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Article *in* Wiener Medizinische Wochenschrift · December 2012

DOI: 10.1007/s10354-012-0163-3 · Source: PubMed

Wien Med Wochenschr (2013) 163:80–88
DOI 10.1007/s10354-012-0163-3

wmw
Wiener Medizinische Wochenschrift

Sulforaphane and related mustard oils in focus of cancer prevention and therapy

Ingrid Herr, Vladimir Lozanovski, Philipp Houben, Peter Schemmer, Markus W. Büchler

Received: 27 August 2012 / Accepted: 29 October 2012 / Published online: 7 December 2012

Sulforaphan und verwandte Senföle im Fokus der Prävention und Therapie von Krebs

Zusammenfassung Die Pflanzenfamilie *Brassicaceae*, mit älterem Namen *Cruciferae,* enthält Senfölglykoside, woraus durch enzymatische Hydrolyse Senföle entstehen, welche Schutz vor Fraßfeinden, Mikroorganismen und Pilzen bieten. Über 120 verschiedene Senföle mit vielfältigen biologischen Funktionen sind bekannt. Seit der Antike kommen diese Substanzen in der Heilkunde als natürliche Antibiotika, Virostatika und Anti-Mykotika zum Einsatz. Der antioxidative Effekt von Senfölen trägt zum Schutz vor DNA-Schäden bei. Epidemiologische und experimentelle Studien belegen eine präventive und therapeutische Wirkung von Gemüse der Kreuzblütlerfamilie und isolierter Substanzen daraus. Besonders gut untersucht ist das Senföl Sulforaphan, das in hoher Konzentration in Brokkoli und seinen Sprossen enthalten ist. Wie an Mäusen nun gezeigt wurde, greift Sulforaphan sogar die besonders bösartigen Krebsstammzellen an, denen konventionelle Tumortherapien nichts anhaben. Aufgrund dieser vielversprechenden Ergebnisse sind nun in den USA erste prospektive Klinische Studien mit Sulforaphan-angereicherten Brokkolisprossen bei Krebspatienten angelaufen.

Schlüsselwörter: Sekundäre Pflanzenstoffe, Brassica, Brokkoli, Glukosinolate, Sulforaphan

Summary The plant family *Brassicaceae,* formerly Cruciferae, contains mustard oil glycosides, from which mustard oils are enzymatically hydrolyzed. Mustard oils offer protection from pests, microorganisms and fungi. More than 120 different mustard oils with various biological functions are known. Since ancient times, these substances are used as natural antibiotics, antiviral drugs and antimycotics. The antioxidative effect of mustard oils contributes to protection from DNA damage. Epidemiological and experimental studies have shown preventive and therapeutic effects of crucifers or isolated substances thereof. Particularly well studied is the mustard oil sulforaphane, which is contained in high concentrations in broccoli and its sprouts. As has been shown in mice recently, sulforaphane also targets the most malignant cancer stem cells, which are not affected by conventional cancer treatments. Based on these promising results, the first prospective clinical studies with cancer patients and sulforaphane-enriched broccoli sprouts have now been initiated in the United States.

Keywords: Phytonutrients, Brassicaceae, Broccoli, Glucosinolates, Sulforaphane

Introduction

Cabbage and other members of plant family *Brassicaceae* are well known as vegetables and food products. What we have largely forgotten is the irrefutable fact that this plant family possesses a tremendous healing power. Since ancient times, the beneficial effects of cruciferous vegetables have been used therapeutically [1]. Cato the Elder (234–149 bc), a Roman statesman, noted in his book about medical plants that cabbage, eaten crude with vinegar or cooked with oil or fat, banishes and cures all, 'from crapulence after exceeding wine consumption up to serious diseases like cancer'. He writes: 'If a cancerous ulcer appears upon the breasts, apply a crushed cabbage leaf and it will make it well' [2]. Physicians took advantage of this traditional knowledge in the Second World War when medicaments were rare. They put sauerkraut or minced cabbage directly to abscesses, festering or open wounds and frostbites or prescribed the intake of

I. Herr, PhD (✉)
Molecular OncoSurgery Group, University of Heidelberg and German Cancer Research Center-G403, Im Neuenheimer Feld 365, 69120 Heidelberg, Germany
e-mail: i.herr@dkfz.de

V. Lozanovski · P. Houben · P. Schemmer · M. W. Büchler
Department of General Surgery, University of Heidelberg and German Cancer Research Center-G403, Heidelberg, Germany

raw cabbage or derived juice to alleviate gastrointestinal disorders, high blood pressure, obesity, diabetes, inflammation and many other diseases [3]. The reported healing effects were overwhelming, and traditional healers still use cabbage and other crucifers as potent remedies.

