Cancer Chemother Pharmacol (2002) 50: 46–52
DOI 10.1007/s00280-002-0465-z

ORIGINAL ARTICLE

A. Munshi · T.J. McDonnell · R.E. Meyn

Chemotherapeutic agents enhance TRAIL-induced apoptosis in prostate cancer cells

Received: 2 November 2001 / Accepted: 14 March 2002 / Published online: 9 May 2002

Abstract *Purpose:* Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a member of the tumor necrosis factor (TNF) family that preferentially kills tumor cells. In this study, we sought to determine whether chemotherapeutic agents augment TRAIL-induced cytotoxicity in human prostate cancer cells, and whether this sensitivity can be blocked by overexpression of bcl-2. *Methods:* Prostate cancer cells, PC3 and LNCaP, were treated with TRAIL alone, drug alone or a combination of both for 24 h. Cytotoxicity was determined by DNA fragmentation and clonogenic survival assay. *Results:* Treatment with the conventional chemotherapeutic agents cisplatin (2 and 5 μg/ml), etoposide (10 μ*M* and 20 μ*M*) and doxorubicin (30 and 60 n*M*) dramatically augmented TRAIL-induced apoptosis in LNCaP and PC3 cells. TRAIL-induced apoptosis was partially abrogated by overexpression of bcl-2 in these two cell lines when it was used in combination with the above agents. Similar results were obtained using clonogenic survival assays where bcl-2 overexpression was also found to marginally protect against TRAIL- and chemotherapy-induced cell killing. *Conclusions:* This study demonstrates that combination treatment of prostate cancer cells with TRAIL and chemotherapeutic agents overcomes their resistance by triggering caspase activation. This greater than additive effect of cotreatment with TRAIL and chemotherapy may provide the basis for a new therapeutic approach to induce apoptosis in otherwise resistant cancer cells.

A. Munshi · R.E. Meyn (✉)
Department of Experimental Radiation Oncology,
Box 066, U T M.D. Anderson Cancer Center,
1515 Holcombe Blvd., Houston, Texas 77030, USA
E-mail: rmeyn@mdanderson.org
Tel.: +1-713-7927328
Fax: +1-713-7945369

T.J. McDonnell
Department of Molecular Pathology,
U T M.D. Anderson Cancer Center,
1515 Holcombe Blvd., Houston,
Texas 77030, USA

Keywords TRAIL · Chemotherapy · Apoptosis · DNA fragmentation · Clonogenic assay

Introduction

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL, or Apo2L) is a novel anticancer agent that has been shown to be capable of inducing apoptosis in a variety of human tumor cells both in vitro and in vivo [1, 11, 44]. TRAIL appears to be nontoxic when given systemically, and this enhances its potential as a cancer therapeutic agent. TRAIL can bind to and interact with five distinct receptors of the tumor necrosis factor (TNF) receptor family: DR4, DR5, DcR1, DcR2 and osteoprotegrin [1, 11]. Binding of TRAIL to its receptors DR4 and DR5 leads to recruitment of the adaptor molecule FADD which then results in cleavage and activation of caspase 8. Recent studies have shown that a number of cancer cell lines are resistant to the apoptotic effects of TRAIL suggesting that soluble recombinant TRAIL alone may be ineffective for cancer therapy [13, 19, 20, 32]. However, many chemotherapeutic agents can synergize with TRAIL to induce significant cell death [30], indicating that combination therapy may be a possibility. Several chemotherapeutic agents as well as TRAIL mediate apoptosis, and may induce apoptosis by engaging the same pathways. In addition, treatment with DNA-damaging agents such as cisplatin and etoposide can upregulate DR4 and DR5 expression, thereby enhancing TRAIL-induced apoptotic cell death [8, 10, 19, 41].

Bcl-2 and Bcl-xL have been shown to block the apoptotic response to cancer therapeutics such as radiation and chemotherapeutic drugs and protect cells from apoptosis induced by FasL, TNF and TRAIL [12, 14, 41]. The present study was designed to evaluate the combined effects of TRAIL and chemotherapeutic agents on prostate cancer cells (PC3 and LNCaP) that are resistant to TRAIL when used alone and to examine the effect of overexpression of bcl-2 on their susceptibility to this combination.

Materials and methods

Cells

The human prostate cancer cell lines PC3 (mutant p53 and androgen-insensitive) and LNCaP (wild-type p53 and androgen-sensitive) were used to study the effect of TRAIL in combination with chemotherapeutic drugs. Neomycin control and bcl-2-expressing counterpart lines were generated as described previously [2, 14] and maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, 10,000 U/ml of penicillin-streptomycin, 2 m*M* L-glutamine and G418 (200 μg/ml).

Recombinant human TRAIL/chemotherapeutic drugs

TRAIL was obtained from R&D Systems (Minneapolis, Minn.). This recombinant bioactive TRAIL is a non-disulfide-linked homotrimer. Etoposide (VP16; Bristol Laboratories Oncology Products, Bristol Myers Squibb Company, Princeton, N.J.), Adriamycin PFS (Doxorubicin hydrochloride, Dox; Pharmacia & Upjohn, Kalamazoo, Mich.), Cisplatin (CDDP; Bristol Laboratories Oncology Products) were used at the concentrations indicated.

