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Are Isothiocyanates from Cruciferous Vegetables Potential Therapeutic Agents for Breast Cancer

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Abstract **Background:** The important role of food bioactive compounds in the reduction of cancer risk has been highlighted by extensive research over the last two decades. Consumption of cruciferous vegetables in particular, rather than vegetables as a group has drawn a great deal of attention in cancer research due to their potential protective properties. The health benefits of cruciferous vegetables have been well reported being attributed to their rich sources of isothiocyanates and indole constituents. This paper focused on the activity of isothiocyanate constituents in breast cancer. **Methods:** Studies and data sources: Medline and Pubmed were searched for studies using key terms isothiocyanate and breast cancer as text words and as exploded subject headings where possible. Other key terms such as sulforaphane, benzyl isothiocyanate, phenethyl isothiocyanate, allyl isothiocyanate and breast cancer were also searched to check for appropriate studies. The search included all studies published from 1995 up to December 2015. Relevant studies cited in the primary-search published before 1995 were also included in the review. Inclusion criteria: The following inclusion criteria were applied in the screening of articles: 1) study published in English; 2) study compared ITC treated group with a control; 3) study examined breast cancer tumour or breast cancer cells; 4) study examined anticancer effect; 5) statistical analysis was provided. The search resulted in 3 human clinical trials and 49 preclinical in vivo and in vitro studies. **Results & Discussion:** There were substantial preclinical data over the last two decades, which reported the activity of various isothiocyanate constituents in breast cancer cell lines and animal models of breast cancer tumour, with a limited number of studies from human clinical trials. Recent studies have also found that isothiocyanates exhibited significant activity against breast cancer stem cells as well as breast cancer bull cells, which is generally thought as a new and innovative approach for targeting breast cancer treatment. The most extensively investigated cruciferous vegetables' active constituents included sulforaphane, benzyl isothiocyanate and phenethyl isothiocyanate, and overall, at pharmacological concentration range of 1-10 μM for sulforaphane and 2.5-5 μM for benzyl isothiocyanate, and phenethyl isothiocyanate with a wide undefined, varied range of concentrations, these food bioactive compounds demonstrated a highly desirable activity at the cellular and molecular levels, therefore they are likely to show great promise for use in humans as anti-cancer therapeutic agents.

Keywords Anti-cancer activity, Cruciferous vegetables, Isothiocyanates, Breast cancer

1. Introduction

Over the last two decades, there has been growing research interest that focused on the health benefits of cruciferous vegetables in the prevention of cancer of various sites, including breast cancer. The cancer protective effects of these vegetables have been well believed to be due to the active constituents glucosinolates as well as the indole compounds, this article will focus specifically on the main isothiocyanate constituents, including sulforaphane, benzyl isothiocyanate, phenethyl isothiocyanate and will also highlight relevant published human studies which examined the association between cruciferous vegetables intake and breast cancer risk in patients. The chemical structures of the main active isothiocyanate constituents from cruciferous vegetables are provided in Figure 1.

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2. Methods

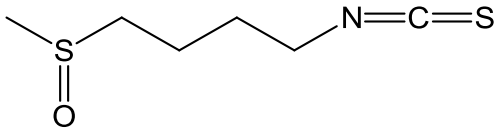
Studies and data sources

Medline and Pubmed were searched for studies using key terms isothiocyanate and breast cancer as text words and as exploded subject headings where possible. Other key terms such as cruciferous vegetables, sulforaphane, benzyl isothiocyanate, phenethyl isothiocyanate, allyl isothiocyanate, and breast cancer were also searched to check for appropriate studies. The search included all studies published from 1995 up to December 2015. Relevant studies cited in the primary-search published before 1995 and clinical studies examined cruciferous vegetables intake and

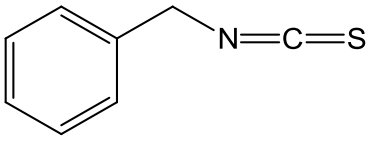
breast cancer risk in patients were also screened and discussed in the review.

Inclusion criteria

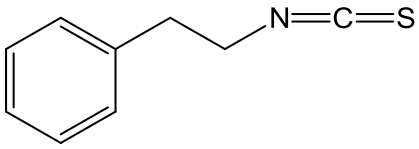
The following inclusion criteria were applied in the screening of articles from published animal models of breast tumour, cancer: 1) study published in English; 2) study compared ITC treated group with a control; 3) study examined breast cancer tumour or breast cancer cells; 4) study examined anticancer effect; 5) statistical analysis was provided.