Brassicaceae family contains more than 330 genera, with the well-known species Brassica oleracea (broccoli, cabbage, cauliflower and Brussels sprouts), Brassica rapa (Chinese cabbage and turnip), Brassica napus (rapeseed and canola), Raphanus sativus (radish), Armoracia rusticana (horesradish), Lepidium sativum (garden cress), Tropaeolum (nasturtium), Nasturtium officinale (watercress), Eruca sativa (rocket and arugula), Sinapis alba (white mustard) and Brassica nigra (black mustard) [4]. Cruciferae is the former name, meaning 'cross-bearing', and describes the four petals of the flowers of Brassicaceae. Latest evidence for the therapeutic effects of crucifers comes from epidemiological studies, in which associations are reported between consumption of cruciferous vegetables and reduction in cancer risk at several sites, including the lung, breast, colon and rectum and prostate [5]. Certain studies have specifically quantified broccoli consumption, as opposed to crucifers in general, and shown that a diet rich in broccoli can reduce cancer risk [6]. Remarkably, among several vegetables, evidence for a cancer protective role is strongest and most consistent for vegetables in the *Brassicaceae* plant family [7–9].

Mustard oil bomb

In addition to vitamins and minerals, such as vitamin C, folate and other B vitamins, β-carotene, potassium, calcium and iron, cruciferous vegetables contain abundant fibre and phytochemicals. The most dominant components of *Brassicaceae* are the mustard oil glucosinolates that contain sulphur and nitrogen and are derived from glucose and an amino acid [10]. The plants have β-thioglucosidase enzyme myrosinase, which cleaves the glucose group from glucosinolate upon hydrolysis (Fig. 1). The remaining molecule then quickly converts to an isothiocyanate, a nitrile or a thiocyanate; these are the active substances and are called 'mustard oils'. These hot, spicy or bitter tasting molecules serve the plant as defence system against insects, mites, rodents and pathogens like bacteria, viruses or fungi. To prevent damage to the plant itself, myrosinase is physically segregated from glucosinolates and released first upon damage to the cells, e.g. by microbial attack, insect predation or mechanical food processing, such as chewing or food preparation. Importantly, myrosinase acts on all major classes of glucosinolates. Intriguingly, a crucifer specialist insect, diamondback moth (*Plutella xylostella*), has the ability to disarm this so-called "mustard oil bomb" by production of glucosinolate sulphatase [11]. Sulphatase activity of this moth enzyme largely prevents the formation of toxic hydrolysis products arising from the plant's defence system.

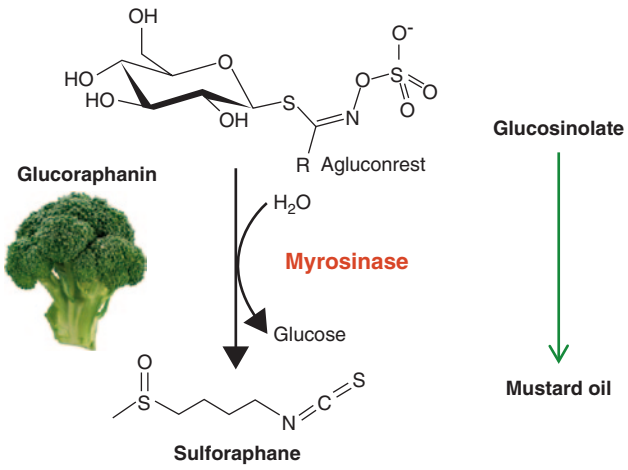

Fig. 1 The Mustard Oil Bomb. Glucosinolates and myrosinase are stored in separate cell compartiments. Break down of the cell, e.g. by chewing, cutting or heating, leads to the release of glucosinolates and myrosinase. Myrosinase causes hydrolytic cleavage of glucosinolates as exemplified for glucoraphanin present in high concentration in broccoli. The resulting mustard oil is the isothiocyanate sulforaphane

