Potentiation by Interleukin 1α of Cisplatin and Carboplatin Antitumor Activity: Schedule-dependent and Pharmacokinetic Effects in the RIF-1 Tumor Model

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ABSTRACT

We have previously demonstrated that the cytokine interleukin 1α (IL-1α) significantly potentiates the antitumor activity of a variety of chemotherapeutic agents, including cisplatin (cDDP). In studies described here, we examined the potential of combining IL-1α and the platinum analogue carboplatin (CBDCA) and compared the schedule-dependent and pharmacokinetic effects for IL-1α combinations with cDDP and CBDCA. RIF-1 tumor-bearing mice (C3H/HeJ) received i.p. injections of varying doses of CBDCA, alone or concurrently with IL-1α (48 or 480 μg/kg). Clonogenic cell kill and tumor regrowth delay were significantly increased when CBDCA was combined with IL-1α, at both doses, compared to either CBDCA or IL-1α alone (P < 0.001 and P < 0.01, respectively). Although pretreatment with IL-1α receptor antagonist blocked the acute tumor hemorrhagic response induced by IL-1α alone, IL-1α receptor antagonist only partially blocked IL-1α enhancement of CBDCA or cDDP-mediated tumor cell kill. The IL-1α enhancement of CBDCA-mediated tumor cell kill was highly schedule dependent, with maximum antitumor activity observed when IL-1α was administered 4–12 h before CBDCA. In contrast, administration of IL-1α from 24 h before or as late as 6 h after cDDP resulted in the same antitumor activity as simultaneous administration of cDDP and IL-1α. Tumor and normal tissue platinum content were significantly increased by IL-1α in animals treated with CBDCA (P < 0.01) but not in those treated with cDDP. The observed differences between cDDP and CBDCA may be explained by their known differential rates of clearance and protein binding affinities and are compatible with an induced alteration in CBDCA pharmacokinetics.

INTRODUCTION

A promising approach in cancer therapy is to combine BRMs with conventional chemotherapeutic agents (1, 2). Such combinations offer the potential of increasing the efficacy of drug-mediated cytotoxic effects, as well as reducing accompanying toxic side effects. IL-1α is a BRM with a wide range of biological activities involving the inflammatory response, immune regulation, and hematopoietic progenitor cell stimulation (3, 4). We have demonstrated in murine tumor model systems that IL-1α induces acute tumor hemorrhagic necrosis, changes in tumor blood flow, and an increase of tumor clonogenic cell kill (5–9). In addition, IL-1α significantly enhances the antitumor effects in vivo of a variety of chemotherapeutic agents, including mitomycin C, porfomycin, and cisplatin (10–13).

The platinum-containing antineoplastic agents, cDDP and CBDCA, are widely used in treating a variety of tumors, but their clinical use is often complicated by significant side effects, including nausea and vomiting, renal toxicity, neurotoxicity, and myelosuppression (14–16). Although the exact mechanism of action of cDDP or CBDCA is not well understood, it is assumed that DNA is the critical target of cytotoxicity for both of these drugs (17, 18). In contrast, the pharmacokinetic behavior of CBDCA differs greatly from that of cDDP. While cDDP has a high clearance due to its avid, covalent binding to protein, the less reactive nature of CBDCA results in a much lower clearance and a much larger percentage of CBDCA being excreted in the urine when compared to cDDP (16, 19, 20).

The mechanism by which IL-1α modulates platinum-mediated tumor cell kill is as yet unknown but could relate to IL-1α effects on tumor vasculature. We have shown acute effects of IL-1α on tumor blood flow, capillary permeability, oxygenation, and pH (5, 7, 8). These effects could enhance tissue damage by cytotoxic agents and/or increase drug accumulation within the tumor. In the studies reported here, IL-1α enhancement of the antitumor activities of the platinum analogue, CBDCA, have been investigated and compared to the combination of IL-1α and cDDP for schedule- and time-dependent effects, pharmacokinetic differences, and overall antitumor efficacy.

MATERIALS AND METHODS

Tumor Model System. The RIF-1 fibrosarcoma model was maintained as described previously (5, 21). Briefly, C3H/HeJ 6–10-week-old female mice (approximately 20 g) were purchased from The Jackson Laboratory (Bar Harbor, ME). Animals were quarantined for 1 week before experimental use and were regularly monitored for the absence of adventitious murine viruses. RIF-1 tumor cells were cultured in RPMI 1640 with 12.5% fetal bovine serum and were regularly monitored for the absence of adventitious murine viruses. RIF-1 tumor cells were cultured in RPMI 1640 with 12.5% fetal bovine serum and were regularly monitored for the absence of adventitious murine viruses. RIF-1 tumor cells were cultured in RPMI 1640 with 12.5% fetal bovine serum and were regularly monitored for the absence of adventitious murine viruses.

