Urol Res (1994) 22:261–266

ORIGINAL PAPER

Y. Mizutani · B. Bonavida · O. Yoshida

Cytotoxic effect of diphtheria toxin used alone or in combination with other agents on human renal cell carcinoma cell lines

Received: 16 February 1994/Accepted: 21 June 1994

Abstract Treatment of renal cell carcinoma (RCC) by conventional chemotherapy and immunotherapy has resulted in minimal remissions. Alternative forms of therapy are therefore being sought. The present study investigated the sensitivity of RCC cell lines to several toxins used alone and in combination with other agents. RCC lines were relatively sensitive to the direct cytotoxic effect of diphtheria toxin (DTX), *Pseudomonas aeruginosa* exotoxin A (PEA) and ricin. Furthermore, DTX in combination with tumor necrosis factor-α (TNF-α) resulted in synergistic cytotoxic activity. The mechanism of synergy was examined. A possible mechanism of resistance to TNF-α in tumor cells is the expression of TNF-α mRNA or protein. R11 cells did not constitutively express mRNA for TNF-α, however, treatment of R11 cells with TNF-α induced the expression of TNF-α mRNA. When DTX was used in combination with TNF-α, the level of TNF-α mRNA induced by TNF-α was markedly reduced. These studies suggest that DTX in combination with TNF-α can overcome the resistance of RCC lines and that the marked downregulation of TNF-α mRNA by DTX may play a role in the enhanced cytotoxicity seen with the combination of DTX and TNF-α. Furthermore, the combination treatment might also potentiate the antitumor host responses. The implications of these findings in clinical therapy are discussed.

Key words Renal cell carcinoma · Diphtheria toxin · Tumor necrosis factor alpha

Currently, surgery remains the most effective form of therapy for renal cell carcinoma (RCC), although efficacy has been only demonstrated for regional resectable disease [4, 13]. Cytotoxic chemotherapy, an integral part of the therapeutic approach for many solid tumors, has shown little or no antitumor activity against RCC and has played no role in either adjuvant or neoadjuvant support therapy [39, 40]. Due to the absence of any significant antitumor response with the use of single therapeutic agents, the use of multidrug combination regimes also proved ineffective when evaluated in prospective trials against a single drug [19, 17]. Endocrine therapy is of only marginal benefit in RCC [14, 19]. Interferons (IFNs) has been proved to be efficacious with remissions in approximately 13% of >400 cases [17, 22]. Almost all responding cases have a small tumor burden and have undergone nephrectomy. Although the use of interleukin-2 with or without lymphokine activated killer cells is a promising new area of therapy for RCC, the response rates vary between 0 and 35%, with complete remission CR being uncommon [3, 40]. Irradiation plays a limited role and has been used primarily for the treatment of central nervous system, spinal cord, osseous and selected soft tissue metastasis [16, 34]. Thus, a new therapeutic approach must be evaluated.

Diphtheria toxin (DTX) is a toxin secreted by *Corynebacterium diphtheriae*, and inhibits protein synthesis by catalyzing the ADP ribosylation of elongation factor-2 (EF-2) [18, 28]. This inhibition results in target cell death [11, 12]. Recent studies have demonstrated that DTX can also lyse target cells by inducing DNA fragmentation and subsequent programmed cell death or apoptosis [8, 9]. Tumor necrosis factor-α (TNF-α) is one of the effective anticancer agents against RCC [1, 36]. Previous studies have indicated that the mechanisms of DTX-mediated target cell lysis are similar to those of TNF-α-mediated lysis [2, 8, 10, 29]. Target cell lysis by DTX is time and concentration dependent and reaches a maximum level at 24 h, similar to the effect of TNF-α. Lysis by DTX or TNF-α may not necessarily be linked to inhibition of protein synthesis, since several inhibitors of protein synthesis have no cytolytic activity against target cells and both DTX and TNF-α still kill in their presence.