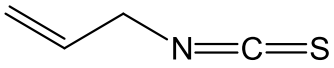
Sulforaphane (SFN)



Benzyl isothiocyanate (BITC)



Phenethyl isothiocyanate (PEITC)



Allyl isothiocyanate (AITC)

Figure 1. Chemical structures of isothiocyanate constituents from cruciferous vegetables

3. Results

The search resulted in a total of 4 clinical studies, 9 animal models and 40 *in vitro* breast cancer cell culture studies. No human randomised control trial that examined the outcomes of in take of cruciferous vegetables or isothiocyanates in breast cancer patients was identified from the literature search. Two of the four clinical studies identified were human case-control trials and these trials overall reported a reduced cancer risk associated with cruciferous vegetables consumption. Specifically, in a study involved 2832 patients, consumption of cruciferous vegetables was found to be inversely associated with breast cancer risk (RR = 0.58, 95% CI (0.42-0.79), P = 0.03) (Terry et al., 2001). In another study of 740 patients, reduced breast cancer risk was reported to be associated with broccoli consumption (OR = 0.60, 95% CI (0.40-1.01), P = 0.058 (Ambrosone et al., 2004). It was worth to note that the finding in the later study did not reach statistical significance at $P < 0.05$. The other two clinical trials were both pilot studies, which examined pharmacokinetics of intake of broccoli and isothiocyanates constituents in healthy women or individuals (Atwell et al., 2015; Cornblatt et al., 2007). Overall, although the findings from published clinical case-control trials were not all significant outcomes; they do provide some good evidence to support the potential protective role of cruciferous vegetables in breast cancer patient.

The activity reported from published animal models of breast cancer appeared to be variable and the reported results seemed to be dependent partly on the experimental model being employed. Three studies, which utilised a transgenic mice model, reported no significant difference in mammary tumour growth between benzyl or phenethyl isothiocyanate treated versus the control groups (Kim et al., 2013; Singh et al., 2012; Warin et al., 2009). However, in another study which employed a different human breast cancer xenograft model of nude mice injected with MDA-MB-231-cells, it was found that benzyl isothiocyanate had activity in reducing the tumour volume (Warin et al., 2010). Another study, which used a rat model of NMU-induced mammary cancer, reported that at concentration of 50 μM/kg animal's body weight for 2 weeks of treatment, the phenethyl isothiocyanate decreased the percentage of rats with mammary tumour phenethyl isothiocyanate was later investigated by and colleagues (Aras et al., 2013). In addition, phenethyl isothiocyanate has also been found to have inhibitory activity in breast tumour metastasis, migration of mammary tumour to other site, the brain, however, this study did not specifically examine the compound's activity on mammary tumour incidence (Gupta et al., 2013). A brief summary of activity investigated in each of the published animal models of breast cancer tumours, which examined sulforaphane, benzyl isothiocyanate, or phenethyl isothiocyanate are provided in Table 1.

The ability of isothiocyanates to target breast cancer stem cells had also been investigated for sulforaphane (Li et al., 2010) and benzyl isothiocyanate (Kim et al., 2013). In these studies, activity against breast cancer stem cells was based on the concept that a small population of breast cancer cells is the stem cells and these cells express a marker, known as $CD44^{high}/CD24^{low}/ESA^{+}$, which can be measured quantitatively. Moreover, the stem cells also display specific enzymatic activity namely aldehyde dehydrogenase 1 ($ALDH1^{+}$), as well as have the ability to form a distinctive morphological structure called mammospheres. The mechanism underlying the activity against breast cancer stem cells of tested compounds has been hypothesised due to inhibition of the receptor tyrosine kinase, known as Ron, this receptor functions to drive stemness in breast cancer cells. In these studies, sulforaphane, and benzyl isothiocyanate significantly reduced population of cells expressing $CD44^{high}/CD24^{low}/ESA^{+}$, decreased the number or size of mammospheres formed, and decreased $ALDH1^{+}$ activity (Kim et al., 2013; Li et al., 2010).