Clonogenic Tumor Cell Survival. The effect of IL-1α, CBDCA, cDDP, and combinations of CBDCA/IL-1α or cDDP/IL-1α on clonogenic tumor cells in vivo was determined by a modification of the excision clonogenic cell survival assay (12). Briefly, RIF-1 tumors (3–5/treatment group) were har...
vested 24 h after treatment. Tumors were weighed, finely minced with scissors, and dissociated with constant stirring at room temperature in an enzyme mixture of 37.5 mg/ml type I collagenase (Sigma Chemical Co., St. Louis, MO), 5 mg/ml DNase (Sigma) and 1% trypsin-EDTA (GIBCO, Grand Island, NY). An additional 200 μg/ml of DNase was added for the last 15 min of the incubation period. Tumor cells were then harvested and washed twice with medium. Viable cells were counted in 0.04% trypan blue and 2% acetic acid, diluted in RPMI 1640 plus 12.5% fetal bovine serum, and seeded in 6-well plates (Costar, Cambridge, MA). After 7 days incubation at 37°C and 5% CO₂, colonies were counted, and the number of clonogenic cells/g of tumor was determined by preincubating monolayers of isolated tumor cells with varying concentrations of IL-1α for 4 h at 37°C. Monolayers were then washed twice with medium and incubated for an additional 1 h with varying doses of CBDCA, after which cells were plated in the clonogenic assay and incubated for 7 days.

Tumor Regrowth Delay. To determine the effect of IL-1α and/or CBDCA on clonogenic tumor cells in vitro was determined by preincubating monolayers of isolated tumor cells with varying concentrations of IL-1α for 4 h at 37°C. Monolayers were then washed twice with medium and incubated for an additional 1 h with varying doses of CBDCA, after which cells were plated in the clonogenic assay and incubated for 7 days.

Quantitation of Tumor Hemorrhage. IL-1α-induced acute tumor hemorrhagic necrosis in RIF-1 tumors was determined by using the 59Fe-labeled red blood cell method as described previously (5, 6).

Quantitation of Platinum Content in Tissue. Blood and tissue samples were collected from experimental groups 24 h after CBDCA or cDDP administration. Blood samples were collected by retro-orbital bleed in heparinized tubes and centrifuged at 1000 × g for 10 min. The plasma was removed, and an aliquot was frozen at −20°C until the time of assay. All the other tissue samples were weighed immediately and frozen at −20°C until platinum content was determined. Sample platinum content was measured by flameless absorption spectrometry as described previously (16). Briefly, plasma was diluted or tissues were homogenized in 0.25% Triton X-100 before being analyzed with a Perkin-Elmer model 1100 flameless atomic absorption spectrometer (Perkin-Elmer, Norwalk, CT) monitoring 265.9 nm. The temperature program used for both plasma and tissue was as follows: ramp over 30 s to 90°C and hold for 60 s; ramp over 10 s to 110°C and hold for 10 s; ramp over 30 s to 300°C and hold for 30 s; ramp over 60 s to 1500°C and hold for 90 s and atomize at 2700°C with no ramping. Argon gas flow was 300 ml/min during all heating steps except atomization when it was interrupted.

Statistical Analysis. ANOVA was used to assess the level of significance between experimental groups.

RESULTS

IL-1α Enhanced CBDCA-mediated Clonogenic Tumor Cell Kill. We have demonstrated previously that IL-1α can synergistically enhance cDDP-mediated antitumor activity in a dose-dependent manner when administered concurrently to tumor-bearing mice (12). To examine the effect of IL-1α on CBDCA-mediated clonogenic tumor cell kill, RIF-1 tumor-bearing mice were treated with concurrent i.p. administration of IL-1α and varying doses of CBDCA. IL-1α, at both 48 and 480 μg/kg, significantly enhanced CBDCA-mediated tumor cell kill (decrease in surviving fraction) even at low doses of CBDCA (10–20 mg/kg; Fig. 1). At the highest dose of CBDCA (100 mg/kg), the addition of IL-1α resulted in greater than 3 logs of cell kill when compared to CBDCA alone. As shown previously (5–9), treatment with IL-1α alone results in significant tumor cell kill when compared to no treatment (P < 0.001).

