Review Article

Sulforaphane Protects against Brain Diseases: Roles of Cytoprotective Enzymes

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Abstract

Sulforaphane (SFN) is a kind of isothiocyanate derived from broccoli and other cruciferous vegetables. Because of its roles of antioxidant, anti-inflammatory, and anti-tumor through multiple targets and various mechanisms, SFN has drawn broad attention of the researchers. One of the most important target of SFN is nuclear factor erythroid 2 related factor 2 (Nrf2), wildly known for its ability to regulate the expression of a series of cytoprotective enzymes with antioxidative, prosurvival, and detoxification effects. Multiple researches have shown that SFN protects against central nervous system diseases through Nrf2pathway. In this article, we list SFN contents in common cruciferous vegetables, and summarize recent advances in the protective effects of SFN against acute brain injuries and neurodegenerative diseases through activating Nrf2 signaling pathway.

Keywords: Sulforaphane; Nrf2; Stroke; TBI; Dementia; Autism

Abbreviations

AD: Alzheimer's Disease; ARE: Antioxidant Response Elements; ASD: Autism Spectrum Disorders; BBB: Blood-Brain Barrier; CNS: Central Nervous System; EGCG: Epigallocatechin Gallate; GSH: Glutathione; GSK-3β: Glycogen Synthase Kinase-3β; GST: Glutathione S-Transferase; HO-1: Heme Oxygenase 1; ICH: Intracerebral Hemorrhages; KEAP1: Kelch-Like Ech Associated Protein 1; NQO-1: NADPH Quinine Oxidoreductase 1; Nrf2: Nuclear Factor Erythroid 2 Related Factor; OGD: Oxygen-Glucose Deprivation; PD: Parkinson's Disease; SFN: Sulforaphane; TBI: Traumatic Brain Injury

Introduction

Central nervous system (CNS) diseases are major health problems because of their high incidence of death and disability around the world. Multiple pathological processes are involved in brains diseases, including oxidative stress, protein misfolding and aggregation, perturbed calcium homeostasis, excitotoxicity, inflammation, and apoptosis [1,2]. In the past decades, there has been a growing interest in a number of dietary phytochemicals for their antioxidant anti-inflammatory effects. Among them 1-isothiocyanate-4-methylsulfinyl butane (sulforaphane, SFN) is one of the most popular one [3-5].

SFN is the hydrolysis product of glucoraphanin, the main glucosinolates in broccoli [6,7]. Glucosinolates are wildly present in cruciferous vegetables (Table 1), such as broccoli, brussel sprouts, kale, cabbage, and cauliflower [8,9], but SFN contents are relatively low (Table 2 and Table 3) [10,11]. Upon consumption, however, glucoraphanin is hydrolyzed by myrosinase, and then converted into SFN [12] has been demonstrated that SFN has neuroprotective effects against brain diseases via a number of mechanisms [3]. In general, SFN inhibits phase I enzymes through directly interacting with cytochrome P450 and, on the other hand, induces phase II enzymes through activating nuclear factor E2-factor related factor (Nrf2). Phase I enzymes are usually involved in oxidation, reduction, or hydrolysis and generally lead to detoxification, while phase II enzymes demonstrate protection effects against CNS diseases due to their antioxidative and detoxifying roles. Here, this review will focus on the protective effects of SFN against various CNS diseases through activating Nrf2.

Bioactivity of SFN

The molecular weight of SFN is 177. Its chemical structure is shown in Figure 1, where its bioactive group is indicated by an arrow. This isothiocyanate group makes SFN an electrophile and readily reacts with nucleophiles, especially cysteine residues in proteins [13-15]. It is lipophilic and can be passively absorbed by cells, where SFN is rapidly conjugated with glutathione (GSH) by glutathione S-transferases (GSTs), leading to maintenance of a concentration gradient and ensuring the passive diffusion into the cell [5]. Then, it is metabolized sequentially by γ-glutamyl-transpeptidase, cysteinyl-glycinease, and N-acetyltransferase, and the derived conjugates are transported into the systemic circulation [14].

Pharmacokinetic studies in both humans and animals show that SFN can achieve micromole concentrations and accumulate in tissues. This process is rapid, with peak plasma concentration between 1 to 3

Table 1: Glucosinolate contents in cruciferous vegetables.

Raw food (100 g)	Glucosinolates (mg)
Broccoli	61.1
Brussels sprouts	247
Cabbage	108.9
Cabbage, red	66.9
Cauliflower	62
Kale, curly	89.4
Kohlrabi	109.3
Turnip-Swede	56

Table 2: SFN contents in vegetable juices.