Apoptosis-inducing treatments

To determine if chemotherapeutic drugs sensitized tumor cells when used in combination with TRAIL, prostate cancer cells were exposed to VP16 (10 and 20 μ*M*), CDDP (2 and 5 μg/ml) or Dox (30 and 60 n*M*) and TRAIL (8 ng/ml) for 24 h. The levels of apoptosis induced were determined using an assay for DNA fragmentation that is based on the solubility of low molecular weight DNA in solutions of low salt concentration [35]. Briefly, cells labeled by ^{14}C-TdR incorporation for one cycle were treated with the chemotherapeutic drug of choice and TRAIL for 24 h to induce apoptosis. After treatment, cells were washed with phosphate-buffered saline (PBS) and then lysed with 0.5 ml lysis buffer (10 m*M* Tris, 1 m*M* EDTA, 0.2% Triton X-100, pH 7.5) on ice for 20 min. The chromatin was pelleted by centrifugation at 14,000 *g* for 10 min. The supernatant (fragmented DNA) was removed and the chromatin pellet was solubilized in 1 ml Soluene (Packard, Meriden, Ct.). Radioactivity was determined using a liquid scintillation counter (Packard Instruments, Downers Grove, Ill.). DNA fragmentation is expressed as the percentage of radioactivity found in the supernatant fraction compared to the total radioactivity (pellet plus supernatant).

Clonogenic survival

The effectiveness of the combination of TRAIL and chemotherapeutic drugs was assessed by clonogenic assays. Briefly, PC3 neo or PC3 bcl-2 cells were treated with TRAIL and chemotherapeutic drug at the indicated concentrations for 24 h. Known numbers of cells were then replated in 100-mm culture dishes and returned to the incubator to allow macroscopic colony development. The surviving fractions following given treatments were calculated based on the survival of untreated cells.

Western blot analysis

Cells were harvested after treatment, rinsed in ice-cold PBS and lysed in lysis buffer containing 50 m*M* HEPES (pH 7.9), 0.4 *M* NaCl, 1 m*M* EDTA, 2 μg/ml leupeptin, 2 μg/ml aprotinin, 5 μg/ml benzamidine, 0.5 m*M* phenylmethyl-sulfonylfluoride and 1% NP-40. The lysed cells were centrifuged at 14,000 rpm to remove any cellular debris. Protein concentrations of the lysates were determined using the Bio-Rad Dc protein assay system (Hercules, Calif.). Equal amounts of protein were separated by 12% SDS-PAGE, transferred to Immobilon (Millipore, Bedford, Mass.) and blocked with 5% nonfat dried milk in Tris-buffered saline/Tween-20 (0.05% v/v) for 1 h at room temperature. The membrane was incubated with the respective primary antibody overnight. Antibodies for caspase 3 and caspase 8 were obtained from Pharmingen (San Diego, Calif.). After washing, the membrane was incubated with an anti-rabbit horseradish peroxidase antibody (diluted 1:2000; Amersham Pharmacia Biotech, Arlington Heights, Ill.) for 1 h. Following several washes, the blots were developed by enhanced chemiluminescence (Amersham, Arlington Heights, Ill.).

Statistical analysis

Data were analyzed using the paired *t*-test (Sigma Plot 5.02v, Richmond, Calif.). Data are presented as the means ± SEM. A difference was regarded as significant if $P < 0.05$.

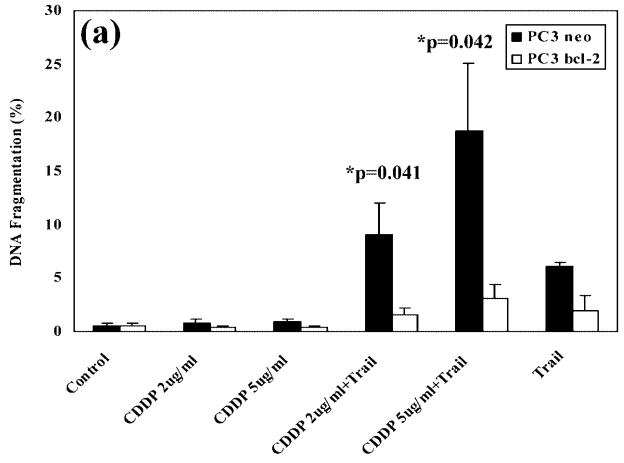
Results

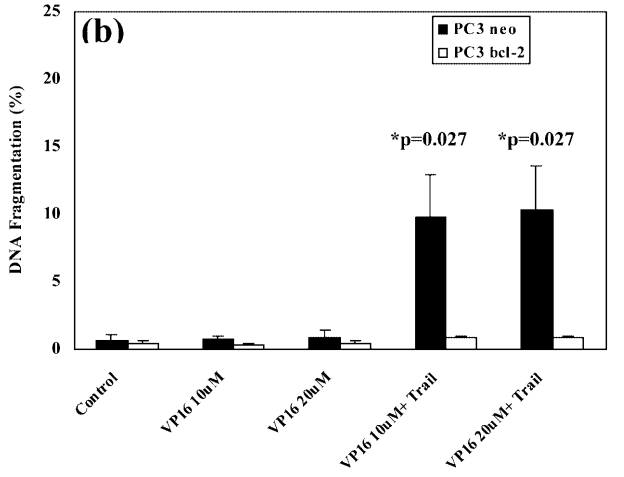
Treatment with a combination of soluble human TRAIL and chemotherapeutic agents enhances apoptosis

