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# Synergistic enhancement by interleukin-1 α of cisplatin-mediated antitumor activity in RIF-1 tumor-bearing C3H/HeJ mice

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**Abstract.** Administration of interleukin-1 α (IL-1 α) plus certain cytotoxic drugs causes substantially greater clonogenic tumor-cell kill and tumor-regrowth delay than does treatment with either agent alone. IL-1 α itself has little effect on tumor growth despite its ability to induce acute hemorrhagic necrosis, restrict tumor blood flow, and cause microvascular injury in a variety of murine model systems. To investigate further IL-1 α's ability to enhance the antitumor activity of cytotoxic drugs, we initiated studies to examine the effect of IL-1 α on cisplatin (cDDP)-mediated cytotoxicity using the RIF-1 tumor system. The antitumor activity of IL-1 α and cDDP was quantitated through standard clonogenic tumor-cell survival assays, a tumor hemorrhagic necrosis assay and tumor-regrowth delay studies, with the interaction between IL-1 α and cDDP being analyzed through median dose-effect. In vitro, IL-1 α had no enhancing effect on the cDDP-mediated tumor-cell kill. For examination of the in vivo efficacy of this regimen, RIF-1 tumor-bearing C3H/HeJ mice (14 days postimplantation) were treated concurrently with single i.p. injections of IL-1 α and/or cDDP at various doses. The increased clonogenic tumor-cell kill obtained with IL-1 α /cDDP was dose-dependent, with significant enhancement by IL-1 α being observed ($P<0.001$), even at the lowest doses tested (2 mg/kg and 6 μg/kg for cDDP and IL-1 α, respectively), but it did not correlate with an increase in tumor hemorrhage. Using median dose-effect analysis, this interaction was determined to be strongly synergistic. When treated animals were monitored for long-term antitumor effects, combinations with IL-1 α significantly increased the tumor-regrowth delay and decreased the fractional tumor volume ($P<0.001$). These results demonstrate that IL-1 α synergistically enhances cDDP mediated in vivo antitumor activity and suggest that the combination of IL-1 α and cDDP may have potential therapeutic application in the design of effective treatment modalities for cancer.

## Introduction

Cytokines and other biological response modifiers (BRMs) are potent physiological effectors with many diverse activities that can be exploited as therapeutics in cancer. They have been evaluated for antitumor efficacy as single agents as well as for their ability to enhance the traditional chemotherapeutic drug-mediated antitumor response. Direct antitumor activities are most often associated with tumor necrosis factor (TNF) [9, 40]. Interferon (IFN) has been reported to have antiproliferative effects on tumor-cells [41], and IL-1 α has been demonstrated to be cytotoxic in vitro against a limited number of human cancer cell lines [23, 33]. These cytokines, as well as interleukin-2 (IL-2), have also been combined with various chemotherapeutic agents to obtain enhanced cytotoxicity both in vitro and in vivo [1, 17, 29, 44, 46, 47].

Studies in our laboratories have demonstrated that IL-1 α has in vivo antitumor activity in the RIF-1 (fibrosarcoma) and $PancO_2$ (adenocarcinoma) tumor-model systems that is characterized by the induction of acute hemorrhagic necrosis, microvascular injury, enhanced clonogenic tumor-cell kill, and a decrease in tumor blood flow

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*Abbreviations:* IL-1 α, interleukin-1 α; cDDP, *cis*-diamminedichloroplatinum (cisplatin); BRMs, biological response modifiers; TNF, tumor necrosis factor; IFN, interferon; Fa, fraction affected; $D_m$ or $ED_{50}$, drug concentration necessary to produce Fa = 0.5 as compared with untreated controls; CI, combination index; SF, surviving fraction; ANOVA, one-way analysis of variance

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[5]. These acute hemorrhagic activities have been characterized by [$^{31}$P]-nuclear magnetic resonance (NMR) [7, 14], can be significantly suppressed by glucocorticoids [6, 7], are not the result of a direct tumor effect, and appear to be T-cell-independent [5]. Despite the close similarities in the biological activities of IL-1 α and TNF, [24], IL-1 α -induced hemorrhagic necrosis is not mediated through TNF [20]. Although significant acute tumor-cell kill is observed in vivo, IL-1 α-induced hemorrhage does not result in substantial or sustained inhibition of tumor growth [5]. However, combinations of IL-1 α with mitomycin C or porfiromycin have greatly enhanced the clonogenic tumor-cell kill and tumor-regrowth delay as compared with those observed for either agent alone [8].

In the studies reported herein, IL-1 α's ability to enhance cytotoxic drug efficacy was further investigated by its combination with the widely used chemotherapeutic drug *cis*-diamminedichloroplatinum (cDDP), and the extent of the interaction between the two agents was formally examined by median dose-effect analysis.

