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Inhibition of DNA Repair and Sensitization of Cisplatin in Human Ovarian Carcinoma Cells by Interleukin-1α

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Interleukin-1α induced an increase in both the cellular accumulation of cis-diamminedichloroplatinum (II) (cisplatin) and DNA platination and significantly reduced the removal of platinum from DNA of human ovarian (NIH: OVCAR-3) carcinoma cells in culture. The combinations of IL-1α and cisplatin were highly synergistic against these ovarian carcinoma cells and maximum levels of sensitization (15-20-fold) were observed during simultaneous exposure of cisplatin and IL-1α. IL-1α specific receptor antagonist decreased this synergy. These results strongly indicate that IL-1α inhibits DNA repair, and this inhibition of DNA repair may explain, in part, a strong synergistic interaction between IL-1α and cisplatin in NIH: OVCAR-3 cells.

Ovarian carcinoma is one of the leading causes of cancer death in females. In recent years, although significant progress has been made in the treatment of this disease using combination chemotherapy, curative therapy is still difficult to achieve. While C-DDP is one of the most commonly used agents for the treatment of ovarian carcinoma (1-3), toxicity as well as emergence of C-DDP-resistant tumors are major problems preventing curative therapy (4, 5). While several mechanisms of resistance to C-DDP

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The abbreviations used are: IL-1α, Interleukin-1α; IL-1RA, Interleukin-1 receptor antagonist; C-DDP, cis-diamminedichloroplatinum (II); MTT, 3-[4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide; AAS, atomic absorption spectroscopy; CI, combination index; FA, fraction affected; FU, fraction unaffected.

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have been identified, the reduction of C-DDP uptake and enhanced DNA repair are important mechanisms contributing to C-DDP resistance (6-9).

Recently, cytokines and lymphokines have been recognized as important biochemical modulators that could either sensitize tumor cells to the effects of other anticancer drugs or exert direct cytotoxic effects on tumor cells. While the mechanism of the antitumor activity of IL-1α is not known, it is now well established that IL-1α has direct antitumor properties both in vitro and in vivo (10,11). Kilian et al (11) have shown that IL-1α inhibited the proliferation of NIH: OVCAR-3 tumor cells in vitro. The human ovarian NIH:OVCAR-3 cells were established in culture from a patient who failed therapy with both Doxorubicin and cisplatin. Previous studies (12) have shown that IL-1α significantly potentiated the antiproliferative activity of Doxorubicin in this cell line, indicating that combinations of IL-1α with other anticancer drugs may be potentially effective against drug-resistant tumors in the clinic.

MATERIALS AND METHODS

Chemicals: Recombinant human IL-1α was kindly provided by Dr. P. Lomedico (Hoffmann-La Roche, Nutley, NJ). Recombinant human IL-1RA was purchased from R & D Systems (Minneapolis, MN). C-DDP was obtained from the Drug Development Branch, National Cancer Institute, NIH, Bethesda, MD.

Cell culture: Human Ovarian Carcinoma (NIH:OVCAR-3) cells (ATCC HTB 161; American Type Culture Collection, Rockville, MD) were maintained in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with antibiotic mixture (5 mg/ml penicillin, 5 mg/ml streptomycin and 10 mg/ml neomycin, Gibco) and 10% fetal bovine serum (Gibco) under standard culture conditions at 37°C in a humidified CO_2 atmosphere.

The cytotoxicities of IL-1α, C-DDP, IL-RA and the combinations were measured by the MTT assay as described previously (12). The analysis for synergy was performed by the combination index median-dose effects methods of Chou and Talalay (13).

For C-DDP accumulation studies, cells were treated with C-DDP (50 μM), harvested by scraping at indicated times following drug treatment, counted and "wet-ashed" according to the method of McGahan et al (14). The Pt concentration in the sample was determined by AAS with Zeeman correction (15,16). For the formation of cisplatin-DNA adducts in DNA following 1 hr C-DDP exposures, cells were grown to 50-60% confluency, and labeled with ^{3}H-thymidine (0.1μCi/ml) for 24 hrs. Cells were washed twice with PBS, supplemented with fresh medium with or without IL-1α, and incubated

