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Combination intraventricular therapy with thiotepa and cytarabine in meningeal carcinomatosis due to breast cancer: *in vitro* evidence for supra-additive cytotoxicity

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Metastatic spread of tumors to the meninges is a frequent complication of many malignancies and is difficult to treat. We describe the case of a patient who developed carcinomatous involvement of the meninges from a breast adenocarcinoma. Despite intrathecal treatment with conventional and experimental agents, the patient's cerebrospinal fluid (CSF) was not cleared of malignant cells until thiotepa and cytarabine were given in combination. This clinical observation led us to assess the *in vitro* activity of the combination of thiotepa and cytarabine in clonogenic cell survival assays. The human breast adenocarcinoma cell line MCF-7^{WT} and its doxorubicin-resistant variant MCF-7^{ADR} were exposed to thiotepa and cytarabine either singly or in combination. We have found that the combination of the two drugs resulted in more than additive cytotoxicity than would have been predicted from the cytotoxicty of either drug given alone. We discuss the implications of these findings on the clinical management of patients with carcinomatous spread to the meninges.

Key words: Breast cancer, cytarabine, meninges, thiotepa.

Introduction

Meningeal involvement by metastatic tumors is a serious problem that may affect 5% of all patients with solid malignancies.[1] Though virtually any tumor can spread to the meninges, meningeal involvement is especially common in patients with lung or breast cancer.[2] Current therapy of cancers involving the meninges includes cranial or cranial–spinal radiation therapy, high-dose systemic chemotherapy, or chemotherapy delivered directly into the cerebrospinal fluid (CSF).[1] Though radiation therapy, of meningeal malignancies is associated with high response rates, long-term neurological toxicity can be severe[3] and short-term toxicity, particularly when cranial–spinal fields are employed, can be formidable.[4] High dose systemic chemotherapy can result in cytocidal concentrations of some drugs within the CSF, but is also limited by systemic toxicities.[1] Instillation of drugs directly into the CSF results in high concentrations of drugs at the tumor site and low systemic levels of drugs with minimal systemic toxicity.[5] Unfortunately, only a few chemotherapeutic agents are currently available for use in the CSF.

Recently we were involved in the care of a patient who suffered from meningeal involvement by a breast adenocarcinoma. Treatment with both conventional and experimental therapies failed to eliminate malignant cells from the patient's CSF. However, intraventricular treatment with thiotepa combined with cytarabine successfully cleared the patient's CSF of tumor cells. This clinical observation led us to investigate the possibility that thiotepa and cytarabine might work in synergy against human tumor cells. We report here the results of *in vitro* studies that confirm that these agents produce more than additive cytotoxicity when given concurrently to human breast adenocarcinoma cells.

Case report

A 49 year old woman was found to have metastatic breast adenocarcinoma in November, 1991. She was treated with paclitaxel and doxorubicin on a protocol at the Medicine Branch of the National Cancer Institute (NCI). After two cycles of therapy she had no evidence of cancer on clinical exam. However, on March 26, 1992, just before receiving her third cycle of paclitaxel and doxorubicin, the patient complained of a headache. Studies, including a lumbar puncture, revealed the presence of malignant cells within the CSF. An Ommaya reservoir was

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inserted and the patient received six doses of intraventricular methotrexate, 12.5 mg each, given twice weekly as well as two doses of intraventricular thiotepa, 10 mg each. The CSF continued to contain malignant cells and the patient was enrolled in a trial of intraventricular diaziquone (AZQ) at the NCI on April 24. The patient received two courses of AZQ but was taken off study in early June because of persistence of malignant cells in the CSF. On June 12, 1992, the patient received thiotepa, 30 mg intravenously as well as 10 mg intraventricularly, together with cytarabine, 10 mg intraventricularly. The patient received additional doses of intraventricular cytarabine on each of the next 2 days. The patient continued to receive intraventricular thiotepa and cytarabine weekly and intravenous thiotepa monthly. By June 25, the patient's CSF was cleared of malignant cells. CSF continued to be monitored weekly for the presence of malignancy. The patient continued to receive thiotepa and cytarabine until September 17, when malignant cells were again detected in the CSF. Treatment with intraventricular 4-hydroperoxy cyclophosphamide was then begun.

Materials and methods

Chemicals

Thiotepa was obtained as powder from the Pharmacy Branch of the Clinical Center of the NIH. Thiotepa was constituted in medium just prior to use. Unused stock solutions of thiotepa were frozen and used within 5 days. Cytarabine was purchased from Sigma (St Louis, MO). Solutions of cytarabine were made in medium just prior to use and were not stored.