## Materials and methods

*Tumor model system.* The RIF-1 fibrosarcoma model was maintained according to the protocol of Twentyman et al. [43] as previously described [4] in 6- to 10-week-old female C3H/HeJ mice obtained from Jackson Laboratories (Bar Harbor, Me.). The mice were age- and sex-matched for experimental use, regularly monitored for the absence of adventitious murine viruses, and housed and cared for in accordance with institution guidelines. RIF-1 tumors were routinely produced by s.c. inoculation of $5 \times 10^5$ log-phase tissue-culture cells in the right flank of the animals. Studies were initiated 14 days later when tumors had reached a weight of 0.5–0.7 g.

*IL-1 α and cDDP therapy.* Recombinant murine IL-1 α was provided by Dr. P. Lomedico (Hoffmann-LaRoche, Nutley, N.J.). The specific activity was $1.3 \times 10^8$ U/mg protein as determined in our laboratory using the D10.G4.1 assay [22]. All solutions were made with pyrogen-free reagents. The endotoxin content of the IL-1 α was 0.05 endotoxin units/mg. IL-1 diluted in 0.9% NaCl with 0.05% bovine serum albumin was injected i.p. at various doses in a total volume of 0.25 ml. No significant toxicity was observed at IL-1 α doses of 240 μg/kg or less. At the highest dose, 480 μg/kg, animals experienced transient toxicities during the first 24 h posttreatment, including a 10%–15% weight loss, hair ruffling, and loss of appetite. cDDP (Bristol-Myers Co., Evansville, Ind.) was diluted in 0.9% NaCl and was injected i.p. at various doses in a total volume of 0.25 ml. At 14 days posttransplantation, RIF-1 tumor-bearing C3H/HeJ mice were treated i.p. with either IL-1 α , cDDP, or concurrent administration of IL-1 α and cDDP were left untreated.

*Clonogenic tumor-cell survival.* The effect of IL-1 α and/or cDDP on clonogenic tumor-cells in vivo was determined by a modification of the excision clonogenic cell-survival assay described by Twentyman et al. [43]. RIF-1 tumors (3–5/treatment group) as described above were removed at 24 h after the i.p. administration of IL-1 α and/or cDDP, weighed, and finely minced with scissors. Aliquots of minced tumor were enzymatically dissociated for 60 min at room temperature under constant stirring in 125-ml flasks containing 37.5 mg type I collagenase/ml (Sigma, St. Louis, Mo.), 5 mg DNAse/ml (Sigma) and 1% trypsin-ethylenediaminetetraacetic acid (EDTA; Gibco, Grand Island, N.Y. An additional 200 μg DNAse/ml was added for the last 15 min of the incubation period. Tumor-cells were then harvested by passage through four layers of sterile gauze placed over a 27-gauge stainless-steel screen, centrifuged, and resuspended in RPMI 1640 plus 12% fetal calf serum (FCS).

Viable tumor-cells were counted in 10% trypan blue and 2% acetic acid and then diluted in RPMI 1640 plus 12% FCS, and various dilutions were seeded in 6-well tissue-culture plates (Costar, Cambridge, Mass.). Smaller identifiable cells such as granulocytes and lymphocytes were not counted. In addition, numbers of other contaminating cells such as mouse stromal cells, macrophages, fibroblasts, and vascular endothelial cells, which are indistinguishable from viable tumor cells, remained constant in both the control and treated tumor groups, and therefore did not interfere with the evaluation of tumor-cell survival.

After 7 days incubation at 37°C in an atmosphere containing 5% $CO_2$, colonies were counted and the numbers of clonogenic cells per gram of tumor were enumerated. The mean (± SD) cell yield, cloning efficiency, and number of clonogenic cells from 17 experiments for control (no treatment) tumors averaged $81.5 \pm 23.5 \times 10^6$ viable tumor cells/g tumor, $25.2\% \pm 0.3\%$, and $20.5 \pm 0.6 \times 10^6$ clonogenic tumor-cells/g tumor, respectively. For accurate comparisons between treatment groups, tumors were always handled and processed identically throughout the assay procedure. The surviving fraction per gram of tumor is defined as the number of clonogenic tumor-cells per gram of treated tumor divided by the number of clonogenic tumor cells per gram of control (untreated) tumor.

The effect of IL-1 α and/or cDDP on clonogenic tumor-cells in vitro was determined by preincubating monoloayers of isolated tumor-cells with 1000 units IL-1 α/ ml for 4 h at 37°C. Monolayers were then washed twice with media and incubated for 1 additional h with varying doses of cDDP, after which cells were plated in the clonogenic assay and incubated for 7 days.

*Quantitation of hemorrhage.* Hemorrhagic necrosis in RIF-1 tumors was determined using the $^{59}$Fe-labeled red blood cell method as described elsewhere [4, 5].