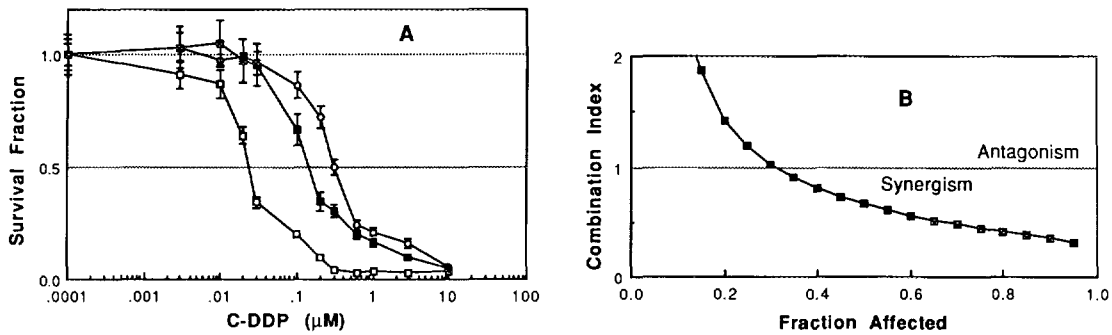

FIGURE 1. Effects of IL-1α and IL-1RA on C-DDP cytotoxicity against NIH:OVCAR-3 cells in vitro during simultaneous exposures for 120 hrs. Effects of IL-1 receptor antagonist on the antiproliferative effects of (A) C-DDP alone (○), in combination with IL-1α (❑), and in the presence of IL-1RA (■) during simultaneous exposures for 120 hrs. IL-1RA was added (200-fold excess) 2 hrs before the addition of other drugs. (B) The combination index was calculated according to the method of Chou and Talalay (13) and the molar ratio between IL-1α and C-DDP was fixed at 1: 100,000. Values are the mean ± SD of 6 replications.

for an additional 24 hrs. For Pt removal studies, following a 1 hr C-DDP exposure, cells were washed twice with ice-cold PBS and harvested at the following time points: 0 hr and 6 hrs later. Total cellular DNA was isolated on cesium chloride density gradients (17), and quantitated by absorbance at 260 nm. Total Pt present in DNA was measured by AAS as described previously (15,16). ^{3}H-Thymidine content was determined by liquid scintillation counter. A decrease in the specific radioactivity of DNA (dpm/μg DNA) at each time point compared to that obtained at 0 hr represents DNA replication. This ratio was used to determine the platinum content of non-replicated DNA.

RESULTS AND DISCUSSION

Cytotoxicity Studies: As shown in Figure-1, simultaneous treatment of NIH:OVCAR-3 cells with IL-1α and C-DDP resulted in a significant (15-20-fold) enhancement of C-DDP cytotoxicity. Similarly, the pretreatment of cells with IL-1α for 24 hrs also reduced the concentration of C-DDP required for 50% cell kill by 20-fold and showed a strong synergy between C-DDP and IL-1α. Pretreatment of cells with IL-1α for 24 hrs followed by 1 hr C-DDP exposure also resulted in a significant enhancement of C-DDP cytotoxicity. While the IL-1 receptor antagonist significantly decreased the cytotoxicity of IL-1α, it had no effect on the cytotoxicity of C-DDP in NIH: OVCAR-3 cells. Furthermore, the IL-1RA also reduced the cytotoxic effects of the combination of IL-1α with C-DDP, however, the combination was still synergistic (Figure-1B).

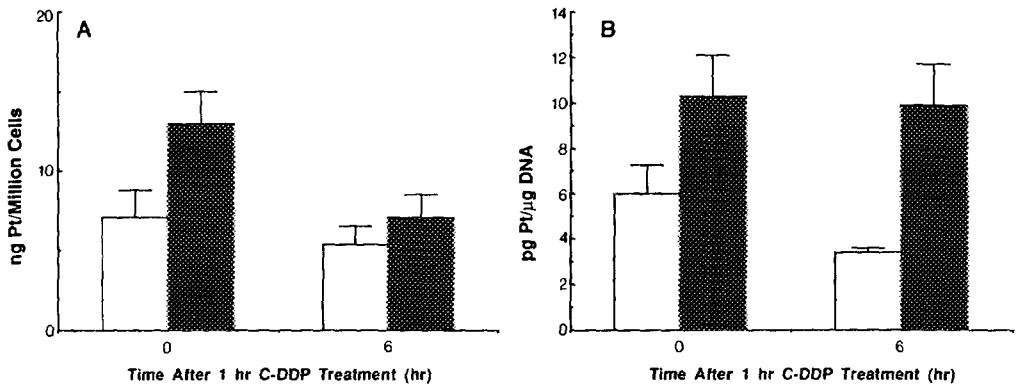

FIGURE 2. Effects of IL-1α on cellular accumulation/efflux of C-DDP (A) and DNA-Pt adduct formation and removal (B) with time. Cells were either pretreated (■) or not treated (□) with 1 nM of IL-1α for 24 hrs before treatment with 50 μM C-DDP for 1 hr. The Pt-DNA adduct formation and the removal of Pt from DNA were carried out as described in the methods section. Values are mean ± SD for at least 3 independent experiments.