Table 1. Anti-breast cancer activity of isothiocyanates from cruciferous vegetables from animal models published from 1995 to December 2015

Study ID	Model utilised	Tested Compound	Duration of treatment and dose	Brief summary of activity investigated in each of the published studies	Statistical Analysis
Kanematsu *et al*, 2011	Human breast cancer cell KPL-1-xenograft N = 10, 5 week old BALB/c mice	SFN	25, 50 mg/kg body wt for 4 week Ip 1-100 μM	Reduced tumour volume: $P < 0.05$ Treated: 351 ±55 mm^3 (50 mg/kg) Control: 686 ±94 mm^3 Reduced tumour weight: $P < 0.01$ Treated: 416 ±63 mg (25 mg/kg) $P < 0.01$ Treated: 338 ±56 mg (50 mg/kg) Control: 571 ±69 mg Decreased tumour proliferation $P < 0.01$ Treated: 1409 ±61; 840 ±41 (25; 50 mg/kg) Control: 1548 ±81 Increased apoptotic ratios $P < 0.01$ Treated: 18 ±2; 24 ±2 (25; 50 mg/kg) Control: 14 ±2 Inhibited cell growth $IC_{50:}$ 19.1 ±1.4 and 17.8 ±0.6 μM (48, 72 h)	Data presented as mean ±SD, N = 10 animals per group Results significant at P < 0.05 and P < 0.01 Analysed by ANOVA and Dunnett's test
Li *et al*, 2010	Human breast cancer cell SUM159-xenograft N = 6, 5 week old NOD/SCID mice Human breast caner cells SUM159, MCF7	SFN	50 mg/kg body wt daily for 2 weeks Ip 0.5. 1, 5, 10 μM	Reduced cancer progenitor $ALDH1^+$ population in mice by >50%, $P = 0.003$ Reduced tumour volume, $P = 0.018$ Inhibited cell proliferation: IC_{50}: 10, 16 μM (SUM159, MCF7) Decreased p-β-catenin, cyclin D1 protein expression (by 85, 77%) In SUM159 cell only: Increased caspase-3 activity (P = 0.005) Inhibited bCSC mammospheres formation $P < 0.01$, reduced by 8~125-fold (size), 45-75% (number) Reduced $ALDH1^+$ population $P <= 0.008$, by 65-80% (1, 5 μM)	Data presented as mean ±SD, N = 6 animals per group Results significant at least at P < 0.005, P < 0.01 and P < 0.05 Analysed by Student's *t* test
Kim *et al*, 2013	Murine mammary carcinoma cell 4T1-xenograft N = 20, 5 week old BALB/c mice	BITC	5, 10 mg/kg body wt for 32 days Oral	Reduced tumour volume: $P < 0.003$ Treated: 1125 ±20 mm^3 (10 mg/kg) Control: 1400 ±60 mm^3 Reduced tumour weight: $P < 0.001$ Treated: 1400 ±40 mg (10 mg/kg) Control: 1660 ±90 mg Reduced lung metastasis of tumour nodules: $P < 0.006$ Treated: 15.0 ±2 (10 mg/kg) Control: 24.5 ±3 Reduced volume of tumour nodules: $P < 0.01$ Treated: 6.4 ±2 mm^3 (10 mg/kg) Control: 16.2 ±4 mm^3 Inhibited tumour proliferation ($P < 0.05$) Induced apoptosis ($P < 0.05$) Suppressed angiogenesis: reduced haemoglobin content, CD31, VEGF expression	Data presented as mean ±SD, N = 20 animals per group Results significant at least at P < 0.001, P < 0.005, P < 0.01 and P < 0.05 Analysed by ANOVA and Duncan's or Student's *t* test

ALDH = aldehyde dehydrogenase; bCSC = breast cancer stem cell; BITC = benzyl isothiocyanate; SFN = sulforaphane; VEGF = vascular epidermal growth factor.

Table 2. Activity of isothiocyanates from cruciferous vegetables in breast cancer from animal models published from 1995 to December 2015(cont)

