

Review

Potential health benefits of sulforaphane: A review of the experimental, clinical and epidemiological evidences and underlying mechanisms

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Extensive epidemiological evidence and animal experimental studies suggest that cruciferous vegetables may prevent or delay various inflammatory disorders, including cancers. Much of this chemopreventive effect has been attributed to the physiological effect of the isothiocyanates, especially sulforaphane (SF). Sulforaphane has been proven as a potent protector against oxidative damage and carcinogens. A plethora of clinical effects are reported in various experimental diseases as well as human clinical studies. This review summarizes the present knowledge about the health effects of sulforaphane with possible underlying mechanisms for these effects based on the reported *in vitro* and *in vivo* studies. These studies suggest that SF has the potential to reduce risk of various types of cancers, diabetes, atherosclerosis, respiratory diseases, neurodegenerative disorders, ocular disorders, and cardiovascular diseases. Traditionally, Nrf2-mediated induction of phase 2 detoxification enzymes has been recognized as the major mechanism by which SF protects cells. However, several recent studies have reported multiple other mechanisms involved in response to SF, including inhibition of cytochrome P450 enzymes, induction of apoptosis and cell cycle arrest, and anti-inflammatory effect. It is suggested that these mechanisms work synergistically to provide the observed health effects of sulforaphane.

Key words: Sulforaphane, CYP enzymes, phase 2 enzymes, cancer, herbal remedies.

INTRODUCTION

Herbal remedies have been used for the treatment of diseases for more than 2000 years and their use is being increased. There is a growing interest in the use of herbal medicine as part of the complementary and alternative medicine (CAM). In the United States, approximately 40% of adults and approximately 12% of children are using some form of CAM (Jung et al., 2002). Public interest in the use of herbal remedies, even though their safety and efficacy have not been established on a rigorous scientific basis, may be attributed to the high cost, side effects, and therapeutic limitations of

conventional medicine as well as availability of these herbal supplements as over-the-counter agents.

Several epidemiological studies have shown that consumption of large quantities of fruits and vegetables, especially cruciferous vegetables (e.g. Broccoli and Brussels sprouts), can protect against carcinogenesis, mutagenesis, drug toxicities, and other chronic diseases (Conaway et al., 2002; Shapiro et al., 2001). Cruciferous vegetables are a rich source of glucosinolates, which, upon chewing, are enzymatically hydrolyzed by the plant-specific myrosinase (β -thioglucoside N-hydroxysulfates; EC 3.2.3.1) and release isothiocyanates (Fahey et al., 1997, 2001; Shapiro et al., 2001). The un-hydrolyzed glucosinolate can be degraded by thioglucosidase activity of the flora present in the human gut, but are not likely to get absorbed into systemic circulation (Vermeulen et al.,

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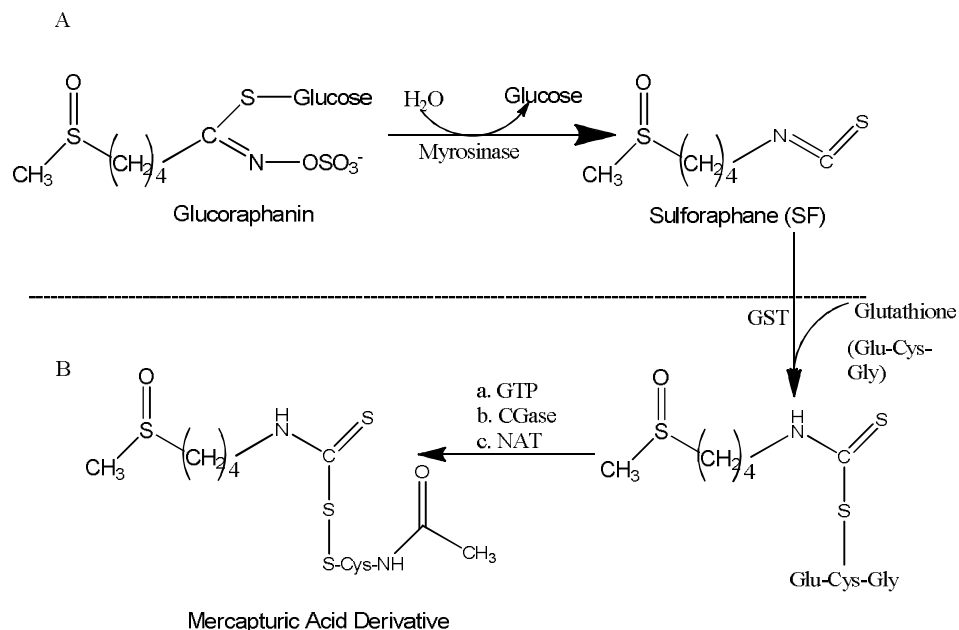


Figure 1. (A) Hydrolysis of glucoraphanin to sulforaphane by myrosinase, and (B) Metabolism of sulforaphane and formation of mercapturic acid derivative.