Properties and biological activity of glucosinolates

About 120 different glucosinolates are known to be present in different amounts in the numerous representatives of *Brassicaceae* [12]. Glucosinolates can be classified by their precursor amino acid and the types of modification to the R group. Compounds derived from alanine, leucine, isoleucine, methionine or valine are called aliphatic glucosinolates, those derived from phenylalanine or tyrosine are called aromatic glucosinolates, and those derived from tryptophan are called indole glucosinolates. In the past decade, certain glucosinolates of the 120 different known ones have been identified as potent anti-inflammatory, cancer-preventive and cancer therapeutic agents or are of agricultural interest (Table 1). In the past 30 years, glucosinolates have gained significant agricultural importance regarding rapeseed, which is suitable not only for the production of biodiesel but also for the production of canola oil for human consumption and of protein-rich seed cake (the residue after crushing for oil extraction) for animal feed [12]. As wild rapeseed contains high amounts of sharp-tasting glucosinolates, plant breeders have drastically reduced the levels of seed glucosinolates to improve the taste and to lower the toxicity. One of the predominant rapeseed glucosinolates, progoitrin, which is also present in broccoli, cabbage and arugula, reduces the production of thyroid hormones, causes goitre and has other harmful effects on animal nutrition after high consumption [13]. However, struma formation due to high intake of cabbage has not been detected in humans to date. The distinct taste and flavour of glucosinolates is primarily due to their isothiocyanate hydrolysis products. Indole glucosinolates are

Table 1. Common mustard oil glucosinolates and derived mustard oils out of the 120 known ones

Mustard oil glucosinolate	Chemical rest	Mustard oil	Sources
Sinigrin	2-Propenyl, Allyl	*Allyl isothiocyanate*	Black mustard, horesradish, wasabi, *broccoli,* Brussels sprouts, cabbage
Sinalbin	4-Hydroxybenzyl	4-Hydroxybenzyl isothiocyanate	White mustard
Glucotropaeolin	Benzyl	Benzyl isothiocyanate, tropaeolin	Nasturtium, garden cress
Gluconasturtiin	2-Phenylethyl	*Phenylethyl isothiocyanate*	Horseradish, water cress, *broccoli*
Gluconapin	3-Butenyl	3-Butenyl isothiocyanate, napin	Rapeseed, Chinese cabbage, cabbage
Glucoraphanin	4-Methylsulfinyl-3-butenyl	Sulforaphane, sulforaphane nitrile	Radish
Glucoraphanin	4-Methylsulfinylbutyl	*Sulforaphane*	*Broccoli,* radish, white cabbage, cauliflower, cabbage, arugula
Glucobrassicin	3-Indolylmethyl	*Indol-3-carbinol*	Cabbage, *broccoli,* cauliflower
Glucobrassicanapin	4-Pentenyl	Brassicanapin	Chinese cabbage
Progoitrin	(2R)-2-Hydroxy-3-butenyl	*Goitrin*	*Broccoli,* cabbage, rapeseed, arugula
Glucoiberin	3-Methylsulfinylpropyl	*Iberin*	*Broccoli,* cabbage
Glucoibeverin	3-Methylthiopropyl	Ibe(r)verin	Cabbage
Glucocapparin	Methyl	Methyl isothiocyanate, capparin	Capers
Glucolepidin	Ethyl	Lepidin	Garden cress
Glucoerucin	4-Methylthiobutyl	Erucin, erucin nitrile	Arugula, cabbage
Glucoraphasatin	4-Methylthiobut-3-enyl	Raphasatin	Radish
Glucoalyssin	Methylsulfinylpentyl	Alyssin	Rapeseed, pok choi, arugula
4-Hydroxy-glucobrassicin	4-OH-3-indolylmethyl	4-Hydroxy-brassicin	Cabbage
4-Methoxy-glucobrassicin	4-Methoxy-3-indolylmethyl	4-Methoxy-brassicin	Cabbage
Glucosativin (dimer)	4-Mercaptobutyl	Sativin	Arugula
6-Methylsulfinyl-hexyl-GLS	6-Methylsulfinylhexyl	6-Methylsulfinylhexyl isothiocyanate	Wasabi