*Assessment of synergy.* The drug interaction was quantitated by median dose-effect analysis [11, 12]. RIF-1 tumor-bearing C3H/HeJ mice (14 days post-treatment) were treated i.p. with various doses of IL-1 α and/or cDDP and, after 24 h, clonogenic tumor cells were enumerated by clonogenic assay. Dose-effect curves were generated for each agent alone and were plotted as the fraction affected (Fa) versus the concentration. The $D_m$ or $ED_{50}$ was defined as the drug concentration necessary to produce Fa = 0.5 as compared with untreated controls. Dose-effect curves were then plotted from experiments of serially diluted combinations using a fixed ratio (interactive ratio) that was based on the $D_m$ of each agent using the median-effects equation:

$$\frac{F(a)}{F(u)} = \left(\frac{D}{D_m}\right)^m,$$

where $F_u$ is the fraction surviving and $m$ is a coefficient of the sigmoidicity of the dose-effect curve. When the dose-effect relationship follows the principle of mass action, the median-effect plot, which is the logarithmic conversion of the median-effects equation, should be linear. The combination index (CI) as determined by the CI equation was then plotted versus Fa. The CI equation is as follows:

$$CI = \frac{(D)_1}{(Dx)_1} + \frac{(D)_2}{(Dx)_2} + \frac{\alpha\,(D)_1\,(D)_2}{(Dx)_1\,(Dx)_2},$$

where $(Dx)_1$ is the dose of agent 1 required to produce $x$% effect alone, $(D)_1$ is the dose of agent 1 required to produce the same $x$% effect in combination with $(D)_2$, $(Dx)_2$ is the dose of agent 2 required to produce $x$% effect alone, and $(D)_2$ is the dose required to produce the same effect in combination with $(D)_1$. If the agents are mutually exclusive (similar mechanism of action), $\alpha = 0$ and the CI is the sum of two terms. If the agents are mutually nonexclusive (distinct mechanism of action), $\alpha = 1$ and the CI is the sum of three terms. For these studies, when the mechanism was unknown, drug interactions were analyzed as involving both mutually exclusive and mutually nonexclusive drugs. CI was solved for 50%, 70% and 90% effects. CI<1 indicates synergy, CI = 1 indicates additivity, and CI>1 indicates antagonism. The IBM-PC program Dose-Effect Analysis with Microcomputers (Elsevier-Biosoft, Cambridge, UK) was used for data analysis [10].

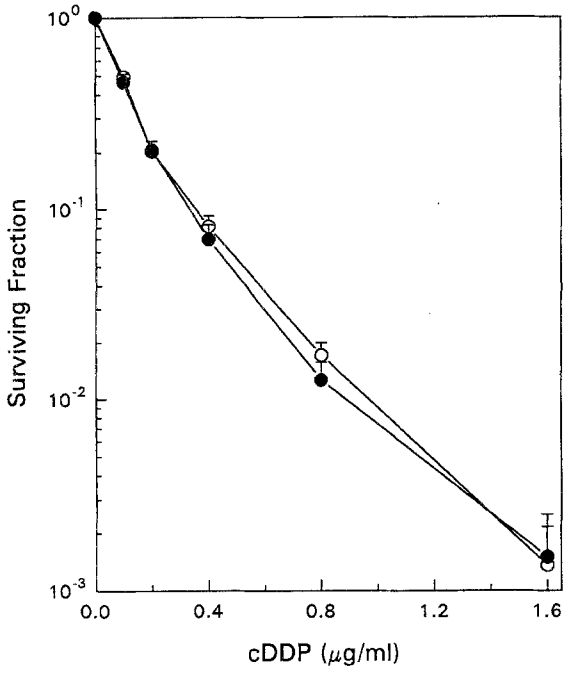


**Fig. 1.** Effect of IL-1α on the in vitro cDDP-mediated RIF-1 tumor-cell kill as determined by modified clonogenic assay. Tumor cells were treated in vitro with varying concentrations of cDDP alone (○) or cDDP plus IL-1α (1,000 units/ml, 4-h pretreatment) (●) and, after 1 h incubation, were plated in a 7-day clonogenic assay. Each point represents the mean value ± SD for the surviving fraction

