Potential role of dietary Sulforaphane in Neuroprotection

Treball Final de Màster Nutrició i Salut

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Juliol 2015

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1. ABSTRACT

Neurodegenerative diseases are illnesses associated with high morbidity and mortality, and few or no effective options are available for their treatment. In the last few years, a number of pharmacological approaches that can potentially prevent and counteract the neuronal dysfunction and death associated with neurodegenerative diseases have been investigated. Intervention strategies using phytochemicals have been proposed as an alternative form of treatment. Among phytochemicals, sulforaphane (isothiocyanato-4-(methylsulfinyl)-butane) (SF) is an isothiocyanate found in broccoli and other cruciferous vegetables with a pleiotropic role modulating different pathways in neuronal/glial cells, showing neuroprotective effects in several studies *in vivo* and *in vitro.*

Thus, SF appears to be a promising compound with neuroprotective properties that may play an important role in preventing neurodegenerative diseases, although further studies will be required to discover SF maximal protective effects.

Based on these considerations, regular intake of cruciferous vegetables in the diet could exert beneficial effects on neurological diseases.

2. INTRODUCTION

Neurodegenerative diseases (NDD), either acute or chronic, such as Parkinson's disease (PD), Alzheimer's disease (AD), traumatic brain injury, are illnesses associated with high morbidity and mortality, and few or no effective options are available for their treatment (Ritchie K, 2002; Akhlaq A, 2010). These diseases result in acute, as well as gradual and progressive neurodegeneration, leading to brain dysfunction and neuronal death.

Recently, there has been a growing interest in the study of the molecular mechanisms involved in their pathogenesis. As possible causes of neurodegeneration, oxidative stress, inflammation, accumulation of proteins, excitotoxicity and apoptosis have been implicated (Mandel S, 2003; Dauer W, 2003).

The adult human central nervous system (CNS) consists of approximately 100 billion neurons and a similar amount of glia cells, namely, astrocytes, oligodendrocytes, and microglia (Azevedo FAC, 2009). The CNS parenchyma is separated from the rest of the body by the blood-brain barrier (BBB), which is formed predominantly by tight junctions of the endothelial cells of the CNS vasculature. The BBB restricts and controls the entry of nutrients and cells, including peripheral immune cells, which are almost completely absent in the healthy CNS. This has led to the concept that the CNS is an immune privileged organ. However, this concept has been modified in recent years since the CNS itself is fully immune competent and quickly responds to injury or infections (Amor S, 2003; Yong VW, 2010). Of particular importance is the excessive generation of reactive oxygen species (ROS), for example, due to mitochondrial dysfunction, which causes neuronal damage and thus the release of cytosolic factors that activate microglia and astrocytes. These cells respond by releasing proinflammatory cytokines as well as ROS and reactive nitrogen species (RNS) thus further promoting the inflammatory response and exacerbating the neuronal damage. Accordingly, persistent activation of glia cells can ultimately result in an amplification loop resulting in chronic neurodegeneration. In spite of the diversity of the neurodegenerative diseases, oxidative stress due to excessive production and release of ROS upon mitochondrial injury and dysfunction has been proposed as a general pathological mechanism of all major chronic neurodegenerative diseases including AD, and PD (Emerit J, 2004; Olanow C W, 1993; Urrutia PJ, 2014).

In the last few years, a number of pharmacological approaches that can potentially prevent and counteract the neuronal dysfunction and death associated with neurodegenerative diseases have been investigated. However, considering that these diseases are multifactorial and no drugs are available to stop their progression, intervention strategies using phytochemicals, organic compounds of foods with several physiological properties, have been proposed. This approach stems from the well-known association between dietary patterns rich in fruits and vegetables and lower ND prevalence (Scarmeas N, 2009; Frisardi V, 2010; Valls-Pedret C, 2015). Moreover, adapting the diet to increase intake of these phytochemicals is an option that can be continued for a lifetime without the risk of side-effects that are often caused by pharmaceuticals.

Among phytochemicals, sulforaphane (isothiocyanato-4-(methylsulfinyl)-butane) (SF) is an isothiocyanate found in broccoli and other cruciferous vegetables after the hydrolisis of glucoraphanin (the major glucosinolate in broccoli) by the myrosinase enzyme (**Figure 1**), with established anticarcinogenic actions. It was first identified as a potent inducer of phase 2 detoxification enzymes (Fahey J, 1997). SF may interact with many molecular targets, such as the nuclear factor (erythroid-derived 2)-like 2 (Nrf2). Other studies have also found other anti-carcinogenic and anti-oxidant mechanisms including induction of caspases, inhibiton of cytochrome P450 isoenzymes, and reduction of the DNA binding of nuclear factor-κB (Fahey J, 1997; Chu W, 2009).

