Eur J Nutr (2009) 48:483–491
DOI 10.1007/s00394-009-0039-5

ORIGINAL CONTRIBUTION

Modulation of detoxification enzymes by watercress: in vitro and in vivo investigations in human peripheral blood cells

Thomas Hofmann · A. Kuhnert · A. Schubert · C. Gill · I. R. Rowland · B. L. Pool-Zobel · M. Glei

Received: 3 March 2009 / Accepted: 2 July 2009 / Published online: 28 July 2009

Abstract
Background Epidemiological studies indicate that consumption of cruciferous vegetables (CV) can reduce the risk of cancer. Supposed mechanisms are partly the inhibition of phase I and the induction of phase II enzymes.
Aim The aim of this study was to investigate in vitro and in vivo effects of watercress (WC), a member of the CV family, on chemopreventive parameters using human peripheral blood mononuclear cells (PBMC) as surrogate cells. We investigated the hypothesis that WC reduces cancer risk by inducing detoxification enzymes in a genotype-dependent manner.
Methods In vitro gene expression and enzyme activity experiments used PBMC incubated with a crude extract from fresh watercress (WCE, 0.1–10 μL/mL with 8.2 g WC per 1 mL extract) or with one main key compound phenethyl isothiocyanate (PEITC, 1–10 μM). From an in vivo perspective, gene expression and glutathione *S*-transferase (*GST*) polymorphisms were determined in PBMC obtained from a human intervention study in which subjects consumed 85 g WC per day for 8 weeks. The influence of WC consumption on gene expression was determined for detoxification enzymes such as superoxide dismutase 2 (*SOD2*) and glutathione peroxidase 1 (*GPX1*), whilst the SOD and GPX activities in red blood cells were also analysed with respect to *GST* genotypes.
Results In vitro exposure of PBMC to WCE or PEITC (24 h) increased gene expression for both detoxification enzymes *GPX1* (5.5-fold, 1 μL/mL WCE, 3.7-fold 1 μM PEITC) and *SOD2* (12.1-fold, 10 μL/mL WCE, 7.3-fold, 10 μM PEITC), and increased SOD2 activity (1.9-fold, 10 μL/mL WCE). The WC intervention had no significant effect on in vivo PBMC gene expression, as high individual variations were observed. However, a small but significant increase in GPX ($p = 0.025$) and SOD enzyme activity ($p = 0.054$) in red blood cells was observed in *GSTM1*0*, but not in *GSTM1*1* individuals, whilst the *GSTT1* genotype had no impact.
Conclusion The results indicate that WC is able to modulate the enzymes *SOD* and *GPX* in blood cells in vitro and in vivo, and suggest that the capacity of moderate intake of CV to induce detoxification is dependent in part on the *GSTM1* genotype.

Keywords Chemoprevention · Gene expression · Enzyme activity · Detoxification enzymes · Watercress

T. Hofmann (✉) · A. Kuhnert · A. Schubert · B. L. Pool-Zobel · M. Glei
Department of Nutritional Toxicology, Institute of Nutrition, Friedrich Schiller University Jena, Dornburger Str. 24, 07743 Jena, Germany
e-mail: th.hofmann@gmail.com

C. Gill
Faculty of Life and Health Sciences, Northern Ireland Centre for Diet and Health (NICHE), Centre for Molecular Biosciences (CMB), University of Ulster, Coleraine BT52 1SA, Northern Ireland, UK

I. R. Rowland
Department of Food Biosciences, University of Reading, Reading RG6 6AP, UK

Introduction

A diet high in cruciferous vegetables (CV) is associated in a number of epidemiological studies with a reduced cancer risk at a number of sites including colon, lung, lymphatic system and possibly prostate [11, 20, 25, 44, 50]. Glucosinolates are sulphur compounds with one group derived

from glucose, which occur mainly in the plants of the family Cruciferae. Approximately, 115 different glucosinolates have been identified to date, which differ only in the chemical composition of the aglucone [16]. Smell, taste, as well as the biological effects of the cruciferous plants, are determined mainly by the active by-products of glucosinolates, e.g. isothiocyanates (ITCs), thiocyanates and nitriles [22]. These are released by hydrolysis of the glucoside by a β-thioglucosidase (myrosinase) found in vacuoles of CV and which is liberated by mechanical treatment (cutting or mastication) of the plant tissue. In humans, ITCs are metabolized primarily through the mercapturic acid pathway. GSTs facilitate the conjugation of ITC to glutathione and these glutathione conjugates are further metabolized to mercapturic acids by the sequential activity of γ-glutamyltranspeptidase, cysteinylglycinase and *N*-acetyltransferase [20]. ITCs and their metabolites show anti-inflammatory activity [19], antibacterial activity [15] and especially cancer-preventive properties, where they are able to influence multiple stages of carcinogenesis. In addition to effects such as the inhibition of the cell growth by cell cycle arrest [45] and the induction of apoptosis [33], ITCs also influence the initiation stage through inhibition of phase I enzymes and induction of phase II enzymes [32, 40].

One important member of the CV and an excellent source for glucosinolates and other bioactive phytochemicals [28] is watercress (WC) (*Nasturtium officinale* or *Rorippa nasturtium-aquaticum*). Its main glucosinolate, gluconasturtiin, is formed via secondary metabolism of the plant from the amino acid phenylalanine and is converted by the myrosinase to phenethyl isothiocyanate (PEITC). The consumption of 57 g WC leads to a release of at least 12–15 mg PEITC measured in the urine [9]. PEITC has been reported to have several anti-carcinogenic effects including the inhibition of phase I enzymes and/or the activation of phase II enzymes [8]. For example, in the rat liver and colon, PEITC leads to an induction of the total GST activity [14, 43], such as the induction of the quinone reductase by 7-methylsulfinylheptyl-ITC and 8-methylsulfinyloctyl-ITC [35].

