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Interleukin-1α-Induced Modulation of Topoisomerase I Activity and DNA Damage: Implications in the Mechanisms of Synergy with Camptothecins *In Vitro* and *In Vivo*

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SUMMARY

Studies have shown that cytokines are directly cytotoxic to tumor cells *in vitro* and *in vivo* and that interleukin-1α (IL-1α) potentiates the cytotoxicity of certain clinically active drugs in a number of human tumor cells, including carcinomas of breast and ovary. We found that interleukin-1α potentiated cytotoxicity of camptothecin (4–5-fold) during simultaneous drug exposure in human ovarian NIH:OVCAR-3 cancer cells *in vitro*. Studies indicated that IL-1α significantly increased topoisomerase I-catalyzed camptothecin-induced DNA cleavable complexes in the ovarian cell line, which was not due increased intracellular camptothecin as IL-1α failed to effect cellular uptake of camptothecin. Pretreatment of the ovarian cells with IL-1α did not result in increased expressions of mRNA for the topoisomerase I gene, whereas a small increase (~1.5-fold) in the expression of topoisomerase I protein was observed, suggesting that IL-1 modulated the activity of topoisomerase I for the observed increase in cleavable complex formation. Treatment of human ovarian tumor cells grown as xenografts in nude mice with IL-1α followed by CPT-11 at minimally toxic doses significantly (5–6-fold) enhanced antitumor activity of either agent alone. Because camptothecins are active against solid tumors *in vivo*, combinations of IL-1α with these active drugs may lead to more effective treatment of ovarian cancers in the clinic.

Camptothecin and its derivatives, e.g., topotecan and CPT-11, have been shown to be effective against human tumor cells *in vitro* (1, 2). The mechanism of action of camptothecins results from its interactions with topo I. Topo I, like topo II, is a nuclear enzyme and is involved in DNA replication and the regulation of DNA topological structures; it has been shown that the inhibition of topo I by camptothecin results in cell death. Topo I, however, unlike topo II, is not a cell cycle-dependent enzyme and therefore is a more desirable target for solid tumors. In this regard, several recent reports have indicated that inhibitors of topo I are effective against solid tumors *in vivo* (3–5).

Selection for drug-resistant phenotypes during antineoplastic chemotherapy continues to be a major obstacle to therapeutic success (6, 7). This is particularly true in the treatment of ovarian cancer, which is one of the leading causes of death in women. Recent phase I and II clinical trials with biological response modifiers alone (e.g., tumor necrosis factor-α) had somewhat disappointing results due to the use of patients who were heavily pretreated with various chemotherapeutic agents, including drugs of the multidrug-resistant family (8, 9). As a result, these patients' tumors may have been selected for drug resistance before therapy.

To treat cancer more effectively, it is important to design and develop new chemotherapeutic agents with improved activity. In addition, design strategies should include optimal combinations of currently effective cytotoxic drugs with cytokines, which may play an important role in killing human tumors. Combinations of cytotoxic agents and biological response modifiers have evolved as an important modality for treating cancers because it is believed that the combinations of these agents may act differently than chemotherapeutic agents (10, 11). IL-1 has shown synergistic antitumor effects in combination with tumor necrosis factor toward malignant melanoma (12) and chondrosarcoma (13).

IL-1α is produced by activated monocytes and macrophages and possesses a wide range of biological activities (14, 15). Several studies suggest that IL-1α may directly inhibit the proliferation of certain tumor cells and exert antitumor activity indirectly by enhancing cellular immune responses. IL-1α is cytocidal/cytostatic *in vitro* against the human melanoma cell line A375 (16) and several human breast carci-

ABBREVIATIONS: IL-1α, interleukin-1α; CPT, camptothecin; CPT-11, 11–7-ethyl-10-hydroxy-(piperidine) ester derivative of camptothecin; topo I, topoisomerase I; PBS, phosphate-buffered saline; DMSO, dimethylsulfoxide; NB, nucleus buffer containing protease inhibitors; SDS, sodium dodecyl sulfate; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; CDDP, cisplatin; PKC, protein kinase C; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

noma cell lines (17) and has shown antitumor activity *in vivo* against murine pancreatic cancer (18). Although the exact mechanism of IL-1α-induced biological effects *in vitro* or *in vivo* is not defined, IL-1α binding to membrane receptors and its subsequent internalization may be necessary for these antiproliferative effects (19). However, very little is known about the relationship between the antitumor effects of IL-1 and the activation of signaling pathways or other intracellular mechanisms triggered by IL-1 receptor occupancy.