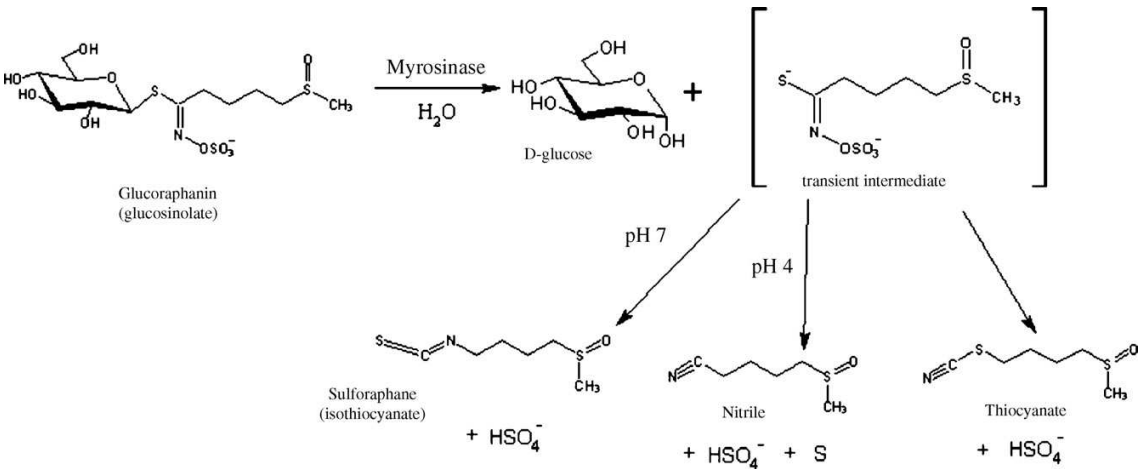

Figure 1. Hydrolysis of glucoraphanin. Glucoraphanin is the major glucosinolate in broccoli and it is hydrolyzed by the myrosinase enzyme to form both d-glucose and sulforaphane. Thiocyanates and nitriles can also be formed under acid conditions (Guerrero-Beltrán CE, 2012).

Reports in the literature have shown a pleiotropic role of this natural compound, modulating different pathways in neuronal/glial cells, showing neuroprotective effects in several studies *in vivo* and *in vitro.* Thus, it has been shown that SF can act as a protector modulating oxidative stress and apoptotic machinery (Morroni F, 2013; Soriano FX, 2008; Kraft AD, 2004; Greco T, 2010).

Recently, it has been shown that bioactive compounds of foods can directly affect enzymes that catalyze methylation and modification of DNA histones (Choi SW, 2010). In particular, SF has a chemo protector role due to its histone deacetylase (HDAC) inhibition (Dashwood R, 2010; Schwab M, 2008), that leads to changes in the expression of various genes, including tumor supressor genes in various cancers (Ho E, 2009).

The link between epigenetics and neurodegenerative disorders has been demonstrated by observing that epigenomic changes lead to alterations in gene readout in cells in the CNS affecting neuronal function and physiology. The role of SF and epigenetic modulation is well known in cancer pathology (Ho E, 2009; Shu L, 2010), mainly through its activity as a histone deacetilase inhibitor. However, there is still insufficient evidence on the role of SF in neurodegenerative diseases. Thus, investigating the impact

of SF on the epigenetic regulation neurodegenerative diseases can open up novel approaches to combat these disturbances.

SF presents many advantages, such as good pharmacokinetics and safety after oral administration as well as the potential ability to penetrate the BBB and deliver its neuroprotective effects in the central nervous system (Shapiro TA, 2006). Based on these considerations, SF appears to be a promising compound with neuroprotective properties that may play an important role in preventing neurodegenerative diseases.

The idea that by simply consuming broccoli may help to protect the brain against neurodegenerative disorders is a tantalizing prospect, and one more reason to eat a healthy, balanced diet, rich in vegetables.

3. OBJECTIVES

The objective of the present work is to review current scientific evidence of the neuroprotective role of SF in the prevention and development of neurodegenerative disorders. The aim is to investigate if adapting the Western diet by increasing dietary SF consumption is a well-founded potential strategy to prevent and delay neurodegeneration.

4. METHODS

In this work articles published in the last 20 years that describe the present knowledge about the health effects of sulforaphane in neurodegenerative disorders, with possible underlying mechanisms for these effects based on the reported in vitro and in vivo studies, have been analysed.

The article have been obtained from different specialized databases (MEDLINE, Web of Science, Elsevier Journal, Science Direct), and included experiments in cell, animals and clinical studies in humans.

Domains evaluated included sulforaphane, neurodegenerative disorders, epigenetics.

5. RESULTS

5.1 Common pathobiochemical alterations in neurodegenerative diseases

The two most common features of neurodegenerative diseases are sustained oxidative stress and inflammation. Of particular importance is the excessive generation of ROS, for example, due to mitochondrial dysfunction, which causes neuronal damage and thus the release of cytosolic factors that activate neighbouring microglia and astrocytes. These cells respond by the release of proinflammatory cytokines as well as ROS and RNS thus further promoting the inflammatory response and exacerbating the neuronal damage. Accordingly, persistent activation of glia cells can ultimately result in an amplification loop resulting in chronic neurodegeneration (Roman Fischer, 2015). (**Figure 2**).