Human intervention studies that investigate the physiological effects of high WC consumption are rare. In pilot studies with smokers by Hecht et al. [17, 18], interventions with WC led to a higher excretion of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and other nicotine-metabolites such as cotinine and 7-hydroxycotinine. The higher excretion of these carcinogens and their metabolites could be attributed to a reduction of cytochrome P450 enzymes or to an induction of UDP glucuronosyltransferases (UGT). Recent investigations by Gill et al. [10] reported that a supplementation with 85 g WC per day for 8 weeks was associated with a reduced level of basal and hydrogen peroxide-induced DNA damage in lymphocytes.

Many studies show that cancer risk reduction by nutritional factor may be linked to specific polymorphisms. For CV, different authors examined the interaction between vegetable intake and *GST* polymorphisms in relation to cancer risk. The protective effect of CV seems to be more pronounced in individuals with a genetic null polymorphism of *GSTM1* and/or *GSTT1* [6, 25, 36]. Because GSTs are involved in the conjugation of ITCs and the rapid excretion in urine, the results suggest that individuals could profit by the absence of certain GST enzyme activities, as this would result in longer circulation of the ITCs in the body. In turn, this could allow further chemical interaction of ITC metabolites with signalling peptides and also enhance the protective effects in vivo [24, 41].

Biomarkers have considerable potential in aiding the understanding of the relationship between diet and disease or health [5]. A number of recent reports show that the latest technologies of genomics have initiated a new era of biomarker development, also for cancer detection, treatment and prevention. Here, different biomarker applications are based on blood cells such as peripheral blood mononuclear cells (PBMC), since they are obtained less invasively than biopsies and can be used as surrogate tissues that may mimic effects occurring in remote target tissues of exposure [34]. In vitro, the cells can be used as a human primary cell model, which, maybe, best mimics the human physiological conditions.

The aim of this study was to investigate in vitro and in vivo effects of WC and one of its main key compounds, PEITC, on chemopreventive parameters in human blood cells as surrogate tissue for target organs, which require more invasive sampling methods [34]. Within the primary prevention of cancers in humans, the modulation of detoxification enzymes by a high consumption of WC could be quite important. In vitro, we investigated the expression of key genes related to biotransformation and detoxification [*GPX1*, *SOD2*, catalase (*CAT*), glutathione *S*-transferase M1 (*GSTM1*), glutathione *S*-transferase P1 (*GSTP1*), UDP glucuronosyltransferase 1A1 (*UGT1A1*)] in PBMC treated with a crude WCE and PEITC. For in vivo investigations, we used biological samples (cryopreserved PBMC) from a previously reported human intervention study with WC [10] and analysed the expression profiles of the aforementioned genes. Additionally, we determined *GST* polymorphisms to assess the link between these genetic factors and the induction of SOD and GPX enzyme activity by a WC intervention. These two enzymes could provide mechanistic explanations for the reported reduction of DNA damage in PBMC after WC consumption.

Materials and methods

Test substances (in vitro)

Phenethyl isothiocyanate (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) was diluted in ethanol to yield a stock solution of 100 mM and was further diluted in cell culture medium to yield the working solutions of 1 and 10 μM. The final ethanol concentration in the two PEITC working solutions was 0.01%.

Preparation of the watercress extract (in vitro)

The WCE used in the in vitro study was produced as described by Boyd et al. [3]. Briefly, fresh WC was chopped into pieces, homogenized in a Cookworks juice maker machine for 10 min at 4 °C; subsequently the juices were centrifuged (9,000*g*, 10 min, 4 °C), and the supernatant was decanted, filter sterilized (0.45 μm and then 0.22 μm) and aliquoted. This single batch preparation with a concentration of 8.2 g WC per 1 mL extract was stored at –70 °C and used for all experiments.

Preparation of PBMC and cell culture (in vitro)

A fraction of mononuclear cells was isolated from several anonymous buffy coat preparations by gradient centrifugation using HISTOPAQUE®-1077 (Sigma, Deisenhofen, Germany) as described earlier [12]. Cells were kept in cell culture medium [RPMI 1640 medium (Invitrogen GmbH, Karlsruhe, Germany) with 10% heat-inactivated FCS, 1% penicillin/streptomycin and 1% L-glutamine (Invitrogen GmbH, Karlsruhe, Germany)] overnight, prior to incubation. Since SOD2 is manganese dependent, we added a "Trace Element Mix" (PromoCell, Heidelberg, Germany) with manganese ($MnSO_4 \cdot H_2O$, 0.17 μg/L) for measuring SOD2 activity. For all experiments, the cell number was adjusted to 4×10^6 cells/mL and PBMC (mean viability 95%) were then incubated with the test substances.

Cytotoxicity (in vitro)

Cell numbers and viabilities were determined before and after treatment of PBMC using a trypan blue solution (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). Cell integrity of the treated PBMC was assessed with the CellTiter-Blue™ assay (Promega, Mannheim, Germany) to estimate the number of viable cells. This assay uses the dye resazurin that only viable cells reduce into the highly fluorescent resorufin. The assay was carried out in 96-well microtiter plates with fluorescence measurements (Tecan, Spectra Fluor Plus, Austria, Em/Ex 520/595 nm) at different time points, namely after 2–72 h.

RNA isolation

Total RNA from PBMC was isolated using RNeasy Mini Kit (Qiagen, Hilden, Germany), dissolved in 50 μL RNase free water and stored at −20 °C. RNA quantification was done spectrophotometrically (Eppendorf BioPhotometer, Hamburg, Germany) and formaldehyde denaturing RNA gel electrophoresis (1.5%) was used to check the integrity of the ribosomal RNA and for DNA contamination before proceeding with real-time PCR.

