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Cisplatin and carboplatin-mediated activation of murine peritoneal macrophages *in vitro*: production of interleukin-1α and tumor necrosis factor-α

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Analysis of tissue culture supernatants collected from cisplatin (10 μg/ml) and carboplatin (50 μg/ml)-treated macrophages show enhanced activity of interleukin-1α and tumor necrosis factor-α. Cytotoxicity of these supernatants was demonstrated using mouse sarcoma-180 cells. These results demonstrate the ability of cisplatin and carboplatin to enhance the immune system suggestive of yet another mechanism of their action in the regression of tumors.

Key words: Carboplatin, cisplatin, IL-1α, *macrophage activation, tumor necrosis factor-α*

Introduction

The immune system plays a role in the regulation of normal and abnormal growth.[1] Secretion of various cytokines from different cells of the immune system like lymphocytes, monocytes, neutrophils and mast cells results from the activation by viruses, parasites, antigens or tumor cells. During recent years, over 35 cytokines have been identified.[2] Because of the regulatory functions of cytokines, some have been tested as therapeutic agents[3] against cancer, infectious diseases, inflammatory processes, autoimmune disorders, developmental defects and aging. Recently, cytokines are being emphasized more and more as anticancer agents, immunomodulators and potentiators of radio and chemotherapy.[4]

Interleukin-1α (IL-1) has been recognized as an important regulator of inflammation and immunity.[5] It has been implicated in macrophage mediated tumor cytotoxicity.[6] Studies have shown IL-1 to be cytostatic for melanoma,[7] ovarian carcinoma,[8] osteogenic sarcoma[9] and MCF-7 breast cancer cells.[10]

Tumor necrosis factor (TNF)-α produced by cells of the monocyte/macrophage lineage is also proposed to play a regulatory role in inflammation and immunological response to tumors.[11] TNF-α activates human monocytes or macrophages to lyse tumor cells although there are some tumors that are insensitive to such treatment.[12]

Macrophages mediate tumor lysis and inhibition of cell growth through the action of cytokines like IL-1 and TNF-α.[13] In addition, primed macrophages release a large number of factors including oxygen radicals, polypeptide hormones, enzymes and coagulation factors which could act alone or in synergy.[14,16] Anticancer drugs cisplatin and carboplatin are able to activate macrophages *in vivo* and *in vitro*.[17,18] These macrophages are induced to transfer lysosomes to the tumor cells, and have enhanced release of cytolytic factors as reactive oxygen species, enzymes and nitric oxide.[19–21] These findings led us to investigate the release of IL-1 and TNF-α in cisplatin and carboplatin-treated macrophages. Though the proposed mechanism of action of these drugs is through the induction of inter and intrastrand crosslinking of DNA[22] or through the depolymerization of microfilaments,[23,24] we now suggest the enhancement of the immune system as yet another possible mechanism of action of these drugs through the release of various cytolytic factors[20,21] including IL-1α and TNF-α.

Materials and methods

Cell cultures

Murine peritoneal macrophages were isolated from 6–8 week old male Swiss Webster mice (Charles Rivers, MA) as previously described.[18] Macrophages were seeded in culture dishes at 2–4 × 10^6 cells/ml in normal medium (Minimal essential media, 10% fetal calf serum, 1% antibiotic-antimycotic, 10 mM HEPES; Gibco BRL, Grand Island, NY), incubated at 37°C overnight before use for the experiments.

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Treatments

Cisplatin (10 μg/ml) and carboplatin (50 μg/ml; gifts from Johnson Matthey Research Laboratories and the National Cancer Institute) dissolved in dimethylsulfoxide (DMSO) (3 μl/ml normal medium; Sigma, St Louis, MO) were used as drug treatments. Macrophages were treated with the drugs for 0.5, 1, 2, 4, 8 and 24 h at 37°C. In addition, macrophages were also treated with phorbol myristate acetate (PMA; 10 ng/ml DMSO; Sigma), lipopolysaccharide (LPS; 10 μg/ml normal medium; Sigma) or normal medium plus DMSO (3 μl/ml normal medium; Sigma) to serve as controls.

