Combinatory effects of docetaxel with the naturally occuring oncolytic virus Coxsackievirus A21 (CVA21)

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Abstract

Recent growth in the field of oncolytic virotherapy has led to an increased number of clinical trials and acceptance of tumour selective viruses as a promising cancer treatment strategy. One such virus is the Coxsackievirus A21 (CVA21), which has demonstrated oncolytic activity not only in *in vitro* settings and *in vivo* murine models, but also in early clinical trials.

Following numerous reports of significant activity in various malignancies, docetaxel has been accepted worldwide as the first line standard of care anti-cancer agent for many types of cancer. Docetaxel, a member of the taxane chemotherapy family acts by disrupting the normal process of microtubule assembly and disassembly. However, like most anti-cancer drugs, it has many undesirable adverse effects at high concentrations, resulting in a narrow therapeutic window. One approach to overcome this is to combine oncolytic virotherapy with mainstream chemotherapies in hopes of reducing toxicity while still achieving a favourable therapeutic index.

To study this, we investigated the oncolytic activity of CVA21 in combination with docetaxel against melanoma. Combination indices (CIs) were determined using the median effect equation at various drug concentrations and ratios (synergy: CI < 0.9, additivity: 0.9 < CI < 1.1, and antagonism: CI > 1.1). Synergy maps were used to identify the dose ratios which led to high synergy and effective cell death. The outcome from this study firmly suggests that the administration of docetaxel does not interfere with the oncolytic effect of CVA21, and in fact enhances it.

Methods

Cells:

SK-Mel-28, Mel-RM, ME4405 and MV3. All cells were maintained in DMEM supplemented with 10 % FBS at 37 °C with 5% CO_2.

Virus:

Coxsackievirus A21 (CVA21) Kuykendall strain (product name: CAVATAK™) was supplied by Viralytics Ltd.

Chemotherapy:

Docetaxel (Taxotere) was obtained from the Department of Clinical Toxicology & Pharmacology (Calvary Mater Newcastle, Newcastle, Australia). Docetaxel was provided as an injectable concentrate of 20 mg / 2 mL.

Cell viability assay:

Cell viability was assessed using a MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay which indicates metabolic activity in live cells. 20 µL of MTT solution (5 mg/mL) was added to the cells at the end of the treatment period. Purple formazan crystals formed after 3 hours of incubation were dissolved using 50 µL of dimethyl sulfoxide. Cell viability was determined by reading the absorbance at 540 nm (Ex) / 620 nm (Em).

CVA21 and docetaxel synergistic interactions:

The unified theory, introduced by Chou, was used to evaluate the relationship of CVA21 and Docetaxel in combination. CI values were determined using the following equation:

$$CI = \frac{[D]_1}{[D_x]_1} + \frac{[D]_2}{[D_x]_2}$$

where CI < 0.9, 0.9 < CI < 1.1, and CI > 1.1 indicates synergism, additive effect, and antagonism respectively. The denominators are doses of drug 1 and drug 2, respectively, each inhibit x %, and the numerators, drug 1 and drug 2 in combination, also inhibit x %.

Results

1. Oncolytic effect and chemosensitivity of melanoma cell lines

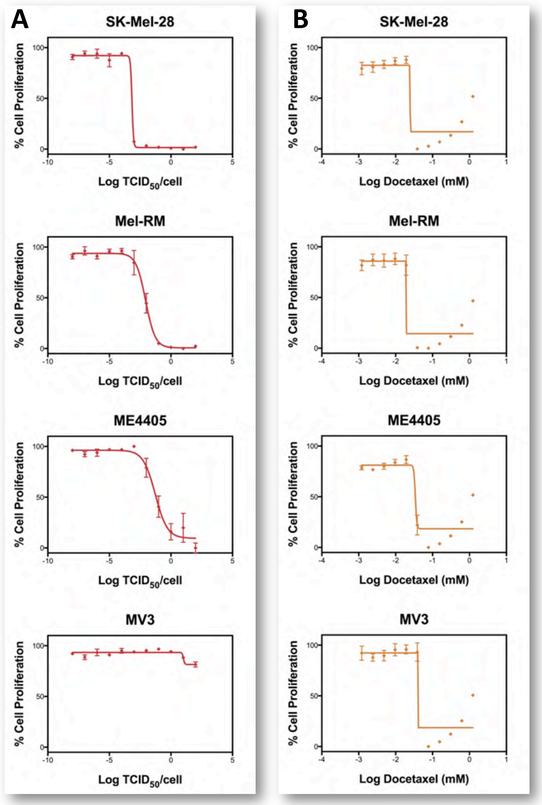

Figure 1. Dose response curves of 4 human cell lines SK-Mel-28, Mel-RM, ME4405 and MV3 treated with serial dilutions of CVA21 and docetaxel, 72 hours post treatment. (A) Oncolytic effects of CVA21 in melanoma cell lines. CVA21 dose is defined as multiplicity of infection (MOI) expressed as tissue culture infectious dose per cell ($TCID_{50}$/cell). SK-Mel-28 was most sensitive to a CVA21 infection followed by Mel-RM and ME4405. MV3 was found to be resistant to CVA21. (B) Chemosensitivity of melanoma cells to docetaxel. Docetaxel concentrations were expressed in logarithmic mM. All four melanoma cell lines had similar chemosensitivy profile to docetaxel, with an unusual resistance at higher concentrations of docetaxel.

