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SULFORAPHANE: A POTENTIAL CHEMOPREVENTIVE AGENT

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ABSTRACT
Chemoprevention of cancer is intended to control the growth of cancer using fruits and vegetables or chemical compounds, in which their mechanism is by reversing or suppressing carcinogenesis. Sulforaphane (4-methysulfinylbutylisothiocyanate, SFN), an isothiocyanate compound contained in Brassicaceae family plants commonly credited to broccoli, is known to exhibit many pharmacology properties, mostly as anticancer. In this review study, we summarize the present knowledge about the chemistry and various pharmacological activities of SFN, furthermore its underlying mechanism as chemopreventive agent. These studies proved that this compound shows 40% bioavailability. Pharmacokinetic studies in human showed that the Cmax of SFN and its metabolites increased rapidly at 1 and 3h after administration. SFN exhibits anti-inflammatory, antioxidant, and anticancer activities, among other minor activities. The mechanism of anticancer activity of SFN was suggested could be attributed to: (1) its inducing capacity on caspase-8 and P21 expression and its down-regulating on hsp90; (2) its ability to selectively decreasing HDAC activity which eventually inducing cell cycle arrest and apoptosis in BPH1, LnCap, and PC3 cells but not PrEC cells; (3) SFN also increased acetylated histone H3 at the promoter for P21 and increased tubulin acetylation in prostate cancer cells; (4) SFN activates the Nrf_2 signaling pathway and protects against H_2O_2-mediated oxidative damage in normal colonic cells.

KEYWORDS: Brassicaceae, Chemoprevention, Isothiocyanate, Glucosinolates, Sulforaphane.

1. INTRODUCTION
Chemoprevention of cancer was intended to control the growth of cancer using fruits and vegetables, noncytotoxic drugs or chemical compounds. The future of these chemopreventive agents depends on the molecular mechanism of carcinogenesis and its related issues.[1,2] According to their mechanisms of action, chemopreventive agents are classified into blocking agents (e.g. flavonoids, oltipraz, indoles, and isothiocyanates) and suppressing agents (e.g. vitamin D and related compounds, NSAIDS, vitamin A and retinoids, etc.). The first class works by preventing the metabolic activation of carcinogens or tumor promoters via enhancing detoxification systems, whereas the other prevents the evolution of the neoplastic process in cells that shows tendency to become malignant.[3]

Sulforaphane (4-methysulfinylbutylisothiocyanate, SFN), an isothiocyanate compound contained in Brassicaceae family vegetables, commonly credited to broccoli, has been the focus of many *in vitro* and *in vivo* studies. SFN inhibits mitochondrial respiratory chain in an antimycin-like fashion, and eventually generates reactive oxygen species (ROS). Thus, SFN cytotoxicity might be attributed to the generation of ROS, which has been identified as playing a central role in promoting apoptosis and autophagy of target cells.[4]

This paper provides a systematic overview on the chemistry of SFN, its pharmacology activities and its underlying mechanism as chemopreventive agent, which were collected from various journals in worldwide accepted scientific database, using *sulphoraphane* as the keyword.

2. CHEMISTRY OF SULFORAPHANE
Many evidences indicated that broccoli sprouts might have important physiological functions for maintaining and improving our health status, and moreover could counteract many diseases, i.e. inflammation, cardiovascular diseases, and cancer.[5,6] Brassicaceae vegetables (e.g. broccoli, Brussels sprouts, cauliflower, daikon, watercress, etc.) are rich in glucosinolates, a large group of sulfur-containing glucosides.[7,8] Glucosinolates in Brassicaceae family are cleaved by myrosinase to yield two phytochemicals, that recently

have been the focus of many researchers, i.e. SFN and indole-3-carbinol (I3C). Young sprouts of broccoli contain 20–50 times more glucoraphanin (GRP) than mature plants.[9-12]