Real-time PCR analysis (in vitro and in vivo)

Gene expression of *CAT*, *GPX1*, *GSTA4*, *GSTP1*, *SOD2*, *UGT1A1* and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was assessed by two-step SYBR Green I relative real-time PCR (iCycler iQ system, Bio-Rad GmbH, Munich, Germany). Briefly, total RNA (1 μg for in vitro and 0.25–1 μg for in vivo experiments) was converted into first-strand cDNA using SuperScript™ First-Strand Synthesis System for RT-PCR (Invitrogen GmbH, Karlsruhe, Germany). Using oligo(dT) leads to specific transcriptions of mRNA. The PCR amplification reactions contained 2 μL of first-strand cDNA mixed with 12.5 μL of iQ™ SYBR® Green Supermix (Bio-Rad GmbH, Munich, Germany) master mixture (2× mix containing SYBR Green I, iTaq DNA polymerase, reaction buffer, deoxynucleotide triphosphate mix, 10 mM $MgCl_2$, 20 nM fluorescein and stabilizers) and 10 pmol stock of each of the specific primers in a final reaction volume of 25 μL. All reactions were performed in duplicate and product-specific amplification was confirmed by melting curve analysis. The fluorescence threshold value (C_T) was calculated using the iCycler iQ optical v3.0a system software. The relative quantification of *mRNA* expression was calculated with the comparative $\Delta\Delta C_T$ ($\Delta\Delta C_T = \Delta C_{T\text{control}} - \Delta C_{T\text{experiment}}$) method. For normalization, ΔC_T values were calculated by subtracting the average of the C_T value in the control for the reference gene from the average of the C_T value for the target gene and subtracting the average of the C_T value in the treated sample of the reference gene (*GAPDH*) from the target gene. Then, the difference between the ΔC_T values of control and treatment ($\Delta\Delta C_T$) was calculated. The fold change was calculated according to the efficiency method ($\Delta\Delta C_T$ method) where it is assumed that the PCR efficiency is 100% [30, 31]. The coefficients of variation for genes reported in this paper were 0.9% for *GAPDH*, 1.0% for *SOD2* and 1.1% for *GPX1*. Gene-specific primer sequences used for the quantification can be seen in Table 1.

Table 1 Primer sequences (F forward, R reverse) for real-time PCR, primers designed by using PerlPrimer v1.1 software

Gene name	Locus ID	Primer sequences 5′–3′		Amplicon size (bp)
CAT	MN_001752	F	TGG ACA AGT ACA ATG CTG AG	144
		R	TTA CAC GGA TGA ACG CTA AG	
GPX1	MN_000581	F	GAC TAC ACC CAG ATG AAC GA	133
		R	ACG TAC TTG AGG GAA TTC AG	
GSTA4	MN_001512	F	CCG GAT GGA GTC CGT GAG ATG G	131
		R	CCA TGG GCA CTT GTT GGA ACA GC	
GSTP1	MN_000854	F	CTG CGC ATG CTG CTG GCA GAT C	149
		R	TTG GAC TGG TAC AGG GTG AGG TC	
SOD2	MN_000636	F	GCC CTG GAA CCT CAC ATC AAC	111
		R	CAA CGC CTC CTG GTA CTT CTC	
UGT1A1	MN_000463	F	TCA TGC TGA CGG ACC CTT TC	145
		R	CTG GGC ACG TAG GAG AAT GG	
GAPDH	MN_002046	F	ACC CAC TCC TCC ACC TTT GAC	110
		R	TCC ACC ACC CTG TTG CTG TAG	

Primer pairs were verified by normal PCR with gene-specific amplicon

Measurement of total protein and of SOD2 activity in PBMC (in vitro)

In vitro changes of the SOD2 activity in PBMC were measured with the Calbiochem® Superoxide Dismutase Assay Kit II (Merck Chemicals Ltd, Darmstadt, Germany), which utilizes a tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. The addition of 3 mM potassium cyanide to the assay inhibits both SOD1 and SOD3, resulting in the detection of only SOD2 activity. Total protein content was measured using the method by Bradford with bovine serum albumin as standard protein [4].

Polymorphisms (in vitro and in vivo)

Null polymorphisms for *GSTM1* and *GSTT1* were determined by multiplex PCR using original procedures [2, 29].

Study design (in vivo)

The study was a single blind randomized crossover trial in 60 subjects as described in detail by Gill et al. [10]. The study received approval of an independent ethical committee and subjects gave their informed consent to participate. Briefly, during the first phase of the study, the volunteers were randomly assigned to either the treatment (WC supplemented) or control group. During the treatment phase, the subjects consumed one portion (85 g) of raw WC daily for 8 weeks in addition to their normal diet. During the control phase (8 weeks), the subjects were asked to maintain their habitual diet. The control and the treatment phases were separated by a 7-week washout phase. The WC used for this intervention study was a commercially available product produced by Vitacress Ltd (Southampton, UK). The subjects were supplied with fresh WC (85 g bag/day) during the supplementation phase. Fasting blood samples were collected before and after each phase (week 0, week 8, week 15 and week 23). PBMC were isolated by using Histopaques-1077, according to the manufacturer's instructions (Sigma Diagnostics, St Louis, MO, USA). Red cell concentrate (RCC) and plasma samples were also prepared.

GPX and SOD activity in red blood cell (in vivo)

Measurements of GPX and SOD activity in RCC were performed by Gill et al. [10] using two commercial kits (RANSEL kit for GPX and RANSOD kit for SOD; Randox Laboritories Ltd, Crumlin, CO Antrim, UK) according to the manufacturer's instructions.

Statistics (in vivo and in vitro)

The Prism software version 5.01 (Graph Pad, San Diego, USA) was used for two-sided paired and unpaired *t* test and one-way or two-way ANOVA with Bonferroni's multiple comparison test with selected pairs.