Y. Mizutani · O. Yoshida (✉)
Department of Urology, Faculty of Medicine, Kyoto University, 54 Shogoin-Kawahara-cho, Sakyo-ku, Kyoto 606, Japan;
Fax: +(75)7613441

B. Bonavida
Department of Microbiology and Immunology, UCLA School of Medicine, University of California at Los Angeles, California, USA

Table 1 Sensitivity of RCC lines to lysis by DTX. The susceptibility of various RCC cell lines to lysis by DTX was determined in a 1-day MTT assay. Results are expressed as mean percentage cytotoxicity ± SD of three different experiments

Tumor cell line	Concentration of DTX (ng/ml)		
	1	10	100
R4	22.9 ± 1.5	50.4 ± 4.7	77.7 ± 7.5
R6	4.4 ± 0.3	48.9 ± 1.3	73.8 ± 5.5
R11	18.5 ± 2.1	46.6 ± 3.4	53.4 ± 3.4

Table 2 Time kinetics of DTX-mediated cytotoxicity against R11 cells. The direct cytotoxic effect of DTX on R11 cells was assessed by the MTT assay. Results are expressed as mean percentage cytotoxicity ± SD of three different experiments

Time of exposure to DTX (h)	Concentration of DTX (ng/ml)		
	1	10	100
4	1.5 ± 0.7	2.6 ± 1.7	4.0 ± 2.6
8	3.1 ± 0.3	3.4 ± 1.8	4.6 ± 0.8
24	18.5 ± 2.1	46.6 ± 3.4	53.4 ± 3.4
48	39.5 ± 3.4	61.1 ± 5.1	66.7 ± 4.4
72	40.1 ± 2.4	62.8 ± 5.1	66.8 ± 3.7

To date, there have been no reports of cytotoxic activity of DTX against RCC when used alone or in combination. The present study investigates the sensitivity of RCC lines to the cytotoxic effect of DTX used alone and in combination with other therapeutic agents (IFN-α, IFN-γ, TNF-α, Adriamycin).

Materials and methods

Tumor cells

The human RCC cell lines, R4, R6 and R11 were supplied by Dr. Hans Stotter, Bethesda, Md., USA (Histopathology, Adenocarcinoma) and were maintained in RPMI-1640 medium (MA Bioproducts, USA) supplemented with 1% L-glutamine (Gibco, Bio-cult, Glasgow, UK), 1% nonessential amino acid (Gibco), 1% Fungibact solution (Irvine Scientific, Calif., USA) and 10% heat-inactivated fetal bovine serum (Gibco), hereafter referred to as complete medium [25, 26].

Reagents

Diphtheria toxin (lot. no. DT-11), *Pseudomonas aeruginosa* exotoxin A (PEA, lot. no. 18), pertussis toxin (PT, lot. no. PT-81A) and ricin (lot. no. 8822) were purchased from List Biological Laboratories, Campbell, Calif., USA. Adriamycin (ADR, lot. no. 61H0815), actinomycin D (Act D, lot. no. A4262), emetine (lot. no. E2375) and cycloheximide (CHX, lot. no. C6255) were purchased from Sigma, St.Louis, Mo., USA. TNF-α (5×10^7 units/mg, lot. no. 4906) was kindly supplied by Pepro Tech Inc., USA. IFN-α (lot. no. R410231) and IFN-γ (Lot. No. R508061) were obtained from Roche, Tokyo, Japan. TNF-α cDNA and β-actin cDNA used in making probes for Northern blot analysis were gifts from Smith-Kline-French.

Cytotoxicity by the MTT assay

Microculture tetrazolium dye (MTT) assay was used to determine tumor cell lysis as previously described [23, 26]. Briefly, 100 µl target cell suspension (2×10^4 cells) was added to each well of 96-well flat-bottom microtiter plates (Corning Glass Works, Corning, N.Y., USA), and each plate was incubated for 24 h at 37°C in a humidified 5% CO_2 atmosphere. After incubation, 100 µl drug solution or complete medium for control was distributed in the 96-well plates and each plate was incubated for 4–72 h at 37°C. Following incubation, 20 µl MTT working solution (5 mg/ml, Sigma) was added to each culture well and the cultures were incubated for 4 h at 37°C in a humidified 5% CO_2 atmosphere. The culture medium was removed from the wells and replaced with 100 µl of isopropanol (Sigma) supplemented with 0.05 N HCl. The absorbance of each well was measured with a microculture plate reader (Titertek Multiskan MCC/340, Flow Laboratories, Finland) at 540 nm. Percentage cytotoxicity was calculated with the following formula: Percentage cytotoxicity = [1 – (absorbance of experimental wells/absorbance of control wells)] × 100.