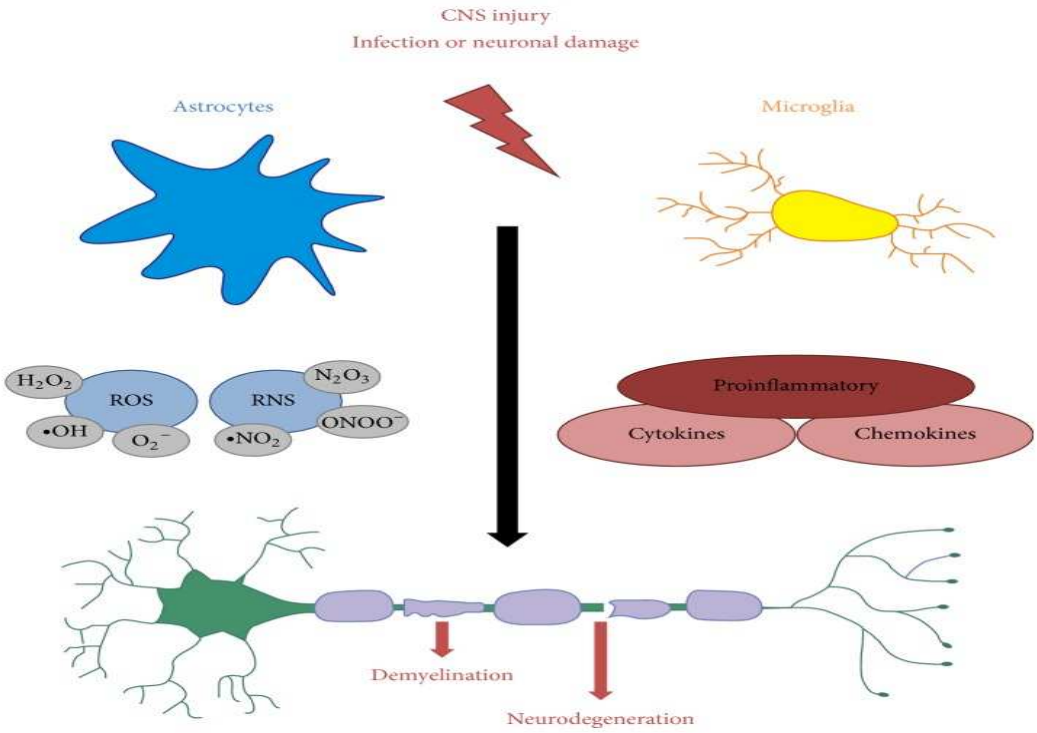

Figure 2. Schematic presentation of the CNS cell mediated demyelination and neurodegeneration. CNS injury, leads to the activation of astrocytes and microglia, that

induces the secretion of ROS, RNS, and proinflammatory cytokines and chemokines leading finally to degeneration (Roman Fischer, 2015).

In the last decade, a growing body of literature suggests that long-term changes in gene transcription associated with CNS's regulation and neurological disorders are mediated via modulation of chromatin structure. The role of epigenetic mechanisms in the function and homeostasis of the CNS and its regulation in diseases is one of the most interesting aspects of contemporary neuroscience.

5.1.1 Oxidative stress

Two main aspects contribute to the vulnerability of the CNS to oxidative stress mediated neurodegeneration: high aerobic metabolism and restricted cell renewal. First, the CNS is a metabolically highly active organ, requiring approximately 20% of the total energy consumption of the body. Therefore the CNS is rich in mitochondria, which are particularly active, resulting in high amounts of ROS (Morató L, 2014).
The brain is particularly vulnerable to oxidative stress because of its high oxygen consumption, high content of oxidizable polyunsatured fatty acids, and low antioxidant defense capacities especially in aging brains (Vincent VAM, 1998; Yun H-Y,1996). Oxidative stress is involved in many neurodegenerative diseases and is a proposed mechanism for age-related degenerative processes as a whole (Federico A, 2012).
A growing body of evidence collected from biochemical and pathological studies in animal models of 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 MF, 2009).
Therapeutic approaches using phase 2 inducers and targeting oxidative stress by enhancing endogenous antioxidant defences are promising treatment strategies in NDD. For instance, feeding a fly model of PD with pharmacological inducers of phase 2 detoxification pathway, including SF, suppresses the neural loss and protects against PD (Trinh K, 2008).

5.1.2 Inflammation

In general, inflammation is a protective response to various cell and tissue injuries to destroy and remove the detrimental agents and injured tissues, thereby promoting tissue repair. However, when inflammation is uncontrolled, it can cause excessive cell and tissue damage ultimately leading to destruction of normal tissue and chronic inflammation (Hsieh HL, 2013). This is especially relevant in chronic neurodegenerative diseases such as PD and AD, which usually last over decades and, the continuous presence of damaged neurons results in the constant activation of microglia and astrocytes. This generates a neuroinflammatory environment which is thought to promote neurodegeneration (Amor S, 2010; Zipp F, 2006).
Human neurodegenerative diseases have been associated with inflammation and dysregulation of the Nrf2 system, in particular sensitizing vulnerable brain regions to additional stresses.