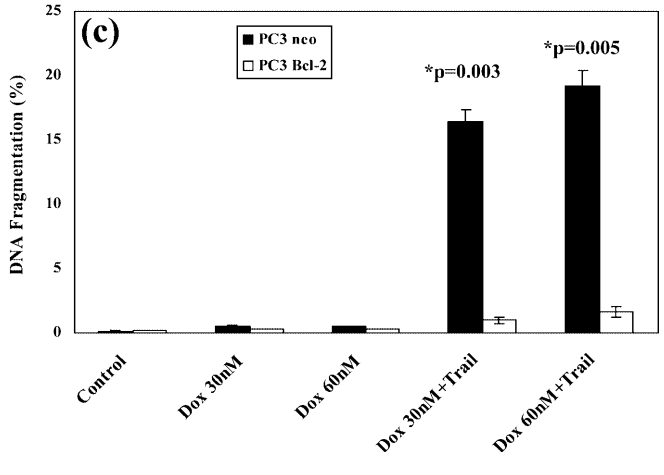
The cytotoxic effect of TRAIL in combination with the commonly used chemotherapeutic agents CDDP, VP16 and Dox in neo control and bcl-2-overexpressing human prostate cancer cell lines, LNCaP and PC3, was evaluated using a DNA fragmentation assay. Both cell lines were relatively resistant to low doses of TRAIL when used alone; however, even these low levels were suppressed in the bcl-2-overexpressing lines (Figs. 1a and 2a).

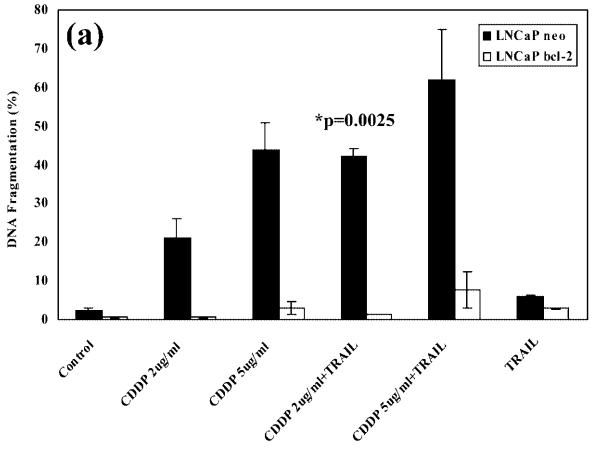
Toxicity was augmented when TRAIL was combined with each of the chemotherapeutic agents. CDDP alone at concentrations of 2 and 5 μ*M* induced only about 1% apoptosis in the PC3 neo cells (Fig. 1a). The toxic effect of the combination was greater than additive at both concentrations of cisplatin in PC3 neo cells yielding 9% and 18% apoptosis, respectively. In LNCaP neo cells exposure to CDDP alone was more toxic than in PC3 cells producing 21% and 44% apoptosis at 2 and 5 μg/ml respectively (Fig. 2a). The combination of TRAIL and CDDP produced a greater than additive effect in the LNCaP neo control cells over either agent used alone; 62% apoptosis was induced by the combination of 5 μg/ml CDDP plus TRAIL ($P = 0.059$).

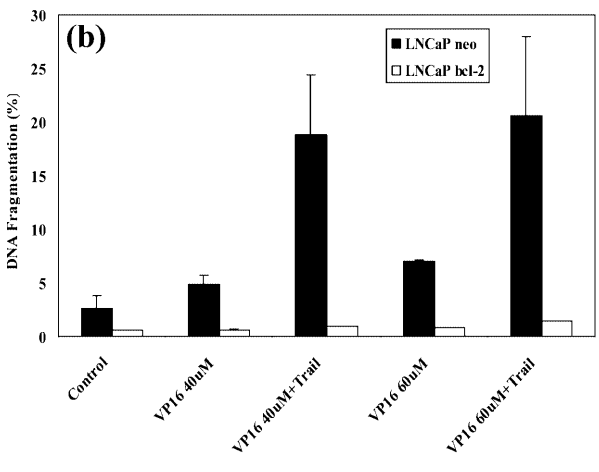
Similar to the findings observed with CDDP, TRAIL-induced apoptosis was found to be augmented in the presence of VP16. VP16 alone had minimal toxicity and induced only 0.73% and 0.86% apoptosis at 10 and 20 μM in the PC3 neo cells whereas combinations of TRAIL and VP16 induced 9.7% and 10.3% apoptosis ($P = 0.027$ and 0.027 at 10 and 20 μ*M*, respectively; Fig. 1b). A greater than additive effect was observed in the LNCaP cells when TRAIL was combined with VP16 (Fig. 2b). Dox at 30 and 60 n*M* enhanced TRAIL-induced apoptosis in the PC3 neo cells from 0.5% and 0.47% to 16.4% and 19%, respectively ($P = 0.003$ and 0.005; Fig. 1c). However, the toxicities of Dox and











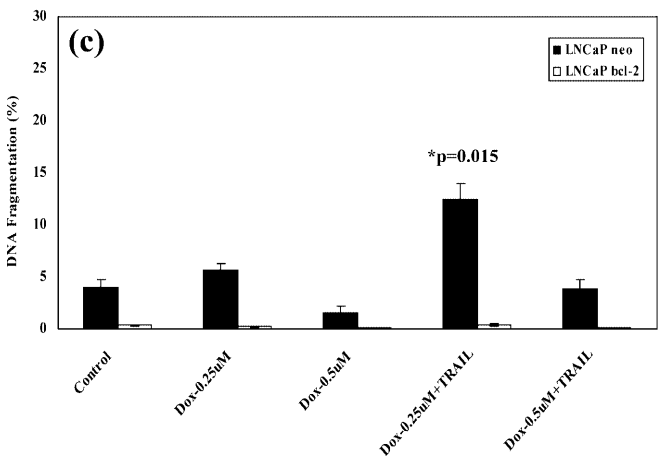


Fig. 1a–c. Dose-response curves for apoptosis induction following administration of TRAIL and chemotherapeutic drugs in PC3 neo and bcl-2-overexpressing cells. Cells were treated with (**a**) CDDP (2 and 5 μ*M*), (**b**) VP16 (40 and 60 μ*M*) or (**c**) Dox (30 and 60 n*M*) alone or in combination with TRAIL (8 ng/ml) for 24 h. Induction of apoptosis was assayed by DNA fragmentation. Overexpression of bcl-2 suppressed the apoptotic response with all three agents

