RAPID COMMUNICATION Jpn. J. Cancer Res. 83, 684–687, July 1992

Potentiation by Tumor Necrosis Factor of Mitoxantrone Cytotoxicity to Human Ovarian Cancer Cell Lines

Patrizia Russo,[1,5] Silvio Parodi,[1] Giovanna Billi,[2] Cristina Oliva,[2] Marco Venturini,[2] Elvira Noviello[3] and PierFranco Conte[4]

[1]Department of Chemical Carcinogenesis, [2]Department of Medical Oncology and [3]Department of Scientific Direction, Istituto Nazionale per la Ricerca sul Cancro, Viale Benedetto XV no. 10, I-16132 Genova, Italy and [4]Department of Medical Oncology, Ospedale Santa Chiara, Via Roma 67, I-56100 Pisa, Italy

The cytotoxic activity of human recombinant tumor necrosis factor (rHuTNF) (from 0.01 to 10000 U/ml) was assayed on six human ovarian cancer cell lines and one human cervical carcinoma cell line using a crystal violet assay. rHuTNF was cytotoxic to four cell lines (A2780, A2774, SW626, PA1), while 3 cell lines (IGROV1, SKOV3, Me180) were marginally sensitive to its activity. However, under the same experimental conditions rHuTNF markedly enhanced the cytotoxicity of mitoxantrone, a chemotherapeutic drug targeted at DNA topoisomerase II, in six cell lines. The potentiation of mitoxantrone cytotoxicity was not caused by increased drug accumulation after rHuTNF treatment. No significant increase in cytotoxicity to Me180 cell line was seen when rHuTNF was added to mitoxantrone.

Key words: TNF — Mitoxantrone cytotoxicity — Ovarian cancer cell

Tumor necrosis factor (TNF) is a peptide with a broad spectrum of biological effects; it is produced by monocytes after endotoxin stimulation as well as by stimulated lymphocytes. This cytokine plays a role in cachexia, endotoxin-induced vascular collapse, acute and chronic inflammatory reactions, septic shock and cytotoxicity to a wide range of human neoplastic cells.[1,2]

Recent studies demonstrated an augmentation of antitumor effects by the combination of human recombinant TNF (rHuTNF) and some topoisomerase I-II-targeted antineoplastic drugs on murine and human tumor cells *in vitro* and *in vivo*.[3–13] In contrast to these results, rHuTNF was unable to synergize the cytotoxic effects of two topoisomerase II-targeted drugs (doxorubicin and etoposide) on nine human lung cancer cell lines.[14]

Six human ovarian cancer cell lines (A2774, SW626, A2780, IGROV1, SKOV3, PA1) and one human cervical carcinoma cell line (Me180) were evaluated here for sensitivity to rHuTNF. The responses were heterogeneous (Table I). A dose-response relationship was seen in most of the cell lines over the wide range (0.01–10000 U/ml) of rHuTNF concentration tested (data not shown).

The effects of rHuTNF, at equiactive concentrations with respect to the drug, on the cytotoxicity of mitoxantrone were also studied in all cell lines. Table I incorporates the IC50 values obtained for mitoxantrone without and with rHuTNF. The dose-response curves for mitoxantrone are shown in Fig. 1 (from panel A to panel F) for the cell lines tested. We have previously demonstrated a strong potentiating effect of rHuTNF on mitoxantrone cytotoxicity to A2774 cell line.[6] When rHuTNF, at equitoxic concentrations with respect to mitoxantrone dosages, was added to the medium simultaneously with the drug, a very significant enhancement of cytotoxicity was seen on A2780 (Fig. 1A), on SW626 (Fig. 1B), on SKOV3 (Fig. 1C) and on IGROV1 cells (Fig. 1D). This enhancement of efficacy is not a simple additive effect, because the effect was much greater than the curve extrapolated for double the concentration of mitoxantrone alone (Fig. 1A–D). On PA1 the enhancement of mitoxantrone cytotoxicity was a simple additive effect, because the effect was the same as the curve extrapolated for double the concentration of mitoxantrone alone (Fig. 1E). On Me180 no potentiation was observed: the effect was less than the curve extrapolated for double the concentration of mitoxantrone alone (Fig. 1F). Only at the highest concentration of mitoxantrone (100 μM) were we able to recover a potentiating effect.