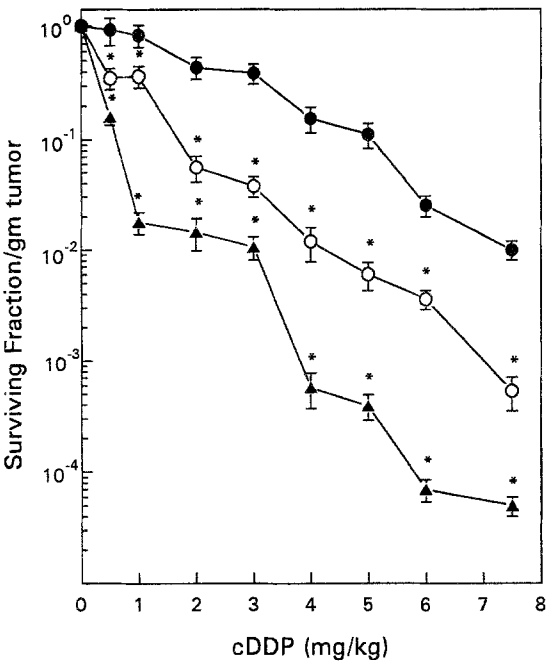


**Fig. 2.** cDDP dose-dependent clonogenic tumor-cell kill in RIF-1 tumors. cDDP was injected i.p. alone (●) or in combination with 48 (○) or 480 (▲) μg/kg IL-1α. Each point represents the mean (± SD) survival fraction for total clonogenic cells per gram of tumor (3–5 mice/treatment group). * Values significantly different from those obtained using cDDP alone ($P < 0.001$, ANOVA)

*Tumor-regrowth delay.* For determinations of the effect of IL-1 α and/or cDDP on RIF-1 tumor growth, mice bearing 14-day-old RIF-1 tumors were treated with single-dose, simultaneous i.p. injections of IL-1 α and/or cDDP and were monitored by measurement of tumor diameters with calipers three times a week. As described previously [5], tumor volumes were calculated using the formula volume = length × width$^2$/2 and were expressed as a fraction of the pretreatment size. The tumor-regrowth delay was determined as the time required for treated tumors to reach four times the pretreatment size minus that required for control (untreated) tumors.

*Statistical analysis.* One-way analysis of variance (ANOVA) was used to assess the level of significance between treatment groups.

## Results

### *Direct effects of IL-1 α and cDDP on RIF-1 tumor cells in vitro*

For determinations of the ability of IL-1 α to increase in vitro sensitivity to cDDP, tumor-cells were plated in the clonogenic cell assay with a set concentration of IL-1 α and varying dilutions of cDDP. As shown in Fig. 1, RIF-1 tumor cells were sensitive in a dose-dependent manner to cDDP in vitro and IL-1 α had no enhancing effect on cDDP-mediated cytotoxicity. The results were the same at IL-1 α concentrations of up to $10^6$ units/ml when cells were treated at various intervals with IL-1 α either before or after cDDP and when IL-1 α was present throughout the entire culture period (data not shown).

### *IL-1 α /cDDP effects on the in vivo clonogenic tumor-cell kill and IL-1 α -induced hemorrhage*

For determinations of the effect of IL-1 α on cDDP-mediated tumor-cell kill in vivo, animals were examined for clonogenic tumor-cell survival using the excision assay from each of the treatment groups. As shown in Table 1, the cell yield was substantially diminished by IL-1 α alone and IL-1 α plus cDDP but not by cDDP alone. In contrast, the surviving fraction (SF) was reduced by cDDP alone and was further reduced when IL-1 α was given concurrently with cDDP.

We have previously shown that IL-1 α alone induces acute hemorrhage in a variety of different tumor types [5–7, 14, 20]. To determine whether cDDP would have any effect on IL-1 α's hemorrhagic activity, treated RIF-1 tumor-bearing mice were examined for vascular hemorrhage using the $^{59}$Fe assay. As shown in Table 1, IL-1 α induced significant hemorrhage in RIF-1 tumors but not in muscle tissue. cDDP alone was not hemorrhagic, nor did cDDP increase IL-1 α -mediated hemorrhage at 6 h after its injection.

To determine whether clonogenic tumor-cell effects were dose-related, we treated tumor-bearing animals i.p. with cDDP at varying doses either alone or in combination with IL-1 α at doses of 480 or 48 μg/kg. As shown in Fig. 2, IL-1 α at both doses significantly enhanced cDDP-mediated cytotoxicity (decrease in the surviving fraction), even at the lowest doses of cDDP, at which little cytotoxicity was observed for cDDP alone. Similarly, animals were treated with 6.0 mg/kg cDDP plus varying doses of IL-1 α.