Previous studies from our laboratory have indicated that IL-1α is highly synergistic with etoposide (VP-16) and adriamycin in human melanoma A375 cells (20–22). Recently, we used human ovarian NIH:OVCAR-3 cell line as our model cell line to further characterize mechanisms of tumor cell killing by IL-1α because OVCAR-3 cells contain a high number of IL-1α receptors per cell and are sensitive to IL-1α (23, 24). NIH:OVCAR-3 cells were obtained and established in culture from a patient undergoing chemotherapy and were found to be clinically resistant to doxorubicin, cisplatin, and melphalan (25). We have shown that simultaneous treatment of NIH:OVCAR-3 cells with IL-1α, adriamycin, and CDDP results in significant enhancements of the drug cytotoxicity (24, 26). We have also found that IL-1α significantly potentiated the antitumor activity (>5-fold) of carboplatin, an analogue of CDDP, in NIH:OVCAR-3 xenografts in nude mice *in vivo* (27). These studies indicate that the use of IL-1α with certain anticancer drugs may provide significant potentiation of antineoplastic activity in the clinic to overcome drug-resistant tumors.

In the present study, we examined the interactions of IL-1α with camptothecins, CPT, and CPT-11 *in vitro* and *in vivo*. We found that IL-1α potentiated CPT cytotoxicity 4–5-fold *in vitro* and significantly enhanced antitumor activity of CPT-11 in NIH:OVCAR-3 xenografts *in vivo*.

Materials and Methods

Chemicals. Recombinant human IL-1α was a gift of Hoffmann-La Roche (Nutley, NJ), and CPT and CPT-11 were obtained from the Drug Development Branch, National Cancer Institute, Nationa Institutes of Health (Bethesda, MD). MTT and DMSO were purchased from Sigma Chemical Co. (St. Louis, MO). CPT and CPT-11 were dissolved in DMSO and diluted to appropriate concentrations with complete media or PBS. Guanidine thiocyanate was obtained from Fluka Biochemicals. RPMI 1640 and PBS were obtained from GIBCO-BRL Laboratories (Grand Island, NY). [*methyl*-^{3}H]Thymidine (specific activity, 20.0 Ci/mmol) was purchased from New England Nuclear (Boston, MA). [^{3}H]CPT (specific activity, 10 mCi/mmol) was obtained from Moraveck Chemical Co. (Brea, CA).

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) supplemented with antibiotic mixture (GIBCO) and 10% fetal bovine serum (GIBCO) at 37° in a humidified CO_2 atmosphere.

Cytotoxicity assays. The antitumor activities of the individual drugs and the combinations were measured with the MTT assay (28). OVCAR-3 cells were plated onto 96-well plates (Costar, Cambridge, MA) at a density of 5000 cells/well and allowed to attach for 24 hr before drug treatment. Cells were exposed to the drugs for up to 5 days; for sequential administration, the first drug was removed after 24 hr, and the cells were washed with drug-free medium before the addition of the second drug. Cytotoxicity results were analyzed for synergism by the median dose/combination index method developed by Chou and Talalay (29). According to this method, values of < 1.0 indicate synergism, values near 1.0 indicate additivity, and values of >1.0 represent an antagonistic interaction between the agents.

Isolation of nuclear extracts. Crude nuclear extracts were prepared after IL-1α treatment according to previously described methods (30). Briefly, cells (~10^8 cells, growing in log phase) were treated with IL-1α (100 pM) for various times, scraped in NB (0.15 M NaCl, 1 mM KH_2PO_4, 5 mM $MgCl_2$, 1 mM EGTA, and 1 mM dithiothreitol), and centrifuged. The cell pellet was suspended in 0.3% Triton X-100 in NB. After incubation on ice for 10 min, the cells were centrifuged at 1200 rpm for 5 min, washed with Triton-free NB once, and resuspended in "high salt" NB containing 0.5 M NaCl. The salt extraction was performed on ice for 30 min, and the cells were centrifuged at 15,000 rpm for 30 min. After the addition of 10% glycerol, aliquots were stored at −70°.

KCl/SDS precipitation assay. The *in vitro* formation of covalent topo I/DNA complexes was quantified by the KCl/SDS precipitation assay described by Liu *et al.* (31) with minor modifications. Briefly, the DNA of cells in logarithmic phase (1×10^5/ml) was labeled by the addition of [*methyl*-^{3}H]thymidine to the medium at a final concentration of 1 μCi/ml. After an overnight incubation, the cells were trypsinized and then diluted in fresh medium to equal counts, seeded into 6-well plates, and incubated overnight. The following day, the cells were pretreated IL-1α (100 pM) for various times, with CPT alone (30 min), and with combinations of IL-1α and CPT (for 30 min). Cells were washed with PBS once and lysed by the addition of 1 ml of prewarmed lysis solution (1.25% SDS, 5 mM EDTA, 0.4 mg/ml salmon sperm DNA, pH 8.0, 65°). After shearing chromosomal DNA by passing the cell lysate through a 22-gauge needle, the sample was transferred to a tube containing 0.25 ml of 325 mM KCl. The sample was mixed vigorously for 10 sec, cooled on ice for 10 min, and centrifuged in an Eppendorf microfuge for 10 min at 4°. The pellet was resuspended in 1 ml of a wash solution (10 mM Tris·HCl, 100 mM KCl, 1 mM EDTA, 0.1 mg/ml salmon sperm DNA) and placed at 65° for 10 min with occasional mixing. The suspension was cooled on ice for 10 min and centrifuged, and the pellet was washed again before resuspension in 0.2 ml of water at 65°. Radioactivity was determined by the addition of 5 ml of scintillation fluid and counting of the samples in a liquid scintillation counter.