The mitoxantrone cytotoxicity-potentiating rate, expressed as the ratio of the mitoxantrone IC50 values without and with rHuTNF, was >10 for A2774 and SW626; >5 for A2780, IGROV1 and SKOV3; $\simeq 2$ for PA1 and 1 for Me180 cell line. This augmentation of tumor cell killing by treatment with the combination of rHuTNF and mitoxantrone (1 h at 37°C) was not caused by increased drug accumulation after rHuTNF treat-

[5] To whom correspondence should be addressed.

Table I. Cytotoxicity of rHuTNF on Six Ovarian Cancer Cell Lines and One Cervical Carcinoma Cell Line (Me180) and Effect of rHuTNF on the Sensitivity to Mitoxantrone

Cell line	rHuTNF		IC50 Mitoxantrone (μM)		rHuTNF potentiation ratio[c]
	IC50 (U/ml)	IC25 (U/ml)	−rHuTNF	+rHuTNF[b]	
A2774	373.8	81.10	0.01	0.0001	100.0
SW626	587.8	12.90	21.50	0.63	34.1
A2780	8.8	1.19	0.002	0.0002	10.0
IGROV1	—[a]	177.80	1.87	0.32	5.8
SKOV3	—[a]	14.70	1.29	0.24	5.4
PA1	3792.7	100.00	0.27	0.13	2.1
Me180	—[a]	0.18	0.12	0.12	1.0

Values represent the average of at least three independent determinations.
a) Not determined since the dose-response curve showed a maximum in the range of concentrations examined with a cellular survival above 50%.
b) The rHuTNF concentrations utilized for these experiments were equiactive with respect to mitoxantrone.
c) Degree of potentiation of each different cell line by rHuTNF expressed as ratio of the mitoxantrone IC50 without and with rHuTNF.
rHuTNF was obtained from KNOLL-BASF (Ludwigshafen, Germany). A stock solution of rHuTNF, containing 0.1 mg/ml of protein was stored at −80°C. Specific activity was 8.74×10^{-6} U/mg protein (48 h L929 bioassay without actinomycin D, as determined in the KNOLL-BASF laboratory). Drugs were diluted in RPMI-1640 with 10% serum to achieve appropriate final concentrations, and immediately used. Cytotoxicity was monitored with the crystal violet assay, as described by Ruff and Gifford[17] with minor modifications.[6] The IC50 (50% inhibitory concentration) was calculated with linear interpolation in the interval of concentrations with a cellular survival immediately above and below 50%. The IC25 (25% inhibitory concentration) was also calculated with linear interpolation.

ment (data not shown). Our findings indicate that rHuTNF potentiates the cytotoxic activity of mitoxantrone toward the 6 ovarian cancer cell lines studied, at concentrations of rHuTNF that are clinically achievable (IC50 <5000 U/ml).[15] Only in the cervical carcinoma cell line (Me180) did rHuTNF not increase the cytotoxic activity of mitoxantrone.

It is possible that rHuTNF may affect the amount and/or the activity of DNA topoisomerase II. We are now investigating this possibility. However, recently we have demonstrated, in A2774 ovarian cancer cell line, potentiation between VP16 or doxorubicin (two other drugs targeted at DNA topoisomerase II) and rHuTNF.[8] This property, however, is not shared by *cis*-platinum, a non topoisomerase II-interactive drug. Furthermore, when A2774 cells were incubated with rHuTNF+mitoxantrone for 1 h at 37°C, an increased number of DNA single-strand breaks was produced, while rHuTNF alone (1000 U/ml) did not induce DNA strand-breaks.[7] This suggests that the enhancing effect of rHuTNF may be related to topoisomerase II-mediated DNA damage and not to other forms of DNA damage.