Results

Cytotoxicity of the test substances

Effects of WCE on isolated PBMC were investigated in vitro. Therefore, we first needed to establish non-cytotoxic concentration ranges. The concentrations of 10 and 50 μL/mL of WCE caused significant time and concentration-dependent reduction of metabolic activity in PBMC (Table 2). The highest concentration at which there were

Table 2 Effects of treatment with the watercress extract on cell viability measured with CTB ($n = 6$) and with trypan blue ($n = 4$)

	Control				0.1 µL/mL WCE				1 µL/mL WCE				5 µL/mL WCE				10 µL/mL WCE				50 µL/mL WCE			
	CTB		Trypan blue		CTB		Trypan blue		CTB		Trypan blue		CTB		Trypan blue		CTB		Trypan blue		CTB		Trypan blue	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
2 h	100	0	89	4	99	2	90	5	96	1	90	4	99	1	90	4	103	3	89	5	111	3	91	4
24 h	100	0	92	3	102	1	92	4	102	2	93	3	99	2	91	4	97	3	90	3	47	3***	89	4
48 h	100	0	92	2	104	5	90	4	102	6	88	4	99	5	91	3	82	5**	89	4	6	5***	85	5
72 h	100	0	89	3	102	3	88	2	95	4	88	3	78	5***	89	2	58	11***	85	3	2	11***	74	3*

For CTB, the controls without test substance (left column) were set at 100%. Statistical analysis was performed with two-way ANOVA and Bonferroni's post-test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

negligible changes in viability (trypan blue) after 72 h was 10 µL/mL WCE. After 24 h, the time period which was used for further experiments, no cytotoxic effects could be observed at 0.1, 1 and 10 µL/mL.

Modulation of gene expression in vitro

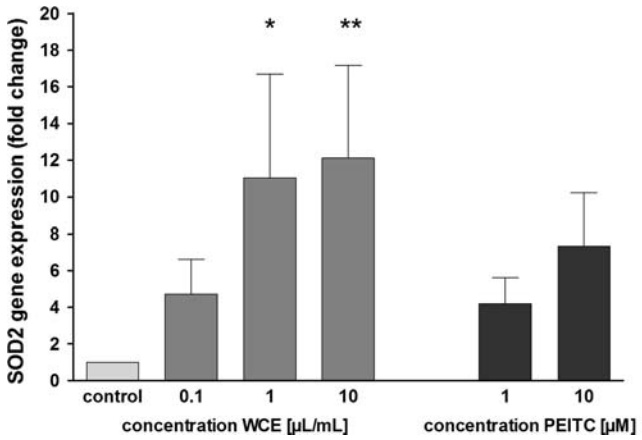
Peripheral blood mononuclear cells were exposed (24 h) to non-toxic concentrations of WCE (0.1, 1, 10 µL/mL) and PEITC (1 and 10 µM). Each independent incubation was performed with PBMC from a different donor. According to the investigations of Kassie et al. [21] the highest concentration of the WCE (10 µL/mL) is equivalent to roughly 1.5 µM PEITC. Incubation of cells with WCE and with PEITC resulted in a dose-dependent increase of *SOD2* gene expression, which was significant for 1 µL/mL WCE (10-fold, $p < 0.05$) and 10 µL/mL WCE (12-fold, $p < 0.01$) of the extract (Fig. 1). Furthermore, PEITC increased *SOD2* up to sevenfold compared to the control, but without reaching significance. *GPX1* could be modulated, but due to the high interindividual differences, the results did not reach statistical significance. For *CAT*, *GSTP1*, *GSTA4* and *UGT1A1*, no changes in gene expression could be detected after treatments (data not shown).

In vitro SOD2 activity

Peripheral blood mononuclear cells showed a basal SOD2 activity of 110 mU/mg protein. After PBMC incubation with the WCE, a dose-dependent increase of SOD2 activity could be detected (Fig. 2). The highest induction (mean fold change 1.96, $n = 4$) could be seen after incubation with 10 µL/mL WCE and an incubation time of 24 h. For PEITC, no regulatory changes in enzyme activity could be observed.

In vivo gene expression

In vitro exposure of PBMC resulted in up-regulation of candidate genes; however, the effect of WC consumption on PBMC expression in vivo was more varied. Gene expression investigations in PBMC were performed from a randomly selected subset ($n = 10$) of all male subjects as a pilot study. The results showed high inter-individual variations for all genes investigated (*CAT*, *GPX1*, *GSTA4*, *GSTP1*, *SOD2*, *UGT1A1*), though intra-individual variation of expression during the course of the study was small. The gene expression profiles for *SOD2* and *GPX1* for the control and the intervention period are shown in Fig. 3. The WC intervention had no significant effect on the expression of all genes, neither when subjects were evaluated as subgroups according to GST genotypes (*GSTM1*0* and



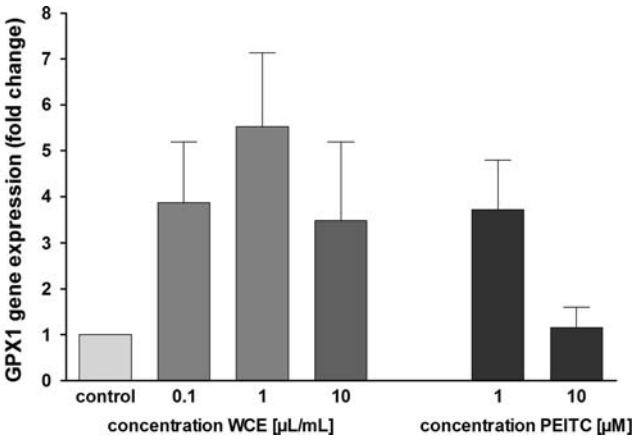


Fig. 1 Changes in gene expression related to *GAPDH* as reference gene for *SOD2* and *GPX1* in PBMC after treatment (24 h) with a watercress extract and with PEITC measured with real-time PCR; mean ± SEM; $n = 5$. Statistical analysis was performed with one-way-ANOVA/Bonferroni post-test, $*p < 0.05$, $**p < 0.01$

*GSTT1*0*) nor to smoking behaviour. On this account, we refrained from analysing the whole study population.