Northern blot analysis

Cytoplasmic RNA from tumor cells was prepared as described in detail elsewhere [23, 24]. Briefly, 10 or 40 µg/lane of tumor cell RNA was electrophoresed in 1.2% agarose-2.2 M HCHO gels in 1×morpholinopropane sulfuric acid (MOPS) buffer [200 mM MOPS, 50 mM sodium acetate, 10 mM sodium ethylenediaminetetra-acetic acid (EDTA)]. The RNA was transferred to Zeta Probe nylon membranes (Bio Rad Laboratories, Calif., USA) in 20×SSC (3 M NaCl, 0.3 M sodium citrate, pH 7.0). A quantity of 50–100 ng cDNA probe was labeled with [α^{32}P] dCTP (NEN, Mass., USA) by random oligo-primer extension. The nylon filters were ultraviolet-cross-linked and then prehybridized at 45°C overnight in 50% formamide (Bethesda Research Laboratories, Md., USA), 5×Denhardt's (Ficoll, polyvinylpyrrolidone, bovine serum albumin), 0.1% sodium dodecyl sulfate (SDS, Bethesda Research Laboratories), 100 µg/ml salmon sperm DNA (Sigma), and 5×SSC. Radiolabeled probe was added at 1×10^6 cpm/ml of hybridized fluid (6× SSC, 0.5% SDS, 5× Denhardts, 100 µg/ml salmon sperm DNA) and blot was incubated overnight at 65°C. Hybridized filters were then washed with 2×SSC and 0.1% SDS twice for 20 min at room temperature and with 0.1×SSC and 0.1% SDS twice at 65°C and exposed to Kodak XAR-5 X-ray film.

Statistical analysis

All determinations were made in triplicate, and the results were expressed as the mean ± standard deviation (SD). Statistical significance was determined by Student's *t*-test. A *P*-value of 0.05 or less was considered significant.

Results

Susceptibility of various RCC cell lines to DTX-mediated cytotoxicity

R4, R6 and R11 RCC cell lines were tested for sensitivity to DTX cytotoxicity by the MTT assay. The tumor cells were lysed by DTX in a concentration-dependent fashion (Table 1). There were some differences noted in the relative susceptibility of the cell lines to DTX cytotoxicity. Treatment of R11 cells with DTX for less than 8 h showed no significant lytic activity (Table 2). However, following 24–72 h incubation, DTX exerted significant cytotoxic activ-

Table 3 Differential sensitivity of R11 cells to various protein synthesis inhibitors. The susceptibility of R11 cells to various protein synthesis inhibitors at a concentration of 2 nM was analyzed by a 1-day MTT assay. Results are expressed as mean precentage cytotoxicity ± SD of three different experiments

Protein synthesis inhibitor	% Cytoxicity (mean ± SD)
DTX	55.4 ± 5.3
PEA	23.2 ± 2.9
PT	16.4 ± 3.1
Ricin	54.9 ± 7.0
Act D	14.6 ± 1.9
Emetine	5.5 ± 1.2
CHX	6.4 ± 2.0

Table 4 Synergistic cytotoxic effect of DTX and TNF-α on R11 cells. The cytotoxic effect of DTX and TNF-α on R11 cells was assessed in a 1-day MTT assay. Results are expressed as means ± SD of three different experiments

DTX (ng/ml)	TNF-α (ng/ml)		
	0	10	100
0	0	7.0 ± 0.7	11.5 ± 1.0
1	18.4 ± 1.7	37.4 ± 5.3[a]	38.0 ± 5.4
10	47.0 ± 8.7	66.7 ± 8.3[a]	69.4 ± 6.9[a]
100	56.8 ± 5.8	77.7 ± 10.1[a]	86.2 ± 7.0[a]

[a] These values are significantly higher than those obtained by treatment with DTX alone plus those obtained by treatment with TNF-α alone at $P < 0.05$

Table 5 Synergistic cytotoxic effect of DTX and TNF- on R4 cells. The cytotoxic effect of DTX and TNF- on R4 cells was assessed in a 1-day MTT assay. Results are expressed as means ± SD of three different experiments

DTX (ng/ml)	TNF-α (ng/ml)		
	0	10	100
0	0	3.5 ± 0.2	8.3 ± 0.7
1	21.6 ± 1.0	33.3 ± 3.4	43.2 ± 3.1[a]
10	50.1 ± 0.8	59.6 ± 1.6	75.4 ± 3.5[a]
100	77.6 ± 1.4	90.4 ± 6.9[a]	95.0 ± 1.4[a]

[a] These values are significantly higher than those obtained by treatment with DTX alone plus those obtained by treatment with TNF-α alone at $P < 10.05$

ity ($P < 0.05$ vs control to which no DTX was added). This cytotoxic effect of DTX reached a maximum plateau after 48 h incubation. Also, the RCC cell lines were more sensitive to DTX than other tumor cell lines tested (data not shown).