In conclusion, our studies suggest that not only is rHuTNF moderately likely to be active against human ovarian cancer cell lines, but also it is synergistic with mitoxantrone, a very promising agent used in the i.p. treatment of ovarian cancer.[16] Keeping in mind these observations, a combination therapy (rHuTNF+mitoxantrone) could be administered, particularly by the i.p. route, to ovarian cancer patients. A phase I study of this combination is in progress in our Institute.

This work was supported by a grant for "Biological response modifiers: effects *in vivo* and *in vitro* in ovarian cancer" awarded by AIRC (Associazione Italiana per la Ricerca sul Cancro), Milano, Italy to P.R. and PF.C. (1991) and by a grant for "Molecular targeting chemotherapy: association between BRMs and DNA topoisomerase I-II inhibitors," S.P.7 "Selective therapies" awarded by CNR (Consiglio Nazionale delle Ricerche), Roma, Italy to P.R. (1991). We are most grateful to Dr. J. Kempeni (Knoll-BASF A.G., Ludwigshafen, Germany) and to Dr. P. Galletti (Knoll-Italia, Milano) for providing us with rHuTNF.

(Received March 3, 1992/Accepted April 21, 1992)

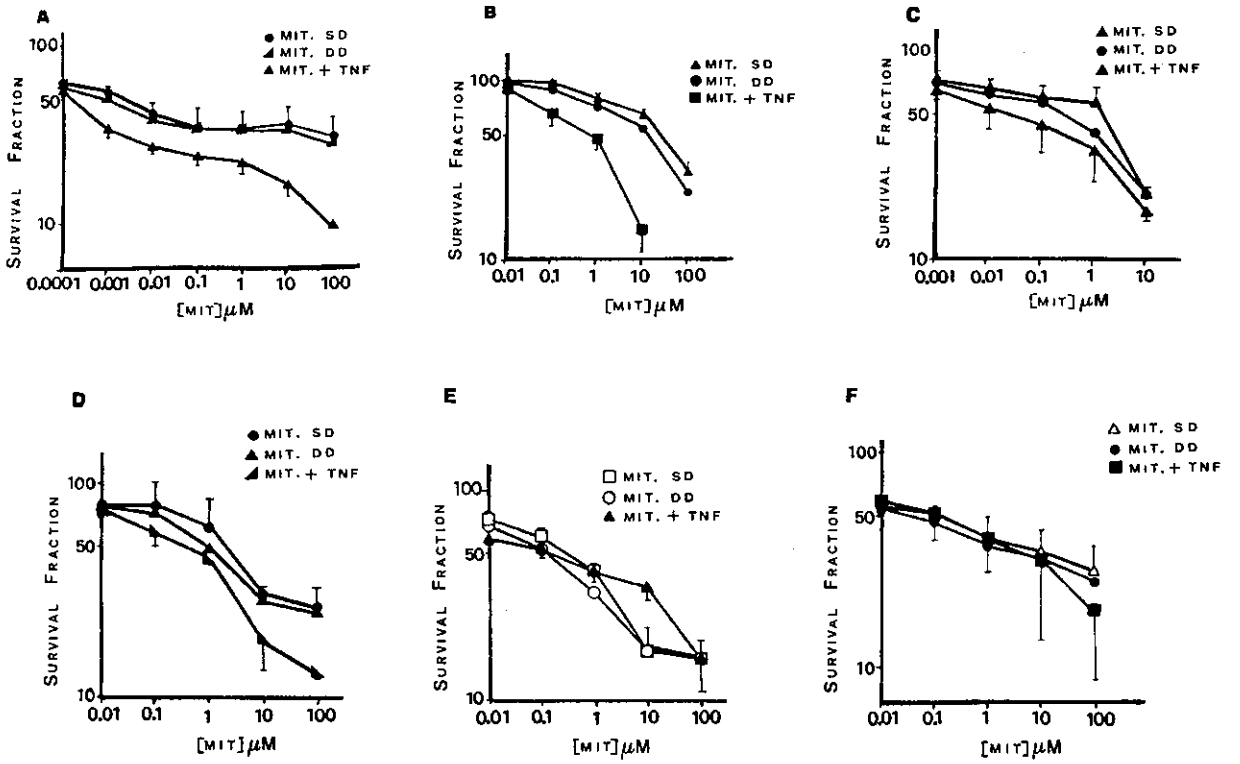

Fig. 1. Effect of rHuTNF on mitoxantrone cytotoxicity toward various human ovarian cancer cell lines and a human cervical carcinoma cell line. Panel A. Effect of mitoxantrone and rHuTNF on A2780 ovarian cancer cell line. A2780 cell line was exposed, 24 h after plating, to various concentrations of mitoxantrone alone ● (single dose, SD) or mitoxantrone + rHuTNF for 20 h. ◢: A2780 cell line, extrapolated curve with a double concentration (double dose, DD) of mitoxantrone. ▲: mitoxantrone and rHuTNF at equitoxic concentrations were added simultaneously to A2780 cells, 24 h after seeding, and for 20 h. The equitoxic dosages of rHuTNF were calculated in the following way: from the results of the dose-response curves for rHuTNF (data not shown) we have interpolated the dosages of rHuTNF giving the same survival fraction as the dosages of mitoxantrone, respectively; these equitoxic dosages are as follows: 1) 2.75 U/ml; 2) 5.20 U/ml; 3) 41.00 U/ml; 4) 