Grower Summary

FV 433

Effect of regular watercress consumption during radiotherapy treatment for early stage breast cancer

Final 2016

Disclaimer

The results and conclusions in this report may be based on an investigation conducted over one year. Therefore, care must be taken with the interpretation of the results.

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Before using all pesticides check the approval status and conditions of use.
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Further information

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Project title:	Effect of regular watercress consumption during radiotherapy treatment for early stage breast cancer
Project number:	FV 433
Project leader:	Dr Jonathan Swann, University of Reading
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Key staff:	Natasa Giallourou, PhD student
Location of project:	Reading, UK
Industry Representative:	Dr Steve Rothwell, Vitacress salads Ltd
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Date project completed (or expected completion date)	October 2016

GROWER SUMMARY

Headline

- Novel anti-cancer properties have been identified for watercress and a watercress-derived compound phenethyl isothiocyanate (PEITC).
- *In vitro* studies show that PEITC enhances the effects of radiotherapy on breast cancer cells and that watercress extract protects healthy cells from the side-effects of radiotherapy.

Background

Breast cancer is a leading cause of cancer-related mortalities globally and epidemiological studies suggest a strong link between healthy nutrition and cancer prevention. Members of the Brassicaceae family like watercress have been extensively studied for anti-cancer properties. Watercress has a complex phytonutrient profile characterised by high levels of carotenoids, flavonols and glucosinolates which all have proven health benefits. Extracts of watercress exhibit strong antioxidant capacity in cells. Watercress and its components have been associated with the inhibition of the three stages of carcinogenesis: initiation, proliferation and metastasis in cancer cell models. Phenethyl isothiocyanate (PEITC) is a glucosinolate breakdown product and watercress is the richest dietary source of it. It has received considerable attention for its anti-cancer properties and has been tested in a number of clinical trials with promising outcomes.

In this study we examined the effect of crude watercress extract and PEITC on breast cancer and healthy breast cells focussing on cancer metabolic pathways as well as DNA damage levels. Radiotherapy is the most common treatment modality for breast cancer patients and functions by killing cancer cells but simultaneously affecting healthy tissue with marked incidents of skin dermatitis. We therefore, proceeded to examine the potential synergistic effect of irradiation exposure and watercress as well as PEITC against breast cancer cells and further investigated whether watercress or PEITC can be protective against radiation-induced collateral damage.

Watercress is becoming increasingly popular in modern cuisines, mainly as a salad crop but also in soups, smoothies and other cooked dishes. We therefore looked at the impact of common cooking methods on the phytonutrient profile of watercress in order to give recommendations on the most appropriate way of preparation.

The aims of the project were to:

1. Investigate the impact of watercress and PEITC on the metabolome (collection of metabolites) of breast cancer and healthy breast cells.
2. Evaluate the interactions between watercress or PEITC with ionising radiation, on markers of cellular function and the metabolism of breast cancer and healthy breast cells.
3. Examine the effects of domestic cooking methods on the phytochemical profile of watercress and formulate recommendations for optimal preparation to maximise nutrient ingestion.