As demonstrated previously, IL-1α did not increase cDDP-mediated tumor cell kill in vitro (12). To determine whether the IL-1α enhanced cytotoxic response of RIF tumor to CBDCA was the result of a direct effect, RIF tumor cells were plated with various combinations of CBDCA and IL-1α in the in vitro clonogenic assay. IL-1α did not have any enhancing effect on CBDCA-mediated cytotoxicity on RIF-1 tumor cells in vitro (Fig. 2). Regardless of dose (10¹–10⁶ units
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Table 1 Effect of IL-1α on cDDP- or CBDCA-induced tumor regrowth delay

<table>
<thead>
<tr>
<th>Treatment groups&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Tumor regrowth delay (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Treatment</td>
<td>–</td>
</tr>
<tr>
<td>IL-1α</td>
<td>4.9 ± 2.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CBDCA</td>
<td>6.3 ± 2.3</td>
</tr>
<tr>
<td>CBDCA/IL-1α</td>
<td>12.0 ± 2.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>cDDP</td>
<td>5.8 ± 2.8</td>
</tr>
<tr>
<td>cDDP/IL-1α</td>
<td>12.6 ± 1.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> RIF-1 3T3/HeJ mice (10–15/group) were treated i.p. with IL-1α 480 µg/kg, cDDP 6 mg/kg, CBDCA 50 mg/kg, and IL-1α 480 µg/kg or cDDP 6 mg/kg and IL-1α 480 µg/kg. Tumors were measured 2–3 times a week, and tumor regrowth delay was calculated by the time it takes for tumor volume to reach 4X pretreatment size minus control.

<sup>b</sup> Mean ± SD days of tumor regrowth delay.

<sup>c</sup> Significantly different from IL-1α, cDDP, or CBDCA alone, P < 0.01 (ANOVA).

Table 2 Effect of IL-1α on IL-1α-induced acute tumor hemorrhage

<table>
<thead>
<tr>
<th>Treatment&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Tumor</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>20.7 ± 3.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.8 ± 2.3</td>
</tr>
<tr>
<td>IL-1α</td>
<td>19.5 ± 2.2</td>
<td>9.1 ± 2.2</td>
</tr>
<tr>
<td>IL-1α (48 µg/kg)</td>
<td>64.6 ± 14.5</td>
<td>14.9 ± 5.0</td>
</tr>
<tr>
<td>IL-1α + IL-1α (48 µg/kg)</td>
<td>22.8 ± 2.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.2 ± 2.1</td>
</tr>
<tr>
<td>IL-1α + (480 µg/kg)</td>
<td>57.5 ± 12.4</td>
<td>12.6 ± 5.7</td>
</tr>
<tr>
<td>IL-1α + IL-1α (480 µg/kg)</td>
<td>30.9 ± 5.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.8 ± 6.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> RIF-1 3T3/HeJ mice (3–5/group) were treated i.p. with IL-1α at 200 µg/mouse 1 h prior to either 48 or 480 µg/kg of IL-1α. Tissues were removed 6 h later.

<sup>b</sup> Mean ± SD µL RBC/g tissue.

<sup>c</sup> Significantly different from mice treated with IL-1α alone; P < 0.001 (ANOVA).

of IL-1α, time of incubation or of pretreatment (0–4 h), IL-1α did not significantly increase CBDCA tumor cell kill in vitro (data not shown).

Tumor Regrowth Delay. To examine the ability of IL-1α to enhance CBDCA- or cDDP-mediated tumor regression in vivo, groups of tumor-bearing mice were treated with either IL-1α, cDDP, CBDCA, concurrent cDDP/IL-1α or concurrent CBDCA/IL-1α and examined for changes in tumor size as expressed in tumor regrowth delay. Tumor regrowth delay was significantly increased when IL-1α was combined with CBDCA as compared to CBDCA alone (Table 1). Similarly, and as shown previously (12), IL-1α significantly increased tumor regrowth delay when administered in combination with cDDP when compared to cDDP alone.

IL-1α Effects on IL-1α-induced Hemorrhage and Enhanced cDDP- or CBDCA-mediated Clonogenic Cell Kill. IL-1α can bind specifically to both IL-1 type I and type II receptors and can block a variety of IL-1-stimulated responses in vitro and in vivo (23, 24). When RIF-1 tumor-bearing mice were injected i.p. with 200 µg/mouse of IL-1α 1 h prior to IL-1α or IL-1α alone, IL-1α blocked the acute tumor hemorrhage induced by IL-1α at both 48 and 480 µg/kg doses, while IL-1α itself did not cause hemorrhage as measured by 59Fe-labeled RBC (Table 2).