Comparison between DTX and other toxins or protein synthesis inhibitors on cytotoxic activity against R11 cells

Since DTX was cytotoxic to RCC cell lines, we examined whether the cytotoxicity was restricted to DTX or was generalized to include other toxins and protein synthesis inhibitors. DTX-mediated cytotoxicity was compared with cytotoxicity mediated by two bacteria toxins, PEA and PT, and one plant toxin, ricin. Ricin was as effective as DTX, but PEA and PT were less cytotoxic (Table 3).

The protein synthesis inhibitor Act D acts at the level of transcription, and CHX and emetine act at the level of translation [35]. These three inhibitors were minimally cytotoxic. These findings demonstrate that other toxins aside from DTX are cytotoxic to RCC and that the cytotoxicity may not be due to protein synthesis inhibition.

Table 6 Synergistic cytotoxic effect of DTX and TNF-α on R6 cells. The cytotoxic effect of DTX and TNF-α on R6 cells was assessed in a 1-day MTT assay. Results are expressed as means ± SD of three different experiments

DTX (ng/ml)	TNF-α (ng/ml)		
	0	10	100
0	0	13.4 ± 1.6	24.2 ± 0.8
1	7.2 ± 2.3	24.0 ± 2.1	42.2 ± 2.1[a]
10	50.3 ± 1.1	62.8 ± 2.1	90.4 ± 4.0[a]
100	71.1 ± 3.0	83.8 ± 2.2	94.7 ± 0.5

[a] The values are significantly higher than those obtained by treatment with DTX alone plus those obtained by treatment with TNF-α alone at $P < 0.05$

Cytotoxic effect of DTX used in combination with other therapeutic agents

We examined the cytotoxic effect of DTX used incombination with TNF-α, IFN-α, IFN-γ or ADR. Treatment with DTX in combination with TNF-α resulted in synergistic cytotoxic activity against R11 cells in a 1-day MTT assay (Table 4). The synergistic cytotoxic effect of DTX and TNF-α was also observed with R4 cells and R6 cells (Table 5, 6). But the synergistic cytotoxic effect on R6 cells was modest. When calculations of synergistic cytotoxicity were estimated according to isobolographic analysis [23], combination treatment of R11 cells, R4 cells or R6 cells with DTX and TNF-α also showed a synergistic cytotoxic effect (data not shown). In contrast, treatment of R11 cells with DTX in combination with IFN-α, IFN-γ or ADR did not overcome the resistance (data not shown). These findings demonstrate that DTX can be used in conjunction with TNF-α for optimal cytotoxic activity under conditions whereby DTX is used in small concentrations.

Specific inhibition of TNF-α mRNA induction following treatment with TNF-α and DTX

It has been reported that one possible mechanism of resistance of tumor cells to TNF-α is the expression of TNF-α mRNA and/or protein [29, 32]. The role of TNF-α mRNA in synergistic cytotoxicity of DTX and TNF-α was examined. Untreated R11 cells were negative for TNF-α mRNA expression (Fig. 1). However, treatment of R11 cells with TNF-α resulted in the induction of TNF-α

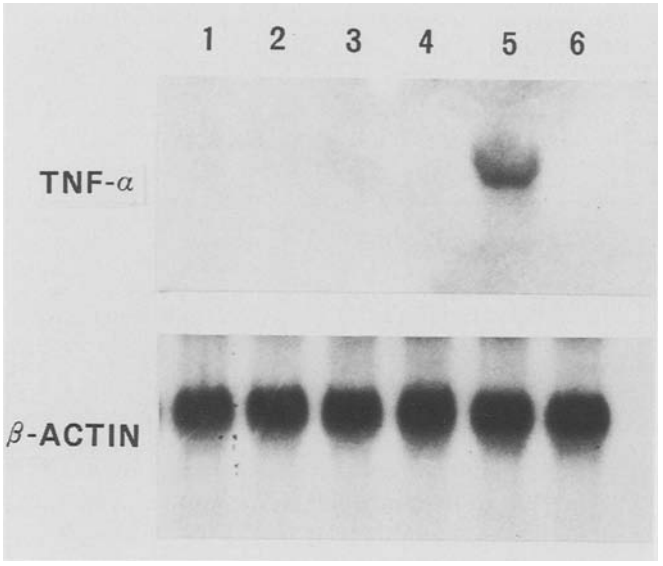

Fig. 1 Effect of DTX and/or TNF-α treatment on the level of TNF-α mRNA in R11 cells: R11 cells were treated with DTX and/or TNF-α at various concentrations for 1 h. Total RNA was then separated, Northern blotted (40 μg/lane tumor cell RNA) and probed for TNF-α and β-actin as described in *"Materials and methods"*. Each lane represents the mRNA for TNF-α and β-actin of R11 cells following these treatments: *lane 1*, medium only; *lane 2*, DTX at 1 ng/ml; *lane 3*, DTX at 10 ng/ml; *lane 4*, DTX at 100 ng/ml; *lane 5*, TNF-α at 10 ng/ml; *lane 6*, DTX at 10 ng/ml and TNF-α at 10 ng/ml simultaneously