Western blotting. Nuclear extracts prepared from 5×10^7 cells after IL-1α treatment were run in 4–20% SDS-PAGE gel and transferred electrophoretically onto nitrocellulose membranes. Nitrocellulose membranes were further incubated with an antibody against topo I (kindly provided by Dr. L. Liu, Rutgers University, New Brunswick, NJ) for 2 hr at room temperature. The membranes were rinsed with PBS, treated with an appropriate secondary antibody, and developed.

Northern blotting. RNA was isolated from NIH:OVCAR-3 cells after IL-1α treatment (up to 24 hr) using the guanidine thiocyanate. Approximately 20 μg of RNA were electrophoresed and transferred onto a nylon filter. Hybridizations were carried out for 2 hr with a ^{32}P-labeled probe (cDNA probe for topo I was obtained from Dr. L. Liu) using QuickHyb (Stratagene, La Jolla, CA) as described before (32, 33). Actin was used for loading and quantification of the RNA.

Uptake of [^{3}H]CPT. Uptake studies with CPT were carried out as previously described (34). Cells were grown to 60–70% confluency in 25-cm^2 flasks and then treated with CPT (0.5 μCi/ml [^{3}H]CPT plus unlabeled CPT dissolved in DMSO) and diluted in fresh, complete tissue culture medium (1 μM, final concentration) in the presence or absence of IL-1α (100 pM). Cells were incubated at 37° under a 5% CO_2 atmosphere for various times, and the uptake of CPT was measured after cells underwent extensive washing with ice-cold PBS and were dissolved in 1 N NaOH. Radioactivity in the samples was measured after neutralization with 1 N HCl.

***In vivo* studies.** The human NIH:OVCAR-3 cells ($1.5–1.8 \times 10^7$) were injected subcutaneously into the intrascapular region of female inbred BALB/c athymic nude mice (6–8 weeks, 20–25 g; Charles River Laboratories). The animals were maintained under sterile conditions in microisolator cages and provided with sterile food and

water *ad libitum*. The tumor volume was calculated as described for solid tumors (35). Sensitivity of OVCAR-3 xenografts to IL-1α, CPT-11, or the combinations were determined by injecting drugs (IL-1α, subcutaneous; CPT-11, intraperitoneal) daily or every other day, in sterile normal saline, starting 7–9 weeks after inoculations of the tumor cells. A total of five treatments with drugs or combinations were used; control mice received vehicle only.

Results

Synergism between IL-1α and CPT *in vitro*. Fig. 1 depicts the cytotoxicity of IL-1α, CPT and combinations against NIH:OVCAR-3 cells *in vitro*. Preliminary experiments showed that a maximally synergistic effect could be achieved when the two agents were present at a molar ratio of IL-1α/CPT of 1:2600. A 5-day exposure to IL-1α and CPT as single agent yielded IC_{50} values of 1.1 ± 0.5 pM and 4.7 ± 1 nM, respectively. IL-1α in combination reduced the IC_{50} value for CPT by ~4–5-fold (1.1 ± 0.5 nM). The combination index plots obtained with simultaneous exposures to IL-1α and CPT indicated a strong synergy, and maximum cell killing was achieved when both agents were present simultaneously throughout the 5-day period (Fig. 1).

Because CPT induces topo I-dependent DNA damage, the mechanism of synergy may involve IL-1α-dependent increase in either the activity of topo I or the level of topo I protein, resulting in increased DNA damage. Alternatively, IL-1α may increase the intracellular concentrations of CPT in NIH:OVCAR-3 cells, resulting in increased DNA damage and cytotoxicity. We examined these possibilities.

