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Augmented cytotoxicity of estramustine and etoposide combination in hormone refractory prostate cancer cell lines and association with TGF modulation

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The combination therapy of oral etoposide and estramustine phosphate (EMP) showed promising results for the treatment of hormone-refractory prostate cancer. Binding of EMP to microtubule-associated proteins, tubulin and proteins of the nuclear matrix are currently considered to be the most likely mechanisms underlying the cytotoxicity in androgen-independent prostatic carcinoma. Combination of EMP with etoposide has produced antitumour responses in 30–60% of patients with metastatic hormone-refractory prostate carcinoma. *In vitro*, EMP and etoposide (VP-16) appeared to act synergistically to inhibit the growth of the metastatic human prostate adenocarcinoma cell line PC3. Transforming growth factor β(TGF-β) is growth inhibitory to many malignant cells, including prostate cancer cells. We have previously shown that TGF-β plays a significant role in PC3 cell growth inhibition. This study aimed to define the possible association of activity of antineoplastic agents with TGF-β protein in hormone-refractory prostate cancer cell lines.

Method

PC3 and DU-145 cells were maintained as monolayer in tissue culture flasks in culture medium consisting of RPMI 1640 supplemented with penicillin/streptomycin, L-glutamine (2 mM final concentration) and 10% fetal calf serum, and incubated at 37 °C in a 5% CO_2-humidified atmosphere. Incubation of cultures was performed in the presence of EMP (10 (g/ml) with or without VP-16 (0.1 (g/ml). Corresponding levels of TGF-b in particular experiments were measured in culture supernatants by ELISA. Cytotoxicity in treatment groups was also investigated by Lactic Dehydrogenase Release Colorimetric Test. Experiments were run in triplicate. Statistical analysis of data was performed by ANOVA test.

Results

Both cell lines secreted TGF-β into the culture medium under basal conditions. PC3 cells revealed 467 ± 58.9 pg/ml ($\pm$s.e.m.) of protein in the control group, 630.8 ± 60.6 pg/ml in EMP ($P = 0.12$) and 936.8 ± 32.5 pg/ml in EMP + VP − 16 group ($P < 0.01$). Corresponding levels of TGF-β were 322 ± 80.7, 473.7 ± 26.7 and 395.5 ± 8.2 pg/ml in the control, EMP and combination groups, respectively.

The LDH cytotoxicity test displayed increased cell death in PC3 cells above control levels: 1%in the EMP and 19% in combination group. Corresponding rates in DU-145 cells were 4% and 56%, respectively.

Conclusion

EMP and VP-16 upregulated TGF-β secretion over control samples and exerted a significant inhibitory effect on hormone-resistant PC3 and DU-145 cell lines. Combination therapy was associated with higher levels of TGF-β protein and increased cell death compared with EMP treatment alone. Our observations are consistent with results obtained in clinical trials, and this combination appears to be relatively efficient in hormone-refractory prostate cancer, at least in part by TGF-β modulation.