GPX and SOD activity in red blood cells

As Gill et al. [10] already demonstrated, there were no changes of GPX and SOD activities in red blood cells in response to the WC intervention in the total population. We determined *GSTM1* and *GSTT1* polymorphisms and subdivided the original data. Out of 60 subjects, 44 had the *GSTM1*0* genotype (73%), seven the *GSTT1*0* genotype (12%) and six subjects were null for both enzymes (10%). Figure 4 summarizes GPX and SOD activities in RCC of totals (already published by Gill et al.) and subgroups. Interestingly, there was a significant up-regulation of the GPX activity ($p = 0.025$) in *GSTM1*0* individuals. The same trend was also detectable for SOD activity ($p = 0.054$). For *GSTM1*1* individuals, a non-significant trend for decreased SOD and GPX activities after WC intervention could be detected. This result is unclear and needs further investigations.

Discussion

In many cell culture experiments and studies in animals, a variety of plant-food constituents have been identified as inducers of phase II enzymes and as potential chemopreventive agents. For WC, it has been reported that constituents from this plant reduced oxidative DNA damage in blood cells and also lowered levels of DNA damage induced by genotoxic agents [3, 10]. In this present study, we have demonstrated that blood cells respond in vitro as well as in vivo to active substances within WC. This resulted in the activation of an apparent oxidative stress

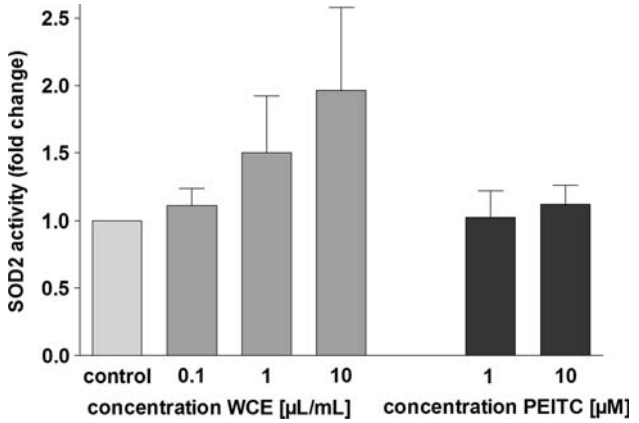


Fig. 2 Changes in SOD2 enzyme activity in PBMC after treatment (24 h) with a watercress extract and with PEITC; mean ± SEM; $n = 4$

response involving up-regulation of GPX and SOD expression, which could also be an explanation for the reported modulations in DNA damage.

High concentrations (50 µL/mL) of WCE strongly suppressed the metabolic activity measured with the cell titre-blue assay in PBMC almost to undetectable levels. Notably, only slight reductions in viability measured with trypan blue at high WCE concentrations were seen and no reduction in cell number could be detected. The highest concentration of the WCE appears not to be physiological for blood cells and was not used for further experiments.

In vitro, important detoxification enzymes such as *SOD2* and *GPX1* were highly up-regulated by the WCE and also by PEITC in our study. Mitochondrial SOD2 is a homotetramer containing one manganese atom per subunit and is indispensable to life [26]. SOD2 is often considered as one of the most effective antioxidant enzymes that has antitumour activity [1, 42]. Furthermore, SOD2 should not only be regarded as an antioxidative enzyme, but also as a

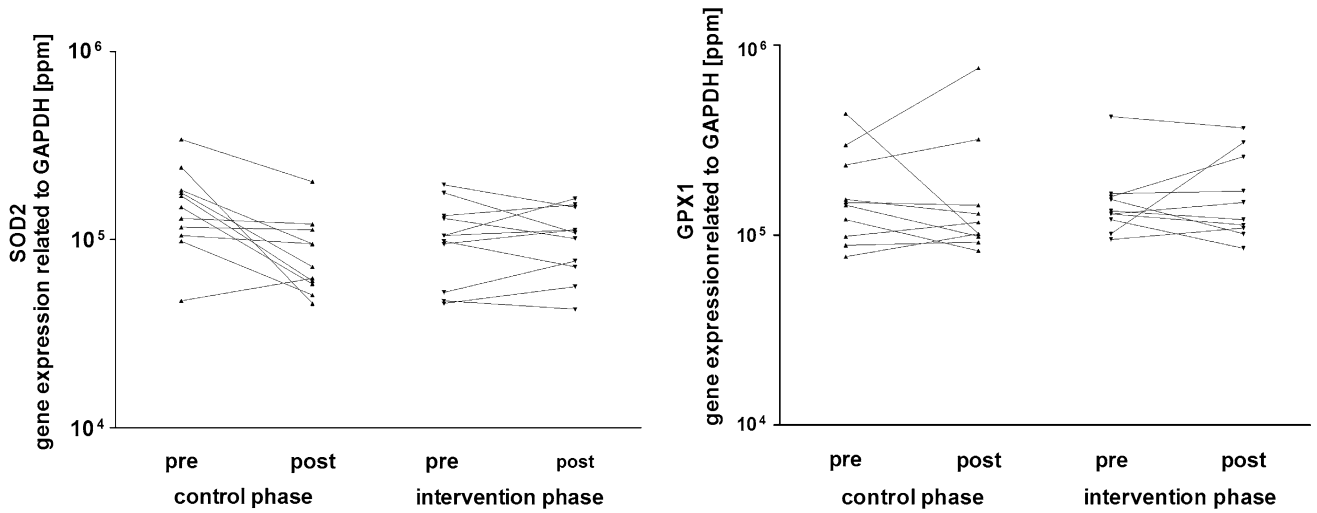


Fig. 3 Changes in gene expression for *SOD2* and *GPX1* in PBMC after a watercress intervention [10] measured with real-time PCR; shown is a randomly selected subset of donors; $n = 10$