Juice from	SFN contents (µg/ml)
White cabbage	2.77
Broccoli	7.77
Red cabbage	8.94
Cauliflower	0.66
Brussels sprouts	4.74

Table 3: SFN contents in vegetable powers (processed at 90℃).

Vegetable	SFN concentration (µg/g)
Broccoli	476.5
Cabbage	168.4
Chinese cabbage	129.1
Red cabbage	91.3
Carrot	85.3
Choy	39.2
Cauliflower	34.6
Radish	28.9
Chinese white turnip	17.7

Figure 1: Chemical structure of SFN. The arrow indicates its bioactive group.

Figure 2: Diagram showing the mechanism that SFN activates Nrf2.
(A) Under normal condition, Nrf2 is inactive due to its sequestration by Keap1 and degradation by the proteasome. (B) After eating cruciferous vegetables, SFN is synthesized and absorbed. It then reacts with several cysteine residues in Keap1, including Cys-38, Cys-151, Cys-368, and Cys-489. These reaction results in conformational changes of Keap1, subsequently releasing Nrf2. Nrf2 then translocate into nucleus, binds to the antioxidant response element (ARE) in the promoters phase II enzyme genes.
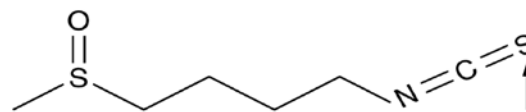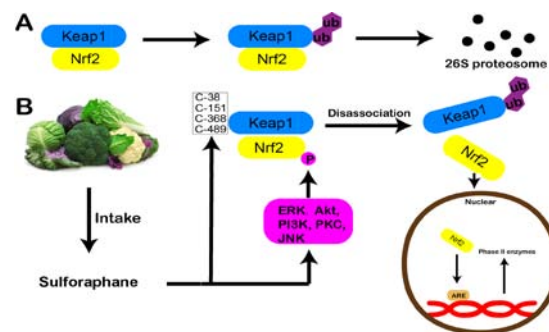
hours after administration of SFN [14]. After a single oral dose of 50 µmol of SFN, the peak plasma concentration in rat was about 20 µM [16]; while a dose of 200 µmol leads to a peak concentration of 1 µM in human plasma or serum [17]. The elimination process of SFN in both plasma and tissue is also rapidly, within 12-24 hours after administration, majorly through urinary excretion.

Blood-brain barrier (BBB) is a highly selective semi-permeable membranes barrier that separates the circulating blood from the brain extracellular fluid in the CNS. The ability to go through the BBB is essential to exert neuroprotective effects for any phytochemicals. Several researches have demonstrated that SFN can traverse the BBB and accumulate in the CNS. After SFN gavage to mice, Jazwa, et al. found that SFN was able to cross the BBB and accumulate in cerebral tissues such as the ventral midbrain and striatum [18]. It reached the peak in 15minutes and disappeared after 2hours. Clarke, et al. also detected SFN in the CNS [19].

Nrf2 signaling pathway

Nrf2 is a transcription factor with a characteristic Cap 'n' collar structure [15,20]. It is the key regulator of cytoprotective phase II enzymes, including glutathione S-transferase (GST), heme oxygenase 1 (HO-1) and NADPH quinine oxidoreductase 1 (NQO-1), all of which play important roles in anti-oxidation and detoxification [20-22]. Kelch-like ECH associated protein 1 (Keap1) and antioxidant response elements (ARE) are another two key factors in Nrf2 signaling pathway.

Under unstressed condition, Nrf2 forms a complex with Keap1, facilitating the ubiquitination and degradation of Nrf2 by the ubiquitin-proteasome system [20,23]. Keap1 is a cysteine-rich protein, and three cysteine residues (Cys151, Cys273, and Cys288) are essential for Keap1-mediated inhibition of Nrf2 activity [20,24]. Under oxidative or electrophilic conditions, however, the cysteine residues of Keap1 will be oxidized, resulting in conformational change of Keap1. This further leads to dissociation of the Nrf2-Keap1 complex, thereby stabilization and nuclear translocation of Nrf2 (Figure 2) [20,23]. Once in the nucleus, Nrf2 forms a heterodimer with a group of nuclear proteins called small Maf proteins [24]. This dimerization increases the binding specificity of Nrf2 to the ARE located in the promoter region of phase II genes [25-27].