Cell Culture

MCF-7^{WT} and MCF-7^{ADR} cells were obtained from ATCC (Rockville, MD). MCF-7^{ADR} is a breast adenocarcinoma cell line derived from MCF-7^{WT}. MCF-7^{ADR} is stably resistant to high concentrations of doxorubicin and expresses high levels of the P170 glycoprotein responsible for mediating multidrug resistance.[6] Both lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and antibiotics. For cell survival experiments, a number of 100 mm Petri dishes were plated with 5×10^5 cells. To establish cytotoxicity parameters for the two drugs individually, exponentially growing cells were exposed to various concentrations of thiotepa or cytarabine. After exposure to a drug for 24 h, the cells were rinsed, trypsinized, counted, plated and incubated for macroscopic colony formation. To determine the interactions of the two drugs on cytotoxicity, cells were exposed to a concentration of one of the agents which was expected to kill 90% of the cells. Concurrently, various concentrations of the second drug were added to the cells. After exposure to both thiotepa and cytarabine for 24 h, the cells were rinsed, trypsinized, counted, plated and incubated for macroscopic colony formation. Following a 10–14 day incubation, colonies were fixed with methanol : acetic acid (3 : 1), stained with crystal violet and colonies with >50 cells counted. All survival points were done in triplicate and experiments were conducted a minimum of two times. Error bars shown in the figures represent SEM and are shown when larger than the symbol. Plating efficiencies for cells were in the following ranges: MCF-7^{WT}, 45–60% and MCF-7^{ADR}, 20–30%.

Results

Figures 1 and 2 show the cytotoxicity of thiotepa and cytarabine in MCF-7^{WT} and MCF-7^{ADR} cells. As noted by others,[7] a plateau in cytotoxicity is present as the concentration of cytarabine is increased. By contrast, no plateau in cytotoxicity is noted for thiotepa at the concentrations used in these studies. These patterns of cytotoxicity are characteristic of antimetabolites and alkylating agents.[7]

The combination of cytarabine and thiotepa resulted in more than additive cytotoxicity in both the parent MCF-7^{WT} and the doxorubicin-resistant MCF-7^{ADR} cell lines. Figure 3 shows the cytotoxicity of cytarabine (Figure 3, top) in the presence of thiotepa and of thiotepa (Figure 3, bottom) in the presence of cytarabine in MCF-7^{WT} cells. Figure 4 shows the cytotoxicity of the two drugs in MCF-7^{ADR} cells. In both cell lines, although cytotoxicity was enhanced, the combination of thiotepa and cytarabine did not change the patterns of cytotoxicity noted when either agent was used singly. A plateau in cell killing with increasing concentrations of cytarabine was seen even in the presence of thiotepa. However, the addition of cytarabine to increasing concentrations of thiotepa did not result in the appearance of a plateau in cytotoxicity.

Discussion

At present the options available for the treatment of carcinomatous involvement of the meninges are