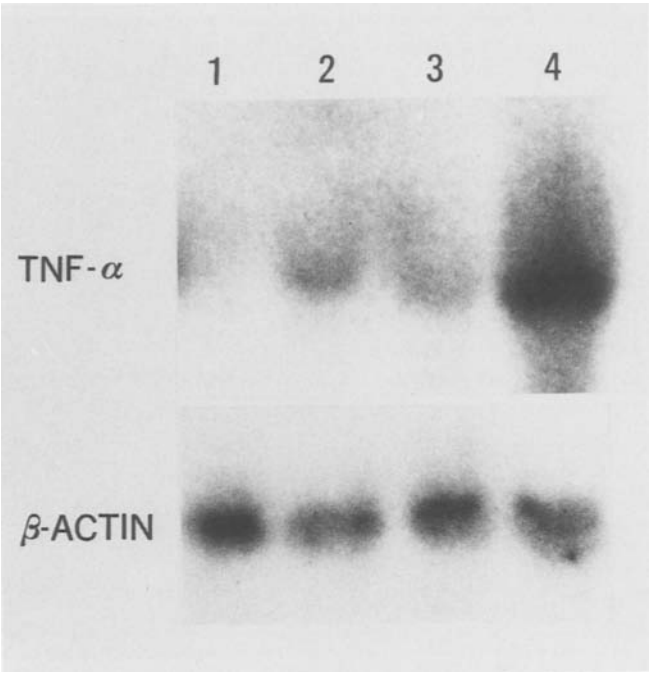

Fig. 2 Effect of CHX and/or TNF-α treatment on the level of TNF-α mRNA in R11 cells: R11 cells were treated with CHX and/or TNF-α at various concentrations for 1 h. Total RNA was then separated, Northern blotted (10 μg/lane tumor cell RNA) and probed for TNF-α and β-actin as described in *"Materials and methods"*. Each lane represents the mRNA for TNF-α and β-actin of R11 cells following these treatment ; *lane 1*, medium only; *lane 2*, TNF-α at 10 ng/ml; *lane 3*, CHX at 1 μg/ml, *lane 4*, CHX at 1 μg/ml and TNF-α at 10 ng/ml simultaneously

mRNA. The optimal induction of TNF-α mRNA was observed following TNF-α treatment at 10 ng/ml for 1 h (data not shown). Treatment of R11 cells with DTX alone did not induce TNF-α mRNA expression. However, treatment with a combination of DTX and TNF-α inhibited the expression of TNF-α-induced TNF-α mRNA. In contrast, treatment of R11 cells with DTX and/or TNF-α had no effect on β-actin mRNA levels. These findings demonstrate that the synergistic cytotoxic activity by DTX and TNF-α against R11 cells may be due in part to the marked downregulation of TNF-α mRNA induction.

DTX inhibits the expression of TNF-α mRNA induced by TNF-α. We examined whether CHX, another protein synthesis inhibitor, also blocks TNF-α mRNA induction. Treatment of R11 cells with CHX resulted in the induction of TNF-α mRNA expression (Fig. 2). Treatment with a combination of CHX and TNF-α enhanced the expression of TNF-α-induced TNF-α mRNA. In contrast, treatment of R11 cells with CHX and/or TNF-α had no effect on β-actin mRNA levels. These findings demonstrate that CHX induces the level of TNF-α mRNA and enhances TNF-α mRNA expression induced by TNF-α in R11 cells and that the block of TNF-α mRNA induction may be specific to DTX.

Discussion

The present study demonstrated that human RCC cell lines were sensitive to DTX-mediated cytotoxicity. The RCC lines were also sensitive to PEA and ricin. Furthermore, treatment of R11 cells with DTX in combination with TNF-α resulted in synergistic cytotoxic activity.