To determine the effect of IL-1α on the ability of IL-1α to enhance CBDCA or cDDP clonogenic tumor cell kill, RIF-1 tumor-bearing mice were pretreated with 200 µg/mouse of IL-1α 1 h prior to IL-1α/cytotoxic drug injection. IL-1α only partially blocked the enhancement of IL-1α on cDDP- or CBDCA-mediated antitumor activity as measured by clonogenic tumor cell kill (Table 3). Similarly, IL-1α was capable of partially abrogating the antitumor effect of IL-1α alone.

Time-dependent Effects of IL-1α on cDDP or CBDCA Antitumor Activity. It has been reported that synergistic effects between IL-1α and a variety of chemotherapeutic drugs are highly time and sequence specific (11, 13). To examine time-dependent effects for IL-1α plus either CBDCA or cDDP, groups of RIF-1 tumor-bearing mice were treated i.p. at time 0 with either CBDCA (50 mg/kg) or cDDP (6 mg/kg) with IL-1α (480 µg/kg), injected either simultaneously or at various times before or after platinum-containing drug. Tumors were harvested 24 h after drug administration, and clonogenic tumor cell kill was determined by clonogenic cell assay. As shown in Fig. 3, IL-1α significantly enhanced CBDCA- or cDDP-mediated clonogenic cell kill when administered simultaneously (time 0) when compared to either drug alone (P < 0.001). However, the antitumor activity of IL-1α and CBDCA was maximal when IL-1α was administered 6–12 h before CBDCA, with a significant decrease in survival.

Table 3 Effect of IL-1α on RIF-1 tumor clonogenic cell kill

<table>
<thead>
<tr>
<th>Treatment&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Surviving fraction (SF&lt;sub&gt;50&lt;/sub&gt; × 10&lt;sup&gt;-7&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Treatment</td>
<td>100.0</td>
</tr>
<tr>
<td>IL-1α</td>
<td>102.1 ± 16.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IL-1α</td>
<td>19.6 ± 2.9</td>
</tr>
<tr>
<td>IL-1α + IL-1α</td>
<td>53.8 ± 8.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>CBDCA</td>
<td>10.1 ± 3.0</td>
</tr>
<tr>
<td>CBDCA/IL-1α</td>
<td>0.09 ± 0.007</td>
</tr>
<tr>
<td>IL-1α + CBDCA/IL-1α</td>
<td>1.18 ± 0.14&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>cDDP</td>
<td>2.6 ± 0.6</td>
</tr>
<tr>
<td>cDDP/IL-1α</td>
<td>0.002 ± 0.001</td>
</tr>
<tr>
<td>IL-1α + cDDP/IL-1α</td>
<td>0.5 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> RIF-1 3T3/HeJ mice (3–5/group) were treated i.p. with IL-1α at 200 µg/mouse 1 h prior to 480 µg/kg of IL-1α or CBDCA/IL-1α 50 mg/kg or cDDP/IL-1α 6 mg/kg. Tumors were harvested 24 h later for clonogenic assay.

<sup>b</sup> Mean ± SD surviving fraction/g tumor.

<sup>c</sup> Significantly different from mice treated with either IL-1α alone, CBDCA/IL-1α or cDDP/IL-1α; P < 0.001 (ANOVA).

Fig. 3. CBDCA or cDDP and IL-1α schedule-dependent clonogenic tumor cell kill. Animals were treated i.p. with either CBDCA (50 mg/kg; ○), cDDP (6 mg/kg; □), IL-1α (480 µg/kg; ▲), CBDCA/IL-1α (■), or cDDP/IL-1α (●) at the same doses (open symbols, drugs alone; closed symbols, combinations). CBDCA and cDDP were administered at time 0 with IL-1α injected at various treatment times before or after platinum-containing drug as each point indicates. Twenty-four h after platinum-containing drug, tumors were harvested, and the numbers of clonogenic tumor cells were enumerated. Points, the mean surviving fraction for total clonogenic tumor cells/g tumor (3–5 mice/treatment group); bars, SD. Control timepoints at +24 h for CBDCA or cDDP alone represent surviving fraction 48 h after injection to compare to drug plus IL-1α with IL-1α given +24 h and clonogenic assay harvested 24 h later. All data points from CBDCA/IL-1α or cDDP/IL-1α, except +24 h, are significantly different than CBDCA, cDDP, or IL-1α alone, P < 0.001 (ANOVA).
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Table 4 Time-dependent effects of IL-1α in combination with either CBDCA or cDDP on clonogenic tumor cell kill in RIF-1 tumor-bearing mice