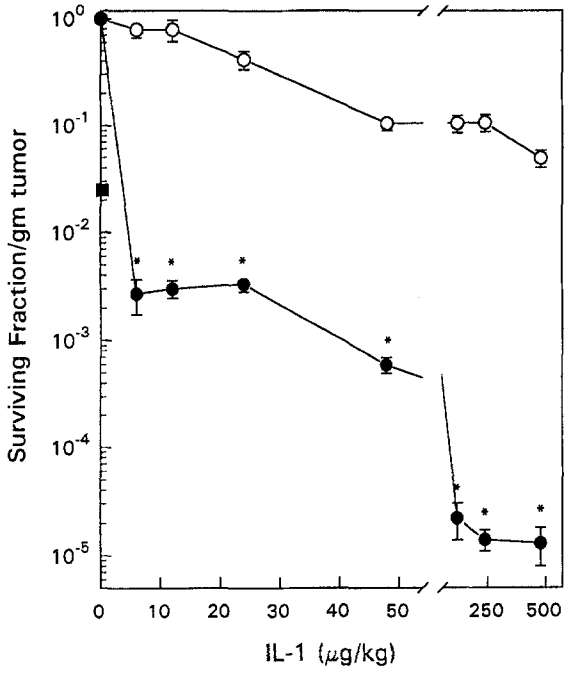


**Fig. 3.** IL-1α-dose-dependent enhancement of the clonogenic tumor-cell kill in RIF-1 tumors. IL-1α was injected i. p. alone (○) or in combination with 6.0 mg/kg cDDP (●), or cDDP (6.0 mg/kg) was injected i. p. in the absence of IL-1α (■). Each point is defined as described in Fig. 2. * Values significantly different from those obtained using either IL-1α or cDDP alone ($P$ <0.001, ANOVA)

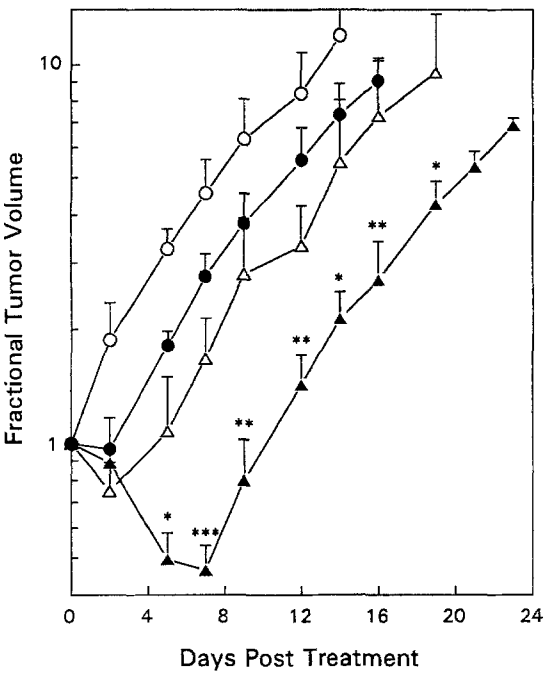


**Fig. 4.** Effect of IL-1 and cDDP on RIF-1 tumor growth. Tumor-bearing C3H/HeJ mice (~0.5–0.7 g tumors) were either left untreated (○) or treated with a single i. p. dose of either cDDP (6.0 mg/kg) (△), IL-1 (480 μg/kg) alone (●), or a combination of IL-1α plus cDDP at the same doses (▲) at time zero. Each point represents the mean value ± SD for 10–12 tumors. Tumor volumes were determined using the formula v = (length × width$^2$)/2 and were expressed as a fraction of the volume on day 0 (fractional tumor volume). Values significantly different from those obtained using cDDP alone are shown with asterisks (* $P$ <0.05, ** $P$ <0.01, *** $P$ <0.001; ANOVA)

As shown in Fig. 3, the combination of cDDP plus IL-1 α was capable of significantly enhancing tumor-cell cytotoxicity in a dose-dependent manner, even at the lowest IL-1 α dose tested (6.0 μg/kg), as compared with IL-1 α alone or cDDP (6.0 mg/kg) alone.

*Tumor-regrowth delay studies*

For determinations of the therapeutic efficacy of the combination regimen, tumor-bearing mice were treated and monitored for tumor growth. In the RIF-1 tumor-model system, the combination of cDDP and IL-1 given simultaneously resulted in fractional tumor volumes that were significantly lower than those obtained in animals treated with either cDDP alone or IL-1 α alone or in control mice (Fig. 4). In addition, IL-1 α alone and cDDP alone resulted in significant antitumor activity as compared with control values. Between day 4 and day 8 posttreatment, when fractional tumor volumes were lowest in the IL-1 α/cDDP group, the tumors were barely measurable and regression appeared almost complete.

To determine the effect of IL-1 α given with a lower dose of cDDP, which itself does not induce significant tumor-regrowth delay, we treated animals bearing 14-day-old RIF-1 tumors concurrently with cDDP and IL-1 α and monitored them for antitumor effects. As shown in Table 2,

**Table 1.** Effect of IL-1α/cDDP on the clonogenic tumor-cell kill and hemorrhage

| Treatment[a] | Cell yield/g[b] (× $10^6$) | SF/g[b] (× $10^{-2}$) | Tumor[c] | Muscle[c] |
|---|---|---|---|---|
| None | 89.5 ± 16.9 | 100 | 22.2 ± 3.6 | 10.0 ± 4.2 |
| IL-1α | 5.7 ± 3.1* | 5.820 ± 1.205* | 54.2 ± 7.1* | 14.8 ± 6.2 |
| cDDP | 66.7 ± 19.5 | 2.291 ± 0.247* | 22.8 ± 4.9 | 15.1 ± 5.2 |
| IL-1α+cDDP | 4.8 ± 2.8*, ** | 0.007 ± 0.002*, ** | 63.6 ± 12.1* | 11.7 ± 4.7 |