In addition to Keap1, recent studies show that there exists another model for Nrf2 ubiquitination and degradation, which is Keap1-independent [28]. In this model, glycogen synthase kinase-3β (GSK3β) phosphorylates two serine residues of Nrf2, Ser342 and Ser347. Phosphorylated Nrf2 can bind with an ubiquitin ligase adaptor β-TrCP. β-TrCP links Nrf2 to the Cullin1/Rbx1 ubiquitination complex, which is subsequently degraded. This GSK-3β and β-TrCP-dependent Nrf2 degradation model is supported by the findings that GSK-3β inhibitors stabilize Nrf2 in Keap1$^{-/-}$ mouse cells [29].

SFN mediate activation of Nrf2

Direct effects of SFN on Nrf2: SFN can enhance Nrf2 activity via several approaches. It has been reported that SFN increases Nrf2 transcription, probably by reducing methylation of the first 15 CpGs of Nrf2 promoters [30]. Consequently, SFN is reported to upregulates Nrf2 expression cardiac cell [31] and epidermal cells [30]. As an Electrophile, SFN reacts with thiol groups of Keap1 to form thionoacyl adducts, especially C-151 [31,32], which is essential for the association of Cul3 ubiquitin ligase [33]. In addition to C-151, several other sensor cysteines in human Keap1 that can be modified by SFN, including C-38, C-368 and C-489 [34,35]. By modifying these cysteines, SFN can affect the Nrf2/Keap1 complex and prevent ubiquitination of Nrf2. A recent study showed that SFN suppressed the activity of GSK-3β and enhanced Nrf2 nuclear translocation [36].

Collectively, SFN stabilizes Nrf2 through antagonizing proteasome-dependent degradation [37].

Long-lasting effects of SFN-mediated upregulation of cytoprotective enzymes: Although absorbed SFN is cleared from the body within a few hours, the SFN-mediated effects exist for a long time. Bergström, et al. studied the kinetics of the SFN-induced Nrf2 response in astrocytes [38]. After a 4-hour SFN exposure, there were increases in NQO1 and HO-1 mRNAs that persisted for 24 hours, and the levels of corresponding proteins remained elevated for over 48 hours. Consequently, peroxide-clearing activity and glutathione levels were elevated, resulting in an increased resistance of astrocytes to superoxide-induced damage [38]. In addition, SFN can be taken for a longer period, as it is a dietary component without toxicity. For instance, Bai, et al. [31] showed that diabetic mice treated with SFN for three months showed a reduced incidence of DCM at the end of the treatment and after 6 months [31]. More evidence has been summarized by Angeloni and colleagues [39], which included long-term decreases of intracellular ROS production, increased cell viability, and decreased DNA fragmentation, accompanied by the induction of antioxidants and phase II enzymes. These features of SFN are critical for long-term neuroprotection.

SFN up regulates Nrf2 target proteins

After Nrf2 pathway activation, over two hundred genes are induced expression and exert detoxification and antioxidant defense. Here our discussion will focus on the phase II enzymes that participate in neuroprotection against common CNS disease.

Antioxidative enzymes: Glutamate cysteine ligase(GCL) is the rate-limiting enzyme in GSH synthesis [40]. In most eukaryotic cells, GCL enzymes are heterodimeric complexes consisting of two distinct gene products, the catalytic subunit (GCLC) and the modifier subunit (GCLM) [41]. SFN induces the expression of both GCLC and GCLM in brain cells, thus enhancing the production of GSH [42]. In addition, Vauzour, et al. [43] observed that SFN could protect against 5-S-cysteinyl-dopamine-induced neurons death, by increasing the expression of GST and glutathione reductase. Similar to these results, Morroni and fellows also suggested SFN could activate Nrf2 signaling pathway, increasing the levels of GHS, GST, and glutathione reductase.

Detoxifying enzymes:

Heme oxygenase 1 (HO-1): HO-1catalyzes the first and rate-limiting step of heme degradation, and HO-1 protects cells through a net reduction in superoxide and other reactive oxygen species [44]. Many researchers have demonstrated that SFN could induce HO-1 expression via activating Nrf2 signaling pathway. Alfier and colleagues demonstrated that SFN treatment activated Nrf2 pathway and increased expression of HO-1 in the brain, which in turn enhanced antioxidant defenses and significantly attenuated neurobehavioral deficits as well as BBB disruption in rat after stroke [45]. Similar results have been reported by Pan and fellows. They showed that SFN pretreatment significantly increased the nuclear accumulation of Nrf2 and the expression of HO-1 in ischemic retinas. And knockdown of Nrf2 with siRNA decreased cell viability and attenuated SFN-induced HO-1 up-regulation [46].

