Student: Eleanor Burgess

Title: Does vitamin C interact with chemotherapy in cancer treatment in vivo

Supervisor(s): Dr Abel Ang and Associate Professor Gabi Dachs

Sponsor: Cancer Society of New Zealand

Introduction:

Ascorbate (Vitamin C) is an anti-oxidant that is necessary in the human body to combat oxidative stressors such as those generated by tumour cells. Vitamin C is also necessary to maintain health in humans as it is an enzymatic co-factor used during cell repair processes. At high doses, vitamin C is a pro-oxidant creating reactive oxygen species which act to kill tumour cells. It is thought that vitamin C treatment can be used in combination with chemotherapy treatment to combat cancer.

However, the use of vitamin C treatment is controversial. While there is thought to be benefit from using vitamin C treatment for cancer patients, there are also clinical concerns that vitamin C may interfere with conventional cancer treatment such as chemotherapy. The use of vitamin C may have different effects on tumour response based on when the treatment is given. We will determine if vitamin C treatment is effective at enhancing or reducing the effects of chemotherapy, and if the timing of vitamin C treatment effects the outcome of the treatment.

Aim:

The project aims to identify the location and measure the level of vitamin C in the tumour following high dose vitamin C injections. This project also aims to determine if the timing of the vitamin C treatment versus the chemotherapy treatment alters the outcome by analysing the level of DNA damage present in tumours, as an indicator of chemotherapy effectiveness

Impact: The use of vitamin C treatment remains controversial. The information from this project will give a good indication of whether vitamin C treatment in combination with chemotherapy is a worthwhile treatment option in a mouse model, while also identifying the most effective timing of treatments for the best treatment outcome. This project is a crucial first step for human trials using a combination of vitamin C and chemotherapy treatment.

Method:

Experimental design: Mice that carry a knock-out mutation in a vital step of the vitamin C synthesis pathway were used as a model. These mice, similar to humans, require vitamin C in their daily diet. A cohort of these mice had previously been implanted with lung cancer tumours and treated with high dose vitamin C with or without chemotherapy. Six treatment groups were analysed: 1) saline controls, 2) high dose vitamin C only, 3) chemotherapy only, 4) vitamin C then chemotherapy, 5) chemotherapy then vitamin C and 6) simultaneous vitamin C and chemotherapy. In our study, tumours harvested from these mice were analysed to determine how much vitamin C were in the tumours and where, as well as how much DNA damage had been caused by the various treatments.

Preparation of tissue: A section from the periphery and core of each tumour sample was harvested and ground to a fine powder on dry ice using a mortar and pestle. The ground samples were then

mixed with phosphate buffer and prepared for vitamin C analysis (high performance liquid chromatography, HPLC assay) and protein analysis (Western blot).

Ascorbate analysis: HPLC was used to measure the concentration of vitamin C present in the samples. Core and periphery sample vitamin C concentrations were compared as well as vitamin C concentrations between the treatment groups.

Protein analysis: Protein samples from the tumour periphery (10 μg of protein) were separated by electrophoresis, blotted onto membranes and detected for specific proteins using antibodies: The β-actin protein served as a housekeeping control, and γH2AX as an indicator of DNA damage. The relative amount of γH2AX to β-actin indicates how much γH2AX is present in the tumour indicating the extent of DNA damage.

Results:

The periphery contained several fold more vitamin C than the core of the tumours (Figure 1). This is likely due to the fact that many tumours have a necrotic core. The levels of vitamin C in the periphery of the tumours varied between the treatment groups, and there were large variations between individual tumours in the chemotherapy and combination groups. We expected to see increased levels of vitamin C in all tumours treated with high dose vitamin C, but only a small increase in the vitamin C treated vs saline treated tumours was evident.

A proportion of all tumours, regardless of treatment, showed signs of DNA damage according to relative γH2AX levels (Figure 2). Chemotherapy treated tumours, either chemotherapy alone or in combination with vitamin C, tended to have more DNA damage than tumours treated with vitamin C alone or the saline-treated control tumours.

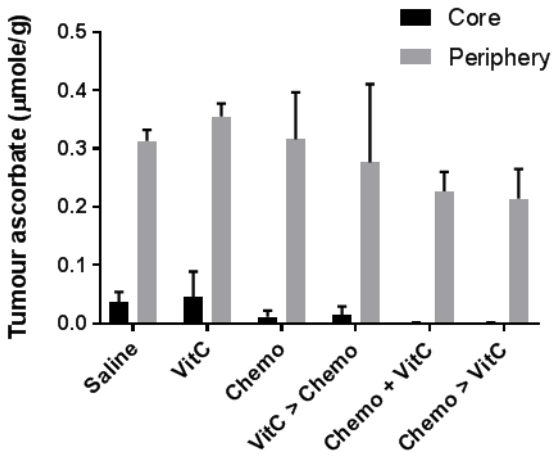

Figure 1: Vitamin C (ascorbate) levels were higher in the periphery than the core of the mouse tumours.

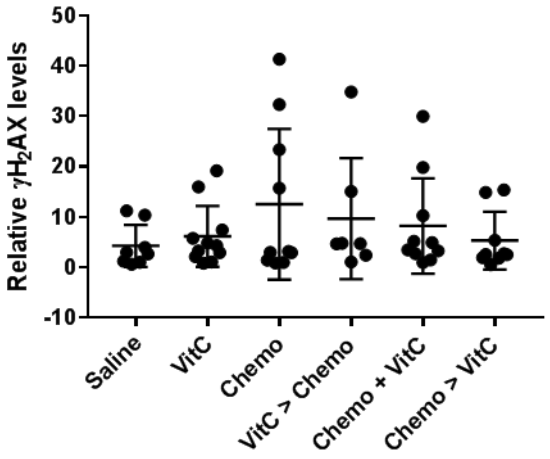

Figure 2: DNA damage, according to γH2AX levels in tumour tissue, varied between treatment groups.

Conclusion:

Vitamin C concentrations were highest in the periphery of the tumours, but did not increase markedly following high dose vitamin C injections. The reasons for this unexpected result are being investigated. DNA damage, as an indicator for tumour cell death, varied between treatment groups

and showed a trend for increased DNA damage in chemotherapy treated tumours. Further research is required to determine whether there are any interactions between vitamin C and chemotherapy.