2008). The most abundant glucosinolate in broccoli is glucoraphanin, which upon hydrolysis by myrosinase or intestinal flora yields the isothiocyanates, sulforaphane (R-1-isothiocyanato-4-methylsulfinyl butane, SF), as shown in Figure 1A. After absorption, sulforaphane (SF) undergoes conjugation to glutathione, a reaction catalyzed by Glutathione-S-Transferases (GST). Subsequently, step-wise cleavage of glutamine and glycine by the enzymes γ -glutamyl transpeptidase (GTP) and cysteinylglycylase (CGase) respectively yields L-cysteine conjugate. This latter conjugate is then acetylated by the enzyme N-acetyltransferase (NAT) to produce N-acetyl-L-cysteine conjugate (mercapturic acid derivative) and subsequently excreted into urine, as shown in Figure 1B. Mercapturic acid derivative present in urine reflect the uptake of SF and the intake of glucoraphanin from cruciferous vegetables (Fahey et al., 1997). Higher amount of SF conjugates in blood and SF-derived mercapturic acid in urine were found when broccoli was eaten raw (bioavailability 37%) versus cooked (bioavailability 3.4%)(Vermeulen et al., 2008). It has been reported that metabolism of SF is significantly affected by the GSTM1 genotype (Gasper et al., 2005). After 24 h of SF consumption, while GSTM1 null individuals excreted almost 100% of the ingested SF, GSTM1-positive individuals excreted only about 60% of ingested SF (Gasper et al., 2005). These observations may indicate that GSTs play an important role in the disposition of SF in humans. Contrary to this hypothesis, a feeding study using a single serving of broccoli (2.5 g broccoli/kg body weight) reported that urinary

concentration of isothiocyanates did not differ by GSTM1, GSTP1, and GSTA1 genotypes (Steck et al., 2007).

Therapeutic effect of sulforaphane in acute and chronic diseases

Sulforaphane is the most extensively studied isothiocyanates, and most studies attribute the health benefits of cruciferous vegetables to their high content of SF (Fahey et al., 1997; Matusheski et al., 2001). Several studies have been conducted, particularly in the past two decade, on the health effect of SF or SF-rich diet on various body systems both in vitro and in vivo. These health benefits may be attributable to a variety of potential mechanisms. This review will attempt to look at specific diseases and conditions and how SF may affect them. The reported health effects of SF, together with proposed underlying mechanisms are shown in Table 1.

Sulforaphane and cancer

Cancer is a complex multi-factorial, multi-step disorder, that manifests itself in uncontrolled cellular reproduction, tissue invasion, and distant metastasis(Jung and Pezzuto, 2002; Patel et al., 2007). Carcinogenesis process can be broadly divided into two principal steps, initiation and promotion/progression. The initiation of carcinogenesis involves the induction of an altered but non-neoplastic cell which is capable of participating in the

Table 1. Reported health benefits of SF and proposed underlying mechanisms.

Disease condition	Proposed mechanism of SF effect
Cancers	Modulation of DME, induction of apoptosis, cell cycle arrest
Respiratory disorders oxidant-induced respiratory diseases	Upregulation of GST and QR in the airway epithelial cells
Neurodegenerative diseases Alzheimer's disease cerebral ischemia	Inhibition of acetyl cholinesterase upregulation of antioxidant gene expression
Kidney diseases, renal fibrosis and ROS-mediated kidney damage	Nrf-2 mediated induction of phase 2 enzymes
Diabetes Diabetic micro- and macro-vascular disorders	Reduction of oxidative stress and restoration of endothelial function
Ocular diseases Retinitis Pigmentosa, Age-related macular degeneration	Upregulation of retinal Trx, TrxR, and Nrf-2 Upregulation of retinal GSH and QR
Cardiovascular disorders hypertension and atherosclerosis	Nrf-2 mediated reduction in the pro-inflammatory state
Gastrointestinal disorders	Nrf-2 dependent eradication of <i>H. pylori</i>

DME: drug metabolizing enzymes, GST: glutathione-S-transferases, QR: quinone reductase, ROS: reactive oxygen species, Nrf-2: Nuclear factor erythroid-derived 2, Trx: Thioredoxin, TrxR: thioredoxin reductase, GSH: glutathione.

neoplastic process. Promotion/progression includes focal proliferation of the initiated cells and transformation into neoplasia (Patel et al., 2007). Despite the advances in early detection and treatment, cancer is still the second leading cause of death in USA with no significant reduction in the death rates from cancer since the early 1970s (Parker et al., 1997). Therefore, research in the last 20 years has been focusing on cancer prevention. Cancer chemoprevention, identified as the use of certain drugs or substances to keep cancer from forming, growing, or coming back, may be achieved through inhibiting these stages of carcinogenesis and may provide a promising approach to reduce cancer burden.

Large number of epidemiological studies have reported association between the consumption of SF-rich vegetables and reduction in cancer risk at several sites including the lung (Spitz et al., 2000), bladder (Zhao et al., 2007), breast (Ambrosone et al., 2004), prostate (Joseph et al., 2004), non-Hodgkin's lymphoma (Zhang et al., 2000), and colorectal (Lin et al., 1998; Seow et al., 2002). Such correlation between consumption of SF-rich diet and reduction of cancer risk was found dependent on the number of servings per day/week. For example, consumption of three or more servings of cruciferous vegetables per week was inversely related to risk of prostate cancer, relative to less than one serving per week especially in more advanced case (Cohen et al.,

2000). Similarly, risk of bladder cancer in males was inversely associated with intake of SF-rich vegetables only when more than five servings per week were consumed (Michaud et al., 1999).