<table>
<thead>
<tr>
<th>Treatment (^{a})</th>
<th>Time of IL-1α administration</th>
<th>Surviving fraction ((SF/g \times 10^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td>IL-1α</td>
<td>0</td>
<td>5.0 ± 1.0 (^{b})</td>
</tr>
<tr>
<td>CBDCA</td>
<td>0</td>
<td>10.1 ± 3.0</td>
</tr>
<tr>
<td>CBDCA/IL-1α</td>
<td>0</td>
<td>0.0057 ± 0.0004 (^{c})</td>
</tr>
<tr>
<td>CBDCA/IL-1α</td>
<td>~3 days</td>
<td>18.4 ± 4.1 (^{d})</td>
</tr>
<tr>
<td>CBDCA/IL-1α</td>
<td>~7 days</td>
<td>42.9 ± 1.2 (^{e})</td>
</tr>
<tr>
<td>cDDP</td>
<td>0</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>cDDP/IL-1α</td>
<td>0</td>
<td>0.093 ± 0.036 (^{f})</td>
</tr>
<tr>
<td>cDDP/IL-1α</td>
<td>~3 days</td>
<td>2.6 ± 0.32 (^{g})</td>
</tr>
<tr>
<td>cDDP/IL-1α</td>
<td>~5 days</td>
<td>9.9 ± 1.5 (^{h})</td>
</tr>
</tbody>
</table>

\(^{a}\) RIF-1 C3H/HeJ mice (3–5/group) were treated i.p. with either 480 µg/kg of IL-1α, CBDCA 50 mg/kg, cDDP 6 mg/kg, or combination of CBDCA and cDDP plus IL-1α at the same doses. CBDCA or cDDP was injected at time 0, and IL-1α was injected concurrent with (time 0) or before CBDCA or cDDP administration. Twenty-four h after treatment with platinum-containing drugs, tumors were harvested for clonogenic assay.

\(^{b}\) Mean ± SD surviving fraction/g tumor.

\(^{c}\) Significantly different from mice treated with CBDCA or cDDP alone; \(P < 0.001\) (ANOVA).

\(^{d}\) Significantly different from CBDCA/IL-1α (0 h); \(P < 0.05\) (ANOVA).

\(^{e}\) Significantly different from cDDP alone; \(P < 0.05\) (ANOVA).

\(^{f}\) Significant difference observed when compared to CBDCA/IL-1α both given at time 0 (\(P < 0.001\)). In contrast, IL-1α enhancement of cDDP-mediated tumor cell kill was not time dependent, with no significant difference observed when IL-1α was given at various times from 24 h before IL-1α to 4 h after, as compared to cDDP/IL-1α administered concurrently. When IL-1α was administered 24 h before either CBDCA or cDDP, the surviving fraction was not significantly different than CBDCA/IL-1α or CDDP/IL-1α administered at time 0 but significantly decreased when compared to CBDCA, cDDP, or IL-1α alone. When IL-1α was given 24 h after CBDCA or cDDP, the surviving fraction was not significantly different compared to CBDCA or cDDP alone. However, when compared to the appropriate control of 48 h after cytotoxic drug (cytotoxic drug given on time 0 with IL-1α administered 24 h later and clonogenic assay harvested after an additional 24 h), a significant, albeit small, enhancement was observed when compared to cytotoxic drug alone (at 48 h; \(P < 0.01\)).

When tumor-bearing mice were treated with IL-1α 3, 5, or 7 days before cytotoxic drug, IL-1α did not significantly enhance clonogenic cell kill mediated by either drug alone but instead significantly increased the surviving fraction (decreased clonogenic tumor cell kill; Table 4).

To determine the time-dependent effects of IL-1α on CBDCA-mediated tumor cell kill at a lower dose of IL-1α (48 µg/kg), animals were treated as described above. As shown in Fig. 4, IL-1α enhancement of CBDCA-mediated tumor cell kill was maximal when IL-1α was administered 4–24 h before CBDCA, even at the lower 48 µg/kg dose of IL-1α. In contrast, when IL-1α at 48 µg/kg was administered 3 and 7 days before CBDCA, clonogenic cell kill was significantly decreased when compared to CBDCA alone.

Platinum Content in CBDCA/IL-1α and cDDP/IL-1α Treatment Groups. We have demonstrated that IL-1α causes vascular injury with changes in tumor blood flow in RIF-1 tumors (5, 6, 7). Previous studies have shown that increasing the dose of CBDCA improves the likelihood of a response in certain tumors (25). Therefore, IL-1α-enhanced clonogenic tumor cell kill may be due to an IL-1α-induced alteration of tumor blood flow or alteration of clearance of CBDCA or cDDP. To examine this hypothesis, tumor and normal tissues were collected 24 h after treatment (a time when maximum clonogenic cell kill is observed), and platinum content was determined by flameless absorption spectroscopy (16). No significant difference was observed in the platinum content of tumor or normal tissues of animals treated with cDDP plus IL-1α when compared to cDDP alone (Table 5). In contrast, platinum content was significantly increased in both tumor and normal tissues in animals treated with the combination of CBDCA/IL-1α as compared to CBDCA alone. When IL-1α was administered 4 h prior to CBDCA, a time when maximum clonogenic cell kill was observed, platinum levels were also significantly increased as compared to animals treated with simultaneous injections of CBDCA and IL-1α. The ratio of platinum content in tumor to plasma, however, was unchanged from that observed with