NAD(P)H: quinone oxidoreductase 1 (NQO1): In cultured astrocytes, SFN administration before or after OGD led to increased NQO1 gene and protein expression through activating Nrf2 signaling pathway; and such increased NQO1 reduced astrocyte cell death [47]. Soane, et al. [42] also found that SFN activated Nrf2 signaling pathway and induce the expression of NQO1, protecting hippocampal neurons against heme toxicity and OGD-induced death. Porritt and colleagues [48] reported that SFN induced up-regulation of NQO1 in photothrombosis-induced infarction in mice.

Other proteins: Bcl-2 is an anti-apoptotic protein [49], and a recent report demonstrated that Bcl-2 is a downstream gene product of Nrf2 [50]. Interestingly, Wu and colleagues reported that SFN protected cultured neurons against OGD, and protective mechanisms included that SFN up-regulated bcl-2 expression in neurons [51].

Protective effects of SFN against brain diseases

Ischemic stroke: Brain stroke is the leading cause of long-term disability and the third-leading cause of death in the entire world, and ischemic stroke is the most common type. The pathological processes of ischemic stroke are multiple, ranging from excitotoxicity, oxidative stress, inflammation, to mitochondrial dysfunction [52,53]. A number of studies have demonstrated the neuroprotective effects of SFN against ischemic brain injury through anti-oxidative mechanism.

Using a neonatal hypoxia-ischemia model, Ping and colleagues observed that SFN significantly increased Nrf2 and HO-1 expression [54]. SFN treatment decreased the number of apoptotic neurons, activated macroglia, and oxidative parameters such as the amount of 8-hydroxy-2-deoxyguanosine and malondialdehyde level, leading to a reduced brain tissue loss. The similar results have also been reported in other models. For example, SFN treatment activated the Nrf2 signaling pathway and protected cultured neurons from delayed death induced by oxygen and glucose deprivation (OGD) [42]. SFN treatment before or shortly after OGD significantly reduced astrocytes death by activating the Nrf2 pathway and antioxidant gene expression [47]. It is also demonstrated that the delayed administration of a single dose of SFN significantly decreased cerebral infarct volume in rat models of ischemia stroke [45,55].

Hemorrhagic strokes: Although not as common as ischemic stroke, hemorrhagic stroke results in higher mortality rate, especially intracerebral hemorrhages (ICH) due to intracranial hypertension and brain herniation caused by hematoma and edema, ischemia and oxidative stress also contribute to brain injury after ICH [56]. SFN also exhibits neuroprotection against hemorrhagic strokes [57].

Yin and colleagues investigated the protective effects of SFN in rat ICH model [58]. They found that SFN could activate Nrf2 signaling pathway and increased HO-1 expression, and could reduce the severity of neurological dysfunction compared to control group. Similar to this study, Zhao and colleagues [59] demonstrated that SFN protected against ICH by activating Nrf2 and reducing oxidative stress and brain edema; and the deficits in Nrf2 led to weakened neuroprotective effects of SFN. It was also reported that Nrf2 activated by SFN played an essential role in the effective cleanup of the hematoma process after ICH. The authors suggested SFN could activate Nrf2 in microglia, resulting in an increase of the anti-oxidative capacity, phagocytosis and hematoma clearance after ICH [57,59]. Moreover, SFN treatment could ameliorate cerebral

vasospasm and early brain injury through activation of Nrf2 signaling pathway, which in turn induced antioxidant and detoxifying enzymes in a model of subarachnoid hemorrhage [60,61].

Traumatic brain injury (TBI): TBI is increasingly becoming an important cause of death, long-term disability, and post head trauma cognitive impairment around the world, with oxidative stress as an integrated pathogenesis [62]. In a rat model of TBI, SFN was able to reduce Evans Blue extravasation in the acute phase of TBI when SFN was applied before injury, and able to reduce the secondary phase of BBB permeability when administrated 6 hours after TBI [63]. The protective effects were mediated by the activation of Nrf2, indicated by increased expression of Nrf2-driven genes such as GST, HO-1, and GSH peroxidases in the cortex and cerebral micro vessels. It was also reported that intraperitoneal administration of SFN resulted in reducing neuronal death, contusion volume, and neurological dysfunction 7d after TBI in rats; importantly, the neuroprotective capacity of SFN was attenuated in Nrf2 KO mice [64]. Zhang and colleagues [65] found that SFN reduced aquaporin-4 (AQP4) channel loss and increased AQP4 protein levels in the peri-lesion region at 24hours and 3days following TBI. They suggest that the neuroprotective effect of SFN may be due to a combination of mechanism, including decreased BBB permeability, enhanced cell survival, and/or increased AQP4 channel levels. SFN also exhibits protection to cognitive function after TBI. Dash and fellows [66] observed that post-injury SFN treatment enhanced learning capacity as well as improved performance of animals in a working memory task. The authors propose that the ability of SFN to protect cognitive function may be due to its ability to protect the neurons and other cell types of the neurovascular unit from the oxidative damage elicited by TBI. Taken together, SFN could protect against various pathophysiological consequences of TBI, in which activating Nrf2 pathway plays a key role [67].