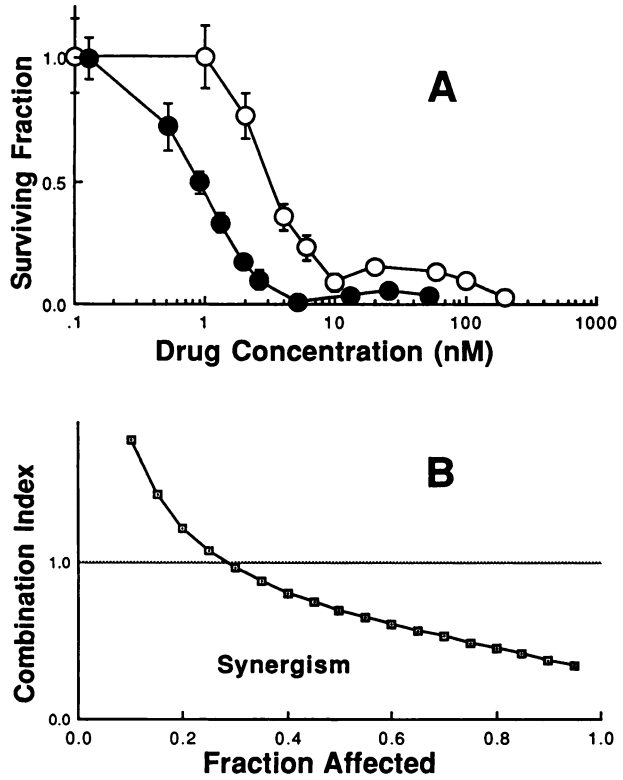

Fig. 1. Dose-response curves (A) of NIH:OVCAR-3 cells to CPT alone (○) and in the presence of IL-1α (●) for 5 days during simultaneous exposures. The molar dose ratio between IL-1α and CPT was fixed at 2600. Values are the mean ± standard deviation of six replications. Similar results were obtained in at least six independent experiments. B, Plots of the combination indices of IL-1α and CPT on NIH:OVCAR-3 cells during simultaneous exposure. The combination indices were calculated according to Chou and Talalay (28) and represent the average of at least three independent experiments.

Effects of IL-1α on topo I protein. Treatment of NIH:OVCAR-3 cells with IL-1α for different times resulted in a small increase in topo I protein levels (1.2–1.5 ± 0.3 versus 1 for treated versus control; five experiments at 30 min and 2 hr); however, these increases were not significant when examined by Western blot analysis with the use of topo I-specific antibody (Fig. 2A). Under these conditions, Northern blot analysis indicated that neither IL-1α nor CPT treatment had any effect on mRNA levels for topo I (Fig. 2B) in the ovarian cells. These observations suggest that pretreatment of NIH:OVCAR-3 cell by IL-1α (up to 24 hr) did not result in increase in the topo I gene expression, nor did it induce the topo I protein expression significantly.

Effects of IL-1α on CPT-induced cleavable complex. The possibility that IL-1α treatment of NIH:OVCAR-3 cells resulted in increased cleavable complex formation, and DNA damage was examined with the SDS/KCl precipitation assay. Results (Fig. 3A) show that treatment of the ovarian cells with CPT resulted in increased formation of SDS/KCl precipitable complexes in a dose-dependent manner, such that 1 μM CPT treatment for 30 min increased the complex formation 2-fold (Fig. 3A). Treatment of NIH:OVCAR-3 cell by IL-1α alone up to 24 hr did not significantly increase SDS/KCl precipitable complex formation; however, combinations of the two agents caused significant increases (3-fold) in the formation of SDS/KCl precipitable complexes compared with both the control and CPT-treated cells alone (Fig. 3B). The increase in DNA damage was observed within 30 min and remained significantly higher up to 2 hr.

Effects of IL-1α on uptake of CPT. Because increased formation of cleavable complex by CPT may have resulted from increased intracellular concentrations of CPT in the presence of IL-1α, uptake studies were carried out with CPT in NIH:OVCAR-3 cells. Pretreatment of the ovarian cells with IL-1α under conditions similar to those described for DNA damage did not significantly alter intracellular CPT concentrations (Fig. 4), indicating the observed increase in DNA damage did not result from higher intracellular CPT in the presence of IL-1α.

Correlation between cleavable complex and cytotoxicity. Because the short (0.5–24 hr) pretreatment of the ovarian cells with IL-1α significantly increased CPT-induced DNA cleavable complex, it was possible that this may result

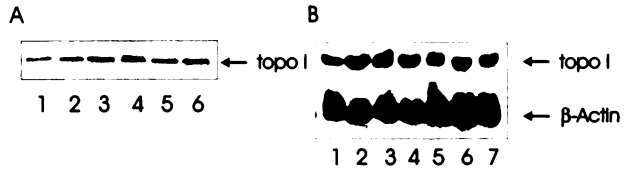

Fig. 2. Western blot analysis (A) for topo I protein expression in the nuclear extracts of NIH:OVCAR-3 cells after IL-1α (100 pM) exposure for 0, 0.5, 1, 2, and 24 hr (*lanes 1–6*; *lane 5* represents untreated cells used as a control for 24 hr). Equal amounts of cellular proteins (100 μg) were loaded. B, Effects of IL-1α on topo I mRNA expressions in NIH:OVCAR-3. Cells were treated with IL-1α (100 pM) for 0, 0.5, 2, and 24 hr (*lanes 1–4*) and in the presence of CPT (1 μM for 30 min after pretreatment with IL-1α at 0.5, 2, and 24 hr (*lanes 5–7*). RNA was extracted and hybridized with ^{32}P-labeled topo I probes as described in Materials and Methods. The β-actin probe was used for normalization of RNA.