**Table 5** Effect of IL-1α on platinum content in tumor and normal tissues of mice treated with cDDP or CBDCA

<table>
<thead>
<tr>
<th>Treatment groups (^{a})</th>
<th>[Pt] plasma (µg/ml) (\times 10^{-4})</th>
<th>[Pt] tumor (µg/g)</th>
<th>T:P Ratio</th>
<th>[Pt] liver (µg/g)</th>
<th>[Pt] kidney (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBDCA</td>
<td>0.19 ± 0.09 (^{b})</td>
<td>1.17 ± 0.56</td>
<td>8.18 ± 5.95</td>
<td>1.96 ± 0.62</td>
<td>2.82 ± 1.02</td>
</tr>
<tr>
<td>CBDCA/IL-1α (0 h)</td>
<td>0.43 ± 0.32 (^{c})</td>
<td>2.59 ± 1.25</td>
<td>7.31 ± 2.86</td>
<td>3.01 ± 1.60</td>
<td>4.60 ± 2.29</td>
</tr>
<tr>
<td>CBDCA/IL-1α (~4 h)</td>
<td>0.97 ± 0.72 (^{d})</td>
<td>5.12 ± 3.76</td>
<td>6.20 ± 3.42</td>
<td>6.45 ± 3.96</td>
<td>10.67 ± 6.30</td>
</tr>
<tr>
<td>cDDP</td>
<td>0.61 ± 0.12</td>
<td>0.21 ± 0.40</td>
<td>3.34 ± 1.46</td>
<td>1.93 ± 0.36</td>
<td>2.53 ± 0.20</td>
</tr>
<tr>
<td>cDDP/IL-1α (0 h)</td>
<td>0.72 ± 0.25</td>
<td>0.23 ± 0.14</td>
<td>3.56 ± 0.98</td>
<td>1.75 ± 0.40</td>
<td>2.85 ± 0.66</td>
</tr>
</tbody>
</table>

\(^{a}\) RIF-1 C3H/HeJ mice were treated i.p. with either cDDP (6 mg/kg) or CBDCA (50 mg/kg) alone or administration of cDDP or CBDCA plus IL-1α (480 µg/kg) at the same indicated. Tissues were removed 24 h later for detecting the level of platinum content as measured by atomic absorption spectroscopy (15–20 mice/group).

\(^{b}\) Mean ± SD.

\(^{c}\) Significantly different from cDDP or CBDCA alone; \(P < 0.05\) (ANOVA).

\(^{d}\) Significant difference from CBDCA/IL-1α (0 h); \(P < 0.05\) (ANOVA).
evaluate therapeutic effects (21, 29). We found significant increases in clonogenic cell kill and tumor regrowth delay when CBDCA or cDDP alone. The enhancement by IL-1α was schedule- and time-dependent.

CBDCA alone, regardless of whether animals were treated with IL-1α or when they were inoculated.

To determine platinum content in relation to dose-response of CBDCA in combination with IL-1α, tumor-bearing mice were treated with varying doses of CBDCA alone or CBDCA in combination with a concurrent injection of 48 or 480 μg/kg of IL-1α. After 24 h, tumors and normal tissues were removed and assayed for platinum concentration.

Platinum concentrations in tumor increased in a dose-dependent fashion in all treatment groups (Fig. 5). The platinum content was significantly enhanced in the tumors from animals treated with both 48 and 480 μg/kg of IL-1α as compared to CBDCA alone. No significant difference was observed in platinum content when the tumors of animals treated with the high (480 μg/kg) or low (48 μg/kg) dose of IL-1α were compared to each other. Similarly, the combination of CBDCA/IL-1α resulted in significantly higher platinum concentrations in the kidneys of treated mice when compared to mice treated with CBDCA alone (0), CBDCA/IL-1α at 48 μg/kg (●), or CBDCA/IL-1α at 480 μg/kg (▲). Points, the mean platinum content (μg/g wt) as measured by atomic absorption spectroscopy (5–7 mice/treatment group); bars, SD. Values for CBDCA/IL-1α that are significantly different from CBDCA alone are shown with asterisks (*). **, P < 0.001; *, P < 0.05 (ANOVA).

