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In Vitro Cytotoxicity of Human Recombinant Tumor Necrosis Factor α in Association with Radiotherapy in a Human Ovarian Carcinoma Cell Line

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It has been speculated that tumor necrosis factor α (TNF- α) may decrease the cytotoxicity of radiotherapy by increasing the scavenging of toxic superoxide radicals. Because of the possible clinical implications, the cytotoxicity of TNF- α in combination with radiotherapy (RT) was compared with that of RT alone in a human ovarian cancer cell line. NIH:OVCAR-3 cells were incubated with TNF- α at 10.0, 1.0, 0.1, and 0.01 $\mu\text{g/ml}$. Plates were divided into two groups; one received 150 cGy of radiotherapy and the other received no further therapy. Seventy-two hours later, supernatants were aspirated and viable cells were stained with a 1% solution of crystal violet. Survival of cells treated with RT plus TNF- α was expressed as a percentage of surviving irradiated controls. Analysis of results revealed minimal additive cell killing effect between TNF- α and radiotherapy at all concentrations of tumor necrosis factor, with the greatest difference noted in the group treated with 10 $\mu\text{g/ml}$ TNF- α . A continued radiotherapy dose-response study with TNF- α showed a similar additive, not radioprotective, effect. This may have implication as a potentiator of RT in some human tumors. © 1990

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INTRODUCTION

Tumor necrosis factor α (TNF- α) has been reported to protect mice from lethal doses when administered prior to radiation [1]. The basis for this reported survival advantage is not well understood. Although TNF- α does not increase hematopoiesis, one of the proposed protection mechanisms may involve the preservation of hematopoietic progenitors [2]. It has also been proposed that this radioprotective effect might result from the induction of messenger RNA for manganous superoxide dismutase (MnSOD), which scavenges toxic superoxide

radicals produced in the mitochondria of normal cells. TNF- α -induced MnSOD mRNA elevation has been observed in all human and murine cancer cell lines studied [2]. Because these findings could have clinical relevance for enhancing sublethal repair, protection of the host from the effects of radiotherapy (RT), and cancer cell cytotoxicity, we studied the effect of TNF- α in association with radiotherapy on a human ovarian carcinoma cell line.

MATERIALS AND METHODS

NIH:OVCAR-3, a human ovarian carcinoma cell line (American Type Culture Collection, Frederick, MD), was seeded into 96-well flat-bottomed microtiter plates (10,000 cells per 0.1-ml well) in 100 μl of RPMI 1640 medium supplemented with 5% fetal calf serum, glutamine (3 mg/ml), insulin (0.2 U/ml), penicillin (100 U/ml), and streptomycin (100 $\mu\text{g/ml}$). All plates were incubated at 37°C in an atmosphere of 5% CO₂ and run in triplicate. Each data point was graphed to represent the average among the triplicate wells. Variations among the triplicate wells within a single data point were found to be between 5 and 10%.

The cells were irradiated (4-MeV linear accelerator) and exposed to different concentrations of recombinant human TNF- α (courtesy of Genentech, South San Francisco, CA). Supernatants were subsequently aspirated and viable cells stained with 50 μl of 1% crystal violet solution as described by Yamamoto *et al.* [3]. Quantification of the staining was assessed by reading the absorbance at 580 nm on a Titertek Multiskan Microplate Reader (Flow Laboratories, McClean, VA). Survival of

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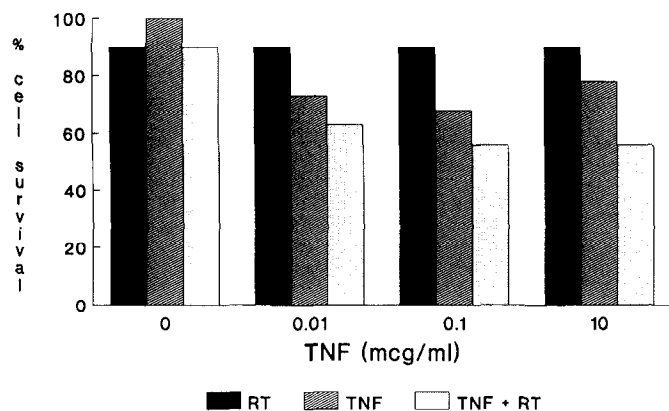


FIG. 1. Cytotoxic effects of tumor necrosis factor α (TNF- α) and radiotherapy on a human ovarian cancer cell line (NIH:OVCAR 3). TNF- α dose-response study, 0–10 $\mu\text{g}/\text{ml}$, in combination with 150 cGy radiotherapy.

cells treated with RT plus TNF- α was expressed as a percentage of surviving irradiated controls. To document synergistic or additive effects, TNF- α and radiotherapy were employed at doses which result in a 15–30% decrease in cell survival when used individually. Comparison of means was performed using Student's *t* test [4].

Experiment 1: TNF- α Dose-Response Study

Twenty-four hours after cells were plated, TNF- α was added to the medium at 0.01, 0.1, 1.0, and 10.0 $\mu\text{g}/\text{ml}$. The medium was replaced in 24 hr with TNF- α -free medium and the plates were subjected to 150 cGy of radiation. The percentage of surviving cells to irradiated-only controls was determined 72 hr later.