key enzyme involved in the maintenance of the cellular redox state and thereby in the biological status of cells and tissues [7]. In several studies, low activity and expression of SOD2 is linked to tumour development and phenotype [38, 39, 47]. The expression of SOD2 is also inducible by many dietary factors such as alcohol, retinoic acid and alpha-tocopherol and is mediated by nuclear factor NF-kappaB [48]. The *SOD2* up-regulation in this study showed high variation between the individual blood donors which ranged from a 1.4- to a 32.0-fold induction, which could in part be due to differences in genetic polymorphisms. Further investigations for SOD2 on protein level (immunoblot, data not shown) and on activity level revealed only slight changes. The WCE increased SOD2 activity almost two-fold, whilst PEITC had no impact. This may be due to the potential influence of further bioactive WC constituents such as quercetin glycosides or hydroxycinnamic acids [3]. Discrepancies between the mRNA and protein levels of the SODs have also been reported by others, where the mRNA induction did not correspond to the augmentation of the protein level or of the enzyme activity [13, 27]. All results were explained by a translational block for the synthesis of SOD2. In contrast to these findings, Yazdanparast et al. [49] observed in vivo that a treatment of hypercholesterolaemic rats, with a WCE, significantly enhanced SOD activities in liver tissues; van Lieshout et al. [43] reported a GPX induction by PEITC in rats. This result is comparable to our findings, since the lower PEITC concentration (1 μM), which can be reached after WC ingestion, induced *GPX1* gene expression 3.7-fold.

In our experiments, we investigated the hypothesis: whether there is a link between *GST* polymorphisms and the induction of SOD and GPX in vivo. GSTs are involved in the conjugation of the bioactive compounds such as the

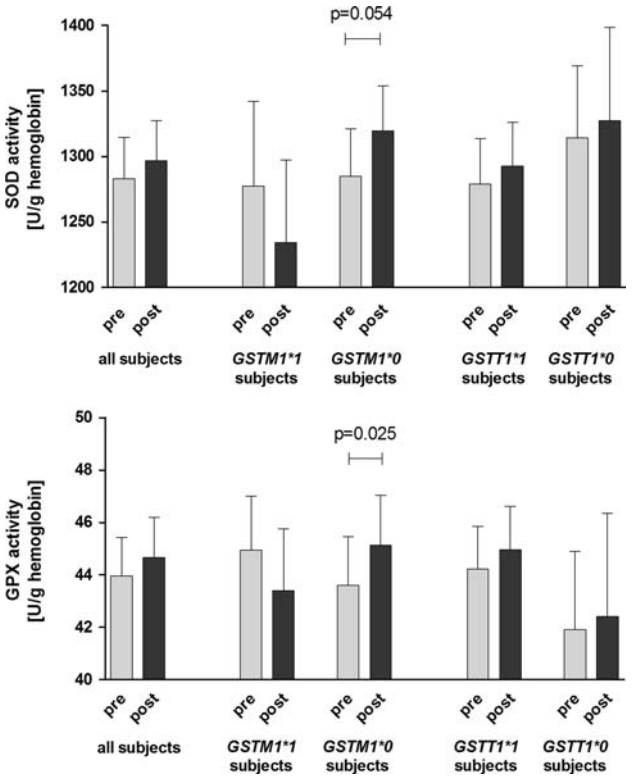


Fig. 4 Changes in enzyme activity for SOD and GPX in red blood cells after a watercress intervention [10]; mean ± SEM from all subjects ($n = 60$) and subjects subdivided for *GST* polymorphisms (*GSTM1*0* $n = 44$, *GSTM1*1* $n = 16$, *GSTT1*0* $n = 7$, *GSTT1*1* $n = 53$). Statistical analysis was performed with two-tailed, paired *t* test. The GPX and the SOD results were standardized to red cell concentrate hemoglobin concentration, and the final results were expressed in U/g hemoglobin

ITCs in CV, and several studies have examined the relationship between CV intake and *GST* polymorphisms. Based on the current understanding, individuals with *GST*

null genotypes, especially with the *GSTM1*0* genotype, conjugate ITCs less readily and excrete bioactive compounds more slowly. Thus, these subjects should have greater amounts at tissue level, with a potentially greater benefit for the individual. But the data are still controversial, since epidemiological studies tend to support this hypothesis by finding the greatest risk reduction in CV consumers with the null or less active *GST* genotypes [25, 51], whilst other studies found a greater risk reduction amongst subjects with the most active or expressed genotypes [37, 46]. In this study, we have seen in vivo an up-regulation of detoxification enzymes only in *GSTM1*0* subjects. It is most likely that this marginal enzyme activity induction of SOD or GPX in blood cells has minor chemopreventive effects. However, the consequence for other tissues is not clear, since effects in blood cells could be smaller than in other tissues. Lampe et al. [23] also reported just slightly elevated GSTα activity after brassica vegetable diets by 26% and total GST activity by 7% in the *GSTM1*0* individuals, particularly women. The higher effects compared to our study could be explained by the more tightly controlled basal diet (vegetable free) and a supplementation at a much higher level.

In summary, our study demonstrates that an extract from WC modulates gene expression in human PBMC in vitro and that this is also reflected in a modulation of enzyme activity in vivo, particularly in individuals with the *GSTM1*0* genotype. Questions for further studies will be to elucidate whether this modulation of SOD and GPX enzyme activity by CV is directly associated with anti-genotoxic effects and if the changes in the blood cells reflect changes that occur in other target tissues.

Acknowledgments The watercress intervention study was supported by Vitacress Ltd, Geest Plc, and the Watercress Company.

