a 1-h exposure to 5.0 and 10.0 µg/ml oxaliplatin led to 10.2 and 28.4% *in vitro* responses in specimens resistant to 1.4 µg/ml carboplatin at 1-h exposure, whereas these concentrations of oxaliplatin in the 14-day exposure schedule led to 32.9 and 55.3% *in vitro* responses, respectively. Oxaliplatin showed similar activity in tumors resistant to protracted exposure to cisplatin and carboplatin (Table 2).

We subsequently compared the effects of oxaliplatin in specimens resistant to 1-h exposure to 5-FU, irinotecan, paclitaxel, doxorubicin, and cyclophosphamide. We observed that a 1-h exposure to 5.0 and 10.0 µg/ml oxaliplatin showed *in vitro* antitumor activity in 17.2 and 34.5% of specimens resistant to 6.0 µg/ml 5-FU, in 10.0 and 20.0% of specimens resistant to 3 µg/ml irinotecan, in 6.7 and 16.7% of specimens resistant to 10 µg/ml paclitaxel, in 11.4 and 34.3% of specimens resistant to 0.04 µg/ml doxorubicin, and in 0.0 and 19% of specimens resistant to 3 µg/ml cyclophosphamide, respectively. The cytotoxic effects of oxaliplatin in drug-resistant specimens was improved when oxaliplatin was given as a 14-day exposure (Table 2).

Although cytotoxic activity of oxaliplatin was observed in some but not all specimens that were resistant to classical anticancer agents, this *in vitro* study was not designed to clearly answer the cross-resistance between oxaliplatin and other anticancer drugs. Therefore, observation of *in vitro* responses in some specimens may lead to an overestimation of the antiproliferative effects of oxaliplatin as compared to other drugs. Moreover, almost all drugs, if given at a sufficiently high concentration, may have activity against specimens that are resistant to classical anticancer agents. To compare the relative antiproliferative effects of oxaliplatin with those of classical anticancer drugs, we performed a scattergram analysis comparing percentages of survival in specimens exposed to several concentrations of oxaliplatin and cisplatin or carboplatin. This approach was proposed as an alternative to the somewhat arbitrary and stringent definition of resistance (*i.e.*, anything less than a 50% colony killing). As can be seen in Fig. 3, even at low, 0.5 µg/ml concentrations, some tumor specimens were resistant to cisplatin but not to oxaliplatin (Fig. 3A) and carboplatin (Fig. 3D). However, when given as a 1-h exposure, data showed that...
oxaliplatin has similar activity to cisplatin and carboplatin in the overall population of specimens. Differences in terms of anti-proliferative effects between oxaliplatin and other platinum compounds appeared for concentrations above 5.0 μg/ml (Fig. 3, B, C, E, and F). Moreover, continuous exposures increased the activity of oxaliplatin in both specimens sensitive and resistant to cisplatin and oxaliplatin. A similar approach has been performed for comparing the antiproliferative effects of several concentrations of oxaliplatin with those of 5-FU, paclitaxel, irinotecan, and doxorubicin (Fig. 4). In this study, we observed that the overall antiproliferative activity of oxaliplatin was higher than that of those compounds only for concentrations above 5 μg/ml.