SFN (4-methysulfinylbutylisothiocyanate) (Figure 1) had been successfully quantified from the ethyl acetate extract of broccoli seed by using RP-HPLC coupled with an evaporative light-scattering detector (ELSD).[13] Optimal temperature and pH for sulforaphane formation from broccoli were 14-25 °C, pH of 5-6, respectively.[12]

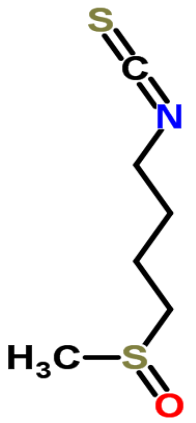

Figure. 1. 2D structure of sulforaphane, molecular formula $C_6H_{11}NOS_2$. Chemspider ID: 5157 (downloaded from http://www.chemspider.com/Chemical-Structure.5157.html, in May 2018).

SFN was easily absorbed. The bioavailability of a single intake of SFN was up to 40%.[14] Pharmacokinetic studies in human showed that the Cmax of SFN and its metabolites increased rapidly at 1 and 3h after administration. The subjects who were given broccoli supplement showed lower bioavailability compared to those consumed fresh broccoli sprouts.[15-19]

3. PHARMACOLOGICAL ACTIVITY: The pharmacological activities of SFN is presented in Table 1.

Table 1. Pharmacological activities of SFN.

Reference	Pharmacological activity	Mechanism of action
Zhao et al., 2018	Alzheimer's disease	Upregulates Nrf2 expression Promotes Nrf2 nuclear translocation.
Zhao et al., 2018	Antioxidative and anti-inflammatory	Reduces the pro-inflammatory cytokines levels.
Jiang et al., 2014	Antidiabetic	Prevents apoptosis and testicular oxidative damage, that may be associated with the continuity of expression and function of testicular Nrf2 under diabetic condition in type 1 diabetes mice.
Patel et al., 2018		Modulates Nrf2, NFκB and PPARγ signaling and crosstalk.
Angeloni et al., 2009	Antihypertensive and Cardioprotective	Decreases production of intracellular ROS, increase cell viability, and after long-term treatment decreases DNA fragmentation which accompanied by antioxidants and phase II enzymes induction in cardiomyocytes.
Choi et al., 2018		Inhibits histone deacetylase (HDAC) activity and showed mild inhibition of HDAC2 and HDAC6 enzymatic activities.
Martins et al., 2018	Antiobesity	Prevents and treats obesity in obese animals.
Kerr et al., 2018	Adjuvant therapy for cancer	SFN-cisplatin treatment suppresses the growth of cancer stem cell spheroids, cell proliferation, migration and Matrigel invasion of HaCaT and SCC-13 cells.
Fimognari et al., 2007		SFN–doxorubicin reduces potential toxicity of doxorubicin.
Milczarek et al., 2011		SFN-5-fluorouracil treatment reduces of the viability of MDA-MB-231 cells and induces autophagy of cell death and premature senescence.
Lenzi et al., 2014	Anticancer	Promotes potent cytostatic and cytotoxic effects by the modulation of different molecular targets.
Royston et al., 2017		Combination of withaferin A (WA) and SFN regulates key epigenetic modifiers and promotes cancer cell death in MDA-MB-231 and MCF-7 cell lines.

		SFN-WA down-regulated HDAC expression at multiple levels, affected the pro-apoptotic BAX and anti-apoptotic BCL-2, decreased BCL-2 and increased BAX.
Kanematsu et al., 2011		Controls breast cancer by cytoprotective role of autophagy in apoptosis.
Qazi et al., 2010		Induces caspase-8 and p21 and down-regulates hsp90.
Arcidiacono et al., 2018		Reduces the antineoplastic effects by the simultaneous presence of other biological elements such as nerve growth factor.
Clarke et al., 2011		Selectively decreases HDAC activity. Induces cell cycle arrest and apoptosis in BPH1, LnCap, and PC3 cells. Increases acetylated histone H3 at the promoter for P21. Induced P21 expression and increases tubulin acetylation in prostate cancer cells.
Abdulah et al., 2009		Combination of SFN and selenium in selenium-enriched broccoli sprouts showed superior in inducing apoptosis, inhibiting cell proliferation, and decreasing prostate-specific antigen secretion of prostate cancer cells compared to normal broccoli sprouts. Selenium-enriched broccoli sprouts induced a downregulation of the survival Akt/mTOR pathway.
Fimognari et al., 2002		SFN produced *in situ* could trigger apoptotic death (and even necrosis at very high doses) and exert G2/M phase cell-cycle arrest of Jurkat tumor cells. These effects can inhibit the leukemic cell growth potentially.