5.1.3 Epigenetic modifications

Over the last two decades, the field of epigenetics, particularly the emerging field of neuroepigenetics, has begun to have a great impact in different areas such as the study of the CNS development, learning behavior, neurotoxicology, cognition, addiction and lately neurological and neurodegenerative pathology (Sweatt JD, 2013). Thanks to these studies, nowadays we know that epigenomic changes allow perpetual alterations in gene readout in cells in the CNS affecting neuronal function and physiology.

At present, studies of epigenetic changes in AD are starting to emerge. Recently, it has been observed that environmental factors even transient ones in early life can induce AD-like pathogenesis in association with aging (Wu J, 2008a). Furthermore, a difference in DNA methylation patterns typical of brain region and aging has been identified in this context (Balazs R, 2014). A variety of studies suggest a genome-wide decrease in DNA methylation present in aging and AD patients (Mastroeni D, 2011;

Bottiglieri T, 1990; Morrison LD, 1996) indicating that in AD patients a hypomethylation is present across the genome.

Regarding PD, the second most common neurodegenerative disorder after AD, most of the studies evaluating the role of epigenetic in pathogenesis have focused on the analysis of promoter methylation of causative PD genes in post-mortem brains and peripheral blood; however, the role of DNA methylation and its links to PD pathogenesis is currently unclear (Coppedè F, 2012).

In recent years, there has been considerable progress in the development of epigenetic-based drugs for the treatment of PD. Some inhibitors of HDACs and DNMTs are currently approved and available for clinical investigation (Xu Z, 2012). In this regard, the targeted downregulation of SIRT2 has been shown to ameliorate α-synuclein toxicity and dopaminergic loss in flies and in primary mesencephalic culture (Outeiro TF, 2007).

5.2 Mechanisms of action of SF in NDD

5.2.1 Oxidative stress and inflammation

The ability of SF to exert neuroprotective effects in different acute and chronic neurodegenerative diseases could be ascribed to its peculiar ability to activate the Nrf2/ARE pathway (**Figure 3**). Nrf2 is a transcription factor essential to the regulation of the cellular redox state that, in non-stimulated cells, remains bound to kelch-like ECH-associated protein 1 (Keap1), forming an inactive complex (Kensler TW,2013). When entering the cell, SF may interact with Keap1 and disrupt the binding between Nrf2 and Keap1, which allows for Nrf2 activation and nuclear translocation (Vomhof-Dekrey EE, 2012).

In the nucleus, Nrf2 binds to the antioxidant response element (ARE), a DNA promoter region of genes codifying antioxidant enzymes, such as NADPH quinone oxidoreductase (NQO1), heme-oxygenase-1 (HO-1), thioredoxin, and superoxide dismutase (Evans PC, 2011; Turpaev KT, 2013). With Nrf2 activation, SF increases the activity of phase 2 enzymes involved in the elimination of xenobiotic compounds, such as glutathione S-transferase (GST) and quinone reductase (Guerrero-Beltrán CE, 2013).

SF has neuroprotective effects in several experimental paradigms. The role of Nrf2 in this protective effect was confirmed by using Nrf2 deficient mice and in vitro studies. For example, in BV2 microglial cells, the protective effect of SF against the oxidative effect of lipopolysaccharide was associated with HO-1 induction (Innamorato NG, 2008). In another cell culture, the dopaminergic cell death, induced by a compound that produces dopamine quinone: 6- hydroxydopamine and tetrahydrobiopterin, was also attenuated by SF preincubation (Han JM, 2007). These experiments demonstrated that SF pretreatment prevented membrane damage, DNA fragmentation, and ROS formation.

Some cross-over from SF's antioxidant actions may influence inflammation through proteins that act in both pathways. New findings have also linked activation of the Nrf2 system to anti-inflammatory effects via interactions with NF-kB.
SF appears to act inhibiting NF-kB translocation attenuaiting the production of IκB-α. Heiss and coworkers observed that SF impairs DNA-binding of NF-kB which was not accompanied by IkBdegradation and nuclear translocation of NFkB (Heiss E, 2005). (**Figure 3**).
SF may also directly prevent NF-kB from forming complexes when in the nucleus. It is supposed that SF interacts with thiol groups, forms dithio- carbamates, and binds directly to redox-regulated cysteinresidues (Cys62 and Cys38) of the p50 and p65 subunits which prevent DNA binding (Anand P, 2008).

The NF-kB subunit p65 has also been shown to function as a negative regulator of Nrf2 activation either by depriving CREB binding protein (CBP) from Nrf2 or by recruitment of histone deacetylase 3 (HDAC3), causing local histone hypoacetylation and down-regulation of Nrf2-ARE signalling (Liu GH, 2008).

Accordingly, Surh and Na described a cross-talk between NF-kB and Nrf2 signalling supported by the fact that most of the phytochemicals, like SF, exhibit both, anti-inflammatory and anti-oxidant properties (Surh YJ, 2008).