[a] C3H/HeJ mice (3–4/group for clonogenic assay and 8–10/group for hemorrhage) bearing 14-day-old RIF-1 tumors were treated i. p. with either cDDP (6 mg/kg) alone, IL-1α ($1.25 \times 10^6$ units/mouse, or 480 μg/kg) alone, or a combination of cDDP plus IL-1. For determination of the clonogenic cell kill, tumors were removed 24 h later and plated in the clonogenic assay. For hemorrhage studies, samples of the tumor and femoralis muscle were taken 6 h after treatment

[b] Mean (± SD) cell yield and surviving fraction (SF)/g tumor

[c] Mean (± SD) volume in μl of packed RBC/g tissue

* Significantly different from control (no treatment; $P$ <0.001, ANOVA)

** Significantly different from cDDP alone ($P$ <0.001, ANOVA)

**Table 2.** Effect of IL-1α on the low-dose cDDP-mediated tumor-regrowth delay

| Treatment[a] | Tumor-regrowth delay[b] (days) |
|---|---|
| IL-1α | 4.90 ± 2.00 |
| cDDP (2 mg/kg) | 3.03 ± 1.85 |
| cDDP (2 mg/kg) + IL-1α | 8.04 ± 2.01* |
| cDDP (6 mg/kg) | 9.42 ± 2.36 |
| cDDP (6 mg/kg) + IL-1α | 13.65 ± 2.56* |

[a] Mice bearing 14-day-old RIF-1 tumors (12–25 animals/group) were treated i.p. with either IL-1α (480 μg/kg), cDDP (2.0 or 6.0 mg/kg), or a combination of the two substances at the same doses

[b] Mean (± SD) tumor-regrowth delay is the difference in the time required for treated versus control tumors (no treatment) to reach a volume 4 times the pretreatment size

* Significantly different from cDDP alone ($P < 0.001$, ANOVA)

**Table 3.** ED values for cDDP and IL-1α given as single agents or in combination[a]

| | Single agent | | Combination (cDDP/IL-1α) |
|---|---|---|---|
| | cDDP | IL-1α | |
| $ED_{50}$ | 649 | 16 | 182/ 5 |
| $ED_{70}$ | 1,235 | 39 | 366/ 9 |
| $ED_{90}$ | 3,445 | 173 | 1,109/28 |

[a] Mice bearing RIF-1 tumors at 14 days postimplantation were treated with a single injection of either cDDP, IL-1α, or a combination of the two agents and were assayed by clonogenic assay 24 h later. The concentration in μg/kg of cDDP and/or IL-1α was predicted at each ED level. The drug ratio used was 40 : 1

IL-1 α at 480 μg/kg was capable of significantly enhancing the cDDP-mediated tumor-regrowth delay in animals treated with 2.0 mg/kg of cDDP, a dose that produces slight antitumor activity when given alone. The antitumor activity as measured by the tumor-regrowth delay obtained with 6.0 mg/kg of cDDP alone was similar to that observed for the lower 2.0-mg/kg dose in combination with IL-1 α . In these studies and others conducted in our laboratories [5–8, 14], a surviving fraction of $<10^{-3}$ as measured by the clonogenic tumor-cell kill always correlated with a significant level of tumor-regrowth delay for the analogous treatment regimen.

### *IL-1* α and cDDP synergistic effects

To determine whether the interaction between IL-1 α and cDDP was additive or synergistic, we applied standard median dose-effect analysis as described by Chou and Talalay [12]. As shown in Fig. 5, the interactions of cDDP and IL-1 α were strongly synergistic (plot below the horizontal line) over the range of concentrations tested and even at the lower doses of each agent. These conclusions remained unchanged, whether mutual exclusivity or mutual nonexclusivity of action was assumed. Similarly, as shown in Table 3, predicted ED values demonstrated a clear synergistic interaction between cDDP and IL-1 α, with a marked reduction in the effective drug concentration being observed at the $ED_{50}$, $ED_{70}$ and $ED_{90}$ levels.