Selection of the most common glucosinolates including those with anti-inflammatory, cancer preventive and therapeutic activity. For references and detailed descriptions, see text

especially known for causing bitterness (cabbage, broccoli and cauliflower) [14]. Sinigrin and the derived allyl isothiocyanate are responsible for the pungent taste of black mustard seeds, horseradish and wasabi. Hence, synthetic allyl isothiocyanate is used in agriculture as an insecticide, a bactericide [15] and a nematocide, and also for certain cases of crop protection [16]. Sinalbin and glucotropaeolin and the derived benzyl isothiocyanates are found in high concentration in seeds of white mustard, nasturtium and garden cress. Gluconasturtiin and the derived phenethyl isothiocyanate are predominantly present in horseradish and watercress. In vitro, benzyl mustard oils have been proven to be effective against bacteria, virus and fungi [17]. In vitro studies demonstrate that a combination of benzyl and phenylethyl mustard oils possesses a broad antibacterial spectrum towards at least 13 clinically relevant bacterial strains [18]. Other experimental studies demonstrate that these mustard oils from nasturtium and horseradish inhibit the increase of influenza virus H1N1 in human lung cells to about 90 % [19, 20]. Therefore, nasturtium is used in combination with horseradish powder as phytotherapeutics for treatment and prophylaxis of respiratory and urinary tract infections (Angocin® Anti Infekt N). Another crucifer-based plant medicament contains glucosinolate gluconasturtiin together with myrosinase purified from watercress (Cressana®). The derived phenethyl isothiocyanate is reported to possess strong anti-infective activity in humans and to act as cancer preventive agents in mouse models [21].

Studies in humans confirm a preventive and therapeutic effect of crucifers in cancer

An extensive review of epidemiologic studies published prior to 1996 reported that the majority (67 %) of 87 case–control studies found an inverse association between some type of cruciferous vegetable intake and cancer risk [22]. Several newer epidemiological studies in humans support these results and demonstrate associations between diets rich in cruciferous vegetables and a reduced risk for different cancer entities. Significant results were obtained for cancer of stomach, colorectum, lung, prostate, breast, bladder and endometrium, as summarized in a recent review article [23]. The systematic literature review published by the World Cancer Research Fund (WCRF)/American Institute for Cancer Research (AICR) in 2007 evaluated two cohort studies and six case–control studies in which there was a tendency for an inverse relationship between cruciferous vegetables and risk of pancreatic cancer [24]. One well-designed large population-based case–control study found that frequent consumption of cruciferous vegetables (i.e. more than four

servings per week) had a 50 % reduction in risk of pancreatic cancer [25]. In another large population-based case control study, the risk of developing pancreatic cancer in people consuming more vegetables (i.e. more than five servings per day), specifically cruciferous vegetables, was half of that in people consuming less vegetables. Data from a prospective Canadian epidemiological study suggest that high consumption of cruciferous vegetables is associated with inhibition of metastasis [26]. In the latter study, dietary patterns of 1,138 men with prostate cancer were evaluated with a median follow up of 4.2 years. While vegetables in general significantly reduced the relative risk (RR) of metastasis to 0.41, cruciferous vegetables had the highest effect among all vegetables (crucifers in general RR = 0.60; cabbage RR = 0.64; cauliflower RR = 0.48; broccoli RR = 0.55). Among all members of the cruciferous vegetables, broccoli and cauliflower were most effective with a significant effect at almost one serving per week; three to five servings were more effective. In a similar study, Richman et al. [27] prospectively examined the post-diagnostic intake of vegetables, specifically cruciferous vegetables, and the relation to risk of prostate cancer progression. These authors reported a 59 % reduced risk of prostate cancer progression after cruciferous vegetable intake after diagnosis in men, while inverse association for total vegetables was non-significant. A recent pilot intervention study in five participants found a significant up-regulation of manganese superoxide dismutase (1.56-fold) along with down-regulation of the heat shock 70-kDa protein (hsp70; 2.27-fold) in white blood cells after consumption of Brussels sprouts [28]. Both proteins play a role in malignant transformation of cells. These findings indicate that the alteration of the synthesis of these proteins may be involved in the anticarcinogenic effects of cruciferous vegetables, which was observed in earlier laboratory studies with animals. Together, these data confirm that bioactive compounds of crucifers have potent cancer preventive and therapeutic effects in humans.