In addition, a number of experimental animal studies have shown strong chemopreventive effects of SF. The administration of SF blocked the formation of mammary tumors in Sprague-Dawley rats treated with the carcinogen 9,10-dimethyl-1,2-benzanthracene (Zhang et al., 1994), reduced the benzo[a]pyrene-evoked forestomach tumors in mice (Fahey et al., 2002a), and resulted in more than 50% reduction in the growth of PC-3 human prostate cancer xenografts in nude mice (Singh et al., 2004). Dietary intake of SF (1.5 and 3 mmole/kg diet) to A/J mice resulted in more than 90% inhibition of the malignant progression of lung adenomas induced by the tobacco carcinogen nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK, nicotine-derived nitrosamine ketone) (Conaway et al., 2005). At a dose of 4.5 mmol/kg, SF inhibited the development of pancreatic tumors in male Syrian hamsters treated with the pancreatic carcinogen, N-nitroso-bis(2-oxopropyl)amine (Kuroiwa et al., 2006). Topical application of 100 nmol of sulforaphane once a day for 14 days prior to 7,12-dimethylbenz(a)anthracene applications decreased the incidence of skin tumor in mice (Xu et al., 2006).

Collectively, the reported human epidemiological studies and animal experiments indicate that SF may inhibit the development of different types of cancers during initiation and/or promotion stages (Juge et al., 2007). Several mechanisms have been proposed for the chemopreventive effect of SF. Although early research focused on the "the blocking activity" of SF through induction of phase 2 detoxification proteins, recent studies have recognized several other mechanisms of chemoprevention by SF. These mechanisms include inhibition of phase 1 enzymes involved in activation of procarcinogens, induction of apoptosis, induction of cell cycle arrest, and anti-inflammatory effect (Juge and Traka, 2007). Most likely, these factors interact together to reduce the risk of carcinogenesis.

Modulation of drug metabolizing enzymes (DME)

Drug metabolism generally plays a key role in the tumor initiation process. Conventionally, drug metabolism takes place by two consecutive processes, phase 1 and phase 2 (Woolf and Jordan, 1987; Woolf, 1999). Phase 1 reactions are principally mediated by cytochrome P450 (CYP) enzymes that are mainly expressed in the liver and catalyze oxidation, reduction and hydrolysis of the drug to generate more polar and rapidly eliminated metabolites (Shimada et al., 1994). Human CYP isoforms that are involved in drug metabolism include CYP1A1/2, CYP2B6, CYP2C8/9/19, CYP2D6, CYP2E1, CYP3A4/5 and CYP4A (Shimada et al., 1994; Elbarbry et al., 2007). Although CYP enzymes normally generate metabolites with diminished biological activity, there are numerous examples where these enzymes mediate the formation of reactive intermediates from chemically inert agents (Yamada et al., 2006). For example, CYP2E1 catalyzes the bioactivation of important environmental chemicals, bioactivates a selected number of drugs (that is acetaminophen (Elbarbry et al., 2007)), industrial solvents (that is carbon tetrachloride) (Elbarbry et al., 2007) and environmental pro-carcinogens (that is nitrosamines (Lin et al., 2001)) to highly reactive intermediates. These reactive metabolites can form adducts with endogenous biomolecules such as DNA, RNA, and proteins, and play a key step in initiating cellular damage and cancers. Therefore, modulation of the CYP activity to decrease the activation of procarcinogens could be a plausible target for chemoprevention through preventing cancer initiation (Patel et al., 2007).

SF was shown to inhibit the activity of CYP1A1 and CYP1A2 in human hepatoma cell line HepG2 (Skupinska et al., 2009a; Skupinska et al., 2009b) and rat hepatocytes (Maheo et al., 1997). The key step in the carcinogenesis of polycyclic aromatic hydrocarbons (PAH) is their biotransformation to the oxyderivatives by CYP1A1 and CYP1A2. Therefore, inhibition of these CYP

enzymes by SF may explain its chemopreventive effect in rats against the carcinogen, aflatoxin B1 (Maheo et al., 1997). Using p-nitrophenol hydroxylase assay, SF was shown to be a potent competitive inhibitor for CYP2E1 enzyme in liver microsomes of acetone-treated rats (Barcelo et al., 1996). In the view of this result, the genotoxicity of N-nitrosodimethylamine (NDMA), a procarcinogen that requires bioactivation by CYP2E1 to cause DNA damage, was inhibited by SF in a dose-dependent manner (Barcelo et al., 1996). On the other hand, SF was unable to inhibit the carcinogenesis of sodium azide, a carcinogen that does not require metabolic activation (Barcelo et al., 1996). Together, these findings suggest that SF may provide protection against carcinogens which are substrates for CYP2E1. Treatment of rats with SF at dietary levels led to a decline in CYP2B activity, as measured by pentoxeresorufin dealkylation, and at higher levels led to a decline in CYP3A2 activity, as measured by erythromycin N-demethylation (Yoxall et al., 2005). Although, the mechanism by which SF inhibits CYP activity has not been extensively studied, it has been reported that isothiocyanates impair CYP activity by acting as competitive inhibitors as well as mechanism-based inhibitors (Goosen et al., 2001; Nakajima et al., 2001). In one study, preincubation of SF in the presence of NADPH resulted in more effective inhibition of the CYP1A isoforms, and may indicate that SF is a mechanism-based inactivator for the CYP1A subfamily (Yoxall et al., 2005).