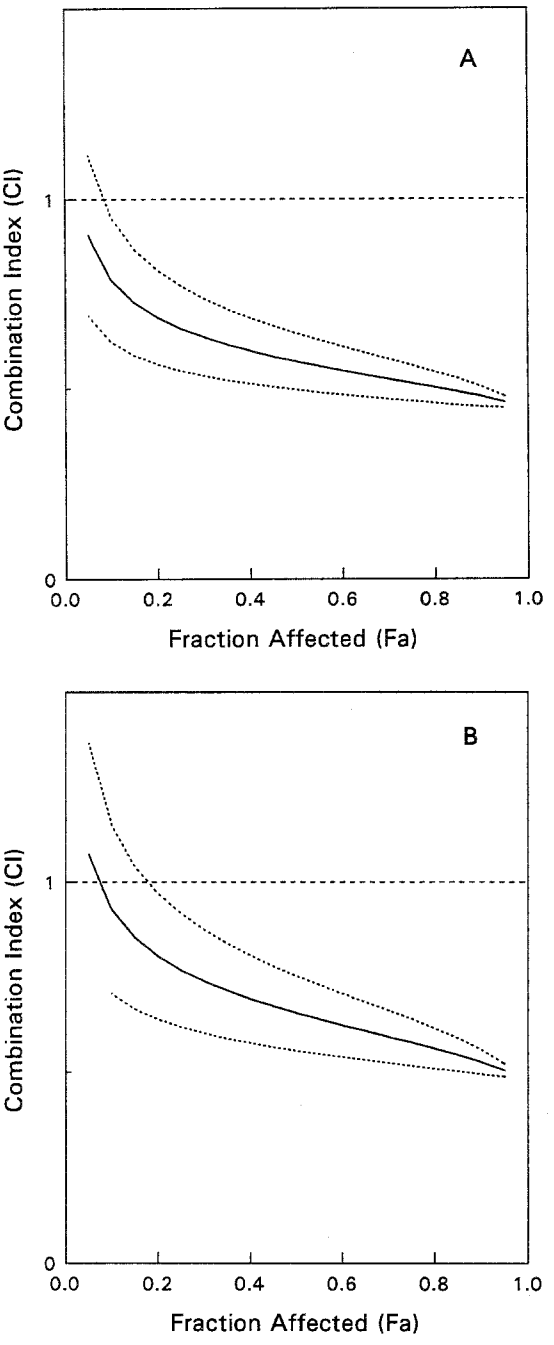


**Fig. 5 A, B.** Fa-CI plot of the mutually exclusive (**A**) and mutually nonexclusive (**B**) interaction of IL-1α and cDDP in the in vivo clonogenic tumor-cell-kill assay. The *solid line* represents the mean value ± SD (*dotted line*) for 3–5 replicate experiments

## Discussion

The addition of BRMs to standard chemotherapy regimens could impact the therapeutic design via three potential mechanisms: (1) an increase in the tumor-cell kill; (2) a reduction in the required maximal effective dose of drug,

resulting in a diminution of drug-induced toxicity; and/or (3) the stimulation of hematopoietic progenitors, leading to an increase in the maximum tolerated dose and thus to the potential for dose escalation.

BRMs have been shown to enhance the antitumor efficacy of a variety of cytotoxic drugs. TNF [1, 47], IL-2 [17], IFN [46] and IL-1 [8, 29, 44] have been shown to increase the antitumor activity of numerous chemotherapeutic agents both in vitro and in vivo. In addition, both the colony-stimulating factors (CSFs) and IL-1 have been shown to ameliorate drug-induced myelosuppression in vivo [3, 26, 39]. IL-1 α can enhance the antitumor activity of etoposide in vitro against human melanoma cells [44]. In the Meth A sarcoma or colon 26 adenocarcinoma model system, IL-1 α significantly increased the antitumor effects of cDDP, carboplatin, or thiotepa [29]. Studies conducted in our laboratories have demonstrated that IL-1 α significantly increases the in vivo tumor-cell kill obtained with the bioreductive drugs mitomycin C or porfiromycin in both the RIF-1 and Panc02 model systems [8]. In the present study, we examined the effect of IL-1 α on cDDP mediated antitumor activity using the RIF-1 fibrosarcoma model system and also determined whether the interaction of the two agents was synergistic by median dose-effect analysis.

IL-1 is generally considered not to mediate direct tumor cytotoxic activity, although cytotoxicity has been observed against selected human tumor-cell lines [23, 33]. North et al. [32] have demonstrated that IL-1 β -induced regression of murine tumors requires intact T-cell function and tumor immunogenicity. IL-1 β has also been reported to inhibit the growth of B16 melanoma in C57/BL6 mice [36]. Nakamura et al. [28] have observed that IL-1 α inhibits the growth of several tumors and that indomethacin enhances this inhibition [30].

Using established RIF-1 tumors, a model used extensively for experimental therapeutic evaluation [21, 43], we report herein that IL-1 α can synergistically potentiate antitumor activity when combined with cDDP as measured by an increase in the clonogenic cell kill, a decrease in the fractional tumor volume, and an increase in the tumor-regrowth delay. These antitumor activities are distinguishable from the acute hemorrhagic effects in that they are observed over a longer period and they result in a measurable decrease in tumor size. Similarly, cDDP alone did not result in acute hemorrhage, nor did cDDP have an effect on IL-1 α -induced hemorrhage. In addition, these effects are observed only in vivo. IL-1 α alone has no in vitro cytotoxic effect [5], and the data presented herein demonstrated no enhancement of cDDP-mediated in vitro cytotoxicity toward RIF-1 tumor-cells in the presence of IL-1 α .