Sulforaphane: a well-examined mustard oil with anticancer properties

Glucoraphanin (4-methylsulfinyl butyl glucosinolate) and the derived isothiocyanate, sulforaphane, are one of the best-studied compounds with high anticancer activity. Sulforaphane was first isolated in 1959 from a weed known as hoary cress [29]. Sulforaphane can also be isolated from broccoli, cauliflower, radish, cabbage and arugula [30]. The current consensus is that flower buds and sprouts of broccoli are the best source of sulforaphane [31, 32]. Prochazka and Koversova [33] identified a potent antimicrobial activity of sulforaphane in 1959, which was much more extensively characterized by Dornberger et al. 1975 [34]. In addition, antioxidant functions of sulforaphane have been detected, which are due to inhibition of phase I enzymes and induction of phase II enzymes [30]. However, in contrast to vitamin C, vitamin E or β-carotene, sulforaphane does not eliminate free radicals directly, but indirectly mainly by raising cellular glutathione levels via induction of phase II enzymes. In this way sulforaphane is involved in detoxification and prevents formation of carcinogen-induced DNA adducts, formed by heterocyclic amines [35]. Diverse in vitro and animal studies identified a protective and therapeutic effect of sulforaphane in a variety of cancer entities [23, 36]. Sulforaphane induces apoptosis and inhibits cell proliferation and angiogenesis [5, 37]. In addition, sulforaphane has been shown to reduce nuclear factor-κB (NF-κB) activity and to affect expression of NF-κB-mediated genes encoding adhesion molecules, inflammatory cytokines, growth factors and antiapoptotic factors [38].

Sulforaphane for targeting of cancer stem cells

More recent experimental studies suggest that sulforaphane may even target the highly aggressive cancer stem cell (CSC) population [39]. This is extremely important because this subpopulation does resist conventional chemo- and radiotherapy [40]. According to the hypothesis, CSCs are a minority population of cancer cells within a tumour mass that possess characteristics associated with normal stem cells. CSCs have the ability to self-renew and differentiate in therapy-sensitive daughter tumour cells, which form the major tumour population. However, only the CSCs are thought to be tumourigenic and responsible for tumour growth and metastasis. CSCs, but not the derived daughter tumour cells, are highly resistant to conventional tumour therapeutics. Thus, shrinkage of a tumour mass after chemotherapy is often observed and may be due to elimination of daughter tumour cells. However, CSCs survive and may be responsible for relapse. It is estimated that with each new cycle of chemotherapy, the population of CSCs is enriched. This explains the often observed failure of chemotherapy after several cycles of chemotherapy, although the tumour was responsive to the first cycles [40]. Development of specific therapies for targeting of CSCs is urgently needed for improvement of survival and quality of life of cancer patients, especially for sufferers of metastatic disease. Sulforaphane may be a candidate substance, which fulfils these requirements. According to recent data, highly tumourigenic pancreatic cancer stem-like cells are protected from chemotherapy by up-regulation of the master transcription factor NF-κB [39]. Sulforaphane down-regulated NF-κB activity and thereby restored sensitivity of CSCs for gemcitabine, sorafenib and other cancer therapeutics [39, 41, 42]. This was associated with a sulforaphane-mediated inhibition of typical CSC features, such as self-renewal potential, in vivo growth in mice and others. Most interestingly, sulforaphane in therapeutic concentrations exhibited no pronounced side effects in non-malignant cells or mice [39, 41]. Targeting of CSCs by sulforaphane has been confirmed in the meantime for prostate and breast cancer by laboratory and animal studies [41, 43, 44], indicating

that sulforaphane may be suited for eliminating CSCs in several tumour entities. It is tempting to speculate that a cheap nutrition strategy may be able to overcome the serious therapeutic problem of efficient treatment of advanced metastatic cancer.