Phase 2 reactions mediate the conjugation of exogenous and endogenous compounds as well as reactive metabolites produced during phase 1 metabolism with molecules such as uridine 5'diphosphate-glucouronic acid, glutathione or sulfate to produce less toxic and readily eliminated metabolites (Woolf, 1999). Recently, this traditional recognition of phase 2 process has extended to include proteins that catalyze reactions that lead to comprehensive cytoprotection against electrophiles and reactive oxygen species (Talalay, 2000). Selected examples of these phase 2 proteins are NADPH: quinone oxidoreductase (QR), glutathione S-Transferases (GST), UDP-glucuronosyltransferases (UGT), glutathione reductase (GR), and heme oxygenase-1 (HO-1). Knockout of one or more of these phase 2 proteins in animals led to a significant increase in carcinogen-induced and spontaneous tumorigenesis (Long et al., 2000, 2002, 2001; Henderson et al., 1998). Therefore modulation of this cellular metabolism by inducing phase 2 enzymes could block or reduce the process of cancer initiation. Research done using *in vitro* and *in vivo* experiments over the past decade have documented that SF is the most potent naturally occurring phase 2 inducer in both animals and human (Fahey et al., 1997; Talalay, 2000).

Using human prostate cancer lines, SF was found to potently induce the activity of QR, GST- α , γ -glutamylcysteine synthetase, and increased the

intracellular glutathione synthesis (Brooks et al., 2001). Such findings may explain the observed association between high consumption of SF-rich diets and lower risk of prostate cancer, the second leading cause of male cancer death. However, additional work is necessary to elucidate the mechanism of phase 2 induction in human prostate by SF. Using human hepatoma HepG2 cells and colorectal adenocarcinoma HT29 cells, sulforaphane and its glutathione conjugate induced significantly both UGT1A1 and GSTA1 mRNA and protein levels (Basten et al., 2002). Similarly, potent induction of GST and QR was observed in rat bladder carcinoma cells treated with SF for 24 h (Zhang et al., 2006). Both SF and its N-acetylcysteine conjugate caused dose-related induction of QR activity and inhibition of cell growth in murine hepatoma cells (Hwang et al., 2005). Induction of phase 2 proteins by SF is not limited only to carcinogenic cells, but also was reported in normal and non-transformed cells. For example, in primary rat and human hepatocytes; SF induced expression of glutathione S-transferase A1/2 isoforms and QR in a dose-response and time-course manner (Maheo et al., 1997; Payen et al., 2001).

Similarly, several *in vivo* studies have shown the effectiveness of SF in inducing the activity of phase 2 proteins reviewed in (Juge and Traka, 2007). Exposure of male Wistar albino rats to SF in drinking water for 10 days, equivalent to doses of 3 and 12 mg/kg, resulted in a dose-dependent induction of the QR activity, but failed to induce GST and UGT activities (Yoxall et al., 2005). Feeding rats for 14 weeks with 200 mg/day of dried broccoli sprouts that contained glucoraphanin, the precursor for sulforaphane, significantly increased glutathione (GSH) content and decreased oxidized GSH, decreased protein nitrosylation, as well as increased GSH reductase and GSH peroxidase activities in cardiovascular and kidney tissues (Wu et al., 2004). In the same study, animals fed sprouts in which most of the glucoraphanin was destroyed did not show any significant upregulation of any of the phase 2 proteins (Wu et al., 2004). When female Sprague-Dawley rats were dosed 40 μ mol/kg/day SF, increased GST and QR activities were observed in the duodenum, fore stomach, and/or urinary bladder, with the greatest effects being seen in the urinary bladder (Munday et al., 2004). Similar studies using higher doses of SF reported also induction of GST, in addition to QR in different animal tissue (Zhang et al., 1992; Matusheski and Jeffery, 2001). Topical application of SF-containing broccoli sprout extracts induced phase 2 response in humans as indicated by a significant elevation in the activity of QR in skin of healthy volunteers (nkova-Kostova et al., 2007). In the same study, topical application of 100 nmol SF/cm² increased the protein levels of QR, GSTA1, and HO-1 in mouse epidermis (nkova-Kostova et al., 2007). These observations could provide a devising strategy for protection against development of skin carcinogenesis,

particularly in populations with higher risk of skin cancers, e.g. solid organ transplant recipients. Finally, consumption of brussels sprouts rich in SF resulted in elevated alpha-class GST levels in human plasma, which may reflect GST-alpha induction in tissues like liver and small intestine (Bogaards et al., 1994).

The gene expression of phase 2 proteins is regulated by three cellular components; Kelch-like ECH-associated protein 1 (Keap 1); Nuclear factor (erythroid-derived 2)-like 2 (Nrf2); and the antioxidant response element (ARE). Under normal conditions, and in absence of SF and other inducers, Nrf2 is sequestered in cytoplasm by Keap 1 and is subject for ubiquitination and proteasomal degradation. Upon exposure to SF, it targets and chemically modifies specific and highly reactive cysteine thiols of Keap 1 resulting in conformational changes and enables dissociation of Nrf2 from Keap 1, and Nrf2 stabilization. Nrf2 undergoes nuclear translocation and binds to ARE and activates the transcription of phase 2 genes (Yu et al., 1999; Keum et al., 2006), reviewed in (Juge and Traka, 2007; nkova-Kostova et al., 2008; Myzak et al., 2006). Several studies have provided compelling evidences for the Nrf2-dependent cytoprotective effect of SF. Although SF blocked the benzo[a]pyrene-evoked forestomach tumors in mice (Fahey et al., 2002), this effect was abrogated in mice lacking the Nrf2 gene (Fahey et al., 2002). In a similar study, topical application of 100 nmol of sulforaphane once a day for 14 days prior to 7,12-dimethylbenz(a)anthracene applications decreased the incidence of skin tumor in the Nrf2(+/+) mice, but no chemoprotective effect was elicited by sulforaphane pretreatment in the Nrf2(-/-) mice group (Xu et al., 2006). Taken together, these results show that the ability of sulforaphane to induce the expression of phase 2 proteins is mediated, at least in part, through Nrf2 as shown in Figure 2B. Collectively, the chemopreventive effect of SF could be attributed to its effect on the balance between procarcinogen activation (by CYP enzymes) and carcinogen detoxification (by phase 2). The aforementioned *in vitro* and *in vivo* studies may indicate that protection against carcinogenesis by SF is attributed to its ability to inhibit the activity of CYP enzymes involved in procarcinogen bioactivation and/or its ability to enhance the capacity for detoxification of electrophiles and oxidants, Figure 2A.