R11 cells were relatively resistant to TNF-α. R11 cells did not express TNF-α mRNA constitutively. However, treatment of R11 cells with a TNF-α induced TNF-α mRNA expression, but treatment with DTX alone did not induce TNF-α mRNA. Treatment of R11 cells with combination of DTX and TNF-α markedly downregulated the level of TNF-α mRNA induced by TNF-α alone. Previous studies have been demonstrated that there is a correlation between the expression of TNF-α mRNA and/or TNF-α protein secretion in tumor cells and their resistance to TNF-α [29, 32]. Thus, one possible mechanism of the synergistic effect of DTX and TNF-α used in combination is the inhibition of TNF-α-induced TNF-α mRNA. Conceivably, treatment of R11 cells with TNF-α induces mRNA for TNF-α and R11 cells may become even more resistant to TNF-α, but the addition of DTX diminishes the TNF-α-induced resistance of R11 cells by marked downregulation of their TNF-α mRNA.

Biosynthesis of TNF-α is regulated at both the transcriptional and post-transcriptional levels [5]. This regulation centers around the presence of a (U + A) exclusive element in the 3'-untranslated region in TNF-α and other cytokines [7]. This element appears capable of directing mRNA degradation as well as suppressing translation [31]. Thus, the protein synthesis inhibitor CHX has been shown to superinduce the level of TNF-α mRNA in tumor cell lines by supposedly inhibiting the production of a labile protein [33]. The current study also demonstrated that CHX induced the expression of TNF-α mRNA in R11 cells. A previous study showed that CHX in combination with TNF-α resulted in no synergistic cytotoxic effect on ovarian cancer cells [27]. These results suggest that the mechanism responsible for the synergistic effect of DTX and TNF-α that involves DTX inhibition of TNF-α mRNA expression induced by TNF-α may be specific to DTX, not a nonspecific effect of protein synthesis inhibition.

It has been reported that the presence of mRNA for TNF-α does not correlate with translation and secretion of TNF-α protein [6, 21]. A preliminary experiment showed that TNF-α-treated R11 cells did not secrete detectable levels of TNF-α protein. Therefore, their resistance may be related to TNF-α mRNA induction in the absence of TNF-α protein secretion.

The precise mechanism of the synergistic cytotoxic effect of DTX and TNF-α on R11 cells is not fully understood, although the inhibition of TNF-α mRNA by DTX is suggestive. It has been reported that the cytotoxic activity of DTX against tumor cells results from its potent inhibitory activity of protein synthesis [11, 12, 18, 28]. The time kinetics of tumor cell lysis resemble those obtained with TNF-α [2, 10]. In addition, like TNF-α, DTX mediates target cell lysis by programmed cell death or apoptosis [9, 10, 15, 30, 38]. The close resemblance of DTX and TNF-α in mediating cell lysis suggests that these cytotoxins may share a common lytic mechanism and may work in concert; thus they may complement each other in the pathway leading to lysis. This possibility may result in the synergistic cytotoxic effect of DTX and TNF-α against R11 cells.

This study suggests that the resistance of R11 cells to TNF-α may be related to TNF-α mRNA induction. Act D, a well known inhibitor of mRNA synthesis, may enhance the sensitivity of tumor cells to TNF-α. However, this possibility has been excluded, since a previous study has demonstrated that Act D does not enhance TNF-α cytotoxicity against ovarian tumor cells [27]. The discrepancy may be due in part to non-specific downregulation of mRNA expression including TNF-α mRNA expression by Act D, or in part to the different tumor cells used.

The findings presented here demonstrating that DTX alone or in combination is cytotoxic to RCC lines offer new scope for the treatment of RCC cells. One possibility is the sequential or simultaneous administration of DTX and TNF-α. DTX can be used in conjunction with TNF-α for optimal cytotoxic activity under conditions whereby DTX is used in small concentrations in vitro, but toxicity needs to be determined in vivo. Clearly, aside from the direct effect of these combinations on the tumor cells, TNF-α is also an immunoregulatory cytokine that can potentiate the antitumor host immune response. DTX may not be the best candidate, since most individuals are preimmunized and upon second exposure they will mount a secondary antibody response that will neutralize the DTX effects. DTX can be administered as shown here.

