Project title:	Watercress: Effects of regular consumption during radiotherapy treatment for early stage breast cancer.
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Project leader:	Jonathan Swann, University of Reading
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Key staff:	Natasa Giallourou
Location of project:	University of Reading, UK
Industry Representative:	Steve Rothwell, Vitacress Salads Ltd
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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headlines

- A higher retention of beneficial nutrients can be achieved by microwaving or steaming watercress instead of boiling it.

- To date, experimental results suggest a dose-dependent response of cancer cells to watercress and phenethyl isothiocyanate (PEITC). PEITC has been studied for its potential for chemoprevention.

Background

This PhD project is based on a collaboration between the Department of Food and Nutritional Sciences, University of Reading, the Institute of Molecular Medicine at the University of Lisbon, the Radiotherapy Department of the University Hospital of Santa Maria, and Vitacress Ltd. All work carried out at the University of Reading is funded by AHDB Horticulture.

The study plans are to look at the health outcomes of 200 early stage breast cancer patients commencing a six-week radiotherapy course and 200 healthy controls; with this phase of work being conducted by the University of Lisbon and the University Hospital of Santa Maria. Blood and urine samples from trial patients are analysed at the Department of Food and Nutritional Sciences at Reading University.

The study aims to find out if the consumption of watercress has beneficial health impacts on breast cancer patients referred for radiotherapy *vs* a second group of breast cancer patients that will maintain their diets whilst receiving the radiotherapy treatment. The potential role of watercress consumption in protecting normal cells from collateral damage, whilst potentially enhancing the susceptibility of cancer cells to radiotherapy treatment will be evaluated. Watercress will be tested in its natural form as a food item that will supplement the usual diet.

Work at Reading includes:

1. Volunteers in the human trial will consume watercress prepared using different methods. Watercress preparation may impact on the abundance of its

phytochemical composition like glucosinolates, flavonols, polyphenols and carotenoids. Watercress samples are and will continue to be prepared in the Food Processing Hall of the Food and Nutritional Sciences Department and analysed for the major phytochemical components.

2. An *in vitro* study looking at the effects of crude watercress extracts and PEITC on Michigan cancer foundation-7 (MCF-7) breast cancer cells will be performed. Cells cultured with watercress extract will be exposed to a source of radiation (mimicking the conditions of radiotherapy). Endpoints used to draw conclusions on the possible synergistic effect of watercress and radiation in cancer will include measuring the degree of DNA damage in the cells via the Comet assay, cell cycle assays and 1H NMR spectroscopy of cell extracts and cell media.

3. Metabonomic approaches primarily based on 1H nuclear magnetic resonance (NMR) spectroscopy and liquid chromatography-mass spectrometry (LC-MS) will be used to characterise the plasma and urinary metabolic phenotypes of the patients before and after radiotherapy. This will allow the biochemical modifications associated with radiotherapy to be explored and the ability of watercress to modulate such responses. This has potential to provide mechanistic insights into how watercress may improve the restorative effects of radiotherapy in breast cancer patients. Blood and urine samples will be collected at the beginning and at the end of radiotherapy treatment (RT), 3 months, 1 year and 3 years after the end of RT. The results will also provide data on the levels of potentially beneficial phytochemicals derived from watercress consumption including folate, carotenoids, flavonoids and glucosinolates. Metabonomics simultaneously measures thousands of low molecular weight metabolites providing holistic information on the biochemical status of the body. High-resolution 1H NMR spectroscopy, mass spectrometry and mathematical modelling approaches will be coupled to determine the metabolic profile in the biological samples from the patients and hence, potentially elucidate the biomolecular mechanisms underlying the watercress-associated improvement in therapeutic outcome.

4. DNA damage in lymphocytes collected from the patients before and three months after the intervention with watercress will be assessed via the Comet assay. DNA damage will be measured using the alkaline version (single-cell gel electrophoresis) of the Comet assay that quantitates basal levels of DNA damage by measuring strand breaks in DNA at alkali labile sites.

