

Enhanced apoptosis of soft tissue sarcoma cells with chemotherapy: A potential new approach using TRAIL

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ABSTRACT

Soft tissue sarcomas are less responsive to conventional chemotherapy when compared to bone sarcomas. We investigated the possibility of enhancing the efficacy of chemotherapy by utilising the recently identified cytokine, tumour necrosis factor-related apoptosis-inducing ligand (TRAIL/Apo2L) in combination with standard chemotherapeutic agents. Fresh human soft tissue sarcomas (rhabdomyosarcoma, fibrosarcoma, malignant fibrous histiocytoma) were obtained at biopsy and dispersed tumour cells were incubated in cell culture with standard cytotoxic agents, either as single agents or in combination with TRAIL. The chemotherapeutic agents were, at best, moderately effective, in terms of induction of cellular apoptosis, although the fibrosarcoma was completely unresponsive to all single agents. TRAIL alone had no effect on any sarcoma cell culture. In contrast, the addition of TRAIL and drug together produced a significant increase in sarcoma cell apoptosis, with TRAIL and doxorubicin the most effective combination.

Key words: Sarcoma, chemotherapy, Apo2L, TRAIL

INTRODUCTION

Chemotherapy is an established treatment modality for high grade bone sarcomas but remains controversial for use with high grade soft tissue sarcomas. The main obstacle to its use is the poor sensitivity of most soft tissue sarcomas to traditional chemotherapeutic agents. The most active agent, doxorubicin, has been reported to have a response rate of only 34% at best and most studies report approximately 26%.^{1,4} Recently, attention has been drawn to the potential use of the TNF family member, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL/Apo2L), as a selective anti-tumour agent.^{2,9} The combination of TRAIL and conventional chemotherapeutic agents has been shown to be synergistic with respect to inducing cell death in several cancer types *in vitro*.^{6,10} We tested this approach, which has not previously been reported for freshly isolated sarcoma cells, in a variety of soft tissue sarcomas in primary culture. We found an increased sensitivity of sarcoma cells to chemotherapeutic agents in the presence of TRAIL, suggesting that this combination may be useful in the future treatment of sarcomas.

MATERIALS AND METHODS

Recombinant soluble human TRAIL/Apo2L was from Pepro Tech Inc. (Rocky Hill, NJ USA). Chemotherapeutic drugs Doxorubicin, Methotrexate, Cisplatin, and Etoposide were obtained from Pharmacia & Upjohn, (Kalamazoo, MI, USA). Cyclophosphamide was purchased from Baxter Health Care Pty. Ltd. (Parkway Deerfield, IL, USA).

Soft tissue sarcomas were harvested from patients undergoing biopsy of a mass. The sarcoma types and patient details are listed in Table 1. Tumour samples were processed within 24 h of surgical removal. Briefly, tissue was dissected using a scalpel blade, then enzymatically digested with 5 ml each of collagenase and dispase (10 mg/mL) and incubated for 3 h at 37°C. Cells were diluted and cultured in Dulbecco's Modified Eagle Medium (DMEM), supplemented with glutamine (2 mM), penicillin (100 IU/ml), gentamicin (160 µg/ml) and 10% fetal bovine serum (Biosciences, Sydney, Aust), in a humidified atmosphere containing 5% CO₂. For determination of TRAIL-mediated cytotoxicity, 1x10⁴ cells per well were seeded in 96 well microtiter plates and allowed to adhere to the plate. Cells were then treated with 100 ng/mL of recombinant soluble TRAIL, in the presence or absence of chemotherapeutic agents, doxorubicin (1 µM), cisplatin (25 µM), etoposide (50 µM), methotrexate (25 µM), or cyclophosphamide (25 µM). These agents were added to the wells alone, or in combination with TRAIL, and incubated for 48 h with assessments of viability undertaken at 48h. Cell viability was determined by staining the cells with crystal violet and the OD₅₇₀ of the cell lysates, which correlated to cell number, was determined. All treatments were performed in quadruplicate and results are given as the mean +/- SD.

Table 1
Tumour demographics

Sarcoma type	Patient age	Patient sex	Sarcoma location
Rhabdomyosarcoma	7	male	calf
Fibrosarcoma	46	female	flank
Malignant fibrous histiocytoma	71	male	pelvis