Broccoli is a rich source of sulforaphane

Experimental sulforaphane concentrations that inhibited growth of human pancreatic cancer xenografts in mice were 4.4 mg/kg/day [39]. Extrapolating this experimental concentration to humans suggests a dose of 0.36 mg/kg according to the body surface area normalization method [45]. This corresponds to 25 mg/70 kg human. The question arises how much intake of cruciferous vegetables is necessary to reach this concentration. This is not easy to answer for the following reasons. First, the bioavailability of sulforaphane upon cabbage consumption is highly variable among different consumers [46]. Second, the presence of glucoraphanin in different *Brassica oleracea* varieties is highly variable [46]. The variation of glucoraphanin in six cultivars of broccoli ranged from 12.2 to 119.4 mg/100 g fresh weight, with an average of 60 mg/100 g serving. Comparable or even higher amounts of glucoraphanin were detected in some but not all cultivars of cabbage and kale, with a special recommendation for black kale of Italian origin (*Cavolo nero*). An ecological cultivation method ('aus kontrolliert biologischem Anbau') seems to enhance the content of indolyl glucosinolates but not of glucoraphanin in broccoli [47]. Therefore, hybrid high-glucosinolate broccoli (HG broccoli) with enhanced levels of glucoraphanin has been developed by introgressing genomic segments from the wild ancestor *Brassica villosa* [48]. These hybrid lines generate about three-fold higher levels of sulforaphane than conventional varieties. Commercial freezing processes and storage of HG broccoli maintains the high level of glucosinolates compared with standard cultivars, although the blanching process denatures the endogenous myrosinase activity [48]. Thus, blanching or cooking destroys myrosinase in fresh vegetables and generation of sulforaphane then depends on thioglucosidase activity of the intact gut microflora [49, 50]. However, this HG broccoli is commercially available only in the United States and the UK so far and is still not a commercially marketed form of broccoli over the world. Nevertheless, consumption of standard broccoli will still provide plenty of glucosinolate phytonutrients necessary for cancer prevention.

Broccoli sprouts may provide highest sulforaphane

Broccoli sprouts and seeds have gained increased scientific attention because they usually contain about 10–100 times more glucoraphanin, on a weight basis, than mature broccoli florets [32]. Subsequently, work with different broccoli seeds has stimulated commercial interest in broccoli sprouts as new food products [51]. Inexpensive open pollinated cultivars (e.g. Calabrese or DeCicco) with highly variable glucoraphanin content due to the genotypically very heterogeneous mixture were used for examination. An alternative source of inexpensive broccoli seed is derived from self-compatible inbreds with a double haploid genotype ensuring genetically homogenous seed and uniform sprouts with uniform glucoraphanin levels. The amount of glucoraphanin in these seeds is influenced significantly by environmental factors such as growth in a greenhouse or an outdoor cage environment. However, the main factor, which determines glucoraphanin levels in broccoli seed, remains the genotype [51]. Broccoli hybrids with high glucosinolate levels in the seeds have been produced, e.g. 'Pinnacle' or 'Marathon'-derived F2 hybrid [48, 51, 52]. However, the authors are not aware that seeds of these improved hybrids are commercially available. What are commercially available are fresh broccoli sprouts derived from such hybrids (e.g. BroccoSprouts®). Fresh broccoli sprouts may be consumed between day 2 and 12, as recent data demonstrate that the glucoraphanin content of broccoli sprouts grown between day 2 and 12 does not differ. Also, growth until day 8 and subsequent cold storage until the day 12 did not lower glucosinolate contents [53]. An alternative to consuming mature broccoli for high intake of sulforaphane may be intake of special freeze-dried broccoli sprouts or broccoli seed extract preparations. Such sprouts, seeds and derived extracts are available from several manufacturers, which promise standardized high sulforaphane levels. Among them are 'Sulforaphan Brokkoli Extrakt' (source: The Nutri Store, 50 mg sulforaphane/500 mg capsule), "Broccoli sprouts extract" (source: Supersmart, 10 mg sulforaphane/500 mg capsule), 'Broccoraphan®' freeze-dried broccoli sprouts (source: Deiters, 9 mg sulforaphane/500 mg sprouts), 'BroccoMax®' broccoli seed extract (source: Jarrow®, 9 mg sulforaphane/500 mg capsule), 'Brassica Nr. 2' freeze-dried sprout extract from a crossbreeding of broccoli×green cabbage×Brussels sprouts (source: Agrinova, 2.5 mg sulforaphane/500 mg capsule). The concentrations given here are taken from the analysis certificates of the manufacturers.