INDUCTION OF APOPTOSIS

Apoptosis is the process of programmed cell death that is a highly regulated and may occur in multi-cellular organisms under several physiological and pathological conditions. Apoptosis is characterized by cell shrinkage, membrane blebbing, chromatin condensation, and DNA fragmentation. Apoptosis is important in eliminating cells under stress or genetically damaged cells, and therefore

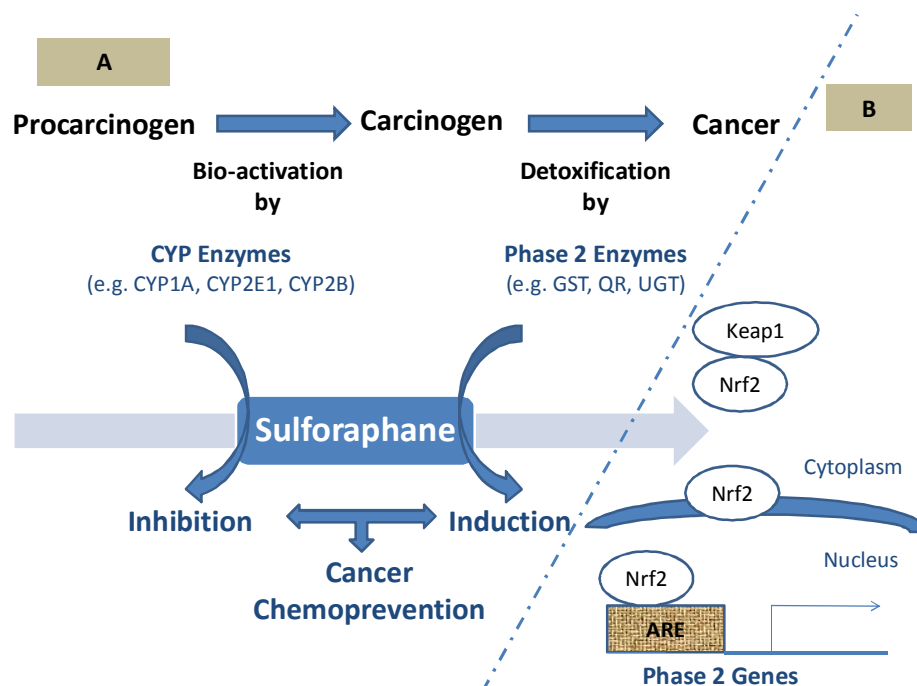


Figure 2. Cancer chemoprevention by sulforaphane via modulation of drug metabolizing enzymes (DME). A: SF interferes with cancer initiation through inhibition of CYP enzymes that are involved in bioactivation of procarcinogens and/or induction of phase 2 detoxification proteins. B: Proposed mechanism for the induction of phase 2 enzymes by SF. SF causes Nrf2 to dissociate of from its inhibitor Keap1 and translocates to the nucleus where it binds to ARE and activates phase 2 genes.

helps in cell protection and maintenance of homeostasis. Inappropriate regulation of apoptosis has been implicated in an extensive variety of diseases, such as ischemic damage, autoimmune diseases and cancers. On the other hand, induction of apoptosis may represent a protective mechanism by which several herbal remedies, including SF, can inhibit these cellular disorders.

Induction of apoptosis is hypothesized to be through intracellular activation of a family of cysteine proteases, named caspases, responsible for initiation and execution of apoptosis. Induction of apoptosis can also be mediated through caspases-independent pathways such as release of the mitochondrial protein apoptosis inducing factor (AIF) into cytosol, activation of a family of Ca^{+2} -activated cytosolic proteases called calpains (Juge et al., 2007), or modulating the activation of transcription factors such as NF- κ B and AP1 family members which are involved in induction of cell survival genes (Mi et al., 2007). The features of apoptosis have been demonstrated in several in vitro studies using SF-treated cancer cell lines from bladder (Shan et al., 2006), brain (Jiang et al., 2010), breast (Telang et al., 2009), colon (Gamet-Payraastre et al., 2000), lung (Mi et al., 2007, 2008), ovary (Bryant et al., 2010), and prostate (Shankar et al., 2008; Chiao et al., 2002). Using a transgenic mouse model of prostate cancer, TRAMP, the viability of TRAMP-C1 cells were

significantly decreased in correlation with apoptosis induction after a 24 h exposure to SF in a dose-dependent manner, as revealed by dose-dependent increase in histone-associated DNA fragmentation (Singh et al., 2009). Such ability of SF to induce apoptosis in various cancerous cells makes it a potential candidate against secondary and possibly recurrent cancers. Several mechanisms, including both caspases-dependent and independent, have been proposed to explain the induction of apoptosis by SF and have been reviewed (Juge et al., 2007) and (Myzak and Dashwood, 2006).