The demonstration that the high level of resistance of RCC cells to chemotherapeutic drugs can be overcome by DTX and TNF-α opens the way to testing such combinations in overcoming drug resistance. In fact, the combined use of TNF-α and drugs was shown to overcome drug resistance in RCC and ovarian cancer [23–26]. Since DTX is highly toxic in humans, the preparation of conjugates genetically engineered to form single-chain DTX-TNF-α or DTX A-chain immunoconjugates is another possibility that needs to be explored. Thus, special delivery systems for DTX to tumor cells or the production of cytotoxic toxins of less toxicity to normal tissues may overcome the toxicity in vivo. Several studies have shown that tumor-specific immunotoxins are produced by coupling the DTX fragment A with the monoclonal antibody against tumor cells and that they are very effective against tumor [20, 37]. The immunotoxins may be effective for the treatment of RCC by achieving relatively high local concentrations. The method of targeting of DTX to tumor cells is not clarified in the present study and awaits further investigation.

Several toxins such as DTX and ricin are cytotoxic to RCC. Furthermore, DTX has a significant augmenting effect on the direct cytotoxicity of TNF-α against R11 cells. Although the mechanisms involved in these phenomena have not been fully elucidated, the present findings suggest that toxins alone or in combination with TNF-α may be beneficial in overcoming the resistance of RCC to conventional forms of therapy.

Acknowledgements This work was supported by grants (no. 3454385 and no. 5671314) from the Japanese Ministry of Education, Science and Culture and in part by a grant from the Boiron Foundation.

References

1. Baisch H, Otto U, Kloppel G (1990) Antiproliferative and cytotoxic effects of single and combined treatment with tumor necrosis factor alpha and/or alpha interferon on a human renal cell carcinoma xenotransplanted into nu/nu mice: cell kinetics studies. Cancer Res 50:6389
2. Baldwin RL, Chang MP, Bramhall J, Graves B, Bonavida B, Wisnieski BJ (1988) Capacity of tumor necrosis factor to bind and penetrate membranes is pH-dependent. J Immunol 141:2352
3. Belldegrun A, Rosenberg SA (1989) Adoptive immunotherapy of urologic tumors. In:Lepor H, Ratliff TL (eds) Urologic oncology. Kluwer Academic, Boston, p 213
4. Belldegrun A, Abi-Aad AS, Figlin RA, deKernion JB (1991) Renal cell carcinoma: basic biology and current approaches of therapy. Semin Oncol 18:96
5. Beutler B (1990) Regulation of cachectin biosynthesis occurs at multiple levels. In: Melli M, Parente L (eds) Cytokines and

