Health Promoting Effects of Brassica-Derived Phytochemicals: From Chemopreventive and Anti-Inflammatory Activities to Epigenetic Regulation

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A high intake of brassica vegetables may be associated with a decreased chronic disease risk. Health promoting effects of Brassicaceae have been partly attributed to glucosinolates and in particular to their hydrolyzation products including isothiocyanates. In vitro and in vivo studies suggest a chemopreventive activity of isothiocyanates through the redox-sensitive transcription factor Nrf2. Furthermore, studies in cultured cells, in laboratory rodents, and also in humans support an anti-inflammatory effect of brassica-derived phytochemicals. However, the underlying mechanisms of how these compounds mediate their health promoting effects are yet not fully understood. Recent findings suggest that brassica-derived compounds are regulators of epigenetic mechanisms. It has been shown that isothiocyanates may inhibit histone deacetylase transferases and DNA-methyltransferases in cultured cells. Only a few papers have dealt with the effect of brassica-derived compounds on epigenetic mechanisms in laboratory animals, whereas data in humans are currently lacking. The present review aims to summarize the current knowledge regarding the biological activities of brassica-derived phytochemicals regarding chemopreventive, anti-inflammatory, and epigenetic pathways.

1. Introduction

Epidemiological studies link a high intake of brassica vegetables with a lower incidence for different kinds of cancers [1–3]. Health promoting effects of brassica vegetables have been attributed to glucosinolates, sulfur containing compounds almost exclusively present in plants of the family Brassicaceae. However, these chemopreventive effects are not mediated by glucosinolates per se but mainly through isothiocyanates, one of the major hydrolysis products resulting from myrosinase cleavage [4–9]. Myrosinase is a thioglucosidohydrolase located apart from glucosinolates in so-called myrosin cells. Upon plant cell disruption enzyme and glucosinolate get in contact and hydrolyzation is initiated. Based on reaction conditions (e.g., pH, temperature) either isothiocyanates, thiocyanates, or nitriles are formed (Figure 1) [10, 11].

Several in vitro and in vivo studies suggest that brassica derived phytochemicals may counteract inflammatory pathways and exhibit chemopreventive activity. Furthermore, impact of glucosinolates and/or their corresponding hydrolyzation products on epigenetic mechanisms including DNA-methylation, histone modification, and microRNAs has been recently described and is in the focus of the present paper. Chemical structures of selected brassica-derived phytochemicals are presented in Figure 2.

2. Brassica-Derived Phytochemicals Target Inflammatory Pathways

Several studies suggest anti-inflammatory properties of brassica-derived phytochemicals [12]. Besides others, these beneficial effects may be mediated through an induction of
Figure 1: Myrosinase-mediated hydrolysis of glucosinolates with main break-down products (modified according to [114]).

Figure 2: Chemical structures of selected aliphatic and aromatic brassica-derived phytochemicals.
antioxidant and phase I/2 genes and the inhibition of proinflammatory signaling pathways via regulation of various transcription factors which may be further controlled by epigenetic modifications and miRNAs [11, 13–15]. Additionally, it has been shown that brassica derived phytochemicals exhibit anti-infective and antiviral activity (e.g. inhibiting Helicobacter pylori) [16–18]. In this context the transcription factor nuclear factor kappa B (NFkB) is a central player in inflammatory processes. In general, NFkB resides inactively in the cytosol as a heterodimer consisting of two subunits, for example, p50 and p65, bound to its inhibitory protein IκBα [19]. NFkB can be activated by a wide variety of proinflammatory stimuli including cytokins and reactive oxygen species resulting in the activation of upstream kinases, phosphorylation, ubiquitination, and consequently the degradation of IκBα [20]. Once p50 and p65 have been released, they translocate to the nucleus and bind to the κB site located in the promoter regions of the DNA of target genes thereby driving gene expression [21, 22]. NFkB target genes include, for example, cyclooxygenase 2 (COX-2), inducible nitric oxide synthase (iNOS) (inflammatory function), Bcl-X, Bcl-2, Bcl-3 (anti-apoptotic function), MYC (cell division), matrix metalloproteinase (MMP), and vascular endothelial growth factor (VEGF) (angiogenesis) [23–25]. Due to NFkB central role in inflammation the transcription factor seems to be an attractive target to treat inflammation related diseases.