IL-1α assay

IL-1 was assayed using ELISA kits from Genzyme (MA). The method utilized the multiple antibody sandwich principle,[25] where a monoclonal anti-murine IL-1 was used to capture murine IL-1 present in the samples. A biotinylated polyclonal antibody binding the captured IL-1 was added and unbound material was washed out. Peroxidase-conjugated avidin was used to bind these biotin tagged complexes, resulting in a color change. The reaction was stopped by acidification and absorbance was read at 450 nm. Standard curves were generated with IL-1 (15–405 pg/ml) provided in the kits and linear regression analysis was performed. Readings were analyzed using the Student's *t*-test (two tailed, $p < 0.05$).

TNF-α assay

TNF-α released from supernatants of the macrophages was assayed using kits purchased from Genzyme. Again, the multiple antibody sandwich principle was utilized with a hamster monoclonal antibody specific for murine TNF-α in the samples.[25] A goat polyclonal anti-murine TNF-α antibody was used to bind the multiple epitopes on TNF-α which in turn was captured by a horseradish peroxidase-conjugated donkey anti-goat Ig. With the use of a peroxidase reaction and OPD (chromagen), a color change was induced and absorbance was measured at 492 nm. Standard curves were generated using 50–800 pg/ml murine TNF-α and linear regression analysis was performed. Readings were analyzed using the Student's *t*-test (two tailed, $p < 0.05$).

Cytotoxicity assay

Measuring the density of mouse sarcoma-180 solid tumor cells (S180; American Type Cell Culture, Rockville, MD) after incubation with the supernatants from the macrophages was used to assess macrophage mediated cell cytotoxicity as previously described.[20,21,26] S180 cells were seeded in 96-well plates at 4×10^5 cells/200 μl in normal medium and incubated for 3 h at 37°C. An equal volume of macrophage supernatants was added and incubated further for 24 h. Direct treatments of the S180 cells with IL-1α (5, 25, 50, 100 pg/ml), TNF-α (100, 300, 500, 1000 pg/ml), cisplatin (1, 5, 10, 20 μg/ml), carboplatin (5, 25, 50, 100 μg/ml) or DMSO (3 μl/ml) were performed for positive control. After the incubation period, cells were fixed in methanol for 30 s, stained with 100 μl 0.5% crystal violet (Sigma) for 20 min and washed with distilled water. Once dry, the optical density of the plates was measured at 620 mm. All treatments were performed in triplicate.

Results

IL-1α release

There was an increase in IL-1 levels detected in the supernatants of the macrophages treated with either cisplatin (10 μg/ml) or carboplatin (50 μg/ml) for the various times tested (Figure 1). However, no significant increases were observed after 4 and 24 h of cisplatin treatment, and a distinct pattern in the release of IL-1 was apparent. Similar increases were observed for PMA and LPS treatments at various time intervals tested.

TNF-α release

TNF-α increases were observed in all the supernatants from macrophages treated with either cisplatin (10 μg/ml) or carboplatin (50 μg/ml) at all times tested (Figure 2), although at 30 min and 2 h of cisplatin treatment, the increases were not so significant. PMA and LPS both enhanced the release of TNF-α in the medium.

Cytotoxicity assay

S180 cells when incubated in supernatants from cisplatin or carboplatin-treated macrophages de-