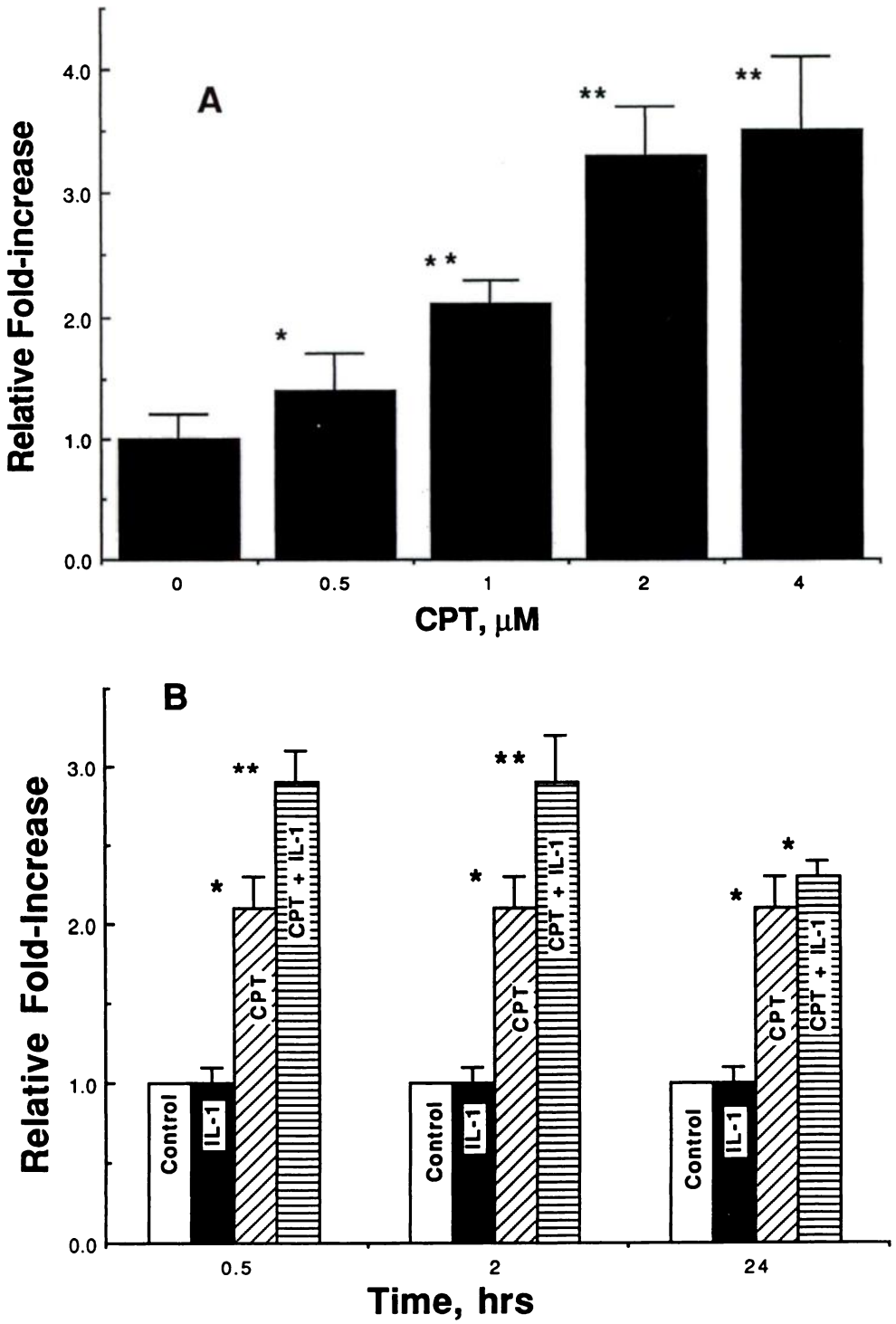

Fig. 3. CPT-induced SDS/KCl precipitable DNA/protein complex formation in the absence (A: *, $p < 0.05$ and **, $p < 0.001$ from the control) and in the presence (B: *, $p < 0.05$ from the control and L-1α alone; **, $p < 0.05$ from CPT, IL-1α, and the control) of IL-1α (100 pM) in NIH:OVCAR-3 cell line. Drug-induced DNA damage was measured with the SDS/KCl precipitation assay; CPT treatment was for 30 min, and the complex was precipitated with SDS/KCl as described in Materials and Methods.

