Phytochemical-rich potato extracts and potential for risk reduction in tamoxifen treatment of breast cancer

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Abstract

Existing data suggest an inverse correlation between breast cancer risk and vegetable consumption, and the anticancer effects of vegetables are attributed to the diversity and abundance of phytochemicals. Standard endocrine therapies for breast cancer are associated with significant side effects and not always effective. Undoubtedly, there is a need for improved treatment of breast cancer. In the quest for better breast cancer treatments with fewer side effects, food and nutrition represent a logical strategy to explore. Potato (*Solanum tuberosum* L.) was chosen for the present project as the target vegetable for investigation. Pigmented potato has recently attracted research attention because of its potential health benefits. Two potato extracts were prepared from a white and purple variety ('Urenika') and referred to as WPE and PPE respectively. Tamoxifen and estradiol exhibited paradoxical effects: each of them was inhibitory at high doses but stimulatory at low to moderate doses, on proliferation of two breast cancer cell lines, MCF-7 and T-47D. In contrast, both PPE and WPE inhibited cell proliferation in a dose-dependent manner without paradoxical effects. The potato extracts also blocked estradiol- or tamoxifen-induced cell proliferation of these two cell lines. These findings imply that both potato extracts may have a role to play in prevention of breast cancer, or complementing tamoxifen to achieve desirable treatment efficacy. Since both PPE and WPE were equivalent in efficacy, one (PPE) was selected for further study, given the intention of developing a nutraceutical or therapeutic product of New Zealand proprietary value. Phytochemical compositions of the potato extracts were identified and quantified using ultra high performance liquid chromatography-mass spectrometry, many of which were reported for the first time in variety 'Urenika'. Several compounds were found at doses which have been reported individually to exert bioactive effects against cancer. It is possible the antiproliferative effects of potato extracts resulted from more than one of these bioactive compounds working together. Dose-dependent apoptotic effects of PPE were observed in T-47D culture, and a combined effect seems to exist between PPE and tamoxifen in modulating the S and G2/M phase. In summary, the key contributions and significance of current thesis are: (1) demonstration

of the "risk" zone for tamoxifen (10^{-8} to 10^{-6} M) and estradiol (10^{-10} to 10^{-8} M) concentrations which may stimulate breast cancer cell growth. Note that these concentrations of tamoxifen or estradiol are physiologically achievable. Furthermore, one key novel finding is regarding the estradiol dependency of tamoxifen action. Specifically, at low to moderate doses (10^{-9} to 10^{-8} M) of tamoxifen, there is a threshold of estradiol (> 10^{-8} M) which allows a significant inhibitory action to occur. The stimulatory action of tamoxifen and complex interaction between tamoxifen and estradiol observed *in vitro* may partially explain the failure of tamoxifen treatment in some patients. Owing to the vast differences between cell culture experiments and the human body, a more systematic *in vivo* investigation of clinical effects of tamoxifen over a range of different doses under various estradiol concentrations is warranted; (2) pioneering data on the efficacy of 'Urenika' extract against breast cancer *in vitro*; (3) a metastatic breast cancer animal model which successfully generated metastasis to distant sites (lymph nodes, lungs, livers and spleens), mimicking advanced stage of breast cancer in humans. This model could be used in future testing of the effect of PPE and the combined treatments (PPE with tamoxifen) on establishment and metastasis; and (4) a 'refined' non-invasive feeding methodology, which is more ethical than oral gavages, for tamoxifen administration in mice was developed and results obtained were comparable to the method of intraperitoneal injection. Using this model and the non-invasive feeding method, a dose-dependent stimulatory effect of tamoxifen on growth of 4T1 tumours was observed in mice. The current thesis has derived a new hypothesis which may be worth clinical investigation: tamoxifen may induce excessive leukocytosis which contributes to tumour invasiveness and growth. This thesis also represents a significant contribution to the potential use of potato extracts in reducing the risk of tamoxifen in stimulating cancer growth.

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I dedicate this thesis especially to my mother and father,
who work extremely hard for my education,
who encourage me to pursue my dreams,
who catch me when I fall,
whose love sustains me till the end.

I also wish to dedicate this thesis to Irene who encourages me throughout my PhD journey.

Lastly, in loving memory, I dedicate this thesis to my late grandmothers and aunt. My aunt was another breast cancer patient whose bravery in battling the disease inspired me to contribute to cancer research.

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Abbreviations

AC	Adriamycin + cyclophosphamide
ACY	Anthocyanins
AI	Aromatase inhibitor
AML	Acute myeloid leukaemia
ANOVA	Analysis of variance
AP-1	Activation protein-1
ATCC	American Type Culture Collection
BMI	Body mass index
BPC	Base peak chromatogram
BRCA1	Breast cancer susceptibility gene 1
BRCA2	Breast cancer susceptibility gene 2
CA	Caffeic acid
CChA	Cryptochlorogenic acid
CFBS	Charcoal-treated fetal bovine serum
CFCS	Charcol-treated fetal calf serum
ChA	Chlorogenic acid
CMF	Cyclophosphamide + methotrexate + fluorouracil
CO_2	Carbon dioxide
CT	Computerized Tomography
DAB	p-dimethylaminoazobenzene
DMEM	Dulbecco's minimal essential medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
E2	Estradiol
EBCTCG	Early Breast Cancer Trialists' Collaborative Group
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EMH	Extramedullary hematopoiesis
EPIC	European Prospective Investigation Into Cancer and Nutrition Italy Study
ER	Estrogen receptor
ERE	Estrogen responsive element
ER-	Estrogen receptor-negative
ER+	Estrogen receptor-positive
ESI	Electrospray ionization
FAC	Fluorouracil + adriamycin + cyclophosphamide
FAO	Food and Agriculture Organization
FCS	Fetal calf serum
FSC	Forward-angle light scatter
GC	Gas chromatography

H&E	Hematoxylin and eosin
HCA	Hydroxycinnamic acids
HER2	Human epidermal growth factor receptor type 2
IC_{50}	Concentration of an agent which shows 50% inhibition of the response measured (e.g. cell proliferation)
IGF	Insulin growth factor
IGFR	Insulin growth factor receptor
IMEM	Improved minimum essential medium
IMEM-ZO	Improved minimum essential medium, zinc option
IP	Intraperitoneal injection
LHRH	Luteinizing hormone releasing hormone
m/z	Mass-to-charge ratio
MAPK	Mitogen-activated protein kinase
MEM	Minimum essential medium
MF	Methotrexate + fluorouracil
µg/mL	Microgram per Litre
µM	Micromole per Litre
MISS	Membrane-initiated steroid signalling
MLN	Mediastinal lymph node
MRI	Magnetic resonance imaging
MS	Mass spectrometry
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NASH	Nonalcoholic steatohepatitis
NChA	Neochlorogenic acid
OA	Ovarian ablation
PBS	Phosphate buffered saline
PI	Propidium iodide
PI3K	Phophoinositide 3-kinase
PPE	Purple potato extract
RNase	Ribonuclease
RPMI	Roswell Park Memorial Institute
SEM	Standard error of mean
SSC	Side-angle light scatter
Tam	Tamoxifen
TD	Diameter
TOF	Time-of-flight
UHPLC	Ultra high performance liquid chromatography
WCRF	World Cancer Research Fund
WPE	White potato extract