2. Combination effects of CVA21 and docetaxel in melanoma cell lines

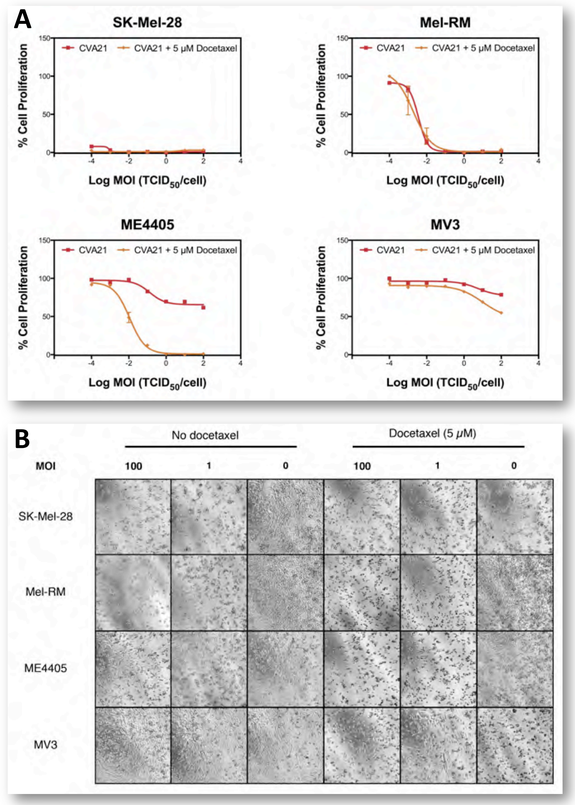

Figure 2. Dose response curves of CVA21 in combination with docetaxel in melanoma cells. (A) The effects of CVA21 in combination with 5 µM of docetaxel, 72 hours post treatment. A concentration of 5 µM was selected for docetaxel as it represents the maximum concentration attainable in patient serum (C_{max}). The highest levels of synergism were observed in the ME4405 cell line followed by the MV3 cell line. The virus-drug combination though not highly synergistic in the SK-Mel-28 and Mel-RM cell line, showed that the addition of docetaxel did not impede the oncolytic activity of CVA21. (B) Photomicrographs of a panel of melanoma cell lines treated with CVA21, with or without docetaxel.

3. Synergy heat map of CVA21 with docetaxel at various concentrations

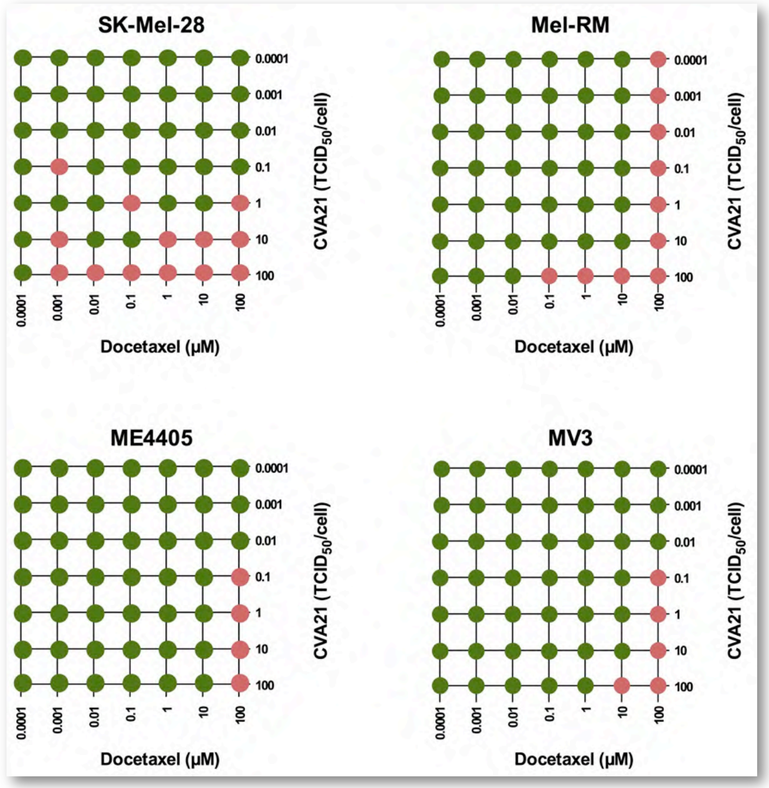

Figure 3. Synergy maps compiling CI values. The dots in the figure indicate antagonism (CI > 1, red), and synergism (CI < 1, green) between CVA21 and docetaxel against a panel of melanoma cells at various concentrations. High synergistic activity was observed in the cell line ME4405 and MV3 where 45/49 and 44/49 drug combinations were tested synergistic. The combination of CVA21 and chemotherapy was mostly antagonistic for cells treated with high levels of docetaxel.

Conclusion

- **Using *in vitro* cell models, three out of four melanoma cell lines were sensitive to CVA21 infection. This panel of melanoma cell lines had similar chemosensitivity profiles and were strongly resistant to docetaxel at higher concentrations (10 – 100 mM).**
- **When used in combination, CVA21 is able to exert its oncolytic activity in the presence of docetaxel at its C_{max}. Our results suggest that the addition of docetaxel does not impede the oncolytic activity of CVA21. This combination significantly improved melanoma cell destruction in the ME4405 and MV3 cell lines.**
- **Photomicrographs taken of ME4405 cells show enhanced tumour cell destruction when treated with CVA21 in combination with docetaxel, compared to single agent CVA21 or docetaxel alone.**
- **After using Chou-Talalay's drug combination analysis to calculate combination index values, a synergy map was used to identify the numerous dose ratios which led to high synergy and effective cell death. The map strongly suggests synergistic activity in most dose ratios except for ratios using high concentrations of docetaxel.**