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Title: Can we slow down breast cancer in mice using vitamin C?

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Introduction:

The role of vitamin C in cancer treatment remains unresolved, despite many cancer patients electing to receive high dose vitamin C treatment from alternative medicine providers. This controversy is largely due to a lack of agreement on a mechanism of action and lack of clinical evidence. Substantial preclinical data from our group suggests that vitamin C acts to reduce tumour growth and aggression by increasing the degradation of the HIF-1 transcription factor, a known contributor to tumour aggression. In support of data from endometrial and colorectal cancer patients, preliminary data in breast cancer patients indicates that higher intratumoral levels of vitamin C are associated with reduced markers of tumour aggression. In addition, increased intratumoral vitamin C in mice is associated with slower growth of melanoma and lung tumours, and reduced HIF-1 pathway markers.

However, the role of high dose vitamin C treatment has not yet been investigated in a breast cancer model. In addition, it is unknown whether a change in vitamin C supply when the tumour is already established, either via dietary supplementation or injection, is sufficient to affect growth.

Aim:

To investigate whether 1) breast tumour levels of vitamin C can be increased and 2) whether breast tumour growth and aggression can be reduced by vitamin C in a mouse model of vitamin C dependency.

Impact:

The value of high dose vitamin C treatment in cancer patients remains controversial. This study provides valuable data on whether breast cancer growth and metastasis can be reduced by high dose vitamin C in a mouse model, and is vital for the design of clinical trials in breast cancer patients.

Method:

Gulo$^{-/-}$ mice, which are unable to synthesise their own vitamin C, and are thus a good representation of the human vitamin C dependency condition, were used in this project. Mice were implanted with breast tumour cells in the mammary fat pad. When tumours reached a size between 50-100 mm^3, mice were randomly assigned to either a control group (maintained on a suboptimal dose of 330 mg/L vitamin C in the drinking water, n=8), high dose vitamin C via the drinking water (3300 mg/L, n=9) or high dose vitamin C via daily IP injection (1 g/kg, n=9). In addition, heterozygote mice (which are able to synthesise vitamin C) were used (n=4). When tumours reached a maximum size of 600 mm^3 or the presence of internal tumours was suspected, mice were sacrificed and tumours and organs harvested for analysis of markers of tumour aggression and measurement of vitamin C concentration.

Results:

Breast cancer tumours in all four treatment groups grew extremely rapidly. No significant difference in tumour growth rate was observed between groups. However, high dose vitamin C treatment was associated with reduced incidence and size of internal metastases. Specifically, 4/8 control mice developed internal tumours, whereas 2/9 mice in the diet group developed internal tumours, and 0/9 mice in the injection group and 0/4 heterozygote mice developed metastases. In addition, the average weight of metastases in the control group was substantially higher than in the diet group (1.05±0.24 g and 0.06±0.04 g, respectively).

Plasma levels of vitamin C were significantly higher in the diet, injection and heterozygote groups compared to controls ($p<0.001$, $p<0.05$, $p<0.001$, respectively). In addition, the diet and heterozygote groups had significantly higher plasma levels of vitamin C than the injection group ($p<0.001$, $p<0.05$, respectively). Intratumoral levels of vitamin C followed a similar trend, with treatment and heterozygote groups having higher levels of vitamin C than the controls, and diet and heterozygote groups having slightly higher levels than the injection group. However, none of the tumour results reached statistical significance. Plasma and intratumoral levels of vitamin C correlated significantly ($p<0.01$, Pearson $R=0.52$).

Intratumoral protein levels of HIF-1α were unchanged between the four groups. No significant difference in GLUT-1 (a downstream target of HIF-1 and thus an indicator of HIF-1 transcriptional activity) protein expression was seen between groups; however, a trend was seen for treatment and heterozygote groups to have lower GLUT-1 expression than controls. A

similar HIF-1 response was previously observed in these breast cancer cells grown in culture, and may point towards a dysfunctional HIF-1 pathway.

Conclusion:

Plasma and intratumoral levels of vitamin C were increased by both dietary and injected high dose vitamin C. This increase in vitamin C was associated with reduced incidence and size of internal metastases, but did not significantly affect tumour growth in this model of breast cancer. Further investigations in additional breast cancer models are required to confirm the observed reduction in tumour spread in the presence of optimal vitamin C levels.