in increased DNA damage and cell death. We examined this possibility by pretreating NIH:OVCAR-3 cells with 100 pM IL-1α for various times and treating the cells with 1 μM CPT for 30 min, conditions similar to those described for the DNA damage assay (Fig. 3). After CPT exposure, cells were washed once with drug-free media and were allowed to grow for 5 days in drug-free media for the MTT assay. Our results (Fig. 5) show that the pretreatment of the cells with IL-1α significantly increased cell death compared with IL-1α or CPT alone at 30 min. Increased cell death was not observed when cells were pretreated for 24 hr followed by CPT treatment, which is consistent with the fact that no significant increase in DNA damage was observed under these conditions. These observations suggest that enhanced DNA damage observed by the combinations of IL-1α and CPT for 30 min resulted in increased cell death compared with the use of single agents.

***In vivo* studies.** As shown in Fig. 6, CPT-11, as a single agent, was also effective in arresting the growth of ovarian xenografts in a dose-dependent manner, and as little as 2.5

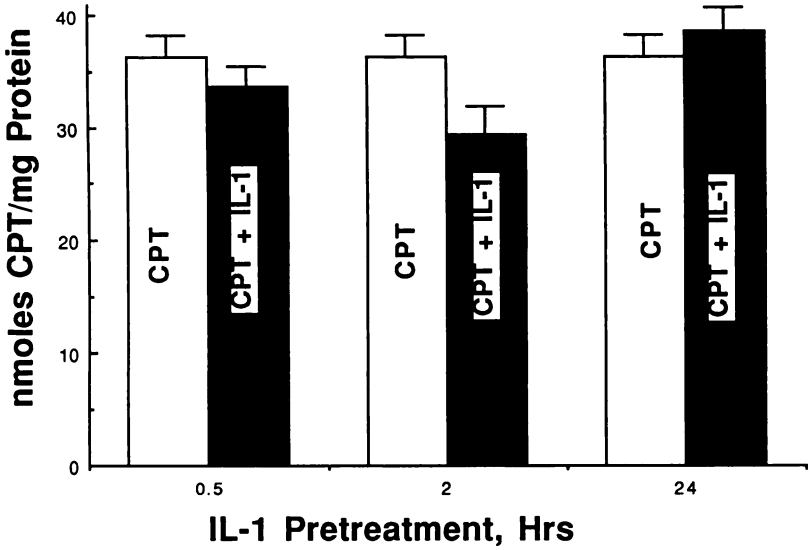

Fig. 4. Effects of IL-1α (100 pM) on the uptake of ^{3}H[CPT] in NIH:OVCAR-3 cells. Cells were pretreated for appropriate times with IL-1α, and the uptake of CPT (1 μM final concentration) was measured at 30 min as described in Materials and Methods.

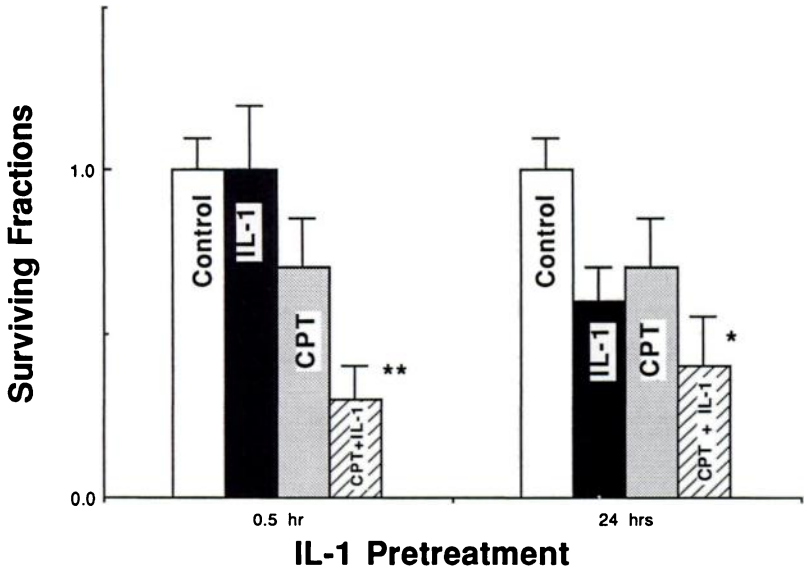

Fig. 5. Effects of IL-1α (100 pM) on cytotoxicity of CPT (1 μM) in NIH:OVCAR-3 cells. Cells were pretreated with IL-1α for 30 min or for 24 hr before CPT was added for 30 min. The media were removed and washed once with media and allowed to grow for 5 days. Cytotoxicity was measured with the MTT assay as described in legend to Fig. 1. *, Additive with CPT and IL-1α. **, Significantly different from both IL-1α and CPT alone ($p < 0.05$).