INDUCTION OF CELL CYCLE ARREST

The progression of cell cycle through the four stages, G1, S, G2 and M, is highly regulated by a number of checkpoint mechanisms that are essential cytoprotective responses to stress including DNA damage and abnormal mitogenic signals. Cyclins, cyclin-dependent kinases (CDKs), and CDK inhibitors (CDKIs) are important factors that regulate the cell cycle progression. A normal cell growth is maintained through a balance between the cyclin/CDK complexes that promote cell growth and CDKIs that promote cell cycle arrest. There is

accumulating evidence that SF can arrest cell cycle at different stages of its progressions, a mechanism by which it can inhibit the proliferation of cancer cells and exert its anti-carcinogenic effect. Block of Go/G1, G2/M, and S phase by SF treatment has been documented in breast, bladder, colon, prostate, and T cells depending on the cell type, with several proposed mechanisms (Singh et al., 2009; Shan et al., 2006; Telang et al., 2009; Bryant et al., 2010; Shankar et al., 2008; Mi et al., 2007; Jiang et al., 2010; Mi et al., 2008; Gamet-Payraastre et al., 2000).

One possible mechanism of SF-mediated cell cycle arrest is through inhibition of cyclins which, together with CDK, drive the cell from one phase to the next. For example, SF inhibited the expression of cyclin D1 and DNA synthesis along with G1 cell cycle block in human prostate and colon cancer cell lines (Chiao et al., 2002; Shen et al., 2006). Another mechanism by which SF affects the cell cycle is through up-regulation of CDKs, which bind and inhibit the activity of cyclin/CDK complexes and regulate cell cycle progression through the four phases. For example, treatment of human bladder cancer T24 cells with 5, 10, and 20 μ M SF for 24 h blocked cells at Go/G1checkpoint, which was associated with up-regulation of CDK1 p27 expression (Shan et al., 2006). Furthermore, treatment of epithelial ovarian cancer cells with SF resulted in dose-dependent decrease in expression of G1 phase cyclins while increasing the expression of CDKs (Bryant et al., 2010). A third mechanism for the arrest in cell cycle progression by SF is through inhibition of tubulin polymerization and disruption of microtubules. Tubulin-containing microtubules play a pivotal role in cell division, motility and intracellular trafficking in all eukaryotes (Nogales, 2000). Therefore, anti-microtubule drugs that disturb tubulin polymerization and interfere with mitosis have gained much attention in the cancer drug discovery. Inhibition of tubulin polymerization by SF has been reported recently in several studies. Treatment of human lung cancer A549 cells with radiolabeled SF revealed covalent binding of SF to tubulin (Mi et al., 2008). Such SF-tubulin adduct could be sufficient by itself to inhibit cell growth.

ANTI-INFLAMMATORY

Accumulating evidence from clinical and epidemiological studies indicate that inflammation plays a critical role in cancer initiation and progression. Accordingly, it is now accepted that chronic inflammation and carcinogenesis are mechanistically-linked (Heiss et al., 2001). Studies on the association between inflammation and cancers have identified several key molecules mediating the inflammatory process in tumor formation and promotion. One of these key molecules is the nuclear factor- κ B (NF- κ B) that is found to be constitutively activated in many human cancers, and leads to induction of cell proliferation, blockade of apoptosis, and promotion of metastasis (Baldwin, 2001). Inhibition of NF- κ B strongly enhances

the apoptotic potential of the chemotherapy treatment, an observation that indicates that Inhibition of NF- κ B could be a new adjuvant approach in chemotherapy (Baldwin, 2001; Clarke et al., 2008).

Several studies have shown down-regulation of NF- κ B activity with SF administration in various cancer cells and have been reviewed (Juge et al., 2007; Clarke et al., 2008). Heiss et al. (2001) proposed that SF could directly inactivate NF- κ B subunits through binding to essential cysteine residues or through interaction with glutathione or other redox regulators like thioredoxin and Ref-1 relevant for NF- κ B function (Heiss et al., 2001).

Inflammatory cytokines such as interleukin-1 (IL-1), tissue necrosis factor (TNF- α), and overproduction of nitric oxide (NO) and prostaglandins (PGE2) have been also associated with chronic inflammation and cancers (Angelo et al., 2007; Rose et al., 2005). It has been shown that lipopolysaccharides (LPS)-mediated expression of NO, PGE2, and TNF- α was attenuated by SF treatment in cultured Raw 264.7 macrophages (Heiss et al., 2001; Woo et al., 2007). Using peritoneal macrophages derived from Nrf2(+/+) and Nrf2(-/-) mice, SF significantly suppressed the LPS-induced inflammation in Nrf2(+/+), but not in Nrf2(-/-) mouse peritoneal macrophages, indicating that activation of Nrf2 may be a key step in anti-inflammatory action of SF (Lin et al., 2008). Additionally, a recent study showed that combination of SF, phenyl isothiocyanate, and curcumin have a synergetic effect in down-regulating inflammation markers like TNF- α , IL-1, NO, and PGE2 (Cheung et al., 2009).