5.2.2 SF targets epigenetic pathways

Several studies suggest that SF can affect epigenetic mechanisms (**Figure 3**). The HDAC inhibitory effects of SF have been shown in various prostate epithelial cells normal prostate epithelial cells (PrEC), benign hyperplasia (BPH1), and cancerous (LnCaP, PC-3) prostate epithelial cells (Clarke JD, 2011; Myzak MC, 2006) as well as in different breast cancer and colon cancer cells (Pledgie-Tracy A, 2007).
The HDAC inhibitory effect of SF has also been confirmed in an in vivo model (Myzak MC, 2006; Myzak MC, 2007).
As mentioned widely in scientific literature, the interaction between diet and epigenetics is best documented in cancer pathology (Ho E, 2009; Shu L, 2010). To date there are no published studies on the epigenetic effects of SF in neurodegenerative disorders.

Aging and age-related diseases are associated with profound changes in epigenetic patterns, though it is not yet known whether these changes are programmatic or stochastic in nature. Future work in this field seeks to characterise the epigenetic pattern of healthy aging to ultimately identify nutritional measures to achieve this pattern.

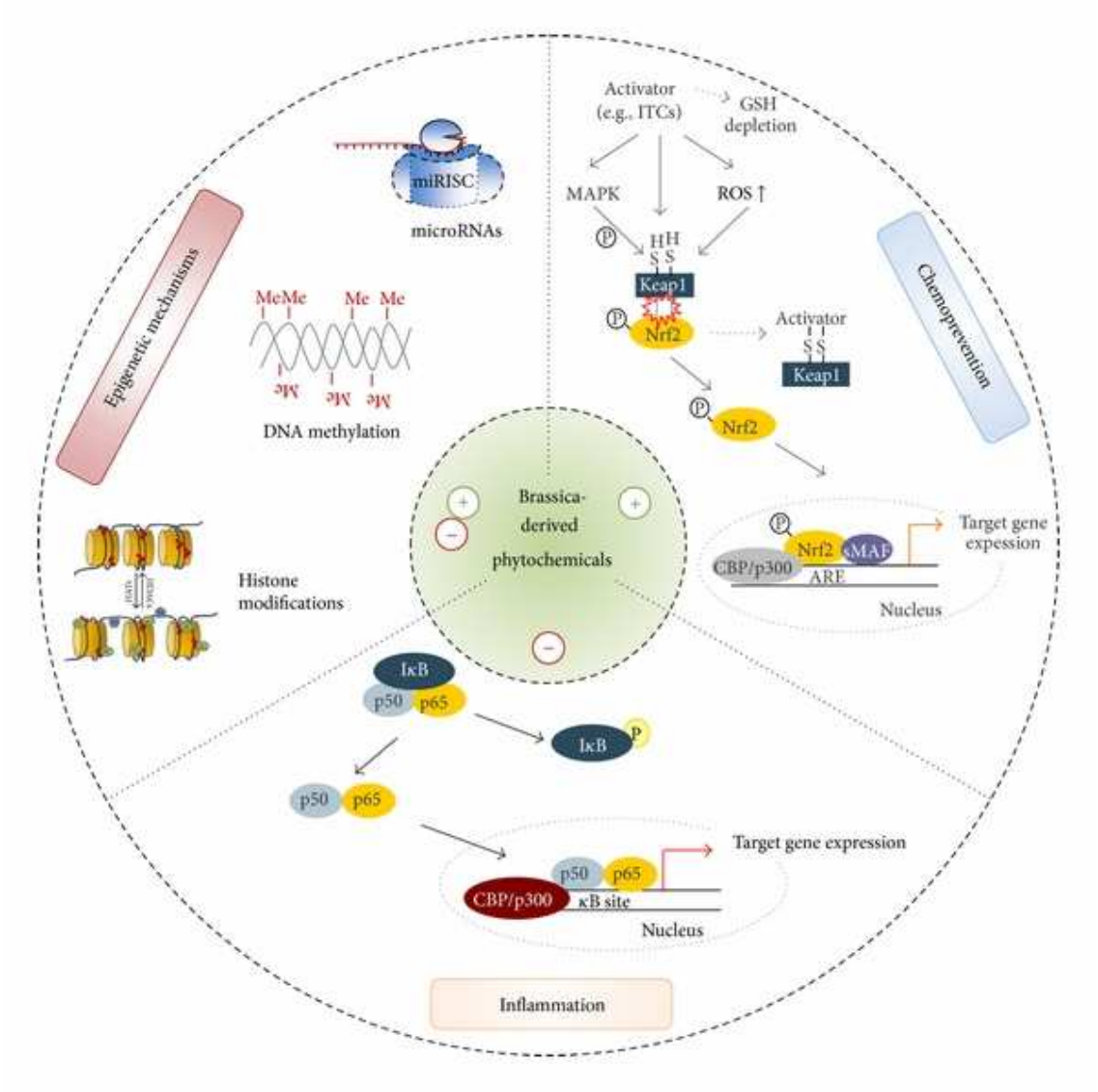

Figure 3. Summary of potential chemopreventive, anti-inflammatory and epigenetic mechanisms by which brassica-derived phytochemicals, like SF, may mediate health benefits (from Wagner AE, 2013).