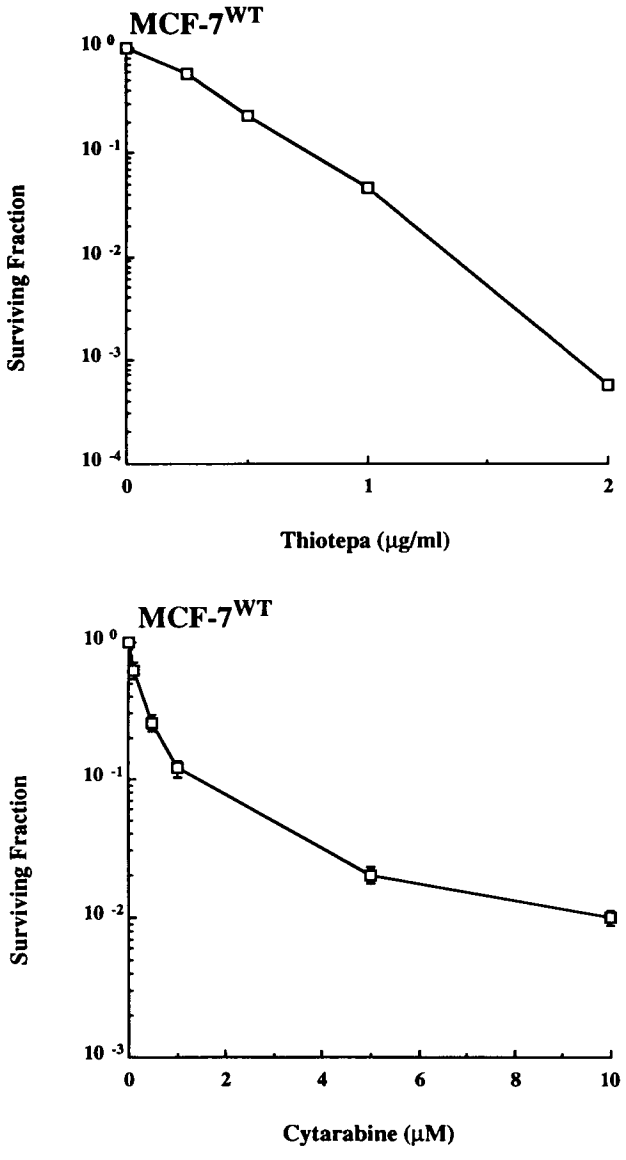


Figure 1. Survival of MCF-7^{WT} cells exposed to thiotepa (top) or cytarabine (bottom) for 24 h. Cells were exposed to various concentrations of the drugs as described in Materials and methods and then plated for clonogenic survival.

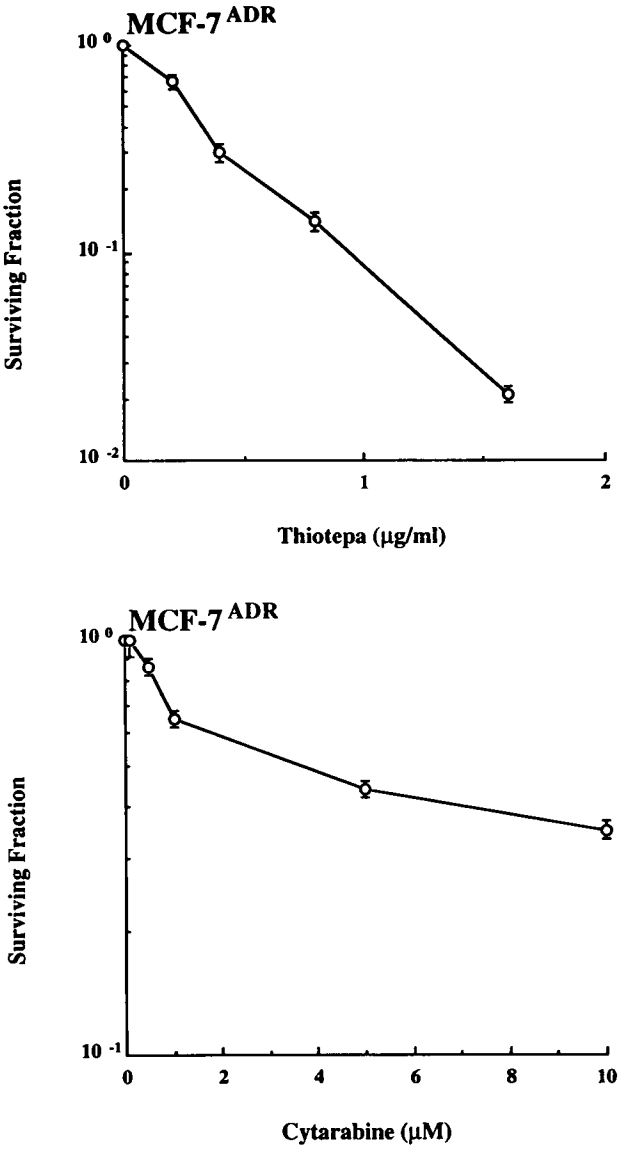
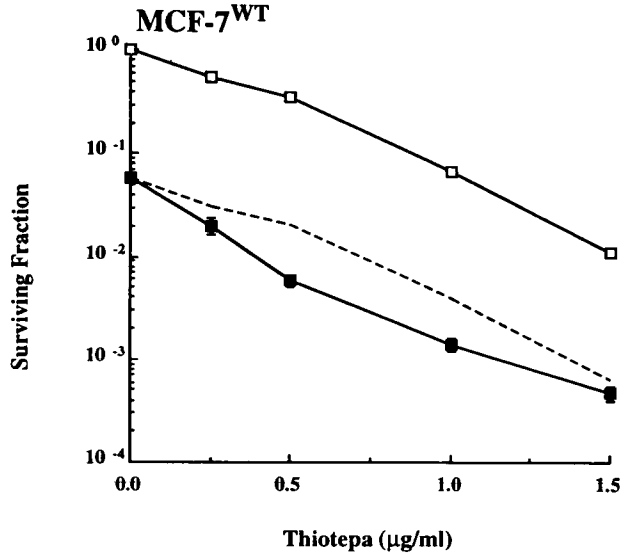
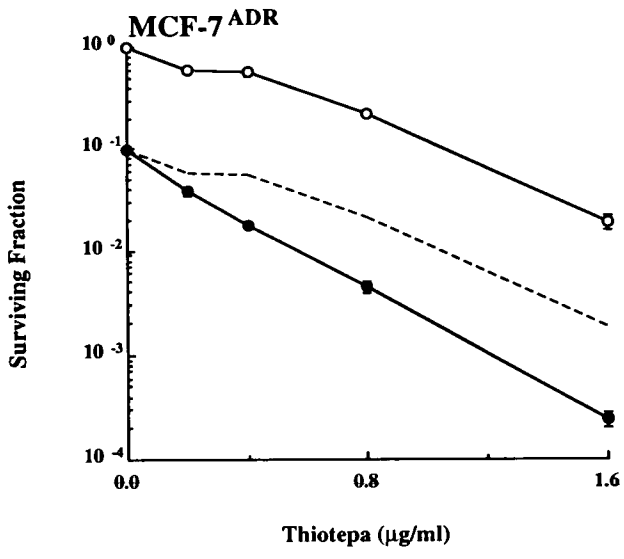