316.20 U/ml; 5) 316.20 U/ml; 6) 316.20 U/ml; 7) 631.00 U/ml. Panel B. Effect of mitoxantrone and rHuTNF on SW626 ovarian cancer cell line. As in Panel A (SW626 cells instead of A2780 cells). The equitoxic dosages of rHuTNF are as follows: 1) 1.00 U/ml; 2) 1.00 U/ml; 3) 10.00 U/ml; 4) 119.40 U/ml. Panel C. Effect of mitoxantrone and rHuTNF on SKOV3 ovarian cancer cell line. As in Panel A (SKOV3 cells instead of A2780 cells). The equitoxic dosages of rHuTNF are as follows: 1) 31.60 U/ml; 2) 147.90 U/ml; 3) 1000.00 U/ml; 4) 3162.00 U/ml; 5) 10000.00 U/ml. Panel D. Effect of mitoxantrone and rHuTNF on IGROV1 ovarian cancer cell line. As in Panel A (IGROV1 cells instead of A2780 cells). The equitoxic dosages of rHuTNF are as follows: 1) 100.00 U/ml; 2) 100.00 U/ml; 3) 1000.00 U/ml; 4) 10000.00 U/ml; 5) 10000.00 U/ml. Panel E. Effect of mitoxantrone and rHuTNF on PA1 ovarian cancer cell line. As in Panel A (PA1 cells instead of A2780 cells). The equitoxic dosages of rHuTNF are as follows: 1) 147.90 U/ml; 2) 1621.80 U/ml; 3) 10000.00 U/ml; 4) 10000.00 U/ml; 5) 10000.00 U/ml. Panel F. Effect of mitoxantrone and rHuTNF on Me180 cervical cancer cell line. As in Panel A (Me180 cells instead of A2780 cells). The equitoxic dosages of rHuTNF are as follows: 1) 467.80 U/ml; 2) 10000.00 U/ml; 3) 10000.00 U/ml; 4) 10000.00 U/ml; 5) 10000.00 U/ml.

REFERENCES

1) Old, L. Tumor necrosis factor (rTNF). *Science,* **230,** 630–636 (1985).
2) Balkwill, F. R., Naylor, M. S. and Malik, S. Tumor necrosis factor as an anticancer agent. *Eur. J. Cancer,* **26,** 641–644 (1990).
3) Alexander, R. B., Nelson, W. G. and Coffey, D. S. Synergistic enhancement by tumor necrosis factor of *in vitro* cytotoxicity from chemotherapeutic drugs targeted at DNA topoisomerase II. *Cancer Res.,* **47,** 2403–2406 (1987).
4) Utsugy, T., Mattern, M. R., Mirabelli, C. K. and Hanna, N. Potentiation of topoisomerase inhibitor-induced DNA strand breakage and cytotoxicity by tumor necrosis factor: enhancement of topoisomerase activity as a mechanism of potentiation. *Cancer Res.,* **50,** 2636–2640 (1990).
5) Coffman, F. D., Green, L. M., Godwin, A. and Ware,