5.3 Clinical Studies in humans

The first direct observation of SF's protective effect against cancer in humans was observed in 200 healthy adults (ages 25-65) from the Jiangsu Province of China, a region with a high rate of hepatocellular carcinoma due to dietary aflatoxin exposure and chronic hepatitis B infection. The primary end-point of this blinded, placebo-controlled trial was to determine if drinking daily broccoli sprout infusions (containing 400 μmol glucoraphanin) for two weeks could reduce urinary excretion of aflatoxin DNA adducts– indicators of DNA damage. A highly significant inverse association was observed for excretion of dithiocarbamates (isothiocyanate metabolites of glucoraphanin) and aflatoxin-DNA adducts in individuals consuming broccoli sprout infusions. Genetic polymorphisms of the glutathione S-transferase enzyme involved in glucoraphanin metabolism may also be partially responsible, affecting dithiocarbamate formation from sulforaphane (Kensler TW, 2005).

From 2004 to 2013 there are published some clinical trials, controlled clinical trials, and randomized clinical trials (RCTs) on the possible effects of the consumption of broccoli, or SF on humans.

The most consistent results in humans are those related to the clinical parameters blood glucose and lipid profile (Bahadoran Z, 2012; Mirmiran P, 2012; Murashima M, 2004) and to molecular parameters of oxidative stress (Bahadoran Z, 2011; Murashima M, 2004, Kensler TW, 2012; Riso P, 2010; Riedl MA, 2009; Traka M, 2008; Gasper AV, 2007), either by increasing antioxidant defenses or by decreasing oxidative damage markers. The findings from these studies also indicated that there was a decrease in low-grade chronic inflammation (Mirmiran P, 2012) and in H. pylori colonization (Galan MV, 2004; Yanaka A, 2009), as well as a higher protection against cancer due to the inhibition of tumorigenesis pathways (Traka M, 2008) or to the excretion of potentially carcinogen metabolites (Kensler TW, 2012; Kensler TW, 2005).

There are two studies that have investigated cellular redox state by measuring the expression or activity of antioxidant enzymes (Riedl MA, 2009) and levels of oxidative stress markers (Murashima M, 2004). The first study found a dose-dependent increase in antioxidant defences. Daily intakes of 200g of broccoli sprouts due to a dose-

dependent increase in the expression of the enzymes glutathione S-transferase M1 (GSTM1), glutathione S-transferase P1 (GSTP1), NQO1, and HO-1, by 119%, 101%, 199% and 121% respectively compared with baseline values (Riedl MA, 2009). The other study evaluated the oxidative stress markers phosphatidylcholine hydroperoxide, 8-isoprostane, and 8-hydroxydeoxyguanosine, and found decreases of 17%, 39% 25% respectively in their levels and an increase of 50% in the reduced/oxidized coenzyme Q ratio compared with pre-intervention values. This study also evaluated the toxicity of bioactive compounds from broccoli by assessing liver function tests (transaminases), uric acid levels, urea levels, and natural killer cell activity, and did not find any difference in their values after treatment (Murashima M, 2004).

It is also important to comment that the anti-inflammatory effect of SF shown by the decrease in IL-6, PCR and TNF-α levels, has been evaluated in one human study (Mirmiran P, 2012).

Although there is consistent epidemiological evidence on the association between the consumption of cruciferous vegetables with a lower risk of cancer, there are few intervention studies in humans. The most convincing evidence comes from two intervention studies that evaluated H. pylori infection (Galan MV, 2004; Yanaka A, 2009).

Regarding epigenetic mechanisms, given the level of HDAC inhibition in PBMCs obtained from mice fed SF (Myzak MC, 2006), a pilot study was conducted in human healthy volunteers to investigate the effect of a single dose of SF-rich broccoli sprouts on HDAC activity in PBMCs. Healthy volunteers in the age range 18-55 years, with no history of non-nutritional supplement use, refrained from cruciferous vegetable intake for 48 h. Each subject consumed 68 g (one cup) of broccoli sprouts, and blood was drawn at 0, 3, 6, 24 and 48 h following sprout consumption. In the PBMCs of all subjects, HDAC activity was inhibited as early as 3 h after broccoli sprout intake, and returned to normal by 24 h. This was the first study to show that a naturally consumed food in humans, namely broccoli sprouts, had such a marked effect on HDAC activity (Myzak MC, 2007).

Although phytochemicals such as SF, have attracted attention owing to their in vitro neuronal potentiating activity, their in vivo and clinical efficacy has yet to be

established in randomised controlled trials. Therefore, further research is necessary to prove the neuroprotective effects in preclinical models and in humans.

5.4 Dietary recommendations

5.4.1 Bioavailability of sulforaphane

Hydrolytic conversion of glucoraphanin to SF occurs through the action of physical damage to the plant, by either the action of plant-derived myrosinase (**Figure 1**) or the microbiota of the human colon. After rapid diffusion into the cells of the intestinal epithelium, SF undergoes metabolism via the mercapturic acid pathway. This process involves its initial conjugation with glutathione, rapidly catalyzed by important glutathione S-transferase (GST) enzymes. The process of N-acetylation (to form sulforaphane-N-acteylcysteine) is important for the subsequent excretion of sulforaphane from the body. The basis for the distribution of sulforaphane is the high degree of binding to glutathione, and its capacity to drive passive diffusion (Fahey J, 1997; Conaway CC, 2001).