Effects of IL-1α on C-DDP Accumulation: The mechanism of the synergy between these agents may involve the modulation of the cellular accumulation of C-DDP by IL-1α . Data show that the pretreatment of NIH: OVCAR-3 cells with IL-1α for 24 hrs, slightly increased (1.5-fold) C-DDP accumulation during a 1 hr C-DDP exposure (Figure-2A). This increased accumulation of C-DDP was dependent upon the concentration of IL-1α used. It should be noted that this increase in C-DDP accumulation was observed with as little as 10 pM IL-1α; the maximum increase was observed when cells were pretreated with 0.1-1 nM IL-1α (Data not shown). While the absolute amounts of cellular Pt were higher, the rates of C-DDP efflux were similar in both groups, indicating that IL-1α did not inhibit C-DDP efflux (Figure 2B).

Effects of IL-1α on Pt-DNA Adduct Formation and Removal: Because IL-1α pretreatment increased C-DDP cellular accumulation, it was possible that IL-1α also increased Pt-DNA adduct formation. Our results, (Table-1) show that pretreatment of NIH: OVCAR-3 cells with IL-1α, significantly increased Pt-DNA adduct formation compared to the non-IL-1α-treated cells. Moreover, IL-1α treatment significantly decreased Pt removal from cellular DNA and as little as 10 pM of IL-1α completely inhibited Pt-DNA adduct removal (Table-1).

Table 1: Dose-dependent effects of IL-1α on Pt-DNA adduct formation and removal at 6 hrs in NIH: OVCAR-3 Cells

IL-1α Dose (pM)	C-DDP Dose (μM)	Amount of Adduct Formed		Amount of Adduct Removed At 6 hrs		
		pg Pt/μg DNA	% of Control Level	pg Pt/μg DNA	% Removed	% of Control
0	50	5.97 ± 1.34	100	2.56 ± 0.21	43.0	100
10	50	7.46 ± 1.74	125	0.17 ± 0.04	2.3	5.3
100	50	8.34 ± 2.85	140	0.41 ± 0.03	4.9	11.4
1000	50	10.3 ± 1.75	173	0.38 ± 0.12	3.7	8.6

The Pt-DNA adduct formation and removal were carried out as described in Methods. Cells were pretreated with IL-1α for 24 hrs prior to the addition of 50 μM C-DDP for 1 hr.

In this report, we examined the interactions of IL-1α with cisplatin in human ovarian NIH:OVCAR-3 cells as a potentially important drug combination for the treatment of ovarian carcinoma. Our results clearly show that the combination of these agents was highly synergistic against this cell line in vitro during both simultaneous and sequential exposures. Interestingly, the presence of IL-1α was necessary for the synergistic interactions and indicate that the binding of IL-1α to its membrane receptor and IL-1α internalization may be necessary for IL-1α-mediated antitumor activity, and also for the potentiation of C-DDP cytotoxicity. This interpretation was further supported by the observation that inclusion of the IL-1α specific receptor antagonist inhibited the cytostatic effects of IL-1α, and decreased the potentiation of C-DDP cytotoxicity.

Studies to define the cellular and molecular mechanisms of synergy between C-DDP and IL-1α in NIH: OVCAR-3 cells indicate that IL-1α had profound effects on cisplatin cellular and subcellular pharmacokinetics. First, pretreatment of cells with IL-1α increased C-DDP uptake in a dose-dependent manner which was observed as low as 10 pM of IL-1α. However, the efflux of C-DDP was not affected. IL-1α significantly enhanced Pt-DNA adduct formation in IL-1-treated NIH: OVCAR-3 cells over untreated cells. More importantly, inhibition of DNA repair induced by IL-1α, at concentrations that are easily achieved in vivo, was observed for up to 24 hrs following C-DDP treatment, resulting in about 3 to 4-fold more DNA-Pt adducts in the IL-1α-treated cells compared to the untreated cells.

While mechanisms of inhibition of Pt-DNA adduct repair in ovarian cancer cells by IL-1α are not clear, decreased removal of these lethal lesions by the cytokine may be one of the reasons for the observed synergy between IL-1α and C-DDP. IL-1α has been reported to decrease high energy phosphate in tumors in vivo (18, 19). It has been postulated that this may lead to disruption of the activities of energy-dependent repair enzymes as well as the synthesis of ATP (18,19). Since the repair of cisplatin-DNA is carried out by excinucleases which are ATP dependent enzymes (20), it is possible that IL-1α-induced inhibition and/or depletion of ATP synthesis may be involved in this inhibition of repair and hence the increased sensitization to C-DDP-induced cytotoxicity. We are currently examining this hypothesis as well as the exact nature of the repair enzymes affected by IL-1α in this ovarian cancer cell line.

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