References

1. Behrend L, Henderson G, Zwacka RM (2003) Reactive oxygen species in oncogenic transformation. Biochem Soc Trans 31:1441–1444
2. Bell DA, Taylor JA, Paulson DF, Robertson CN, Mohler JL, Lucier GW (1993) Genetic risk and carcinogen exposure: a common inherited defect of the carcinogen-metabolism gene glutathione *S*-transferase M1 (GSTM1) that increases susceptibility to bladder cancer. J Natl Cancer Inst 85:1159–1164
3. Boyd LA, McCann MJ, Hashim Y, Bennett RN, Gill CI, Rowland IR (2006) Assessment of the anti-genotoxic, anti-proliferative, and anti-metastatic potential of crude watercress extract in human colon cancer cells. Nutr Cancer 55:232–241
4. Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein–dye binding. Anal Biochem 72:248–254
5. Branca F, Hanley AB, Pool-Zobel BL, Verhagen H (2001) Biomarkers in disease and health. Br J Nutr 86:S55–S92
6. Brennan P, Hsu CC, Moullan N, Szeszenia-Dabrowska N, Lissowska J, Zaridze D, Rudnai P, Fabianova E, Mates D, Bencko V, Foretova L, Janout V, Gemignani F, Chabrier A, Hall J, Hung RJ, Boffetta P, Canzian F (2005) Effect of cruciferous vegetables on lung cancer in patients stratified by genetic status: a Mendelian randomisation approach. Lancet 366:1558–1560
7. Buettner GR, Ng CF, Wang M, Rodgers VG, Schafer FQ (2006) A new paradigm: manganese superoxide dismutase influences the production of H_2O_2 in cells and thereby their biological state. Free Radic Biol Med 41:1338–1350
8. Canistro D, Croce CD, Iori R, Barillari J, Bronzetti G, Poi G, Cini M, Caltavuturo L, Perocco P, Paolini M (2004) Genetic and metabolic effects of gluconasturtiin, a glucosinolate derived from Cruciferae. Mutat Res 545:23–35
9. Chung FL, Morse MA, Eklind KI, Lewis J (1992) Quantitation of human uptake of the anticarcinogen phenethyl isothiocyanate after a watercress meal. Cancer Epidemiol Biomarkers Prev 1:383–388
10. Gill CI, Haldar S, Boyd LA, Bennett R, Whiteford J, Butler M, Pearson JR, Bradbury I, Rowland IR (2007) Watercress supplementation in diet reduces lymphocyte DNA damage and alters blood antioxidant status in healthy adults. Am J Clin Nutr 85:504–510
11. Giovannucci E, Rimm EB, Liu Y, Stampfer MJ, Willett WC (2003) A prospective study of cruciferous vegetables and prostate cancer. Cancer Epidemiol Biomarkers Prev 12:1403–1409
12. Glei M, Liegibel UM, Ebert MN, Böhm V, Pool-Zobel BL (2002) ß-Carotene reduces bleomycin-induced genetic damage in human lymphocytes. Toxicol Appl Pharmacol 179:65–73
13. Gomi F, Matsuo M (2002) Effects of 60% oxygen inhalation on the survival and antioxidant enzyme activities of young and old rats. Mech Ageing Dev 123:1295–1304
14. Guo Z, Smith TJ, Wang E, Sadrieh N, Ma Q, Thomas PE, Yang CS (1992) Effects of phenethyl isothiocyanate, a carcinogenesis inhibitor, on xenobiotic-metabolizing enzymes and nitrosamine metabolism in rats. Carcinogenesis 13:2205–2210
15. Haristoy X, Fahey JW, Scholtus I, Lozniewski A (2005) Evaluation of the antimicrobial effects of several isothiocyanates on *Helicobacter pylori*. Planta Med 71:326–330
16. Hayes JD, Kelleher MO, Eggleston IM (2008) The cancer chemopreventive actions of phytochemicals derived from glucosinolates. Eur J Nutr 47(suppl 2):73–88
17. Hecht SS, Carmella SG, Murphy SE (1999) Effects of watercress consumption on urinary metabolites of nicotine in smokers. Cancer Epidemiol Biomarkers Prev 8:907–913
18. Hecht SS, Chung FL, Richie JP Jr, Akerkar SA, Borukhova A, Skowronski L, Carmella SG (1995) Effects of watercress consumption on metabolism of a tobacco-specific lung carcinogen in smokers. Cancer Epidemiol Biomarkers Prev 4:877–884
19. Heiss E, Herhaus C, Klimo K, Bartsch H, Gerhauser C (2001) Nuclear factor kappa B is a molecular target for sulforaphane-mediated anti-inflammatory mechanisms. J Biol Chem 276:32008–32015
20. Higdon JV, Delage B, Williams DE, Dashwood RH (2007) Cruciferous vegetables and human cancer risk: epidemiologic evidence and mechanistic basis. Pharmacol Res 55:224–236
21. Kassie F, Laky B, Gminski R, Mersch-Sundermann V, Scharf G, Lhoste E, Kansmuller S (2003) Effects of garden and water cress juices and their constituents, benzyl and phenethyl isothiocyanates, towards benzo(a)pyrene-induced DNA damage: a model study with the single cell gel electrophoresis/Hep G2 assay. Chem Biol Interact 142:285–296
22. Keck AS, Finley JW (2004) Cruciferous vegetables: cancer protective mechanisms of glucosinolate hydrolysis products and selenium. Integr Cancer Ther 3:5–12