Study ID	Model utilised	Compound tested	Duration of treatment and dose	Brief summary of activity investigated in each of the published studies	Statistical Analysis
Warin *et al*, 2010	Human breast cancer cell MDA-MB-231-x enograft N = 8/12, 6-7 week old nude mice	BITC	2.5. 5, 7.5 μM 5 day per week for 2 week + 50 day Ip 6, 9, 12 μM for 2 weeks + 30 day Oral	Reduced tumour volume: $P < 0.05$ Treated: 526 ±50 mm^3 (2.5 μM Ip) 632 ±200 mm^3 (7.5 μM) Control: 1581 ±240 mm^3 Reduced tumour weight: $P = 0.002$ Treated: 400 ±30 mg (2.5 μM) Control: 1100 ±170 mg Reduced tumour volume $P < 0.05$ Treated: 290 ±40 mm^3 (9 μM Oral) Control: 620 ±230 mm^3 Inhibited tumour proliferation ($P = 0.04$) (Ip) Suppressed angiogenesis: reduced haemoglobin, CD31, VEGF expression	ANOVA and Dunnett's test or Student's *t* test Tumour weight ($P = 0.067$), growth ($P > 0.05$) for high ip, oral, low oral dose: not significant
Warin *et al*, 2009	MMTV - *neu* expressed mammary cancer N = 32, 6 week old transgenic mice	BITC	1, 3 μM/kg in diet for 25 weeks	No significant difference in tumour incidence ($P > 0.05$) Reduced cumulative incidence of tissue abnormal structures ($P = 0.01$) Suppressed angiogenesis: reduced vessel area, increased $CD3^+$ T cells, E-cadherin protein expression Inhibited tumour proliferation ($P < 0.05$) Induced apoptosis ($P < 0.05$)	Fisher's or Student's *t* test; Tumour incidence: 72% vs 54%, 48.2%, $P = 0.15, 0.07$; Hyperplasia incidence, area: 60%, 6.6 ±5.1 mm^2 vs 34.8%, 37.8%, 0.12 ±0.09/ 0.13 ±0.08 mm^2 ($P = 0.06, 0.09, 0.06, 0.08$)
Gupta *et al*, 2013	Human breast cancer cell MDA-MB-231-x enograft, 4-6 week old mice	PEITC	10 μM daily for 10 days (metastasis) or 25 days (tumour) Oral	Reduced brain metastatic tumour by 50% ($P < 0.05$) Reduced metastatic tumour growth by 50-55% ($P < 0.05$) Suppressed cell invasion: reduced HER2, EGFR, VEGF protein expression by 90%, 50%, 60% ($P < 0.05$) Increased survival of mice bearing tumours ($P < 0.05$)	Student's *t* test
Aras *et al*, 2013	NMU-induced mammary cancer N = 20/30, 8 week old SD rats	PEITC	50, 150 μM/kg body wt every 2 days for 2 weeks +18 weeks Oral	Reduced % animals developed tumour $P < 0.05$ Treated: 25.0% (50 μM/kg) $P < 0.05$ Treated: 17.2% (150 μM/kg) Control: 56.6% Reduced number of tumour per animal: $P < 0.05$ Treated: 0.21 ±0.12 (50 μM/kg) $P < 0.05$ Treated: 0.25 ±0.11 (150 μM/kg) Control: 1.03 ±0.24 Increased tumour free survival time ($P < 0.05$) Inhibited angiogenesis: decreased capillary density ($P < 0.05$), increased plasma oestrogen (high dose) ($P < 0.05$)	Fisher's or Kruskal Wallis and Duncan's test or Log rank test Or ANOVA and Newman Keul's test
Singh *et al*, 2012	MMTV - *neu* expressed mammary cancer N = 32/35, mice	PEITC	3 μM/kg in diet for 29 week	No significant difference in tumour growth ($P > 0.05$) Inhibited tumour proliferation ($P = 0.03$) Induced apoptosis ($P < 0.01$) Suppressed angiogenesis: reduced blood vessel number ($P = 0.05$)	Fisher's or Student's *t* test Tumour incidence, lesions: 40%, 42.8%/8.5% *vs* 18.7%, 18.7%/21.8% ($P = 0.07, 0.04, 0.18$)

BITC, benzyl isothiocyanate; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; PEITC, phenethyl isothiocyanate; VEGF, vascular epidermal growth factor.

Anticancer activity of isothiocyanates from cruciferous vegetables has also been studied extensively in several types of breast cancer cell lines, with MCF-7 and MDA-MB-231 cells being the most commonly used. Collectively, at pharmacological concentration range of 1-10 μM for sulforaphane and 2.5-5 μM for benzyl isothiocyanate, and phenethyl isothiocyanate with a wide undefined, varied range of concentrations, the three most investigated food bioactive isothiocyanate constituents demonstrated a highly desirable activity at the cellular and molecular levels, therefore they are likely to show great promise for use in humans as anti-cancer therapeutic agents.

In summary, although limited clinical evidence is available at present, substantial published preclinical data exists on anticancer activity of isothiocyanates from cruciferous vegetables sulforaphane, benzyl isothiocyanate and phenethyl isothiocyanate. Among these most extensively investigated constituents, the activity of sulforaphane was most consistently reported and benzyl isothiocyanate appeared to be relatively the most potent compound, with the least required concentration or concentration range for its anticancer activity pharmacologically. Based mainly on cell culture studies and animal models of breast cancer tumours, isothiocyanate compounds clearly demonstrated highly desirable activity in both *in vitro* and *in vivo* and show great promise for use in humans as potential chemopreventive agents for breast cancer.

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