DISCUSSION

Our data show that oxaliplatin exerts potent in vitro cytotoxic activity against a large variety of human tumor colony-forming units taken directly from patients. In this study, in vitro responses were observed in non-small cell lung, breast, and gastric cancers. Moreover, responses were also observed in colon cancer, and despite a limited number of specimens, activity was shown in melanoma, renal cell carcinoma, and sarcoma, which are commonly considered highly resistant to conventional anticancer drugs. Our results are fully consistent with previous reports showing that oxaliplatin has antiproliferative activity in vitro against human cancer cell lines (12) and in vivo against several types of human tumor xenografts including L1210 leukemia (29, 30), LGC lymphoma (31), MA-16c mammary carcinoma (6, 31), M5076 sarcoma (30, 32), B16 melanoma (30, 32), C38 colon carcinoma (30, 32), P388 leukemia (32), L40AkR leukemia (31), and Lewis lung carcinoma (30, 32). Interestingly, the spectrum of activity of oxaliplatin in vitro and in vivo seems to differ from that of cis-diammine-platinum compounds. In a recent study using the National Cancer Institute’s anticancer drug screen database in a panel of 60 human cancer cell lines, investigators have compared, using the Pearson correlation coefficient, the sensitivity profiles of dach-platinum compounds, including oxaliplatin versus cis-diammine compounds (12). They showed that dach-platinum compounds, including oxaliplatin, belong to a family separated from cis-diammine-platinum compounds that includes cisplatin and carboplatin. The authors suggested that oxaliplatin may have one or more different cellular targets, mechanisms of action, and/or mechanisms of resistance from those of cisplatin. In our study, we showed that oxaliplatin has in vitro antiproliferative effects against colon cancer cells that are usually considered resistant to cisplatin. The specific activity of oxaliplatin in colon cancer is the best-documented example of the different spectrum of activity of oxaliplatin. More recent evidence suggests that the loss of mismatch repair that occurs early in the acquisition of cis-diammine-platinum resistance in colon cancer cells displays a strong carrier ligand specificity for cis-diammine versus dach-platinum adducts (16). The activity of oxaliplatin against colorectal cancer has previously been well documented in clinical trials showing overall response rates of 20 and 10% in patients with previously untreated and 5-FU-resistant colorectal cancer, respectively (23–25). Although modest, those results are clearly superior to cisplatin and compared favorably with clinical results obtained with the best modulations of 5-FU and with single-agent irinotecan in patients with advanced colon cancer.

Fig. 3 Scattergrams comparing survival following exposures to 0.5, 5.0, and 10.0 μg/ml oxaliplatin with that of 0.2 μg/ml cisplatin and 1.4 μg/ml carboplatin, respectively. Each point represents data from one individual specimen. Points were generated by calculating the percentage survival in individual specimens exposed to either oxaliplatin (X axis) and other compounds (Y axis), such as cisplatin (A, B, and C) or carboplatin (D, E, and F), respectively. Activity of short-term (○) versus long-term (■) exposures to oxaliplatin along with other drugs is also shown. Graphs are presented with the best-fitted linear regressions that show a tendency for concentration-related (-----) and time-related (-----) cytotoxic effects with oxaliplatin at 0.5, 5.0, and 10.0 μg/ml concentrations.
platin accumulated progressively in RBCs with a long terminal half-life close to that of erythrocytes (17). It is therefore possible that oxaliplatin rapidly accumulates and remains in cells after a single bolus injection. Further animal studies may be necessary to determine whether sequestration of oxaliplatin can be maintained in tumor xenografts after a single injection and whether a protracted infusion can increase the antitumor activity of this drug.

Our data showed that oxaliplatin has in vitro activity in some tumors that were spontaneously resistant to cisplatin and carboplatin. To date, there are several reports showing that acquired resistance to cisplatin does not systematically confer cross-resistance to dach-platinum compounds such as oxaliplatin in cisplatin-resistant cancer cell lines selected by prolonged culture in the presence of the drug (12). However, there is little information regarding the activity of oxaliplatin in human cancers primarily resistant to cis-diammine-platinum compounds (35). In this study, we showed that concentrations between 5.0 and 10.0 μg/ml can induce in vitro response in tumors that were primarily resistant to cisplatin and carboplatin. Moreover, we showed that both concentration and duration of exposure are important factors for oxaliplatin cytotoxicity against cisplatin- and carboplatin-resistant cancers. Recent studies have suggested that defects in mismatch repair can lead to cisplatin resistance (16, 36). Supporting the importance of mismatch repair in oxaliplatin cytotoxicity, it has been shown that colon carcinoma cell lines, defective in either the hMLH1 or hMSH2 mismatch repair enzymes, are 2-fold resistant to cisplatin but display little or no resistance to oxaliplatin (16). Thus, the current data suggest that loss of mismatch repair activity may be a frequent occurrence in the acquisition of cisplatin resistance (37, 38). The difference in the mismatch repair of platinum DNA lesions induced by cisplatin and oxaliplatin may also be consistent with differences in the spectrum of activity between those two drugs in colorectal and ovarian cancers cells.