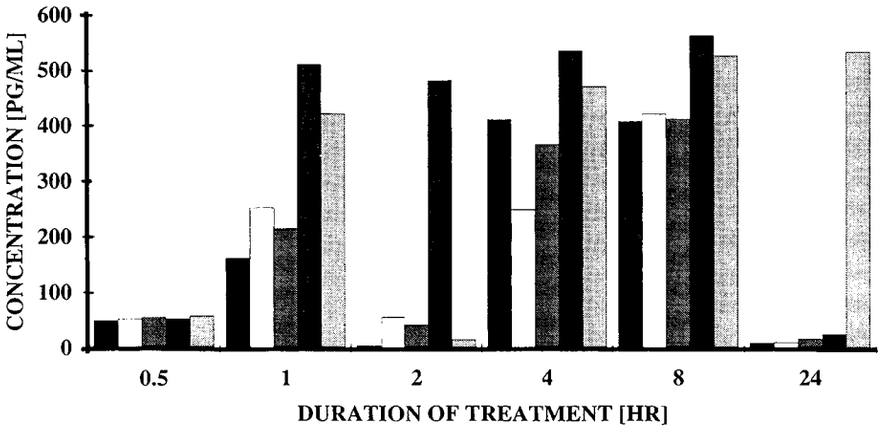

Figure 1. Bar graph showing IL-1 release in the supernatants of macrophages treated with either cisplatin (10 μg/ml), carboplatin (50 μg/ml), LPS (10 μg/ml) or PMA (10 ng/ml) at various times. Note the increase in the release of IL-1 for all treatments except for cisplatin treatment at 4 and 24 h. Standard deviation was less than 10% of the mean. ■, Control; □, cisplatin; ▩, carboplatin; ■, PMA; ▨, LPS.

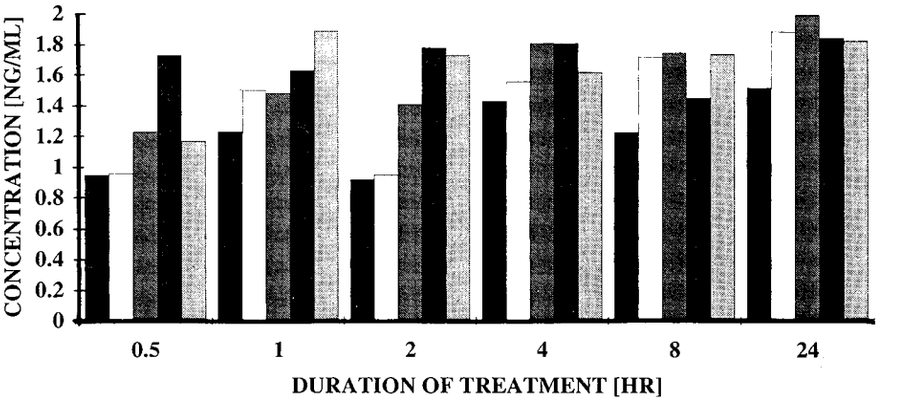

Figure 2. Bar graph showing TNF-α release in the supernatant of macrophages treated with either cisplatin (10 μg/ml), carboplatin (50 μg/ml), LPS (10 μg/ml) or PMA (10 ng/ml) at various times. Note the increase in TNF-α for all treatments except for cisplatin treatment for 30 min and 2 h. Standard deviations were less than 10% of the mean. ■, Control; □, cisplatin; ▩, carboplatin; ■, PMA; □, LPS.

monstrated a decrease in cell density until 12 h after which a gradual increase was observed. On the other hand, S180 cells incubated in carboplatin-treated macrophage supernatants induced a consistent decrease in cell numbers. S180 cells directly treated with cisplatin (1, 5, 10, 20 μg/ml) demonstrated a constant decrease in cell numbers, whereas carboplatin-treated (5, 25, 50, 100 μg/ml) S180 cells demonstrated an initial decrease in cell number at 5 μg/ml after which a gradual increase was observed. Similarly, IL-1 (Figure 3) and TNF-α (Figure 4) treatments of S180 cells demonstrated a continuous decrease in cell densities.