Sulforaphane and kidney diseases

Clinical and experimental evidences have shown the important role that reactive oxygen species (ROS) play in renal damage including glomerular, tubulointerstitial, and endothelial alteration (Ragheb et al., 2009). Accordingly, SF is expected to protect against these ROS-mediated deleterious effects on the kidney through its indirect antioxidant effect. Treatment of HK2 renal tubular epithelial cells with SF dramatically increased phase 2 enzymes and effectively protected cells against cytotoxicity induced by hypoxia-reoxygenation (Yoon et al. 2008). In other set of experiments using a renal ischemia-reperfusion model, SF induced Nrf2-mediated up-regulation of phase 2 enzymes and improved ischemia-reperfusion-induced changes in the lipid hydroperoxides, glutathione, creatinine clearance, kidney weight, and histologic abnormalities (Yoon et al., 2008). Promoting epithelial-mesenchymal transition (EMT) has been implicated in renal fibrosis induced by cyclosporine A (CsA) (McMorrow et al., 2005). In a recent study, pretreatment of rat tubular epithelial NRK-52E cells with SF prevented, through activation of Nrf2 and induction of HO-1 in these cells, EMT gene changes (Shin et al., 2010). Such protective effect of SF against CsA-induced

renal fibrosis was not observed in Nrf2-deficient mice (Shin et al., 2010). Similarly, SF effectively prevented the cisplatin-induced increase in reactive oxygen species production and the decrease in QR and γ -glutamyl cysteine ligase (γ GCL) activities and in glutathione (GSH) content. The protective effect of SF on ROS production and cell viability was prevented by buthionine sulfoximine, an inhibitor of γ GCL, and by dicoumarol, an inhibitor of QR (Guerrero-Beltran et al., 2010).

Sulforaphane and diabetes

Diabetic micro- and macro-vascular diseases are associated with dysfunction of the endothelial cells in hyperglycemia. Oxidative stress and formation of reactive oxygen species by mitochondria are common features in the endothelial cell dysfunction in hyperglycemia (Nyengaard et al., 2004). When incubated with human microvascular endothelial cells under low and high glucose concentrations, SF resulted in activation of Nrf2 and prevented the hyperglycemia-induced activation of hexosamine and protein kinase C (PKC) and prevented increased cellular accumulation of the glycating agent methylglyoxal (Xue et al., 2008). In another interesting study, pretreatment with SF blocked the development of type 1 diabetes in streptozotocin-treated mice and resulted in restoration of normal insulin secreting responses to glucose in cytokine-treated rat pancreatic islets (Song et al., 2009).

Sulforaphane and ocular disorders

Retinitis Pigmentosa is a hereditary disease that kills photoreceptor cells and causes blindness to approximately 100,000 Americans (Bird, 1995). Several novel observations suggested that impaired expression of thioredoxin (Trx), thioredoxin reductase (TrxR), and Nrf2 is involved in the pathogenesis of retinal degeneration in this disease. Systemic administration of SF to a mouse model of Retinitis Pigmentosa significantly up-regulated the retinal levels of Trx, TrxR, and Nrf2, and effectively protected photoreceptor cells (Kong et al., 2007). Induction of phase 2 response, as reflected in GSH content and NAPQ1 activities, by SF was found to provide protection of the retinal pigment epithelial cell (RPE cell) against photooxidative damage and impairment of vision in age-related macular degeneration (Gao et al., 2001, 2004).

Sulforaphane and respiratory disorders

Generation of ROS and oxidative stress have been shown to be critical in the induction of robust inflammatory response and pathogenesis of allergic airway inflammation and asthma (Bowler et al., 2002;

Boldogh et al., 2005). A placebo-controlled dose escalation trial concluded that oral SF safely and effectively induced the mucosal phase 2 enzymes (GST, QR, NADPH quinone reductase) expression in the upper airway of human subjects (Riedl et al., 2009). Due to up-regulation of phase 2 enzymes (e.g. GST and QR) in the airway epithelial cells, SF was found to mitigate the effect of airborne particulate pollutants such as diesel extract using airway epithelial cell line (Ritz et al., 2007). Such observations demonstrate the potential of SF as a novel therapeutic strategy for oxidant-induced respiratory disorders.

Sulforaphane and neurodegenerative disorders

A growing body of evidence collected from biochemical and pathological studies in animal models of neurodegenerative diseases (NDD) as well as postmortem brain tissue and genetic analysis in humans suggest that free radical toxicity, oxidative stress, and mitochondrial dysfunction are key pathological mechanisms in NDD (Beal, 2009). Therefore, therapeutic approaches using phase 2 inducers and targeting oxidative stress suggests a promising treatment strategy in NDD. Feeding a fly model of Parkinson's disease (PD) with pharmacological inducers of phase 2 detoxification pathway, including SF, suppresses the neural loss and protects against PD (Trinh et al., 2008). Future research in this area will be required to identify the specific phase 2 pathway component(s) most responsible for the neuroprotective effect of SF in PD.

Stroke is the third leading cause of death and disability in the USA. Several biochemical mechanisms have been proposed to contribute to the pathophysiology of stroke, with reactive oxygen species and inflammatory responses being the most studied and may be effective targets for intervention. Systemic pretreatment of SF or SF-containing diet was found to significantly reduce the cerebral infarct volume following focal ischemia (Zhao et al., 2006), provide neuroprotection in a neonatal hypoxia-ischemia rat model (Ping et al., 2010), and decrease the aging-related degenerative changes in the spontaneously hypertensive stroke-prone rat (SHRsp) CNS (Noyan-Ashraf et al., 2005). Using an *in vitro* model of ischemia/reperfusion, SF was found to stimulate the Nrf2 pathway of antioxidant gene expression in astrocytes and protect them from cell death when given before and after oxygen and glucose deprivation (Danilov et al., 2009). Moreover, SF improves cognitive function in rats when administered 1 h following traumatic brain injury (Dash et al., 2009). Such observations indicate that pharmacological stimulation of antioxidant gene expression by SF is a promising approach to neuroprotection after cerebral ischemia.