This study also found that concentrations above 5 μg/ml oxaliplatin have activity in some tumors that are primarily resistant (or not sensitive) to conventional concentrations of 5-FU, irinotecan, paclitaxel, doxorubicin, and cyclophosphamide. This was observed using both a stringent definition of resistance (less than 50% colony killing) and a more sophisticated scattergram analysis comparing the antiproliferative effects of drugs. For example, 10.0 μg/ml oxaliplatin (1 h) induced in vitro response in 34.5, 20.0, 16.7, 34.3, and 19.0% of tumors primarily resistant to 5-FU, irinotecan, paclitaxel, doxorubicin, and cyclophosphamide, respectively. These data are of particular interest for clinical applications considering the importance of 5-FU, irinotecan, and oxaliplatin in the treatment of advanced colon cancer (24, 33). As indicated previously, Phase II studies have shown that single-agent oxaliplatin has activity in patients with colorectal cancers that acquired resistance to 5-FU (23). Little preclinical and clinical evidence is actually available concerning the activity of oxaliplatin in cancer cells expressing primary resistance to irinotecan, paclitaxel, doxorubicin, or cyclophosphamide. Therefore, our results encourage preclinical and clinical studies evaluating the antitumor effects of oxaliplatin in tumors resistant to 5-FU, irinotecan, paclitaxel, or doxorubicin and suggest that the addition of oxaliplatin to combination chemotherapy containing those classical anticancer drugs may improve the antitumor effects.

(33). Because clinical experience with oxaliplatin remains modest, our results showing in vitro responses in non-small cell lung, ovarian, breast, and gastric cancers and melanomas encourage further clinical investigation with oxaliplatin in those tumor types.

In our study, we showed that the duration of exposure is an important parameter in oxaliplatin cytotoxicity in vitro. In this study, a 2-fold increase in the in vitro response rate was obtained by increasing the duration of exposure to oxaliplatin. This finding may be important to consider in future studies investigating the mechanism of action of oxaliplatin in cultured cancer cells. However, this in vitro study does not pretend to provide any schedule design for a clinical trial, and there is no clinical information supporting the notion that concentrations of oxaliplatin above 5.0 μg/ml may be sustainable for 14 days in patients. Although only one Phase I study showed that the toxicity profile of oxaliplatin was not affected when given in protracted infusion over 5 days (34), no pharmacokinetic data investigating protracted-infusion oxaliplatin are currently available. Moreover, whether the favorable cytotoxic effects of prolonged exposures to oxaliplatin in vitro may be translated into benefits in clinical trials is obviously questionable. Pharmacokinetic data showed that oxaliplatin has a very large volume of distribution after short-term i.v. injection (7). Moreover, oxaliplatin accumulated progressively in RBCs with a long terminal

Fig. 4 Scattergrams comparing survival following exposures to 0.5 (+), 5.0 (○), and 10.0 μg/ml (●) oxaliplatin with that of 6.0 μg/ml 5-fluorouracil (A), 2.5 μg/ml paclitaxel (B), 1.5 μg/ml irinotecan (C), and 0.04 μg/ml doxorubicin (D), respectively. Each point represents data from one individual specimen. Points were generated by calculating percentage survival in individual specimens exposed to oxaliplatin (X axis) and other compounds (Y axis), such as 5-fluorouracil (A), paclitaxel (B), irinotecan (C), and doxorubicin (D), respectively. Activity of 1-h exposure to 0.5 (+), 5.0 (○), and 10.0 μg/ml oxaliplatin (●) versus a single concentration of each of the other drugs is also shown. Graphs are presented with the best-fitted linear regressions that show a tendency for concentration-related cytotoxic effects with oxaliplatin at concentrations ranging from 0.5 (— — —), 5.0 (—— —), and 10.0 μg/ml (— — —).
In summary, we showed that the duration of exposure is an important parameter in oxaliplatin cytotoxicity in vitro. In addition, oxaliplatin shows potent in vitro cytotoxic activity against a large variety of human tumors in the human tumor cloning assay, including colon cancer, non-small cell lung cancer, breast cancer, and melanoma. Moreover, oxaliplatin displayed activity in some tumors that showed initial resistance to cisplatin, carboplatin, 5-FU, and irinotecan. Those results encourage further clinical investigation of oxaliplatin alone or in combination with these drugs in patients with advanced cancers refractory to conventional chemotherapy.

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