Key studies in patients using broccoli sprouts and derived extracts

Broccoli sprouts have been used in patient studies, which may give hints to the therapeutically active doses. One of these studies demonstrates inhibition of *Helicobacter pylori* after consumption of 56 g glucoraphanin-rich broccoli sprouts daily for a total of 1 week in three of nine gastritis patients [54]. More promising results were found in another small clinical trial with 48 patients, in which a significant reduction of the *H. pylori* infection after consumption of daily 70 g glucoraphanin-rich 3-day-old broccoli sprouts corresponding to 183 mg glucoraphanin [55]. Eight weeks after daily intake, it became evident

that patients from the test group had fewer markers for *H.pylori* in the breath and faeces test compared with the placebo group. Simultaneously, reduction of the gastritis was noticed, as concluded from the amount of detectable pepsinogen I and II [55]. An actual and ongoing pilot study performed at the University of Pittsburgh, USA, evaluates the effect of broccoli sprout extract to atypical nevi, which are precursor lesions and risk markers of melanoma (ClinicalTrials.gov identifier: NCT01568996). In this study, eighteen individuals in total receive oral broccoli sprout extract rich in sulforaphane in concentrations of 50 μM (22 mg), 100 μM (44 mg) or 200 μM (88 mg) capsules for 28 days. A different ongoing pilot study at the Roswell Park Cancer Institute, Buffalo, New York, USA, examines the effect of broccoli sprout extract in patients with transitional cell bladder cancer undergoing surgery (National Library of Medicine (NLM) identifier: NCT01108003). Two hundred micromoles per day (88 mg/day) is given for 14 days to patients destined to undergo definitive bladder resection for bladder cancer. Another ongoing clinical trial at the OHSU Knight Cancer Institute, Portland, Oregon, USA, evaluates the effect of broccoli sprout extract in patients with recurrent prostate cancer (ClinicalTrials.gov identifier: NCT01228084). A total of 200 μmol (88 mg) of sulforaphane is given daily via four 50 μmol capsules taken once daily during 20 weeks. Results from these latter studies are not available yet but are awaited with great interest.

Bioavailability of broccoli sprout extracts

One of the several challenges in design of clinical studies using broccoli sulforaphane is the selection of the dose, formulation, and dose schedule, whose optimum in humans are unknown so far. Therefore, a recent clinical trial compared the bioavailability and tolerability of sulforaphane from two broccoli-sprout-derived beverages: one glucoraphanin-rich (G-rich) and the other sulforaphane-rich (S-rich). Sulforaphane was generated either by the gut microflora of probands or formed by pre-treatment of the G-rich drink with myrosinase from daikon sprouts [56]. Fifty healthy participants randomized into two treatment arms participated and consumed either the G-rich or the S-rich drink for 7 days. Bioavailability was measured by urinary excretion of sulforaphane and its metabolites 12 h after dosing. Bioavailability of sulforaphane was substantially greater with the S-rich drink (70 %) than with the G-rich drink (5 %). However, clearance of glucoraphanin from the body was considerably slower with the G-rich drink, allowing for achievement of steady-state dosing as opposed to bolus dosing with the S-rich drink. The conclusion from this study is that optimal dosing formulations should consider blends of a mixture of sulforaphane and glucoraphanin.