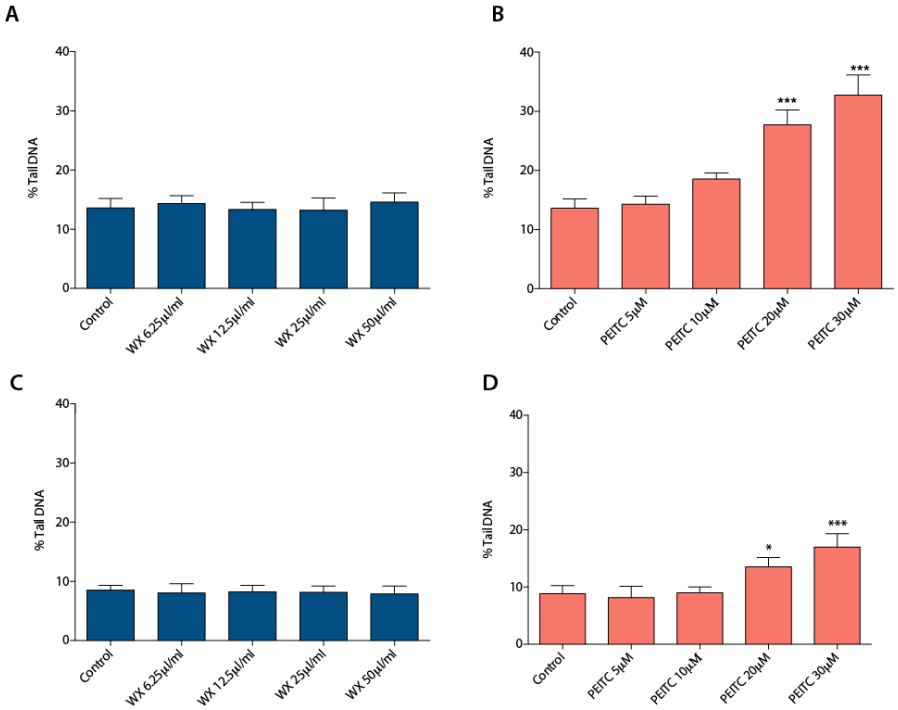
Summary

The impact of watercress and PEITC in cancer and healthy cells

We performed cell cycle experiments that helped us understand at which point of the cancer cell's life cycle can watercress and PEITC inhibit growth and proliferation. Cell cycle can be seen as the quality control station in the life of a cell. If a cell fails this control it initiates its suicide processes. The earlier the watercress or PEITC stop the cell cycle, the better the chances of cancer cell death are. Our results suggest that watercress and PEITC cause a significant dose dependant arrest in the cancer cell's life cycle without having a major impact in healthy cells.

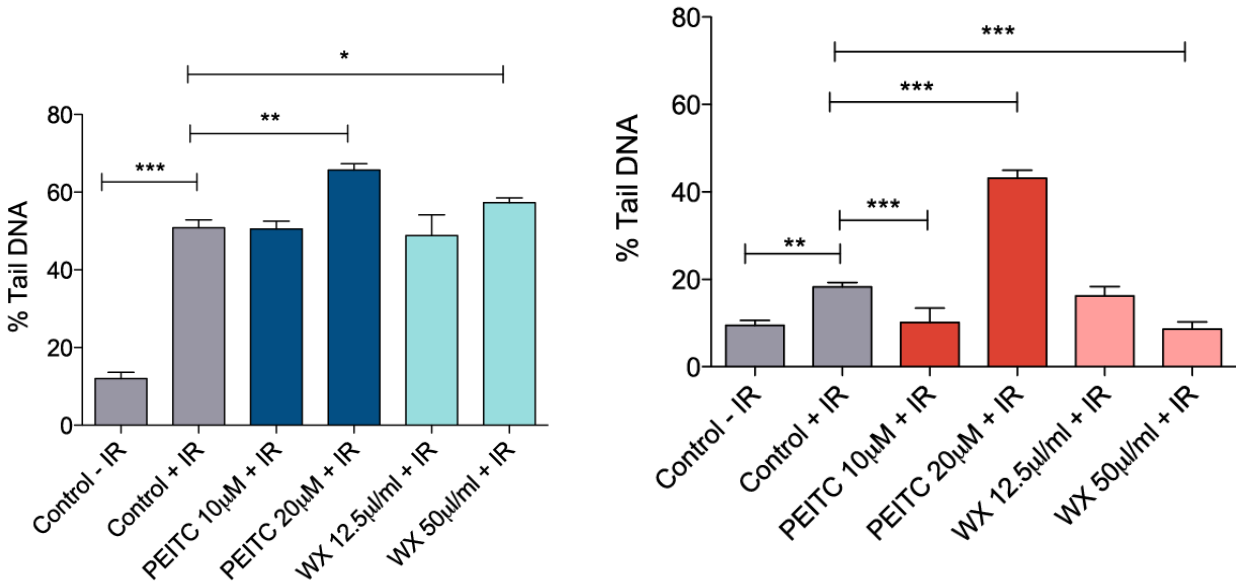
DNA damage is a very important step in cancer initiation and progression. The higher the level of DNA damage in a cancer cell the better the chances of the cell undergoing programmed cell death (apoptosis). PEITC contributes to cancer cell death by depleting the cells of their major antioxidant molecule, glutathione, increasing the amounts of harmful reactive oxygen species inside the cells and essentially increasing the levels of DNA damage, leading to cell death. Watercress extract does not cause DNA damage to cancer cells but affects their antioxidant status and significantly limits the cancer cells' nutrient and energy sources necessary for cell proliferation and survival. PEITC on the other hand is highly damaging to cancer cells but it also harms healthy cells at high doses (Figure i).