Alzheimer's disease (AD): AD is the commonest cause of dementia without a cure. It is characterized by memory loss and cognitive dysfunction, and its pathological hallmarks are the cerebral deposition of amyloid-beta (Aβ) peptides in senile plaques and the neurofibrillary tangles of hyper-phosphorylated tau aggregates [4,68,69]. Aβ is believed to bean important reason for neuronal cell death in AD, and Aβ 40 and Aβ 42 peptides can exist in multiple aggregation forms, including soluble oligomers or protofibrils, to insoluble fibrils, responsible for various pathological effects [70]. Aβ has been reported to induce the production of hydrogen peroxides in cultured cells [71]; on the other hand, oxidative production increases Aβ production [72]. As discussed below, SFN demonstrates its protective effects against AD, due to its ability to activate Nrf2 and consequent antioxidant.

SFN was reported to be able to ameliorate cognitive deficit in various models of AD [69,73]. Lee and colleagues [74] demonstrated that SFN protected Aβ-induced oxidative death of cultured neuronal cells. Park, et al. confirmed the ability of SFN to protect the neuronal cells from Aβ 42-mediated cytoxicity [75]. In an Aβ-induced mouse model of AD, Kim, et al. reported that SFN could ameliorate cognitive impairment and protect the brain from amyloidogenic damages [69]. Aβ peptides are degraded, at least in part, through autophagy. A recent study showed that Nrf2 promotes autophagy by upregulation of p62 protein, an adaptor for selective autophagy [76]. By administration of

SFN, autophagy genes was up regulated in cultured cells; when Nrf2 was knocked out, SFN exhibited a reduced induction of these genes [77].

However, it is not clear whether SFN can protect the brain against vascular cognitive impairment and dementia, the second commonest type of dementia.

Parkinson's Disease (PD): Oxidative stress is an important factor in the pathogenesis of PD [78]. A number of studies showed that SFN was able to significantly protect dopaminergic cells from the cytotoxicity of 6-OHDA [79,80]. Vouzour, et al. [43] have found that SFN could protect primary cortical neurons against 5-S-cysteinyl-dopamine-induced injury, which is formed by DA quinone reacting with cellular thiols. It was reported that SFN inhibited 6-OHDA-induced cytotoxicity in dopaminergic-like neuroblastoma SH-SY5Y cells through increasing Nrf2 nuclear translocation, and increasing the levels of HO-1 expression and GSH contents [81,82]. Other studies confirmed that Nrf2 activation by SF might play an important role in DA neuron protection against 6-OHDA-induced toxicity in rat organo typical nigrostriatal coculture [83]. *In vivo*, SFN could also reduce neurotoxicity induced by rotenone, where Nrf2 played a key role [84]. In summary, SFN protects against PD, mainly through the Nrf2 signaling pathway.

Autism Spectrum Disorders (ASD): ASD refers a group of neurodevelopmental disorders, characterized by deficits in communicating with others and repeated behaviors staring in early childhood. The pathogenesis of ASD is not clear now; however, several causes have been suggested for ASD, including increased oxidative stress and decreased cellular defense [85,86]. A recent clinical study suggested SFN treatment improved the social interaction, abnormal behavior, and verbal communication of the patients [87]. In addition, a phase 2 clinical trial (NCT02561481) is ongoing to detect the effects on SFN on ASD.

Conclusion

Along with other beneficial effects of SFN, such as anti-inflammation [88]. Multiple *in vitro* and *in vivo* studies have demonstrated the ability of SFN to prevent various brain diseases, including stroke, TBI, AD, PD, and ASD. SFN exerts neuroprotective effects, at least in a part, through activating Nrf2 signaling pathway and its downstream antioxidative as well as antitoxic enzymes. Existing in various vegetables especially in broccoli, SFN can be easily consumed as a phytochemical inducer of Nrf2. Because of its BBB permeability, SFN can reach effective concentration in the CNS. Considering its other beneficial effects, such as anti-inflammation [88], SFN is a promising dietary and medical agent for neuroprotection.

Acknowledgement

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