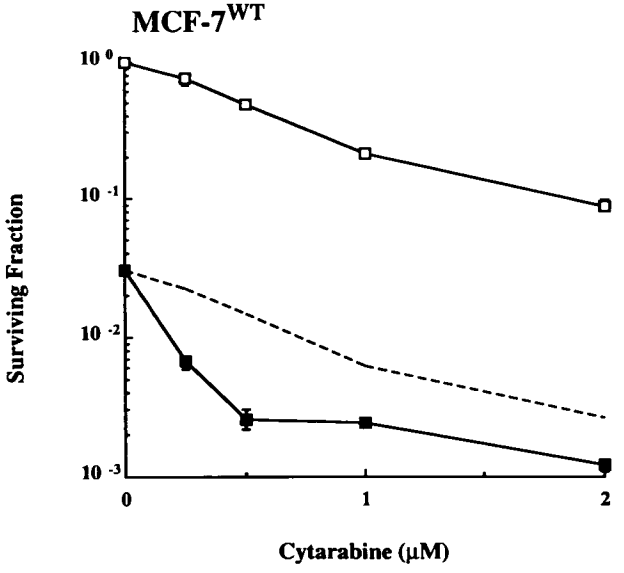
Figure 2. Survival of MCF-7^{ADR} cells exposed to thiotepa (top) or cytarabine (bottom) for 24 h. Cells were exposed to various concentrations of the drugs as described in Materials and methods and then plated for clonogenic survival.

limited. In the US, only three drugs—methotrexate, cytarabine, and thiotepa—are available for intrathecal administration. The responses to intrathecal therapy of tumors metastatic to the meninges vary widely.[2] However, even tumors that initially respond to therapy will often eventually relapse. There is a need for more effective therapies for metastatic disease within the central nervous system (CNS).

Pre-clinical studies have suggested that cytarabine can potentiate the effects of thiotepa.[8] Cytarabine can interfere with repair of DNA lesions induced by alkylators.[8] In addition to cytarabine's effects on lesions induced by thiotepa, it is conceivable that thiotepa might enhance cytarabine toxicity. Cytarabine is a cell cycle active drug which preferentially kills cells in S phase. Thiotepa and other alkylators can induce a cell cycle delay in late S.[9] It is possible that thiotepa might enhance cytarabine cytotoxicity by prolonging the duration