These results will provide metabolic and genetic information on the mechanism of action of watercress exposure in breast cancer cells and preliminary indications on whether it can potentially be used as a therapeutic adjuvant in early breast cancer treatment. Moreover, this study will contribute to the development of a multidisciplinary nutritional intervention protocol, feasible and adequate to patients and researchers. It will also promote awareness of other health professionals to the relevance of nutrition as a supplementary therapy of cancer patients.

Summary

In this PhD project, breast cancer patients referred for radiotherapy are consuming watercress following their treatment as a part of their normal diet. To date the number of patients recruited in the study is 42, which is far lower than the 200 hoped for at the beginning of the study. This is due to several issues in the hospital where the study is taking place which is not allowing a higher rate of volunteer recruitment. Therefore as far as this PhD is concerned, any samples that have or will be collected in Portugal until the end of January 2016 will be sent to Reading for all the analyses. Metabonomic analysis, in addition to clinical measurements, will be used to evaluate the effect of watercress consumption on disease outcomes and therapeutic efficiency. Metabonomics is a powerful approach because metabolites and their concentrations directly reflect the underlying biochemical activity and state of cells / tissues. Metabonomics allows holistic information on the biochemical status of breast cancer patients to be obtained and the ability of watercress to improve the toxic effects of radiotherapy to be explored. DNA damage parameters will also be assessed.

The volunteers in the human intervention trial are using a variety of preparation methods for watercress, which could impact on the abundance of the beneficial phytochemicals. Tests to determine the effects of a range of preparation methods were carried out in Year 1. Watercress was processed using different domestic

cooking methods and the major phytochemical components were quantified using a range of analytical techniques. The results to date suggest that boiling of watercress has a detrimental effect on the levels of beneficial phytochemical whereas microwaving and steaming have little effect suggesting that they should be used as the preferred method of watercress preparation. A manuscript with the results from this study has been submitted for publication at the Journal of Food Chemistry.

At the molecular level, the *in vitro* study being performed is looking at the effects of watercress extracts on a breast cancer cell line exposed, or not, to a radiation source to mimic the radiotherapy conditions. To date the experiments looking at the metabolic effects of different doses of watercress and PEITC have been performed and analysed and the results suggest a dose dependent response of cancer cells to watercress and PEITC. Watercress and PEITC cause the release of different metabolites, which are related to the detoxification mechanisms, and regulation of the oxidative status of the cancer cells. Modification of these pathways by watercress and PEITC is of great importance as they are considered to be hallmarks of cancer progression.

Cell cycle experiments that help us understand at which point of the cancer cell's life cycle watercress and PEITC can inhibit growth and proliferation have also been performed and the results are being analysed. The earliest the watercress or PEITC stop the cell cycle the better the chances of cancer cell death are. Preliminary data suggest that PEITC is a strong inhibitor of cell cycle early on.

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 apoptosis, the programmed cell death. We are measuring the levels of DNA damage using the Comet assay.

All of the above experiments are also being performed in a healthy breast cell line to examine whether any observed effects of watercress and PEITC are specific to cancer cells or not.

Collectively, these studies will further our understanding of the biomolecular mechanisms of radiotherapy in breast cancer and the potential for its effectiveness to be improved by watercress consumption.

Financial Benefits

None to date but growers may benefit if peer reviewed science which substantiates the health properties of watercress is generated. Such information can be used in PR campaigns aimed at raising consumer awareness of the nutritional benefits of watercress and its potential cancer fighting properties. Past campaigns based upon research demonstrating anti-cancer properties have seen sales increases of up to 40% with a sustained uplift for months afterwards. This is significant to the watercress industry whose sales exceed £40M at Retail Sale Value (RSV). In addition the shelf space of multiple retailers is under intense competitive pressure; watercress needs to maintain its exposure and national publicity to keep it on retail shelves.

Action Points

None at this stage.