RESULTS

Doxorubicin (DOX), Cisplatin (CDDP) and Etoposide (ETP) were found to be, at best, moderately effective

in inducing apoptosis in the sarcoma cells and were differentially cytotoxic in the different tumours. Methotrexate (MTX) and cyclophosphamide (CPM) were found to have no significant activity. The most sensitive sarcoma to single agent treatment was the rhabdomyosarcoma, followed by the malignant fibrous histiocytoma (Table 2). TRAIL alone had no effect on the viability of sarcoma cells cultured from any of the tumours under the conditions of culture (Table 2). In contrast, the addition of TRAIL to the single agents produced a dramatic response in most cases, with most of the combinations producing a highly significant enhancement of cell death compared with the drugs alone (Table 2). TRAIL in combination with doxorubicin was the most efficacious in terms of cytotoxicity, leaving 24%, 35% and 17% of cells viable after 48h treatment, compared with untreated control, in the rhabdomyosarcoma, fibrosarcoma and malignant fibrous histiocytoma, respectively. This was significantly different from treatment with doxorubicin alone. CDDP in combination with TRAIL decreased cell viability to 52%, 63% and 32% of untreated control, in the rhabdomyosarcoma, fibrosarcoma and malignant fibrous histiocytoma, respectively. An independent experiment with the rhabdomyosarcoma cells yielded results identical to those shown. A weaker response was also seen with etoposide, methotrexate and cyclophosphamide in combination with TRAIL (Table 2).

DISCUSSION

Bone sarcoma treatment was revolutionized with the advent of chemotherapy. This revolution has not extended to the treatment of soft tissue sarcomas, which often prove resistant to conventional chemotherapy. The tumor necrosis factor (TNF) is the prototype of a rapidly growing superfamily of cytokines to be considered as potential new therapeutics for the treatment of malignant disease because of their ability to induce cell death by apoptosis.⁸ Tumor necrosis factor-Related Apoptosis-Inducing Ligand (TRAIL/Apo2 ligand) is a member of the tumor necrosis factor cytokine family. It induces cell death through interactions with its cognate death domain containing receptors in a wide variety of tumor cell lines but does not seem to be cytotoxic to normal cells.^{2,9} The differential sensitivity of cells to TRAIL seems to be achieved by mechanisms that include the expression of 'decoy' receptors by normal cells and/or the overexpression of intracellular inhibitory proteins such as FLICE-inhibitory protein, FLIP.⁵

The sarcomas reported in this paper belong to a

Table 2
Dose response of soft tissue sarcomas to chemotherapeutic agents as single agents or in culture with TRAIL.
Results are the mean percentage viability of the cells, compared with control, \pm standard deviation of cells,
treated in quadruplicate. ND = treatment not done.

Chemotherapeutic agent	Rhabdomyosarcoma	Fibrosarcoma	Malignant fibrous histiocytoma
Untreated	100 \pm 1.7	100 \pm 4.7	100 \pm 5.6
TRAIL	100 \pm 3.1	110 \pm 5.9	100 \pm 7.7
Doxorubicin	38 \pm 5.6	103 \pm 11.3	58 \pm 6.1
DOX + TRAIL	24 \pm 1.7 *	35 \pm 6.1 *	17 \pm 11.1 *
Cisplatin	57 \pm 3.7	125 \pm 12.9	102 \pm 19.3
CDDP + TRAIL	52 \pm 3.5 *	63 \pm 26.8 *	32 \pm 5.9 *
Etoposide	72 \pm 1.0	111 \pm 12.0	104 \pm 7.3
ETP + TRAIL	75 \pm 2.6	66 \pm 8.5 *	69 \pm 14.7 *
Methotrexate	ND	99 \pm 1.4	104 \pm 21.5
MTX + TRAIL	ND	79 \pm 20.1	68 \pm 19.6 *
Cyclophosphamide	ND	103 \pm 8.6	129 \pm 2.7
CPM + TRAIL	ND	74 \pm 7.4 *	61 \pm 15.6 *

* Values for the combined treatment were significantly lower than for treatment with TRAIL alone, drug alone and from untreated controls ($p < 0.05$).

class of tumours that have variable sensitivity to the common chemotherapeutic agents, ranging from fair to no response. The impetus to test the combined effect of TRAIL and chemotherapeutic agents in these tumours was based on the promising results obtained in several other tumour types^{6,10} and by ourselves in a panel of osteogenic sarcoma cell lines (manuscript in preparation). It was found that the addition of TRAIL significantly increased the effects of chemotherapeutic agents in soft tissue sarcomas *in vitro*. The mechanisms for this have not been explained but in other tumour types include an up-regulation of the death receptors DR4 or DR5 by the chemotherapeutic agents.⁷ This

increase in apoptosis with TRAIL was seen when sub-optimal concentrations of chemotherapeutic agents were used that alone had little effect on the cells. Furthermore, these concentrations were within the range clinically achieved in patients.³ These results therefore suggest the potential for TRAIL to increase the effectiveness of chemotherapeutic agents in soft tissue sarcoma, while concurrently allowing a reduction in the exposure to drugs such as doxorubicin, and consequently, reduced toxicity. This synergistic action has yet to be tested *in vivo* but may prove clinically relevant in the treatment of this usually refractory class of malignancies.

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