23. Lampe JW, Chen C, Li S, Prunty J, Grate MT, Meehan DE, Barale KV, Da Dightman, Feng ZPJD (2000) Modulation of human glutathione *S*-transferases by botanically defined vegetable diets. Cancer Epidemiol Biomarkers Prev 9:787–793
24. Lampe JW, Peterson S (2002) Brassica, biotransformation and cancer risk: genetic polymorphisms alter the preventive effects of cruciferous vegetables. J Nutr 132:2991–2994
25. London SJ, Yuan JM, Chung FL, Gao YT, Coetzee GA, Ross RK, Yu MC (2000) Isothiocyanates, glutathione *S*-transferase M1 and T1 polymorphisms, and lung-cancer risk: a prospective study of men in Shanghai, China. Lancet 356:724–729
26. Mates JM, Perez-Gomez C, Nunez de Castro I (1999) Antioxidant enzymes and human diseases. Clin Biochem 32:595–603
27. Niu CS, Chang CK, Lin LS, Jou SB, Kuo DH, Liao SS, Cheng JT (1998) Modification of superoxide dismutase (SOD) mRNA and activity by a transient hypoxic stress in cultured glial cells. Neurosci Lett 251:145–148
28. Palaniswamy UR, McAvoy RJ, Bible BB, Stuart JD (2003) Ontogenic variations of ascorbic acid and phenethyl isothiocyanate concentrations in watercress (*Nasturtium officinale* R.Br.) leaves. J Agric Food Chem 51:5504–5509
29. Pemble S, Schroeder KR, Spencer SR, Meyer DJ, Hallier E, Bolt HM, Ketterer B, Taylor JB (1994) Human glutathione *S*-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. Biochem J 300:271–276
30. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29:e45
31. Pfaffl MW, Horgan GW, Dempfle L (2002) Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res 30:e36
32. Pool-Zobel B, Veeriah S, Bohmer FD (2005) Modulation of xenobiotic metabolising enzymes by anticarcinogens: focus on glutathione *S*-transferases and their role as targets of dietary chemoprevention in colorectal carcinogenesis 1. Mutat Res 591:74–92
33. Pullar JM, Thomson SJ, King MJ, Turnbull CI, Midwinter RG, Hampton MB (2004) The chemopreventive agent phenethyl isothiocyanate sensitizes cells to Fas-mediated apoptosis. Carcinogenesis 25:765–772
34. Rockett JC, Burczynski ME, Fornace AJ, Herrmann PC, Krawetz SA, Dix DJ (2004) Surrogate tissue analysis: monitoring toxicant exposure and health status of inaccessible tissues through the analysis of accessible tissues and cells. Toxicol Appl Pharmacol 194:189–199
35. Rose P, Faulkner K, Williamson G, Mithen R (2000) 7-Methylsulfinylheptyl and 8-methylsulfinyloctyl isothiocyanates from watercress are potent inducers of phase II enzymes. Carcinogenesis 21:1983–1988
36. Seow A, Yuan JM, Sun CL, Van Den Berg D, Lee HP, Yu MC (2002) Dietary isothiocyanates, glutathione *S*-transferase polymorphisms and colorectal cancer risk in the Singapore Chinese Health Study. Carcinogenesis 23:2055–2061
37. Spitz MR, Duphorne CM, Detry MA, Pillow PC, Amos CI, Lei L, Andrade M, Gu X, Hong WK, Wu X (2000) Dietary intake of isothiocyanates: evidence of a joint effect with glutathione *S*-transferase polymorphisms in lung cancer risk. Cancer Epidemiol Biomarkers Prev 9:1017–1020
38. St Clair D (2004) Manganese superoxide dismutase: genetic variation and regulation. J Nutr 134:3190S–3191S
39. St Clair DK, Holland JC (1991) Complementary DNA encoding human colon cancer manganese superoxide dismutase and the expression of its gene in human cells. Cancer Res 51:939–943
40. Talalay P, Fahey JW (2001) Phytochemicals from cruciferous plants protect against cancer by modulating carcinogen metabolism. J Nutr 131:3027S–3033S
41. Traka M, Gasper AV, Melchini A, Bacon JR, Needs PW, Frost V, Chantry A, Jones AM, Ortori CA, Barrett DA, Ball RY, Mills RD, Mithen RF (2008) Broccoli consumption interacts with GSTM1 to perturb oncogenic signalling pathways in the prostate. PLoS ONE 3:e2568
42. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 160:1–40
43. van Lieshout EMM, Peters WHM, Jansen JB (1996) Effect of oltipraz, alpha-tocopherol, betacarotene and phenethylisothiocyanate on rat oesophageal, gastric, colonic and hepatic glutathone, glutathione *S*-transferase and peroxidase. Carcinogenesis 17:1439–1445
44. van Poppel G, Verhoeven DT, Verhagen H, Goldbohm RA (1999) Brassica vegetables and cancer prevention. Epidemiology and mechanisms. Adv Exp Med Biol 472:159–168
45. Visanji JM, Duthie SJ, Pirie L, Thompson DG, Padfield PJ (2004) Dietary isothiocyanates inhibit Caco-2 cell proliferation and induce G2/M phase cell cycle arrest, DNA damage, and G2/M checkpoint activation. J Nutr 134:3121–3126
46. Wang LI, Giovannucci EL, Hunter D, Neuberg D, Su L, Christiani DC (2004) Dietary intake of cruciferous vegetables, Glutathione *S*-transferase (GST) polymorphisms and lung cancer risk in a Caucasian population. Cancer Causes Control 15:977–985
47. Xu Y, Krishnan A, Wan XS, Majima H, Yeh CC, Ludewig G, Kasarskis EJ, St Clair DK (1999) Mutations in the promoter reveal a cause for the reduced expression of the human manganese superoxide dismutase gene in cancer cells. Oncogene 18:93–102
48. Xu Y, Porntadavity S, St Clair DK (2002) Transcriptional regulation of the human manganese superoxide dismutase gene: the role of specificity protein 1 (Sp1) and activating protein-2 (AP-2). Biochem J 362:401–412
49. Yazdanparast R, Bahramikia S, Ardestani A (2008) *Nasturtium officinale* reduces oxidative stress and enhances antioxidant capacity in hypercholesterolaemic rats. Chem Biol Interact 172:176–184
50. Zhang SM, Hunter DJ, Rosner BA, Giovannucci EL, Colditz GA, Speizer FE, Willett WC (2000) Intakes of fruits, vegetables, and related nutrients and the risk of non-Hodgkin's lymphoma among women. Cancer Epidemiol Biomarkers Prev 9:477–485
51. Zhao B, Seow A, Lee EJ, Poh WT, Teh M, Eng P, Wang YT, Tan WC, Yu MC, Lee HP (2001) Dietary isothiocyanates, glutathione *S*-transferase-M1, -T1 polymorphisms and lung cancer risk among Chinese women in Singapore. Cancer Epidemiol Biomarkers Prev 10:1063–1067