C. F. Cytotoxicity mediated by tumor necrosis factor in variant subclones of Me-180 cervical carcinoma line: modulation by specific inhibitors of DNA topoisomerase II. *J. Cell. Biochem.*, **39**, 95–105 (1989).
6) Vigani, A., Chiara, S., Miglietta, L., Repetto, L., Conte, P. F., Cimoli, G., Morelli, L., Billi, G., Parodi, S. and Russo, P. Effect of recombinant human tumor necrosis factor on A2774 human ovarian cancer cell line: potentiation of mitoxantrone cytotoxicity. *Gynecol. Oncol.*, **41**, 52–55 (1991).
7) Russo, P., Billi, G., Cola, S., Rosso, R., Venturini, M., Chiara, S., Parodi, S., Kempeni, J., Vigani, A. and Conte, P. F. Potentiation of mitoxantrone-induced DNA single-strand breaks and cytotoxicity by tumor necrosis factor on human ovarian cancer cell lines *in vitro* and *in vivo*. *Proc. Am. Assoc. Cancer Res.*, **32**, 335 (1991).
8) Billi, G., Venturini, M., Piccini, D., Parodi, S., Rosso, R., Conte, P. F. and Russo, P. Synergistic cytotoxic effects of recombinant human tumor necrosis factor and etoposide (VP16) or doxorubicin on A2774 human epithelial ovarian cancer cell line. *Int. J. Gynecol. Cancer*, **2** (1992), in press.
9) Orengo, G., Billi, G., Cimoli, G., Venturini, M., Rosso, R., Vigani, A., Conte, P. F. and Russo, P. Synergistic enhancement by tumor necrosis factor of *in vitro* cytotoxicity from chemotherapeutic drugs targeted at DNA topoisomerase I and II. *Eur. J. Cancer Res.*, **27** (Suppl. 3), 54 (1991).
10) Alexander, R. B., Isaacs, J. T. and Coffey, D. S. Tumor necrosis factor enhances the *in vitro* and *in vivo* efficacy of chemotherapeutic drugs targeted at DNA topoisomerase II in the treatment of murine bladder cancer. *J. Urol.*, **138**, 427–429 (1987).
11) Bahnson, R. R. and Ratliff, T. L. *In vitro* and *in vivo* anti-tumor activity of recombinant mouse tumor necrosis factor (TNF) in a mouse bladder tumor (MTB-2). *J. Urol.*, **141**, 172–175 (1990).
12) Das, A. K., Walther, P. J., Buckley, N. J. and Poulton, S. H. M. Recombinant human tumor necrosis factor alone and with chemotherapeutic agents. *Arch. Surg.*, **124**, 107–110 (1989).
13) Krosnick, J. A., Mulé, J. J., McIntosh, J. K. and Rosemberg, S. A. Augmentation of antitumor efficacy by combination of recombinant tumor necrosis factor and chemotherapeutic agents *in vivo*. *Cancer Res.*, **49**, 3729–3733 (1989).
14) Giaccone, G., Kadoyama, C., Maneckjee, R., Venzon, D. V., Alexander, R. B. and Gadzar, A. F. Effects of tumor necrosis factor, alone or in combination with topoisomerase-II-targeted drugs on human lung cancer cell lines. *Int. J. Cancer*, **46**, 326–329 (1990).
15) Salmon, S. E., Young, L., Scuderi, P. and Clark, D. Antineoplastic effects of tumor necrosis factor alone and in combination with gamma-interferon on tumor biopsies in clonogenic assay. *J. Clin. Oncol.*, **5**, 1816–1821 (1987).
16) Markman, M., George, M., Hakes, T., Reichman, B., Hoskins, W., Rubin, S., Jones, W., Almandrones, L. and Lewis, J. L., Jr. Phase II trial of intraperitoneal mitoxantrone in the management of refractory ovarian cancer. *J. Clin. Oncol.*, **8**, 146–150 (1990).
17) Ruff, M. R. and Gifford, G. E. Tumor necrosis factor. *Lymphokines*, **2**, 235–272 (1981).