A variety of naturally occurring NFκB inhibitors have been described including brassica derived phytochemicals like sulforaphane (SFN), phenethyl-isothiocyanate (PEITC), 8-methylsulphinoctyl isothiocyanate (MSO), and indole-like sulforaphane (SFN), phenethyl-isothiocyanate (PEITC), [50]. Once p50 and p65 have been released, they translocate to the nucleus and bind to the κB site located in the promoter regions of the DNA of target genes thereby driving gene expression [21, 22]. NFκB target genes include, for example, cyclooxygenase 2 (COX-2), inducible nitric oxide synthase (iNOS) (inflammatory function), Bcl-X, Bcl-2, Bcl-3 (anti-apoptotic function), MYC (cell division), matrix metalloproteinase (MMP), and vascular endothelial growth factor (VEGF) (angiogenesis) [23–25]. Due to NFkB central role in inflammation the transcription factor seems to be an attractive target to treat inflammation related diseases.

3. Brassica-Derived Phytochemicals Target Chemopreventive Pathways

Nrf2 is a transcription factor playing a crucial role in regulating inflammation and chemoprevention. Under basal conditions Nrf2 is bound to its cytosolic inhibitor, the Kelch like ECH-associated protein 1 (Keap1) [40]. In the presence of activating agents including isothiocyanates and other electrophiles Nrf2 may be activated through two distinct cellular signaling pathways resulting in the liberation of NFκB from its inhibitor Keap1. Nrf2 can either be phosphorylated through an activation of upstream protein kinases which causes the destruction of the Nrf2-Keap1 complex or in the presence of pro-oxidants the cytochrome thiols of Keap1 may be modified which promotes dissociation of Nrf2 from Keap1. Liberated Nrf2 translocates to the nucleus where it binds together with several cofactors including small Maf proteins (MafE, MafG, and MafK), c-Jun, and CAMP response element-binding (CREB) protein (CBP) to the antioxidant response elements (AREs) in the promoter regions of genes encoding antioxidant and detoxifying phase II enzymes like NADPH quinone oxidoreductase 1 (NQO1), NADH manganese superoxide dismutase (SOD), glutamyl cysteine ligase (GCL), and GST [20, 41]. Khor et al. [2006] have reported that Nrf2-deficient mice suffer under a more severe dextran-sulfate-sodium- (DSS-)induced colitis than Nrf2+ mice which was accompanied by a decreased expression of antioxidant and phase II enzymes and an increased level of proinflammatory mediators like COX-2, iNOS, interleukin 1β (IL-1β), and tumor necrosis factor α (TNFα) [42]. Different phytochemicals can lead to nuclear accumulation of Nrf2 [43, 44]. In particular, the effect of SFN on Nrf2 pathways has been intensively investigated, showing that SFN in vitro and in animal studies successfully activates phase 2 and antioxidant enzymes via induction of Nrf2 [45–48]. Also other ITCs such as allyl-isothiocyanate (AITC), butyl-isothiocyanate (BITC), and PEITC have been shown to exhibit similar Nrf2 inducing activity in vitro [48, 49]. PEITC has been proven to be an even stronger inducer of Nrf2-ARE mediated signaling pathways than SFN [50].

There is evidence that overexpression of Nrf2 can modulate NFκB expression, since NFκB competes with Nrf2 for binding to the transcriptional coactivator CREB-binding protein [51, 52]. Accordingly, Surh and Na described a cross-talk between NFκB and Nrf2 signaling supported by the fact that most of the phytochemicals exhibit both, anti-inflammatory and anti-oxidant properties [20].
4. Brassica Derived Phytochemicals Target Epigenetic Pathways

Epigenetics describe a heritable change in gene expression that is not mediated through a change in DNA-sequence [53]. Epigenetic changes comprise DNA-methylation, histone modifications, and microRNA expressions [53–55]. Several studies suggest that brassica derived phytochemicals affect epigenetic mechanisms. Epigenetic aberrations take place in the early stages of carcinogenesis and partly represent an initiating process of cancer development. Phytochemicals may intervene in this process and incarnate potential targets for cancer prevention [56]. Posttranslational modifications at the N-terminal tail of histones are one epigenetic mechanism that plays a role in gene regulation and carcinogenesis [56, 57]. Histones can be post-translationally modified by acetylation, deacetylation, phosphorylation, ubiquitinylation, sumoylation, and ADP ribosylation [58, 59]. Biotinylation has also been suggested to mediate histone modification. However, recent studies conducted by Li and coworkers as well as by Xue and colleagues discovered that it is not a biotinylation per se but an assembly of different proteins including biotin ligase and holocarboxylase synthetase interceding histone modification [60, 61]. Histone acetylation and deacetylation are the most analysed modifications mediated through a coaction of histone acetyl transferases (HAT) and histone deacetylases (HDAC) [56] resulting in gene activation and inhibition of gene activity, respectively [57, 62]. HATs transfer acetyl groups from acetyl-CoA onto lysine residues at the histone [63] while HDACs detach histone acetyl group transferring them onto CoA [56]. Chromatin acetylation by HATs opens the chromatin structure providing a possibility for transcription factors to approach the DNA which may lead to gene activation [57]. HATs are divided into four families on the basis of their structure homologues. At present, however, there is no literature data available presenting effects of brassica derived phytochemicals on HAT activity. Several studies suggest an effect of these phytochemicals on HAT activity.