Although, significant oxidative damage is observed in Alzheimer's disease and leads to extracellular deposition of β -amyloid as senile plaques, very little, if any, research

has been conducted to investigate the beneficial effect of SF in this NDD. A recent review by Mukherjee et al. (2007) showed that broccoli's glucosinolate content has an anti-acetylcholinesterase (AChE) activity and could have a potential to treat symptoms of Alzheimer's disease (Mukherjee et al., 2007).

Sulforaphane and cardiovascular disorders

Cardiovascular diseases account for approximately 20% of all annual worldwide deaths, and remain the leading cause of death in both developed and developing countries (Wattanapitayakul et al., 2001). Several studies have shown that oxidative stress plays a critical role in the different forms of cardiovascular disorders, including congestive heart failure, atherosclerosis, myocardial ischemia, and chemical-induced cardiac toxicity (Molavi et al., 2004). Therefore, xenobiotics with potent antioxidant properties are expected to protect against oxidative cardiovascular disorders.

In an *in vitro* study using rat neonatal cardiomyocytes, long-term treatment with SF was shown to have a potential cardioprotective effect as confirmed by the decrease in production of intracellular reactive oxygen species, increase in cell viability, and decrease in DNA fragmentation (Angeloni et al., 2009). Such observations were accompanied by induction of antioxidants and phase 2 enzymes in cardiomyocytes (Angeloni et al., 2009). Consumption of broccoli sprouts rich in SF was found to decrease oxidative stress in both male and female spontaneously hypertensive stroke-prone rats (SHRsp) and, thus, improves blood pressure as well as decreases inflammation in all tissues examined, including heart, arteries, kidney and central nervous system (Wu et al., 2004; Noyan-Ashraf et al., 2006; Noyan-Ashraf et al., 2005). Furthermore, if pregnant SHRsp are fed with this source of SF, then their adult offspring regardless of the diet they were placed on had lower blood pressures and less inflamed organs than the parental generation (Noyan-Ashraf et al., 2006).

Several studies have indicated that release of proinflammatory mediators influence atherosclerosis by inducing adhesion molecules (eg, VCAM-1) on endothelial cells through signaling intermediaries including p38 MAP kinase. Treatment with sulforaphane activated Nrf2 and suppressed p38-VCAM-1 signaling at the susceptible sites (Zakkar et al., 2009). Such activation of Nrf2 prevented endothelial cells at the atheroprotected sites from exhibiting a proinflammatory state and was shown only in wild-type but not Nrf2(-/-) mice, indicating that SF suppresses endothelial cells activation via Nrf2 pathway (Zakkar et al., 2009).

Sulforaphane and gastric diseases

Daily intake of SF-rich broccoli sprouts for 2 months

reduces *Helicobacter pylori* (*Helicobacter pylori*) colonization in mice and improves the consequence of infection in infected mice and in humans (Yanaka et al., 2009). This therapeutic effect was not observed in mice in which the Nrf2 gene was deleted, strongly indicating the important role of Nrf2-dependent antioxidant effect (Yanaka et al., 2009). Several recent human studies have also reported the potential effect of SF for the eradication of *H. pylori* infection (Galan et al., 2004; Fahey et al., 2002).

CONCLUDING REMARKS

The use of herbal remedies and their active ingredients became an increasingly attractive approach for the prevention and treatment of variety of acute and chronic disorders. Among these food derivatives, SF is the main active isothiocyanate component in cruciferous vegetables. *In vitro* experiments, *in vivo* animal studies and a smaller number of clinical trials with humans have suggested that the dietary SF is a safe, cheap and effective strategy to reduce the risk of atherosclerosis, cancer, diabetes, gastric, heart, neurodegenerative, ocular and respiratory diseases. These findings are consistent with epidemiological observations that high intake levels of SF and SF-rich diets are correlated with low rates of these diseases. These health benefits may be attributable to a variety of potential mechanisms. Majority of reports focused on Nrf-2 mediated induction of phase 2 detoxification enzymes that protect cells against oxidative damage. However, recent studies have indicated several other mechanisms in response to SF. These mechanisms include inhibition of CYP enzymes involved in carcinogen activation, induction of apoptosis and cell cycle arrest, and anti-inflammatory activity.

Although, current evidences indicate that SF intake has many potential health benefits, some questions and issue still remain to be resolved. As indicated under disposition of SF, there is some contradiction regarding the effect of GST polymorphism on pharmacokinetics of SF. Most of these studies were conducted using a single serving of SF or SF-rich diets. However, more prolonged feeding studies should be conducted to reflect the routine dietary practice among population. Such studies should examine dose effect relationship, effect of SF source, and population genotype to accurately understand the underlying mechanisms leading to inter-individual variations in response to SF intake.

SF-drug interactions should be carefully addressed in the context that SF is a potent inhibitor for CYP enzymes and inducer for phase 2 enzymes involved in drug metabolism. Studies in our lab show that SF is a potent inhibitor for human CYP3A4, an enzyme involved in metabolism of almost 50% of drugs available on the market (data not published). Such decrease in metabolism of these drugs can result in toxicity problems when drug concentrations get too high.

While the major function of CYP enzymes is drug metabolism, some CYP enzymes are involved in maintenance of the homeostasis of endogenous molecules such as cholesterol and vaso-active agents and play a critical role in the regulation of vascular function. The potential inhibitory effect of SF on these CYP enzymes should be investigated as it may affect the normal cellular functions, or could be used as a therapeutic approach in case of upregulation of such CYP enzymes.

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