mg/kg CPT-11 caused moderate growth delay (Fig. 6A). At the end of treatment, however, tumor began to grow. IL-1α was effective in arresting the growth of NIH:OVCAR-3 cells in nude mice, and as little as 0.25 μg/mouse (total of 2.5 μg/mouse) reduced tumor volume 1.5–2-fold up to 2 weeks after treatments. However, tumor grew after this time period, and there was no significant difference in rates of tumor growth between the control and the treated groups (Fig. 6B). Combinations of IL-1α (0.25 μg/mouse × 5) with CPT-11 (2.5 mg/kg × 5 and 5.0 mg/kg) were significantly more effective than single agents (Fig. 6, B and C). These combinations of CPT-11 and IL-1α were as effective as 15.0 mg/kg CPT-11 alone (Fig. 6A); a dose modification of 5–6-fold was observed with IL-1α *in vivo*.

Discussion

It is well established that certain cytokines (IL-1α and tumor necrosis factor) are cytotoxic to some tumor cells *in vitro* and *in vivo*. Combinations of anticancer drugs with cytokines have emerged as an important modality for treatment of cancers. Our previous studies have shown that combinations of anticancer drugs with IL-1α resulted in significant enhancement of antiproliferative activity against melanoma and ovarian tumor cells. Ovarian carcinoma is one of the leading causes of cancer death in females. Selection for drug-resistant phenotypes during antineoplastic chemotherapy is a major obstacle to therapeutic success. This is also true in the treatment of ovarian cancer, where the development of resistance to one agent often confers cross-resistance to a broad class of antineoplastic agents. The human ovarian carcinoma cell line NIH:OVCAR-3 was established from the malignant ascites of a patient with progressive papillary adenocarcinoma of the ovary after combination chemotherapy. Ovarian cancer is clinically noted for the rapid development of primary drug resistance characterized by a broad cross-resistance pattern; accordingly, this cell line has been found to be resistant *in vitro* to clinically achievable concentrations of melphalan, cisplatin, and adriamycin (25). NIH:OVCAR-3 cells differ from several other human ovarian carcinoma cell lines in that they do not produce endogenous IL-1 (36), express relatively high levels of IL-1R (80 kDa), and respond with growth arrest to exposure to this cytokine. We