Pharmacokinetic studies in both humans and animals showed that the plasma concentration of SF and its metabolites increased rapidly, reaching a maximum between 1 and 3 h after administration of either SF, glucosinolate, or broccoli (Gasper AV, 2005; Veeranki OL, 2013).The SF metabolites are distributed throughout the body and accumulate in different tissues, with unpublished data from Franklin and coworkers after a whole body autoradiographic study in rats suggesting that high concentrations of isothiocyanate metabolites are present in the gastrointestinal tract, liver, kidneys, and blood.

There are many factors that may affect the bioavailability, and therefore overall therapeutic benefit, of dietary SF, including pharmacokinetic properties, genetic variation, and food preparation (Clarke JD,2011).

Glucoraphanin is relatively stable under chemical and thermal conditions, and, therefore, hydrolysis is mainly enzymatic (myrosinase mediated). Cooking and/or blanching (during freezing process) of cruciferous vegetables inactivates myrosinase, and has been shown to decrease the bioavailability of SF (Clarke JD, 2011). In general, results suggest that only about 30%–50% of the initial administered dose is excreted after these preparation processes. Boiling for more than 1 min, or steaming for more than 4–5 min has been shown to lead to the loss of myrosinase activity (Clarke JD, 2011).

The in vivo bioactivity of each SF metabolite is still unclear, although many in vitro studies have shown the ability of SF-Cys, and SF-NAC metabolites to exert some bioactivity (Clarke JD, 2011). These data suggest the hypothesis that repeated consumption of SF or cruciferous vegetables is required to maintain the SF metabolite concentration in tissues.

In order to exert protective effects towards neurodegenerative disorders or improve brain function, SF must traverse the blood-brain barrier (BBB) and accumulate in the central nervous system (CNS). Several studies in animal models of neurodegeneration suggest the ability of SF to reach CNS and to display protective effects at this level. For instance, SF is able to cross the BBB and to accumulate in cerebral tissues such as the ventral midbrain and striatum, with a maximum increase and disappearance after 15min and 2 h, respectively (Jazwa A, 2011). These results show the ability of SF to quickly reach the CNS and the potential contribution of SF metabolites to prolong the presence of SF at this level because they are unstable under physiological conditions and readily dissociate back to SF (Veeranki OL, 2013; Jazwa A, 2011).

5.4.2 Safety of the SF

Several studies have been conducted to assess the safety of SF in humans. A randomized, placebo-controlled, double-blind study showed broccoli sprout extracts were without significant side effects at doses of 25 and 100 μmol glucoraphanin for seven days. Another randomized, placebo-controlled study involving 200 healthy adults consuming broccoli sprout infusions daily for two weeks (400 μmol or approximately

175 mg glucoraphanin) showed no adverse effects (Kensler TW, 2005). In a dose escalation safety study, broccoli sprout extracts containing SF doses as high as 340 nmol were topically applied three consecutive times to forearm skin. Researchers reported significant induction of phase II enzyme activity in biopsied tissue without any adverse reactions (Dinkova-Kostova AT, 2007).

5.4.3 Dietary lifestyles and measures

Phytochemicals present in vegetables and fruits are believed to reduce the risk of several major diseases including cardiovascular diseases, cancers as well as neurodegenerative disorders. Therefore people who consume higher vegetables and fruits may be at reduced risk for some of diseases caused by neuronal dysfunction (Selvam AB, 2008; Lobo V, 2010).

In general, many organizations, including the National Cancer Institute, recommend eating a variety of fruit and vegetables daily (serving number depends on age, sex, and activity level; see www.fruitsandveggiesmatter.gov). In particular, consumption of cruciferous vegetables such as broccoli, collard greens, Brussels sprouts, kohlabi, red cabbage, and kale which can provide SF, can provide some beneficial effects for the health, although separate recommendations for cruciferous vegetables have not been established.

The most sources of SF and/or glucoraphanin include:

- Broccoli (44-171mg/100g dry weight (Nakagawa K, 2006)
- Broccoli sprouts (1153mg/100g dry weight (Nakagawa K, 2006)

Although an ideal dosage is not known, supplementation of 0.1-0.5mg/kg SF to rats has been noted to be bioactive. This is an estimated human dose of:

- 1.1-5.5 mg for a 150lb person
- 1.5-7.3 mg for a 200lb person
- 1.8-9.1 mg for a 250lb person

While, in diets, the whole broccoli is consumed, yet most data supporting the protective potential of broccoli against different cancers and several other diseases have focused on purified or semipurified SF, or a water extract of broccoli sprouts, rather than the whole broccoli.

Regular consumption of sprouts of broccoli, because its phytochemical properties and that the intake of these occurs in its natural matrix, increases the bioavailability of the bioactive compounds, stimulating mechanisms defense of the organism of more efficiently than commercial inflorescences of broccoli. Thus, there is approximately 15-fold more glucoraphanin in 3-day-old broccoli sprouts (cv Saga) than in the florets of mature cultiva (Fahey, 1997).