FIG. 5. Dose-response of platinum content in tumors of RIF-1 tumor-bearing mice treated with CBDCA alone (○), CBDCA/IL-1α at 48 μg/kg (●), or CBDCA/IL-1α at 480 μg/kg (▲). Points, the mean platinum content (μg/g wt) as measured by atomic absorption spectroscopy (5–7 mice/treatment group); bars, SD. Values for CBDCA/IL-1α that are significantly different from CBDCA alone are shown with asterisks (*). **, P < 0.001; *, P < 0.05 (ANOVA).

**DISCUSSION**

BRMs can be exploited for the treatment of malignant diseases because of their ability to directly reduce tumor cell growth and because of their capacity to improve the therapeutic index or efficacy of many antineoplastic drugs. In this regard, TNF, IL-1 (α and β), IL-2, and IFN have been reported to increase the efficacy of many chemotherapeutic drugs (1, 2, 10–13, 26–28). The exact mechanisms for cytokine or BRM enhancement of chemotherapeutic efficacy are currently unknown. The studies presented here have evaluated the use of IL-1α in combination with the platinum-containing agents CBDCA and cDDP to treat RIF-1 tumors, a model system extensively used to evaluate therapeutic effects (21, 29). We found significant increases in clonogenic cell kill and tumor regrowth delay when CBDCA or cDDP was combined with IL-1α as compared to either CBDCA or cDDP alone. The enhancement by IL-1α was schedule- and time-dependent when given in combination with CBDCA with much less of a time-dependent effect observed with cDDP. Maximum enhancing effects of IL-1α were observed on CBDCA-mediated clonogenic cell kill when IL-1α was given 4 to 12 h before CBDCA administration as compared to when both agents were given at the same time. cDDP/IL-1α-mediated clonogenic cell kill, while significantly enhanced as compared to cDDP alone, was not significantly different when IL-1α was given anytime between 24 h before and 4 h after cDDP injection. In addition to enhancing CBDCA-induced cytotoxicity, IL-1α treatment significantly increased the platinum content of the tumors and normal tissues when compared to treatment with CBDCA alone. In contrast, no differences were observed in the platinum content of tumors and normal tissues from animals treated with cDDP versus those from animals treated with the combination of cDDP and IL-1α. These results extend the range of clinically important agents whose activity is significantly enhanced by IL-1α but also underscore the complexity of the interactions and of the mechanisms likely to be involved.

Although cDDP and CBDCA belong to the same class of drugs, they have certain distinct biological and pharmacokinetic properties. cDDP is highly reactive and binds quickly to plasma and other proteins with little elimination by urinary excretion (16, 19). In contrast, CBDCA is much less reactive, binds protein much more slowly, and is primarily excreted via the kidneys. Our results suggest that IL-1α may alter the pharmacokinetics of CBDCA and deposit more CBDCA in the tissues including kidney, liver, and tumor. While a decrease in renal function has not been reported as a dose-limiting toxicity in any of the clinical trials using IL-1 (30–33), it is possible that IL-1α may change blood flow in the kidney, thereby altering glomerular filtration, resulting in decreased excretion of CBDCA and increased drug content in plasma as well as normal and tumor tissues.

The time-dependent antitumor effects observed with CBDCA/IL-1α are consistent with IL-1α-induced changes in CBDCA pharmacokinetics. Although these changes are likely secondary to IL-1α-induced renal impairment, resulting in higher drug levels and an enhanced tumor cell kill, our studies also suggest that other mechanisms may be involved. While CBDCA and cDDP both kill tumor cells in a similar manner (17, 18), IL-1α did not have the same time-dependent effect on cDDP-mediated tumor cell kill as it did on
CBDCA-induced toxicity. Also, in contrast to its effect on tissue platinum content in mice treated with CBDCA, IL-1α did not increase the platinum content of the tumor or normal tissue of animals treated with cDDP/IL-1α when compared to those of mice treated with cDDP alone. While IL-1α may have distinct mechanisms for enhancing the activity of these two drugs, it is more likely that an alternative mechanism(s) exists, with IL-1α-induced changes in CBDCA pharmacokinetics contributing only partially to the enhancement of antitumor activity.

IL-1α is a member of the IL-1 family which competes with IL-1 for occupancy of IL-1 cell surface receptors but not for the triggering of the cellular responses typical of IL-1 (23, 24). Administration of IL-1α blocked IL-1α-induced acute tumor hemorrhage as measured by 51Fe-labeled RBC. IL-1α-induced enhancement of cDDP or CBDCA-mediated clonogenic cell kill could only be partially inhibited by IL-1α injection. While cDDP does not significantly increase the hemorrhagic response induced by IL-1α (12), acute hemorrhage contributes to the antitumor effects of IL-1α and may play a role in the ability of IL-1α to enhance the clonogenic tumor cell kill of cDDP or CBDCA.