Discussion

Macrophages can be activated to mediate cytolytic processes against malignant cells. The mechanism by which these primed macrophages destroy tumor cells appears to involve direct cell–cell contact as well as the secretion of cytolytic factors, that include reactive oxygen species, reactive nitrogen intermediates, arginases, proteases, IL-1 and TNF-α.[28–33] Recently, the antitumor agents, cisplatin and carboplatin, have been demonstrated to enhance the immune system.[17,34] These agents activate the macrophages by inducing an increase in

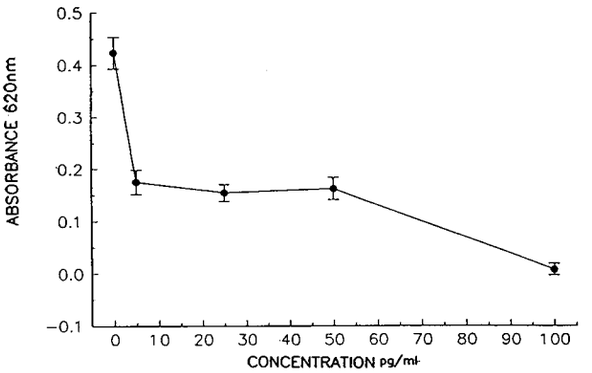

Figure 3. Graph showing cell density of S180 tumor cells after incubation in IL-1 for 24 h at various concentrations. Note the decrease in cell density in a dose-dependent manner.

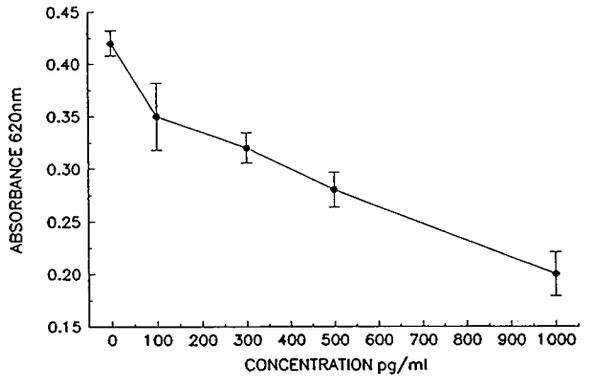

Figure 4. Graph showing cell density of S180 tumor cells after incubation in TNF for 24 h at various concentrations. Note the decrease in cell density in a dose-dependent manner.

their lysosomes, promoting extension formations that establish close contact with the tumor cells. Such macrophages transfer lysosomes to the tumor cells resulting in their lysis.[18] In addition, release of various cytolytic factors has been demonstrated.[20,21]

Both IL-1 and TNF-α have been demonstrated to possess direct antitumor activity,[35] and immunomodulating properties including an increase in MHC antigen expression in tumor cells[36], macrophage and T lymphocyte activation.[37,38] In addition a synergy in the antitumor activity of cisplatin and TNF-α has been found in sensitive and resistant mouse solid tumors.[39]

The mechanism by which IL-1 could destroy tumor cells may be through several pathways. One is via the production of superoxide, nitric oxide and hydrogen peroxide,[40,41] resulting in lipid peroxidation,[42] mitochondrial membrane depolarization and calcium mobilization, reduction in ATP synthesis,[43] and DNA base alterations.[44]

TNF-α is recognized as a prime mediator of a variety of immune functions.[45] Its ability to influence the synthesis of other cytokines like IL-1 has implicated its role in macrophage mediated cytotoxicity. This study demonstrates the increased release of TNF-α in cisplatin and carboplatin activated macrophages which probably plays a direct role in the cytotoxicity. Some of the possible mechanisms of action proposed for TNF-α are via nitric oxide which induces iron loss, and inhibits DNA synthesis, mitochondrial respiration and the citric acid cycle.[46–50] In addition, the ability of TNF-α to induce apoptosis is yet another process by which tumor cells could be affected.[51] Although studies on the induction of apoptosis by cisplatin and carboplatin-treated macrophages are underway, evidence already exists for the induction of apoptosis after cisplatin treatments.[52,53]

Conclusion

The major mechanism of action proposed for the anticancer agents cisplatin and carboplatin is through the inhibitors of DNA synthesis and the disruption of microfilaments. Our studies further support the activation of the immune system via the mediation of cytolytic factors, as yet another possible mechanism of action. We propose that cisplatin and carboplatin-treated macrophage mediated cell cytotoxicity involves various cytolytic factors including IL-1α and TNF-α.

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