Experiment 2: Radiotherapy Dose-Response Study in Combination with TNF- α

Twenty-four hours after a second group of cells were plated, TNF- α was added at a concentration of 0.01 $\mu\text{g}/\text{ml}$. The culture medium was replaced in 24 hr with TNF- α -free medium and the plates were divided into four sets which were subjected to radiotherapy at doses of 100, 150, 200, and 250 cGy. The percentage of surviving cells was determined 72 hr postradiotherapy.

RESULTS

Experiment 1

When cells were preincubated with TNF- α , then exposed to 150 cGy radiation, a minimal decrease in cell survival was detected in comparison to nonirradiated cells (Fig. 1). This additive effect was slightly larger when

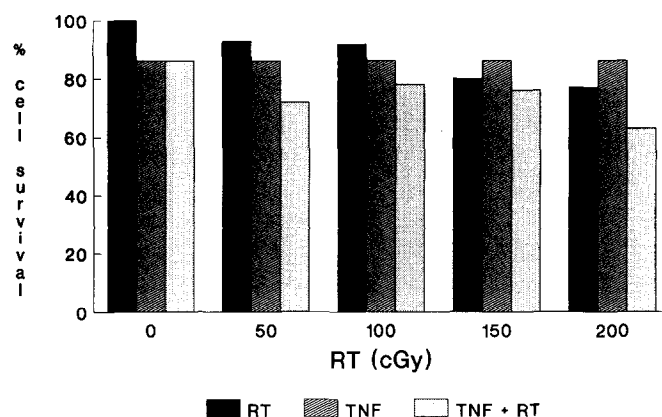


FIG. 2. Cytotoxic effects of tumor necrosis factor α (TNF- α) and radiotherapy on a human ovarian cancer cell line (NIH:OVCAR 3). Radiotherapy dose-response study, 0–200 cGy, in combination with 0.01 $\mu\text{g}/\text{ml}$ of TNF- α .

cells were treated with 10 $\mu\text{g}/\text{ml}$ TNF- α . The difference did not achieve statistical significance: a radioprotective effect of TNF- α was not demonstrated.

Experiment 2

When plates were preincubated with 0.01 $\mu\text{g}/\text{ml}$ TNF- α , then exposed to radiation at 0–250 cGy, a minimal decrease in cell survival was noted in comparison to cells not exposed to TNF- α (Fig. 2). This difference was not statistically significant and a radioprotective effect of TNF- α was not demonstrated. Similar results were obtained using TNF- α at 0.1 $\mu\text{g}/\text{ml}$ and radiotherapy within the range 0–400 cGy (Fig. 3).

On the basis of our results, we were not able to reject our null hypothesis, that cells treated with TNF- α and radiotherapy have a different surviving fraction than cells treated with either TNF- α or radiotherapy alone.

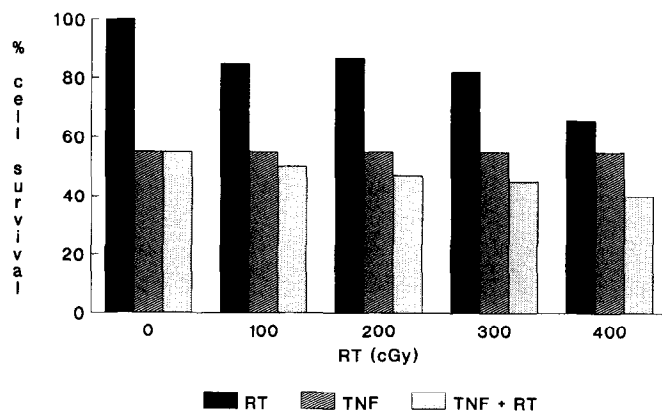


FIG. 3. Cytotoxic effects of tumor necrosis factor α (TNF- α) and radiotherapy on a human ovarian cancer cell line (NIH:OVCAR 3). Radiotherapy dose-response study, 0–400 cGy, in combination with 0.1 $\mu\text{g}/\text{ml}$ TNF- α .

DISCUSSION

Cytotoxicity of TNF- α against a variety of murine and human tumors has been demonstrated [5]. Increased survival of nude mice bearing intraperitoneal human ovarian cancer treated with TNF- α , either alone or in combination with interferon γ , has been shown by Balkwill *et al.* [6] and Manetta *et al.* [7]. Since radiotherapy has been reported to be successful in the management of selected groups of ovarian cancer patients [8], a combination of TNF- α and radiotherapy could be considered suitable for clinical experimentation.

TNF- α has been reported to have an additive or synergistic effect with chemotherapeutic agents such as *d*-actinomycin [9], with biologic response modifiers such as interferon γ [10], and with hyperthermia [11], the latter used very often in association with radiotherapy [12]. Clinical protocols under consideration will examine combinations of hyperthermia, radiotherapy, and TNF- α .

The question of the possible radioprotective effect of TNF- α should be addressed prior to the initiation of clinical trials using combined TNF- α and radiotherapy. Our study reveals a lack of radioprotective effect of TNF- α in an ovarian cancer cell line. To the contrary, a small additive effect has been detected, although this did not achieve statistical significance.

Despite hypothetical considerations, on the basis of these data, preclinical studies associating TNF- α and radiotherapy should continue. In addition, the correlation of hyperthermia, TNF- α , and radiation should be explored in a suitable animal model.

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