In the clonogenic tumor-cell studies, the disparity in the effects on tumor-cell yield versus cell survival observed for IL-1 α and cDDP probably relates to differences in the effects of these agents on cell lysis or recovery versus clonogenicity. The SF observed in the present study for murine IL-1 α given alone or in combination was somewhat lower than that reported from previous studies [6, 7, 14] that used recombinant human IL-1 α . Although IL-1 α lacks species specificity [34], we have previously shown that murine IL-1 α also has a greater effect on mouse hematopoietic progenitors in vivo as compared with human IL-1 α [19].

Regrowth-delay studies in animal tumor-model systems are a measure of both tumor-cell death and posttreatment growth rate [2]. This measurement of antitumor activity in vivo may not correlate with median survival studies in which the endpoint is quite different. In many ways, a similar situation exists in the clinical evaluation of new treatment regimens, whereby chemo-therapeutic drugs have been developed on the basis of their tumor-response rates (complete or partial). In both the animal-model and clinical settings, tumors respond only temporarily by regression, which is usually followed by recurrence or a rebound of tumor growth. The data presented herein focused initially on the effects of a single administration of IL-1 α /cDDP on the clonogenic cell kill and tumor-regrowth delay. Studies are currently in progress to determine whether further cycles of treatment every week or 5 days can prolong this "regressed" state, ultimately increasing median survival.

According to median dose-effect analysis, the enhancement of cDDP's clonogenic cell kill mediated by IL-1 α was clearly synergistic. The mechanism for the observed synergistic interaction of IL-1 α and cDDP is unknown. The lack of demonstrated IL-1 α activity in vitro on the tumor-cell kill strongly suggests an indirect effect. IL-1 induces other cytokines as well as a variety of cell-regulatory molecules [24, 35]. Growth factors such as epidermal growth factor (EGF) have been shown to increase the sensitivity of tumor-cells to cDDP [13]. IL-1's ability to induce changes in the cell cycle [31] may ultimately sensitize tumor-cells to the effects of cDDP. It is also possible that these effects are related to an antitumor immune function. The effect of the immune response on IL-1 α /cDDP-mediated antitumor activity is unknown. We have demonstrated that the acute antitumor effects of IL-1 α are not dependent on intact T-cell function [5]. The antitumor activity of IL-1 β alone has been shown to exhibit a requirement for intact T-cell function [32]. Although in vivo immunologic reactivity has not always correlated with a positive therapeutic response, the effects of IL-1 on the immune system are not trivial [16, 18, 24]. In addition, natural killer-cell (NK) activity has been shown to be significantly increased by cDDP in vitro and in vivo [25].

Another potential focus for IL-1 α effects is the endothelial cell. Recent studies indicate that many endothelial cell activities can be regulated by IL-1 and TNF through modulation of cell surface molecules [27, 37]. Both of these cytokines enhance procoagulant and prostacyclin activity and increase plasminogen-activator inhibitors. IL-1 and TNF also cause endothelial cells to become markedly adhesive for neutrophils, monocytes, and lymphocytes [37, 38], thus providing an important early step for the establishment of leukocyte infiltration. These vascular effects culminate in hypotension, diffuse intravascular coagulopathy, and vascular leakage. We have shown significant acute effects on tumor blood flow following IL-1 α injection as well as capillary damage, hypoxia, and reduced tumor pH in vivo [5–7, 14]. These effects could increase cDDP accumulation in the tumor. As a result, studies are currently in progress to examine IL-1 α's effect on the total

platinum content of tumor versus normal tissue as measured by atomic absorption spectroscopy.

Although cDDP is an effective chemotherapeutic agent, its toxicity can be dose-limiting [45]. Pertinent to the clinical application of IL-1 α /cDDP is the ability of IL-1 α to enhance the clonogenic tumor-cell kill and tumor-regrowth delay when combined with lower doses of cDDP that demonstrate little antitumor activity when given alone. IL-1 β has been used in phase I clinical trials primarily as a bone-marrow-sparing or stimulatory agent [15, 42]. Of significance to its clinical application was that patients in both of those studies experienced a rise in platelet counts, with hypotension being the predominant dose-limiting toxicity. As a direct result of the studies reported herein, phase I clinical trials of IL-1 α with concurrent administration of carboplatin are in progress.

The use of combinations of biological and cytotoxic agents to maximize antitumor effects followed by determination of the mechanisms of synergistic action could have significant impact on the design of effective therapies for cancer. BRMs have primarily been used to boost or enhance the host's immune response to malignancy. Although IL-1 α may have significant effects on the immune system, it may have greater therapeutic applications as a cytokine with antitumor activity that targets specific cytotoxic drug interactions with either the tumor-cell or the endothelium.

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