By other hand, aqueous extracts of broccoli sprouts is an excellent vehicle for delivering the chemopreventive activity of SF and this has also been demonstrated in several studies (Dinkova-Kostova AT, 2007; Zhang Y, 2006).

So there is also a need to design more experimental and clinical studies to evaluate the health effects of whole broccoli, specifically in the context of neuropathological conditions, to supplement the existing reporting on bioactive components and plant extracts. The results of some epidemiological studies suggest that adults should aim for at least five weekly servings of cruciferous vegetables (Feskanich D, 2000; Giovannucci E, 2003).

These low quantities are likely attainable via raw broccoli or cruciferious vegetable products, while higher doses may be further beneficial. However, the optimal supplemental dose of SF is still unknown.

5.4.4 Dietary advices

The Healthiest Way of Cooking Broccoli

To cook broccoli is better with a low cooking temperature in a range that includes the steaming temperature of 212°F (100°C), with a cooking times of 5 minutes at the most. Since the fibrous stems take longer to cook, they can be prepared separately for a few minutes before adding the florets.

There may be some special advantages for the digestive tract when broccoli is eaten in uncooked form. With fresh raw broccoli, simple slicing a few minutes prior to eating or thorough chewing of unsliced pieces will help activate sulfur-metabolizing enzymes.

Another form of broccoli to enjoy raw broccoli is broccoli sprouts. Some of the nutrients found in broccoli—like vitamin C—are especially concentrated in broccoli sprouts.

A Few Quick Serving Ideas

- Toss pasta with olive oil, pine nuts and steamed broccoli florets. Add salt and pepper to taste.
- Purée cooked broccoli and cauliflower, then combine with seasonings of your choice to make a simple, yet delicious, soup.
- Add broccoli florets and chopped stalks to omelets.

6. DISCUSSION

The study on SF has been increasing due to accumulating evidences about its beneficial effects on health. Today, SF shows diverse therapeutic actions making it a strong candidate for human therapeutic application.

In the context of neurodegenerative disorders, the discovery of the fact that SF induces cytoprotective proteins via Nrf2 pathway has prompted the study of this compound in several experimental models associated with oxidative damage and inflammation. Thus several in vitro and in vivo studies have demonstrated the ability of SF to exert neuroprotective effects activating the Nrf2/ARE pathway (Ma Q, 2012; Calkins MJ, 2009). This pathway has been shown to be neuroprotective in many different paradigms of neuronal injury or neurodegeneration (Siebert, A, 2009; Jakel, R.J, 2007). Given the striking neuroprotective effects of Nrf2 activation, it is reasonable to assume that nutraceutical Nrf2 inducers, like SF, may provide significant therapeutic benefit against neurodegeneration.

A novel neuroprotective mechanism of SF could be through its HDAC inhibitory activity (Dashwood R, 2008; Myzak MC, 2007). SF-mediated HDAC inhibition activity causes a wide range of epigenetic alterations in many genes which are actively involved in malignant progression of cancer cells. The SF-mediated HDAC inhibition might be due to the possible direct interaction with SF on the HDAC active site (Myzak MC, 2004).
In the context of neurodegenerative disorders, the promising results obtained with HDAC inhibitors in Huntington's disease, epilepsy, and bipolar disorder (Butler R, 2006; Phiel CJ,2001) suggest that 'epigenetics' will likely impact upon multiple disease areas, not simply cancer therapeutics.

There is a need to better define the precise mechanisms involved, such as the specific HDAC targets and the downstream pathways affected. These mechanisms could be cell-type specific, due to the unique epigenetic marks laid down in each tissue; thus, protection theoretically might be achieved with the same dietary agent against motor

neuron loss in neurodegenerative disorders, or aberrant vascular changes leading to stroke.

Nutrients can act as the source of epigenetic modifications and can regulate the placement of these modifications. While DNA sequences cannot be changed and aging cannot be avoided, individuals have the ability to change their diet. There is significant impetus to continue research within the field of nutritional epigenetics as the findings may support significant public health applications.

Aging and age-related diseases are associated with profound changes in epigenetic patterns, though it is not yet known whether these changes are programmatic or stochastic in nature. Future work in this field seeks to characterise the epigenetic pattern of healthy aging to ultimately identify nutritional measures to achieve this pattern.

7. CONCLUSIONS

The study on SF has been increasing due to accumulating evidences about its beneficial effects on health. Today, SF shows diverse therapeutic actions making it a strong candidate for human therapeutic application.

The discovery of the fact that SF has the ability to exert neuroprotective effects in different acute and chronic neurodegenerative diseases appears to making it a promising compound with neuroprotective properties that may play an important role in preventing neurodegenerative diseases.

Today, further studies will be required to discover SF maximal protective effects, the involving players and the way it acts on different human disease models.

Because cruciferous vegetables provide SF, regular intake of cruciferous vegetables in the diet, could exert beneficial effects on neurological diseases.

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