Figure (i) DNA damage levels from effects of the crude watercress extract and PEITC on cancer cells (A&B) and healthy cells (C&D) after a 24-hour treatment. Statistically significant differences between control and treated cells are indicated *p<0.05, ***p<0.001). Data shown represent the average of three independent experiments + SEM with two replicates per sample. WX, watercress.



The combined effect of watercress or PEITC with radiation treatment in cancer and healthy cells

To mimic the conditions of radiotherapy during breast cancer in real life we irradiated cancer and healthy cells that had previously been treated with either PEITC or watercress extract. The results from the radiation experiments suggest that PEITC causes additional DNA damage and cell cycle arrest in cancer cells adding to the irradiation induced cancer-killing process. The fact that PEITC is depleting cancer cells of their major antioxidant renders these cells prone to damage such that they cannot survive the cancer killing effects of irradiation. Pre-treatment of the healthy cells with the watercress extract reduces DNA damage levels caused by irradiation, by up-regulating these cells' antioxidant mechanisms as observed by increased glutathione levels in these cells (Figure ii).



CANCER CELLS **HEALTHY CELLS**

Figure (ii) DNA damage levels in cancer and healthy cells exposed to 5 Gy of IR following 24 hour pre-treatment with PEITC or crude watercress extract (WX). Statistically significant differences between groups are indicated as follows *p<0.05, **p<0.01, ***p<0.001. Data shown represent the average of three independent experiments + SEM with two replicates per sample. In 'Control – IR' treatments, cells were not irradiated and not pre-treated with watercress or PEITC; in 'Control + IR' treatments, cells were irradiated but not pre-treated with watercress or PEITC.

Overall, *in vitro* studies indicated that PEITC can enhance the sensitivity of cancer cells to irradiation making the cancer killing process more effective, and watercress extract can protect healthy breast cells from radiation-induced damage.

Effect of cooking on watercress nutrients

The impact on the phytonutrient profile of five different common watercress preparation methods was examined namely, microwaving, steaming, boiling, chopping as well as blending into a smoothie. In the experiments performed, changes in the phenolic, carotenoid and glucosinolate content were quantified as well as the overall antioxidant activity. Our results suggest that microwaving and steaming ensure better retention of all the phytonutrients measured. On the other hand, boiling has detrimental effects on the levels of phytonutrients and should be avoided to ensure maximum ingestion of beneficial compounds.

Limitations

One limitation of this study is that our results have not been validated in a human trial where breast cancer patients would consume watercress during their radiotherapy treatment. Our experiments in breast cells provide a substantial body of evidence for the potential efficacy of watercress against radiation-induced damage. Future work that includes the performance of a human clinical trial with watercress as a nutritional intervention is highly recommended. Dietary guidance is very limited during radiotherapy therefore such a study could generate useful information for the efficacy of antioxidant diets against damage.

Financial Benefits

There are no financial benefits for growers at this stage. However, if further *in vivo* studies are conducted and reported that support the results of these *in vitro* studies, then growers may see increased growth in watercress sales.

Action Points

None to date