Lymphocortins in inflammation and differentiation. Wiley-Liss, New York, p 229
6. Beutler B, Krochin R, Milsark IW, Leudke C, Cerami A (1986) Control of cachectin (tumor necrosis factor) synthesis: mechanisms of endotoxin resistance. Science 232:977
7. Caput D, Beutler B, Hartog K, Brown-Shimer S, Cerami A (1986) Identification of a common nucleotide sequence in the 3'-untranslated region of mRNA molecules specifying inflammatory mediators. Proc Nat Acad Sci USA 83:1670
8. Chang MP, Wisnieski BJ (1990) Comparison of the intoxication pathways of tumor necrosis factor and diphtheria toxin. Infect Immun 58:2644
9. Chang MP, Baldwin RL, Bruce C, Wisnieski BJ (1989) Second cytotoxic pathway of diphtheria toxin suggested by nuclease activity. Science 246:1165
10. Chang MP, Bramhall J, Graves S, Bonavida B, Wisnieski BJ (1989) Internucleasomal DNA cleavage precedes diphtheria toxin-induced cytolysis. Evidence that cell lysis is not a simple consequence of translation inhibition. J Biol Chem 264:15261
11. Collier RJ, Kandel J (1971) Structure and activity of diphtheria toxin. J Biol Chem 246:1496
12. Collier RJ, Pappenheimer AM Jr (1964) Studies on the mode of action of diphtheria toxin. II. Effect of toxin on amino acid incorporation in cell-free system. J Exp Med 120:1019
13. Cronin RE (1991) Southern internal medicine conference: renal cell carcinoma. Am J Med Sci 302:249
14. deKernion JB, Ramming KD, Smith RB (1978) The natural history of metastatic renal carcinoma. A computer analysis. J Urol 120:148
15. Duck RC, Chervenak R, Cohen JJ (1983) Endogeneous endonuclease-induced DNA fragmentation: an early event in cell-mediated cytolysis. Proc Natl Acad Sci USA 80:6361
16. Halperin E, Harisiadis L (1983) The role of radiation therapy in the management of metastatic renal cell carcinoma. Cancer 51:614
17. Harris DT (1983) Hormonal therapy and chemotherapy for renal cell carcinoma. Semin Oncol 10:422
18. Honjo T, Nishizuka Y, Hayaishi O (1968) Diphtheria toxin-dependent adenosine diphosphate ribosylation of aminoacyl transferase II and inhibition of protein synthesis. J Biol Chem 243:3553
19. Hrushesky WJ, Murphy GP (1977) Current status of therapy for advanced renal cell carcinoma. J Surg Oncol 9:277
20. Johnson VG, Wrobel C, Wilson D, Zovickian J, Greenfield L, Oldfield EH, Youle R (1989) Improved tumor-specific immunotoxins in the treatment of CNS and leptomeningeal neoplasia. J Neurosurg 70:240
21. Kronke M, Hensel G, Schluter C, Scheurich P, Schutze S, Pfizenmair K (1988) Tumor necrosis factor and lymphotoxin gene expression in human tumor cell lines. Cancer Res 48:5417
22. Krown SE (1985) Therapeutic options in renal cell carcinoma. Semin Oncol 4:13
23. Mizutani Y, Bonavida B (1993) Overcoming CDDP resistance of human ovarian tumor cells by combination treatment with CDDP and TNF-α. Cancer 72:809
24. Mizutani Y, Bonavida B (1993) Overcoming TNF-α and CDDP resistance of a human ovarian cancer cell line (C30) by treatment with buthionine sulfoximine in combination with TNF-α and/or CDDP. Int J Oncol 3:229
25. Mizutani Y, Yoshida O (1994) Overcoming TNF-α resistance of human renal and ovarian carcinoma cells by combination treatment with buthionine sulfoximine and TNF-α. Role of TNF-α mRNA downregulation in tumor cell sensitization. Cancer 73:730
26. Mizutani Y, Bonavida B, Nio Y, Yoshida O (1994) Overcoming TNF-α and drug resistance of human renal cell carcinoma cells by treatment with pentoxifylline in combination with TNF-α or drugs. J Urol 151:1697
27. Morimoto H, Safrit JT, Bonavida B (1991) Synergistic effect of tumor necrosis factor-α and diphtheria toxin-mediated cytotoxicity in sensitive and resistant human ovarian tumor cell lines. J Immunol 147:2609
28. Ness VBG, Howard JB, Bodley JW (1980) ADP-ribosylation of elongation factor-2 by diphtheria toxin: NMR spectra and proposed structures of ribosyl-diphthamide and its hydrolysis products. J Biol Chem 255:10710
29. Rubin BY, Anderson SL, Sullivan SA, Williamson BD, Carswell EA, Old LJ (1986) Nonhematopoietic cells selected for resistance to tumor necrosis factor produce tumor necrosis factor. J Exp Med 164:1350
30. Rubin BY, Smith LJ, Hellermann GR, Lunn RM, Richardson NK, Anderson SL (1988) Correlation between the anticellular and DNA fragmentation activities of tumor necrosis factor. Cancer Res 48:6006
31. Shaw G, Kamen R (1986) A conserved AU sequence from the 3' untranslated region of GM-CSF mRNA mediated selective mRNA degradation. Cell 46:659
32. Spriggs DR, Imamura K, Rodriguez C, Horiguchi J, Kufe DW (1987) Induction of tumor necrosis factor expression and resistance in a human breast tumor cell line. Proc Nat Acad Sci USA 84:6563
33. Spriggs DR, Imamura K, Rodriguez C, Sariban E, Kufe D (1988) Tumor necrosis factor expression in human epithelial tumor cell lines. J Clin Invest 81:455
34. Van der Werf-Messing B (1973) Carcinoma of the kidney. Cancer 32:1056
35. Vazquez D (1979) Inhibitors of protein synthesis. Mol Biol Biochem Biophys 30:136
36. Wirth M (1991) The value of interferons, interleukin-2 and tumor necrosis factor in the therapy of renal cell cancer. Urologe 30:77
37. Wrobel CJ, Wright DC, Dedrick RL, Youle RJ (1990) Diphtheria toxin effects on brain-tumor xenografts. J Neurosurg 72:946
38. Wyllie AH, Kerr JFR, Currie AR (1980) Cell death: the significance of apoptosis. Int Rev Cytol 68:251
39. Yagoda A (1989) Chemotherapy of renal cell carcinoma: 1983–1989. Semin Urol 7:199
40. Yagoda A, Bander NH (1989) Failure of cytotoxic chemotherapy, 1983–1988, and the emerging role of monoclonal antibodies for renal cancer. Urol Int 44:338