Fig. 2a–c. Dose-response curves for apoptosis induction following administration of TRAIL and chemotherapeutic drugs in LNCaP neo and bcl-2-overexpressing cells. Cells were treated with (**a**) CDDP (2 and 5 μ*M*), (**b**) VP16 (40 and 60 μ*M*) or (**c**) Dox (30 and 60 n*M*) alone or in combination with TRAIL (8 ng/ml) for 24 h. Induction of apoptosis was assayed by DNA fragmentation. Overexpression of bcl-2 suppressed the apoptotic response with all three agents

TRAIL in combination were only additive in LNCaP neo cells where Dox appeared to suppress DNA fragmentation at the higher concentration (Fig. 2c). In contrast to the neo controls, apoptosis following any TRAIL and drug combination was uniformly suppressed in the bcl-2-expressing lines.

Overexpression of bcl-2-enhanced clonogenic survival in PC3 cells treated with TRAIL and chemotherapeutic agents

The apoptosis assays described above were carried out at a single time-point following treatment and therefore may not have reflected the total toxicity that may have been expressed over time. Therefore, we also determined the survival of prostate cancer cells exposed to combinations of chemotherapeutic agents and TRAIL using clonogenic assays. Lower drug concentrations were used for the clonogenic assays due to the higher degrees of toxicity measured by this assay compared to the DNA fragmentation assay. CDDP, VP16 and Dox caused a dose-dependent decrease in clonogenic survival (Fig. 3). TRAIL alone had only slight toxic effects, reducing cell survival to 62% and 88% in the neo and the bcl-2-overexpressing lines, respectively. The survival assays shown in Fig. 3 in which TRAIL was used were normalized to the plating efficiency obtained with TRAIL alone. In relation to the neo control cells, the bcl-2-overexpressing cells showed a reproducible enhancement of clonogenic survival for at least two of the three drugs ($P = 0.027$ for both 0.5 and 1 μg/ml of CDDP and $P = 0.002$ and 0.047 at 5 and 10 μ*M* of VP16). The addition of TRAIL to the treatments sensitized the neo control cells to a small but reproducible degree for all drugs at both concentrations. This effect of TRAIL was even smaller or completely absent in the bcl-2-expressing cells.

Chemotherapeutic agents augment TRAIL-induced toxicity through caspase activation

Caspase activation is considered to be the final common pathway leading to induction of apoptosis in many systems [38]. To identify whether apoptosis induced by TRAIL in combination with chemotherapeutic agents was mediated by caspase activation, PC3 cells were incubated with TRAIL alone, chemotherapeutic agents alone or their combination at the concentrations listed above. Activation of caspase 3 was detected both as cleavage of the proform and formation of the cleavage product using Western blot analysis. Caspase 3 was partially cleaved in cells treated with CDDP, VP16, Dox, or TRAIL when used alone compared to untreated controls (Fig. 4). The combination of TRAIL and chemotherapeutic agents appeared to result in an enhanced activation of caspase 3. Bcl-2 expression in the PC3 cells blocked activation of caspase 3 (Fig. 4). Similar results were obtained with caspase 8, where cells treated with TRAIL in combination with chemotherapeutic agents (Fig. 5) produced cleavage of the pro-caspase form and formation of the cleavage product. As was observed in the case of caspase 3, bcl-2 overexpression blocked caspase 8 activation. Interestingly, TRAIL alone was able to partially activate both caspase 3 and caspase 8 even though it did not induce apoptosis by itself.