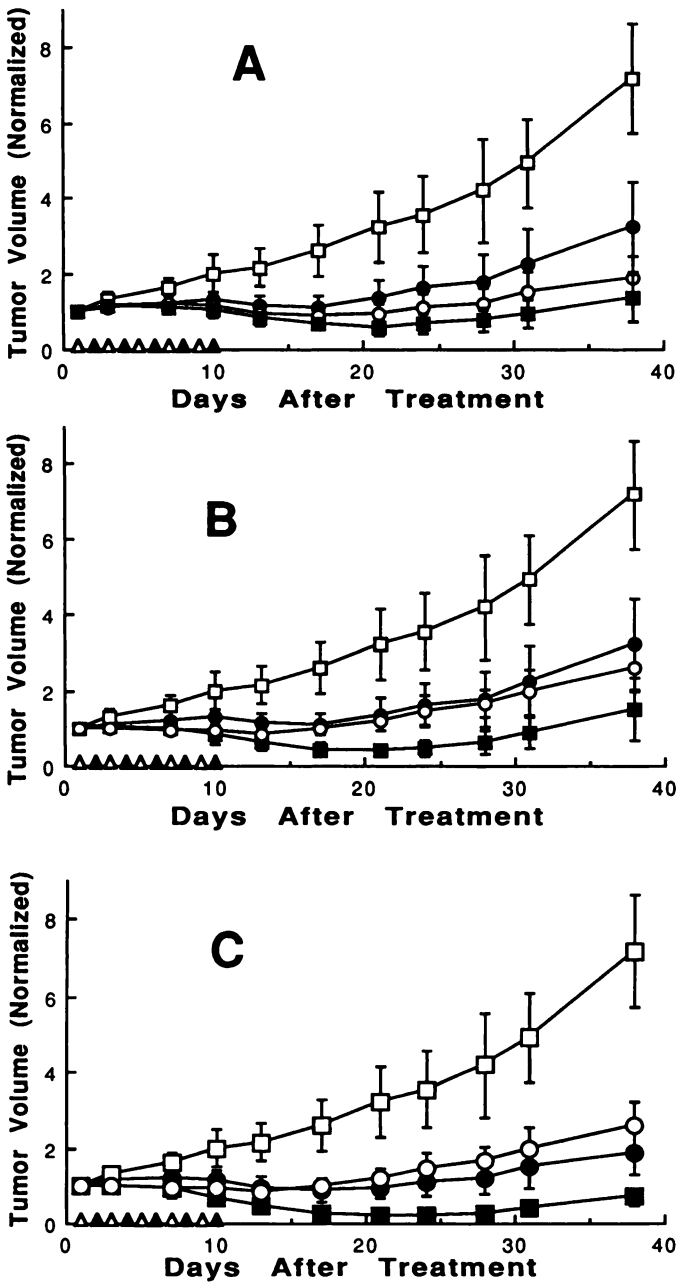

Fig. 6. Relative response of CPT-11 [A: 0 (□), 2.5 (●), 5 (○), and 15 (■) mg/kg; B: IL-1α 0.25 μg/mouse (○)], CPT-11 (●, 2.5 mg/kg), combination of IL-1α and CPT-11 (■), IL-1α [C: 0.25 μg/mouse, (○)], CPT-11 [(●), 5 mg/kg], and combination of IL-1α and CPT-11 (■) on the growth of NIH:OVCAR-3 xenografts (□) in nude mice. Values are mean ± standard deviation (>3 experiments).

found that simultaneous treatment of NIH:OVCAR-3 cells with IL-1α and CDDP resulted in a 15–20-fold enhancement of CDDP cytotoxicity.

Because camptothecins have been reported to be clinically active against solid tumors (37, 38), it was of interest to investigate interactions of combinations of camptothecins and IL-1α in NIH:OVCAR-3 tumor cells. Evidence indicates that CPT and CPT-11 kill tumor cells through inhibition of topo I (1, 2, 39), a nuclear enzyme often implicated in the replication mechanism. Our studies suggest that combinations of IL-1α and CPT are synergistic against NIH:OVCAR-3 cells *in vitro*.

Mechanisms to define the molecular and biochemical bases for this *in vitro* synergy between IL-1α and CPT suggested that IL-1α did not significantly modulate topo I at the mRNA level, whereas a small increase in the levels of topo I protein was detected with the Western blot analysis in this ovarian cell line. However, pretreatment of the cells by IL-1α significantly increased CPT-induced topo I-catalyzed cleavable complex formation, which was not due to an increase in intracellular CPT as IL-1α had no effect on CPT uptake. This discrepancy between a small increase in topo I expression and a large increase in the cleavable complex formation suggests that pretreatment by IL-1α increased the activity of the topo I in NIH:OVCAR-3 cells. Furthermore, the IL-1- and CPT-dependent increases in cleavable complex formation were rapid and detected within 30 min of pretreatment. Phosphorylation of topo I by PKC has been reported to increase topo I the activity, resulting in increased formation of cleavable complexes (40). IL-1 has been shown to increase PKC activity in some cell lines (41). It is possible that IL-1α pretreatment of NIH:OVCAR-3 cell induced PKC activity, resulting in the phosphorylation of topo I and increased cleavable complex. This hypothesis is under investigation.

This is interesting to note that studies carried out under the conditions of increased cleavable complex (i.e., 30 min of pretreatment) indicated that IL-1α also caused a significant enhancement of cell death compared with the use of either agent alone, and this enhancement in cell killing was more than additive. These observations suggest that the synergistic cell killing of NIH:OVCAR-3 cells by combinations of IL-1α and CPT resulted from the increased topo I-catalyzed CPT-induced DNA damage in the presence of IL-1α.

Our results also indicate that IL-1α modulated the antitumor activity of CPT-11 *in vivo* and resulted in a 5–6-fold increase in CPT-11 cytotoxicity. Although the exact mechanism of IL-1α-induced biological effects *in vivo* is not known, synergistic interactions between CPT-11 and IL-1α may also involve increased DNA damage, as noted in our *in vitro* studies. Because CPT-11 has been reported to be metabolized *in vivo* to its active form, SN-38, and the activity of CPT-11 has been found to correlate with the presence of SN-38 *in vivo* (42), it is possible that IL-1α increased the metabolism of CPT-11 by cytochrome P-450. This possibility is under investigation in our laboratory. IL-1α has been shown to induce transient hypoxia in tumors *in vivo*, resulting in enhancement of cytotoxicity of SR 4233, mitomycin C, and porfiromycin, drugs that are preferentially cytotoxic under hypoxic conditions (43, 44). Although these biological effects of IL-1α were not examined in the present study in ovarian xenografts, it is possible that other factors may also contribute to the synergistic antitumor effects observed with IL-1α and camptothecins.

Because IL-1 has been reported to stimulate growth and differentiation of bone marrow stromal cells, simultaneous treatment with IL-1 would allow patients to be treated with higher amounts of cytotoxic drugs, like CPT and CPT-11, resulting in better clinical response against solid tumors. In this regard, IL-1α has been demonstrated to decrease some of the acute side effects produced by certain anticancer drugs in mice (45)

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