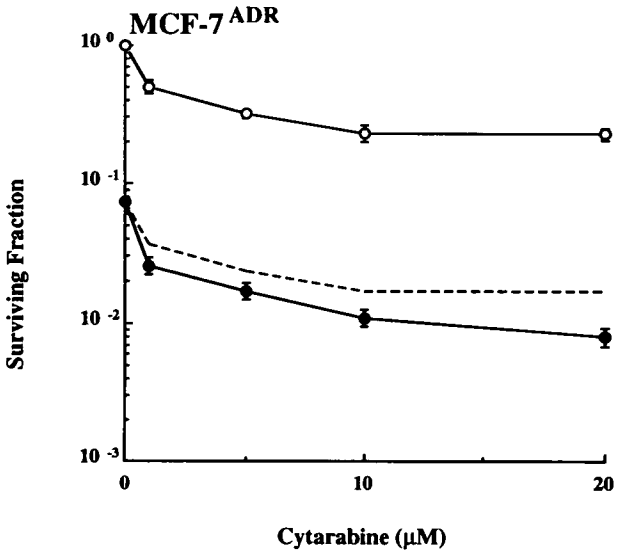


Figure 3. Survival of MCF-7^{WT} cells exposed to increasing concentrations of thiotepa (top) or cytarabine (bottom) in the presence or absence of cytarabine (Ara-C, 1 μM, top) or thiotepa (1 μg/ml, bottom). Open squares denote survival of MCF-7^{WT} cells exposed to thiotepa or cytarabine alone. Closed squares denote cell survival in the presence of cytarabine (Ara-C, 1 μM, top) or thiotepa (1 μg/ml, bottom). The dashed lines show the expected survival if the drug combinations produced additive cytotoxicity.

Figure 4. Survival of MCF-7^{ADR} cells exposed to increasing concentrations of thiotepa (top) or cytarabine (bottom) in the presence or absence of cytarabine (Ara-C, 5 μM, top) or thiotepa (0.5 μg/ml, bottom). Open squares denote survival of MCF-7^{ADR} cells exposed to thiotepa or cytarabine alone. Closed squares denote cell survival in the presence of cytarabine (Ara-C, 5 μM, top) or thiotepa (0.5 μg/ml, bottom). The dashed lines show the expected survival if the drug combinations produced additive cytotoxicity

that cells spend in the cytarabine-sensitive phase of the cell cycle.

Our current laboratory studies demonstrate that cytarabine and thiotepa, when given together, result in supra-additive cytotoxicity in human breast cancer cell lines. It is particularly gratifying to note that the drug combination was effective against the doxorubicin resistant variant of MCF-7 cells. Most patients with breast cancer will have been treated with systemic chemotherapy before they develop clinically overt meningeal metastases. Many of these patients will have received doxorubicin and their

tumor cells may express the P170 glycoprotein responsible for multi-drug resistance.[10] Though neither cytarabine or thiotepa are substrates for the P170 pump, it is of interest that the MCF-7^{ADR} cells are less sensitive to cytarabine than the parent MCF-7^{WT} cells (*cf.* Figures 1 and 2). The MCF-7^{ADR} cell line has been found to differ from MCF-7^{WT} cells in a number of other characteristics, including expression of enzymes that scavenge reactive oxygen species[6] and in doubling time. Regardless of the mechanism(s) by which MCF-7^{ADR} cells might be somewhat resistant to cytarabine compared to MCF-7^{WT} cells, the supra-additive effect of the drug combination was present in both cell lines.

Peak concentrations of thiotepa and cytarabine within CSF after intrathecal or intraventricular administration are about 100 μg/ml and 1 mM, respectively.[5] Thiotepa levels of at least 1 μg/ml are maintained in the CSF for 8 h after a single 10 mg intraventricular dose.[11] CSF levels of cytarabine exceed 1 μM for 24 h after a 30 mg intraventricular dose.[5] The drug concentrations which we chose for these studies are in the range of concentrations of both drugs which would be found in CSF after clinical administration. These studies have not defined the optimal scheduling of drug administration for clinical use. The use of animal models of carcinomatous meningitis would likely be the most appropriate way to address issues of drug schedule in preclinical testing.

Virtually all chemotherapeutic agents which have been administered into the CSF have been associated with some neurological side effects.[1] There has been the suggestion of enhanced drug toxicity in patients who were treated with intraventricular drug combinations which included thiotepa and cytarabine.[12,13] Such reports serve to underscore the need for additional *in vivo* preclinical testing as well the importance of carefully conducted clinical trials of this combination.

In summary, as the result of an encouraging response to intraventricular administration of thiotepa and cytarabine in a patient with refractory breast cancer with meningeal spread, we have conducted *in vitro* studies of this drug combination in human breast adenocarcinoma cell lines. We have found that when cells are exposed simultaneously to thiotepa and cytarabine, more than additive cytotoxicity results from this drug combination than would be expected from either drug alone. Given the paucity of options for treating patients with carcinomatous meningitis, these results warrant further preclinical testing of this drug combination. If indicated from the results of such additional studies, clinical trials should be conducted to assess the efficacy of thiotepa and cytarabine combinations in patients with carcinomatous spread to the meninges.

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