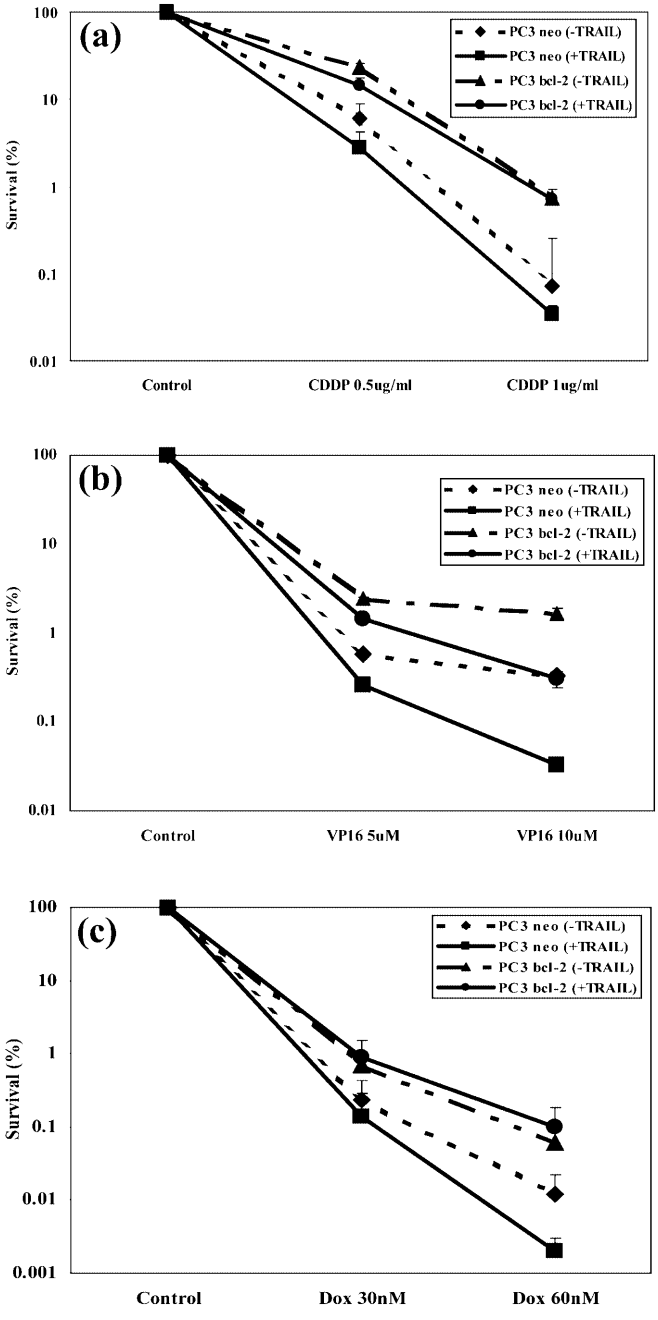


Fig. 3a–c. Clonogenic survival curves for PC3 neo and bcl-2-overexpressing cells following exposure to TRAIL (8 ng/ml) in combination with (**a**) CDDP (0.5 and 1 μg/ml), (**b**) VP16 (5 and 10 μ*M*) or (**c**) Dox (30 and 60 n*M*). Survival was assessed by colony forming assays following 24-h exposures to both TRAIL and each drug. Each data point represents the mean ± SE of two or three independent experiments each done in triplicate

Discussion

The cytokine TRAIL is a promising agent for cancer therapy and is presently under investigation for future clinical studies. A critical aspect of TRAIL as a potential anticancer agent is that its cytotoxic activity appears to

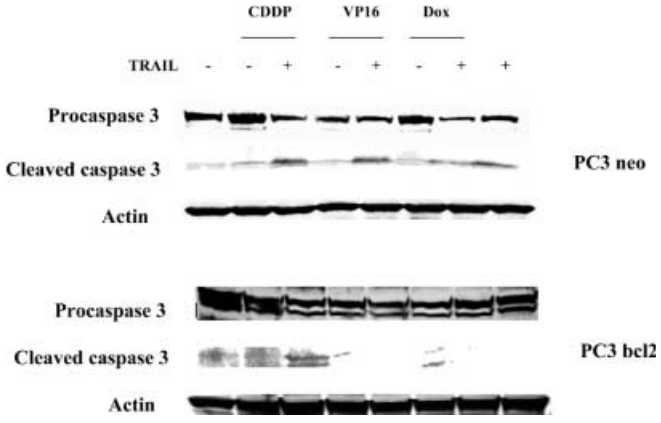


Fig. 4. Combination treatment with TRAIL plus chemotherapeutic drugs results in caspase 3 activation in PC3 cells. Activation of caspase 3 was detected in neo control cells when TRAIL was combined with CDDP, VP16 or Dox on the basis of processing of the pro-caspase form and generation of the cleavage product. TRAIL alone had some ability to activate caspase 3 processing as observed by the generation of the cleavage product. Actin was used as a loading control

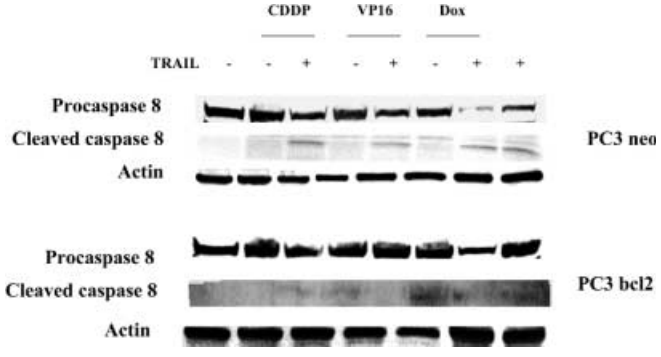


Fig. 5. Combination treatment with TRAIL plus chemotherapeutic drugs results in caspase 8 activation in PC3 cells. Activation of caspase 8 was detected in neo control cells when TRAIL was combined with CDDP, VP16 or Dox on the basis of processing of the pro-caspase form and generation of the cleavage product. TRAIL alone had some ability to activate caspase 8 processing as observed by the generation of the cleavage product. Actin was used as a loading control

be specifically directed towards cancer cells resulting in less systemic toxicity by sparing normal cells [20]. In spite of this fact, TRAIL will probably not be used clinically as a single agent. This has spurred considerable effort to examination of combinations of TRAIL and other modalities including chemotherapy and radiation therapy [10, 24, 26, 29, 33]. There are several reports that point to the fact that TRAIL-induced cell death is enhanced in combination with chemotherapeutic agents. Lacour et al. [22] have demonstrated that CDDP and Dox can sensitize colon cancer cells to TRAIL-induced cytotoxicity by increasing TRAIL-mediated caspase 8 and caspase 3 activation. Hepatocellular carcinoma (HCC) cell lines are also significantly sensitized to TRAIL-induced apoptosis by chemotherapeutic agents such as Dox, camptothecin, 5-fluorouracil and paclitaxel. Sensitization of these HCC cell lines has been found to be independent of the p53 status [42].