The lack of ability of IL-1α to induce direct cytotoxicity against RIF-1 tumor cells in vitro (12) suggests an indirect mechanism(s). One possible mechanism is the involvement of nitric oxide. Cytokines, especially TNF, IFN, and IL-1 are known to be strong inducers of the enzyme, nitric oxide synthetase (34, 35). IL-1 has the ability to induce IFN in vivo and, together IL-1 and IFN have been shown to induce nitric oxide production, which could contribute to tumor cell kill in vivo. Preliminary studies have demonstrated that, although IL-1α itself has no direct cytotoxic effect on RIF-1 tumor cells in vitro, tumor cell survival was significantly decreased in the presence of low doses of IFN-γ (data not shown). Further studies on the role of nitric oxide in the antitumor activities of IL-1α with and without CBDCA or cDDP are currently in progress.

Benchekroun et al. (36) has reported that in vitro IL-1α induced an increase in both the cellular accumulation of cDDP and DNA platination with a significant inhibition of DNA repair, resulting in an increase of cDDP sensitization in human ovarian carcinoma cells. Another BRM, IFN, has been shown to potentially enhance the antitumor activity of 5-fluorouracil through effects on both de novo thymidylate synthesis and thymidine salvage (2). As a result of the lack of the ability of IL-1α to potentiate in vitro CBDCA or cDDP-mediated tumor cell kill in our model system, it is unlikely that IL-1α mediates antitumor activity through effects on DNA platination, DNA repair, or biochemical modulation.

When IL-1α was administered >72 h before CBDCA or cDDP, enhancement of CBDCA- or cDDP-mediated cytotoxicity was significantly reduced. These results suggest that IL-1α may protect tumor cells from platinum-induced cytotoxic effects. IL-1 stimulates the biosynthesis of MT (37, 38). Studies in both cell culture and animal tumor models demonstrate that increases in MT can provide protection against the cytotoxic action of platinum compounds (39). The activation of macrophages and other cells by IL-1 during the acute-phase response results in the massive release of oxygen-free radicals (40). These free radicals result in toxic effects to tumors, with MT an efficient free radical scavenger (37). Other molecules including glutathione are also involved in the scavenger of free radicals and protection from platinum cytotoxic action (41).

An important aspect of therapeutic approaches that combine BRMs and chemotherapeutic agents is whether to administer the BRM before or after the cytotoxic drug. A determination of schedule usually depends on the mechanism of action of the specific BRM involved. Because IL-1 has been shown to stimulate hematopoietic progenitor cells (2, 3), it has been administered most often after cytotoxic drugs (30, 33), which may themselves kill proliferating bone marrow cells and hence minimize its potential to enhance myelotoxicity. Our studies demonstrate that IL-1α was most effective in potentiating the activity of CBDCA or cDDP when administered concurrently or before IL-1α. In contrast, another study has reported maximal antitumor activity for the combination of cDDP and IL-1 to be strictly time dependent, with enhancement of cDDP only when cDDP was given 6 h before IL-1α but not 8 h before or 2–6 h after IL-1α (13). Those studies, however, performed clonogenic assays 24 h after IL-1α treatment and not after cDDP administration as reported here. In addition, cDDP was injected i.v. as opposed to i.p., which could change the time course of plasma concentration and tissue distribution of the drug.

A major benefit for combining IL-1α with CBDCA or cDDP is the effect of IL-1α on the host’s rapid hematopoietic recovery, allowing for a higher amount of chemotherapeutic drug to be given to patients. In Phase I clinical trials, IL-1α has resulted in an increase in platelet counts within 6 days (31, 32), and when administered in combination with 5-fluorouracil, fewer days of neutropenia have been observed, with a dose-dependent increase in neutrophils and platelets 14 days following IL-1 (30). IL-1α has also been shown to decrease the duration of thrombocytopenia following administration of CBDCA (33). We have demonstrated in vivo that injection of IL-1α alone significantly increases megakaryocyte progenitors in both the spleen and bone marrow of normal mice (42), and when given in combination with CBDCA in tumor-bearing mice, the duration of thrombocytopenia is significantly shortened when compared to CBDCA alone (43).

In conclusion, our studies demonstrate that IL-1α significantly enhanced CBDCA/cDDP-mediated tumor cell kill in vivo with schedule-specific patterns and differences in pharmacokinetics, suggesting that IL-1α may be a potential candidate for combination therapy with the platinum analogues.

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