DNA methylation is regulated by various DNA-methyltransferases (DNMT 1, 3a, 3b) that transfer methyl groups from the methyl precursor S-adenosyl-L-methionine (SAM) to the C5-position of certain cytosines and influence gene transcription [81, 82]. Gene promoter regions rich in CpG sequences—are generally unmethylated in normal cells [56], whereas transformed cells often show hypermethylated promoters and/or genome wide hypomethylation [57]. Predicted CpG islands in the promoters of Nrf2 and Keap1 are shown in Figure 3. Besides carcinogenesis changes in DNA methylation patterns are also associated with ageing and the development of chronic degenerative diseases [56, 83]. Currently only little information regarding DNMT inhibition by ITCs is available in the literature. The main investigated ITC regarding its DNMT inhibiting activity is SFN. Studies conducted by Meeran and colleagues [84] in the human breast cancer cell lines, MCF-7 and MDA-MB-231, revealed an inhibiting effect of SFN on DNMT1 and DNMT3a. SFN has been reported to inhibit DNMT-expression in the human colorectal cancer cell line CaCo-2 [85], in LnCaP human prostate cancer cells [86], and in porcine satellite cells [87]. Furthermore, effects of the naturally occurring ITC iberin [85] and the synthetic ITC PHI on DNMT inhibition in colorectal cancer cells and in the acute lymphoid leukemia cell line Molt-4 [88] have been described. However, in vivo data for DNMT inhibition by brassica derived phytochemicals is lacking and needs further investigation.

MicroRNAs (miRNA) represent a class of evolutionary conserved small noncoding RNAs with a length of ~22 nucleotides that control gene expression at the posttranscriptional level [89–91]. MicroRNAs bind to the 3' untranslated region (3' UTR) of target mRNAs and lead, depending on
Figure 4: Predicted binding sites of conserved microRNAs of Nrf2 (a) and Keap1 (b) as identified by http://www.microrna.org/microrna/home.do.
Figure 5: MicroRNAs being involved in the regulation of Nrf2 and Keap1.

The expression of Nrf2 regulating microRNAs is modulated by several environmental insults including diesel exhaust [112] and the mycotoxin ochratoxin A [108]. Interestingly, a study conducted by Singh and coworkers suggests that Nrf2 itself targets microRNAs. The authors observed an increase of miR-1 and miR-206 levels while Nrf2 is simultaneously upregulated [113]. Figure 5 gives an overview of how microRNAs are involved in Nrf2/Keap1 signaling. Table 1 lists confirmed microRNAs that target the promoter region of Nrf2 and Keap1.
5. Summary and Conclusion

ITCs are one of the major degradation products of glucosinolates derived from brassica vegetables and are partly held to be responsible for the health-promoting effects observed in populations with a high consumption of Brassicaceae. The health-promoting effects of a diet rich in Brassica vegetables have been known for several decades and seem to be a promising starting point for, for example, the development of chemopreventive and anti-inflammatory functional foods, dietary supplements, and drugs. Figure 6 gives an overview of how brassica-derived phytochemicals may mediate their health-promoting activity. An increasing number of studies deal with ITC effects on the chemopreventive transcription factor Nrf2 and its corresponding target genes including antioxidant and phase 2 enzymes. In addition to studies in cultured cells beneficial effects of ITC have also been confirmed in vivo in laboratory rodents and humans. Also the anti-inflammatory effects of ITCs are well documented in both cell culture and in vivo studies. The applied concentrations of brassica derived phytochemicals range between 0.25 and 100 µmol/L in vitro studies and up to 90 mg/kg BW in mouse studies. However, with regard to ITC effects on epigenetic mechanisms including histone modifications, DNA methylation, and microRNAs only a limited number of studies are currently available. In this context several studies have been performed in cultured cells and have revealed an increase in epigenetic targets connected with an inhibition of HDAC and DNMT as well as on microRNA expression. However, data concerning the modulation of epigenetic targets by ITCs especially in humans are, by and large, lacking even though epigenetic targets seem to play an
essential role in the development of different kinds of cancer and may depict a promising target to prevent and/or treat chronic diseases.

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