TRAIL and chemotherapeutic agents have also been shown to act cooperatively to induce apoptosis in malignant mesothelioma cells, and ovarian, breast, bladder, renal and pancreatic cell lines [5, 6, 9, 19, 23, 26, 42]. In a recent study Nimmanapalli et al. [33] have demonstrated that pretreatment with paclitaxel enhances Apo-2L/TRAIL-induced apoptosis of prostate cancer cells by inducing death receptors 4 and 5 protein levels. As compared with treatment with each agent alone, the sequential treatment with paclitaxel followed by Apo-2L/TRAIL resulted in greater processing of caspase 8, Bid, procaspase 9, and caspase 3 as well as greater cytosolic accumulation of cytochrome c.

In the present study, we demonstrated that the combinations of chemotherapeutic drugs (CDDP, VP16 and Dox) and TRAIL resulted in enhanced cell killing in the human prostate cancer lines tested. This was evident using two different assays for tumor cell inactivation, apoptosis induction and loss of clonogenic survival. Use of these different assays allowed a wider range of drug concentrations to be examined. Based on these results, it appears that certain chemotherapeutic agents may abrogate TRAIL resistance. The mechanisms underlying the resistance to TRAIL are not completely known. The loss of p53 function has been related to the loss of chemosensitivity and poor prognosis of carcinomas [28]. We found no clear correlation between p53 status and a greater than additive interaction of TRAIL and chemotherapy. For example, PC3 harbors mutant p53 whereas LNCaP is wild type for p53. In addition, since LNCaP cells are androgen-sensitive and PC3 cells are not, there was no correlation with androgen sensitivity. A greater-than-additive effect was observed in both these cell lines with at least two of the three chemotherapeutic agents tested. Toxicity was observed in LNCaP neo cells treated with VP16 but the toxicity of the combination with TRAIL appeared to be only additive. Thus, there were some differences in response to this combination of agents between LNCaP and PC3 lines. This may reflect some fundamental differences in the effects of these drugs on these cell lines.

One possible mechanism that could explain an interaction between TRAIL and chemotherapeutic agents is an enhanced activation of caspases. We observed that when chemotherapeutic drugs or TRAIL were used alone, only a weak activation of caspases was detected. However, when used in combination a greater degree of caspase activation was detected in the form of decreases in the amount of procaspase forms and release of active caspase for both caspase 3 and 8. This is consistent with the results of several recent studies showing caspase cleavage in cancer cells derived from different organs upon treatment with TRAIL and chemotherapeutic agents [5, 19, 20]. However, since activation of caspases is downstream of apoptosis initiation, the exact mechanism responsible for enhanced caspase activation in this context has yet to be elucidated.

One factor that does appear to have a substantial effect on TRAIL-induced apoptosis is bcl-2 expression [27]. The bcl-2 oncoprotein is abnormally expressed in a variety of human malignancies. Overexpression of bcl-2 has been shown to delay apoptosis both in vitro and in vivo in many cell and tissue types [4, 15, 18, 31, 40] and to block chemotherapeutic drug-induced apoptosis [34, 37, 39]. Whereas cytotoxic drugs damage tumor cells by a variety of mechanisms (e.g. DNA cleavage, DNA alkylation, topoisomerase II inhibition), ultimately such damage must be translated into signals for apoptosis. In this regard, overexpression of bcl-2 or bcl-xL has been shown to prevent proteolytic processing and activation of caspases in response to chemotherapy-induced damage [16, 17]. The two prostate cancer lines examined in this study displayed lower levels of apoptosis and suppressed caspase processing when bcl-2 was ectopically expressed.

Although it is widely accepted that bcl-2 is a survival factor for many cell types [21] and that overexpression of bcl-2 results in chemoresistance through blocking of apoptosis [25, 36], the ability of bcl-2 to protect against apoptosis induced by TRAIL in combination with chemotherapeutic drugs has been examined to a lesser extent. Since most studies on apoptosis do not include assessment of clonogenic survival, we investigated the ability of bcl-2 to enhance clonogenic cell survival as assessed using a colony forming assay in cells treated with TRAIL and chemotherapeutic agents. Clonogenic survival assays complement assessment of apoptosis because they are sensitive to all modes of cell death and sum these effects over a longer period of time. Despite profound inhibition of apoptotic cell death as assessed by DNA fragmentation, for all agents tested, bcl-2 overexpression led to a less-dramatic but nonetheless reproducible and significant enhancement of clonogenic survival following a 24-h exposure to TRAIL and/or any of the chemotherapeutic agents tested.

The results of several previous studies indicate that bcl-2 overexpression does not enhance clonogenic survival following exposure to chemotherapeutic agents such as etoposide or doxorubicin [7]. A previous study in our laboratory has shown that overexpression of bcl-2 among small cell lung cancer cell lines suppresses apoptosis but does not correlate with loss of clonogenic survival following treatment with etoposide [3]. This previous finding is similar to those of other studies concerning bcl-2 expression in human tumor cells of epithelial origin. For example, Yin and Schimke [43] have examined the response of HeLa cells, in which bcl-2 expression is under the control of a tetracycline-repressible promoter, to aphidicolin. Aphidicolin induces apoptosis that is blocked when bcl-2 expression is allowed, but bcl-2 expression does not enhance clonogenic survival in this cell system following aphidicolin treatment. The conclusions from these studies was that although bcl-2 is able to block the appearance of apoptotic features following exposure to chemotherapeutic drugs, it does not enhance clonogenic survival. Therefore, our observation here of an increase in clonogenic survival in the bcl-2-overexpressing human prostate cancer lines treated with TRAIL and chemotherapeutic drugs suggests that the ability of bcl-2 to suppress apoptosis may impact tumor response in this case.