Tips for preparing and consumption of broccoli

To reach the highest possible levels of glucoraphanin, florets and peeled stems should be cut into small pieces for quick and even cooking. For heating broccoli, the use of a steamer with a cooking time of 5 min is recommended [57]. Microwaving should be avoided since the bioactive ingredients of broccoli may be largely inactivated and the water-soluble glucosinolates are leached into the cooking water [58]. For that reason, the amount of cooking water should be as low as possible and may be used for preparation of a sauce or soup to save the glucosinolates. There is a study of broccoli stir frying that produced some fairly encouraging results with respect to nutrient retention in broccoli [59]. Results showed that during stir frying, phenolics and vitamin C were more affected than glucosinolates and minerals. Stir fry cooking with extra virgin olive, soybean, peanut, or safflower oil did not reduce the total glucosinolate content of the cooked broccoli compared with that of the uncooked sample. The stir frying took place for 3.5 min in a frying pan heated to 120–140 . Approximately two-thirds or more of the nutrients examined (including vitamins, minerals, phenols and glucosinolates) were retained after stir frying. When calculating the amount of sulforaphane derived from glucoraphanin-enriched cabbage, it should be considered that a maximum of 20 % of the glucoraphanin precursor is bioavailable and transferred to sulforaphane in the body [60–62]. Conversion of glucoraphanin can be increased by well chewing uncooked cruciferous vegetables to ensure release of myrosinase.

Side effects of broccoli florets and broccoli sprouts consumption

Almost everyone knows the flatulent effect of cabbage. Other known effects of cabbage to digestion are due to the two atoms of sulphur in glucosinolates [12]. During break down of cabbage by gut bacteria hydrogen sulphide is produced and is responsible for a ‘rotten egg’ odour. Additionally, sulforaphane acts as an indirect antioxidant. Antioxidant supplements may help protect normal cells from oxidative damage and reduce the short- and long-term harmful effects of cancer treatment [63]. However, concern has been raised that antioxidant supplements may also protect tumour cells during radiotherapy and chemotherapy, thereby compromising treatment efficacy [63, 64]. This has resulted in controversy over guidelines for the use of vitamin supplements during cancer treatment. We tested this issue in an experimental human pancreatic cancer xenograft model in mice. We found that sulforaphane increased the effect of gemcitabine, cisplatin, doxorubicin, 5-fluorouracil, sorafenib, TRAIL and quercetin for growth inhibition of tumour xenografts [39, 41, 42, 65]. These laboratory experiments are promising but cannot be directly transferred to the clinic. For a definite statement, we need the data of a patient study in which sulforaphane is admin-

istered together with chemotherapy. Regarding broccoli sprouts, it should be considered that the sprouts may be contaminated with dangerous gut bacteria or fungi because of the warm and humid germination. In addition to glucoraphanin, broccoli contains the goitrogenic glucosinolates glucobrassicin and progoitrin. However, cabbage does not alter thyroid function in humans unless massive amounts are consumed [66]. Notably, sprouts of many broccoli cultivars contain negligible quantities of glucobrassicin, which predominate in the mature vegetable [32].

Conclusions

It is increasingly clear that mustard oils and especially sulforaphane possess cancer preventive and therapeutic properties, of which the anti-CSC activity is of special interest. However, sulforaphane from crucifers is not the only naturally occurring plant substance with anti-CSC activity, as recent studies suggest that additional foods contain substances with anti-CSC activity. One is polyphenol quercetin, present in broccoli and many other fruits and vegetables, which eliminates pancreatic CSC characteristics and thereby enhances the effect of sulforaphane [65, 67]. Latest experimental data identified anti-CSC activities in legumes (genistein from soybeans), curcuma (curcumin, e.g. in curry), tomatoes (lycopene), grapes, berries, plums and peanuts (resveratrol, e.g. in grape seed oil, red wine), black pepper (piperin), green tea (EGCG), and fish, egg yolk and cod liver oil (vitamin D) [44]. Regarding vitamin D, it should be considered that more than 80 % of this hormone is produced by solar radiation on the skin [68]. The importance of sufficient vitamin D levels in the body has been underlined by a recent study, which clearly demonstrates that sufficient plasma levels of 25-hydroxyvitamin D >75 nmol/L were associated with a lower risk for pancreatic cancer among participants in five large prospective cohorts [69]. The actual available data reveal that a frequent intake of vegetables of the cruciferous family along with a colourful selection of many other fruits and vegetables combined with sufficient exposure to the sun may lower cancer risk and prolong the life of patients.

Acknowledgements
This manuscript was supported by grants from the German Cancer Aid (Deutsche Krebshilfe 109362), German Research Community (DFG HE 3186/11-1) and German-Israeli Foundation for Scientific Research and Development (GIF 1058-7.11/2008).

Conflict of interest
The authors declare that there is no conflict of interest.

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