In summary, our results indicate that chemotherapeutic agents can sensitize cells to TRAIL-mediated cell killing in human prostate cancer cells, thereby raising hopes that such strategies may be useful in the treatment of this disease. However, continued efforts should be made to elucidate the biochemical mechanism responsible for the inhibition of apoptosis by bcl-2 and develop strategies for reversing this resistance to enhance tumor response to drug and cytokine-based therapies.

Acknowledgement This study was supported by grants from the National Cancer Institute (RO1 CA69003) to R.E.M. and the American Cancer Society (RPG-CDD-101054), National Institute of Health (PO1 CA 78778) and the SPORE (P50 CA90270) to T.J.M.

References

1. Ashkenazi A, Dixit VM (1999) Apoptosis control by death and decoy receptors. Curr Opin Cell Biol 11:255
2. Beham A, Marin MC, Fernandez A, Herrmann J, Brisbay S, Tari AM, Lopez-Berestein G, Lozano G, Sarkiss M, McDonnell TJ (1997) Bcl-2 inhibits p53 nuclear import following DNA damage. Oncogene 15:2767
3. Breton C, Story MD, Meyn RE (1998) Bcl-2 expression correlates with apoptosis induction but not loss of clonogenic survival in small cell lung cancer cell lines treated with etoposide. Anticancer Drugs 9:751
4. Bruckheimer EM, Cho S, Brisbay S, Johnson DJ, Gingrich JR, Greenberg N, McDonnell TJ (2000) The impact of bcl-2 expression and bax deficiency on prostate homeostasis in vivo. Oncogene 19:2404
5. Cuello M, Ettenberg SA, Nau MM, Lipkowitz S (2001) Synergistic induction of apoptosis by the combination of trail and chemotherapy in chemoresistant ovarian cancer cells. Gynecol Oncol 81:380
6. Dejosez M, Ramp U, Mahotka C, Krieg A, Walczak H, Gabbert HE, Gerharz CD (2000) Sensitivity to TRAIL/APO-2L-mediated apoptosis in human renal cell carcinomas and its enhancement by topotecan. Cell Death Differ 7:1127
7. Elliott MJ, Stribinskiene L, Lock RB (1998) Expression of bcl-2 in human epithelial tumor (HeLa) cells enhances clonogenic survival following exposure to 5-fluoro-2′-deoxyuridine or staurosporine, but not following exposure to etoposide or doxorubicin. Cancer Chemother Pharmacol 41:457
8. Gibson SB, Oyer R, Spalding AC, Anderson SM, Johnson GL (2000) Increased expression of death receptors 4 and 5 synergizes the apoptosis response to combined treatment with etoposide and TRAIL. Mol Cell Biol 20:205
9. Gliniak B, Le T (1999) Tumor necrosis factor-related apoptosis-inducing ligand's antitumor activity in vivo is enhanced by the chemotherapeutic agent CPT-11. Cancer Res 59:6153
10. Gong B, Almasan A (2000) Apo2 ligand/TNF-related apoptosis-inducing ligand and death receptor 5 mediate the apoptotic signaling induced by ionizing radiation in leukemic cells. Cancer Res 60:5754
11. Griffith TS, Lynch DH (1998) TRAIL: a molecule with multiple receptors and control mechanisms. Curr Opin Immunol 10:559
12. Guo BC, Xu YH (2001) Bcl-2 overexpression and activation of protein kinase C suppress the trail-induced apoptosis in Jurkat T cells. Cell Res 11:101

13. Hernandez A, Wang QD, Schwartz SA, Evers BM (2001) Sensitization of human colon cancer cells to TRAIL-mediated apoptosis. J Gastrointest Surg 5:56
14. Herrmann JL, Beham AW, Sarkiss M, Chiao PJ, Rands MT, Bruckheimer EM, Brisbay S, McDonnell TJ (1997) Bcl-2 suppresses apoptosis resulting from disruption of the NF-kB survival pathway. Exp Cell Res 237:101
15. Hockenbery D, Nunez G, Milliman C, Schreiber RD, Korsmeyer SJ (1990) Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. Nature 348:334
16. Ibrado AM, Huang Y, Fang G, Bhalla K (1996) Bcl-xL overexpression inhibits Taxol induced Yama protease activity and apoptosis. Cell Growth Differ 7:1087
17. Ibrahim SM, Ringel J, Schmidt C, et al (2001) Pancreatic adenocarcinoma cell lines show variable susceptibility to TRAIL-mediated cell death. Pancreas 23:72
18. Jacobson MD, Burne JF, Raff MC (1994) Programmed cell death and bcl-2 protection in the absence of a nucleus. EMBO J 13:1899
19. Keane MM, Ettenberg SA, Nau MM, Russell EK, Lipkowitz S (1999) Chemotherapy augments TRAIL-induced apoptosis in breast cell lines. Cancer Res 59:734
20. Kim K, Fisher MJ, Xu SQ, el-Diery WS (2000) Molecular determinants of response to TRAIL in killing of normal and cancer cells. Cancer Res 6:335
21. Korsmeyer SJ (1992) Bcl-2 initiates a new category of oncogenes: regulators of cell death. Blood 80:879
22. Lacour S, Hammann A, Wotawa A, Corcos L, Solary E, Dimanche-Boitrel MT (2001) Anticancer agents sensitize tumor cells to tumor necrosis factor-related apoptosis-inducing ligand-mediated caspase-8 activation and apoptosis. Cancer Res 61:1645
23. Liu W, Bodle E, Chen JY, Gao M, Rosen GD, Broaddus VC (2001) Tumor necrosis factor-related apoptosis inducing ligand and chemotherapy cooperate to induce apoptosis in mesothelioma cell lines. Am J Respir Cell Mol Biol 25:111
24. Matsuzaki H, Schmied BM, Ulrich A, Standop J, Schneider MB, Batra SK, Picha KS, Pour PM (2001) Combination of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and actinomycin D induces apoptosis even in TRAIL-resistant human pancreatic cancer cells. Clin Cancer Res 7:407
25. Miyashita T, Reed JC (1993) Bcl-2 oncoprotein blocks chemotherapy-induced apoptosis in a human leukemia cell line. Blood 81:151
26. Mizutani Y, Nakao M, Ogawa O, Yoshida O, Bonavida B, Miki T (2001) Enhanced sensitivity of bladder cancer cells to tumor necrosis factor related apoptosis inducing ligand mediated apoptosis by cisplatin and carboplatin. J Urol 165:263
27. Munshi A, Pappas G, Honda T, McDonnell TJ, Younes A, Li Y, Meyn RE (2001) TRAIL (APO-2L) induces apoptosis in human prostate cancer cells that is inhibitable by bcl-2. Oncogene 20:3757
28. Murakami I, Hiyama K, Ishioka S, Yamakido M, Kasagi F, Yokosaki Y (2000) p53 gene mutations are associated with shortened survival in patients with advanced non-small cell lung cancer: an analysis of medically managed patients. Clin Cancer Res 6:526
29. Nagane M, Pan G, Weddle JJ, Dixit VM, Cavenee WK, Huang HJ (2000) Increased death receptor 5 expression by chemotherapeutic agents in human gliomas causes synergistic cytotoxicity with tumor necrosis factor-related apoptosis-inducing ligand in vitro and in vivo. Cancer Res 60:847
30. Nagane M, Huang HJ, Cavenee WK (2001) The potential of TRAIL for cancer chemotherapy. Apoptosis 6:191
31. Newmeyer DD, Farschon DM, Reed JC (1994) Cell free apoptosis in *Xenopus* egg extracts: inhibition by bcl-2 and requirement for an organelle fraction enriched in mitochondria. Cell 79:353
32. Nguyen T, Zhang XD, Hersey P (2001) Relative resistance of fresh isolates of melanoma to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis. Clin Cancer Res 7 [3 Suppl]:966s
33. Nimmanapalli R, Perkins CL, Orlando M, O'Bryan E, Nguyen D, Bhalla KN (2001) Pretreatment with paclitaxel enhances Apo-2 ligand/tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis of prostate cancer cells by inducing death receptors 4 and 5 protein levels. Cancer Res 61:759
34. Reed JC (1995) Bcl-2: prevention of apoptosis as a mechanism of drug resistance. Hematol Oncol Clin North Am 9:451
35. Sellins KS, Cohen JJ (1987) Gene induction by gamma-irradiation leads to DNA fragmentation in lymphocytes. J Immunol 139:3199
36. Tang C, Willingham MC, Reed JC, Miyashita T, Ray S, Ponnatupur V, Huang Y, Mahoney ME, Bullock G, Bhalla K (1994) High levels of p26BCL-2 oncoprotein retard Taxol-induced apoptosis in human pre-B leukemia cells. Leukemia 8:1960
37. Thompson CB (1995) Apoptosis in the pathogenesis and treatment of disease. Science 267:1456
38. Thornberry NA (1997) The caspase family of cysteine proteases. Br Med Bull 53:478
39. Tu SM, McConnell K, Marin MC, Campbell ML, Fernandez A, von Eschenbach AC, McDonnell TJ (1995) Combination adriamycin and suramin induces apoptosis in bcl-2 expressing prostate carcinoma cells. Cancer Lett 93:147
40. Vaux DL, Cory S, Adams JM (1988) Bcl-2 gene promotes hematopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. Nature 335:440
41. Wen J, Ramadevi N, Nguyen D, Perkins C, Worthington E, Bhalla K (2000) Antileukemic drugs increase death receptor 5 levels and enhance Apo-2L-induced apoptosis of human acute leukemia cells. Blood 96:3900
42. Yamanaka T, Shiraki K, Sugimoto K, Ito T, Fujikawa K, Ito M, Takase K, Moriyama M, Nakano T, Suzuki A (2000) Chemotherapeutic agents augment TRAIL-induced apoptosis in human hepatocellular carcinoma cell lines. Hepatology 32:482
43. Yin DX, Schimke RT (1995) Bcl-2 expression delays drug-induced apoptosis but does not increase clonogenic survival after drug treatment in HeLa cells. Cancer Res 55:4922
44. Zhang XD, Franco A, Myers K, Gray C, Nguyen T, Hersey P (1999) Relation of TNF-related apoptosis-inducing ligand (TRAIL) receptor and FLICE-inhibitory protein expression to TRAIL-induced apoptosis of melanoma. Cancer Res 59:2747