3.1. Anti-inflammatory: SFN was reported as an alternative candidate for Alzheimer's disease (AD) treatment. SFN upregulated nuclear factor erythroid 2-related factor 2 (Nrf2) expression that exerts neuroprotective effects and promoted Nrf2 nuclear translocation through decreasing methylation levels of DNA in a cellular model of AD. SFN was leading to antioxidative and anti-inflammatory effects by reducing the pro-inflammatory cytokines levels, interleukin 1β (IL-1β) and IL-6, and decreased cyclooxygenase-2 (COX-2), phosphorylated nuclear factor-κB (NF-κB) p65, and iNOS protein expression levels in N2a/APPswe cells.[20,21] SFN inhibits IL-23 and IL-12 in dendritic cells.[22] Moreover, a combination of phenethyl isothiocyanate and SFN or curcumin and SFN resulted synergistic anti-inflammatory effect.[23]

3.2. Antidiabetic

SFN prevents apoptosis and testicular oxidative damage, that may be associated with the continuity of expression and function of testicular Nrf2 under diabetic condition in type 1 diabetes mice.[24] A combination of SFN and pyridoxamine could normalize endothelial dysfunction associated with type 2 diabetes.[25] Recent studies of SFN suggest that protective actions of SFN mediated by its various pro-inflammatory modulation (e.g. NFκB) and metabolic (e.g. PPARγ) signaling pathways. Nrf2, NFκB and PPARγ signaling and crosstalk were modulated by SFN and a critical evaluation the evidence linking provided these transcriptional pathways with cardiometabolic and diabetic complications and cytoprotection is mediated by SFN.[26]

3.3. Antiobesity

SFN has the potential in preventing and treating obesity which was demonstrated in *in vitro* studies. Recently, the few *in vivo* studies also shown that SFN could have a therapeutic effect in obese animals.[27]

3.4. Antihypertensive and Cardioprotective: SFN was confirmed as potential cardioprotective by decreasing production of intracellular reactive oxygen species, increase cell viability, and after long-term treatment decrease DNA fragmentation which accompanied by antioxidants and phase II enzymes induction in cardiomyocytes.[28] SFN was reported to inhibit histone deacetylase (HDAC) activity and showed mild inhibition of HDAC2 and HDAC6 enzymatic activities. SFN inhibited HDAC2 activity stronger than piceatannol.[29] This compound also indicated a protection against ischemic injury of the heart through antioxidant pathway and mitochondrial K(ATP) channels.[30]

3.5. Adjuvant Therapy for Cancer

The efficacy of SFN as adjuvant therapy monitored with cisplatin in squamous cell carcinoma. The combination SFN-cisplatin treatment is more efficient to suppress the growth of cancer stem cell spheroids, cell proliferation, migration and Matrigel invasion of HaCaT and SCC-13 cells. SFN treatment increases apoptosis of tumors or cultured cells and p21Cip1 level, and the combination increases the tumor apoptosis.[31]

Treatment of a doxorubicin-resistant phenotype of fibroblasts by a combination of SFN-doxorubicin showed that the resistance to doxorubicin was reversed by SFN. The combination of SFN–doxorubicin may

allow lower doses of doxorubicin administration and reduces potential toxicity of doxorubicin.[32]

The combination of SFN-5-fluorouracil increased the cell number in the S phase, but not statistically significant.[33] In MDA-MB-231 breast cancer line, SFN-5-fluorouracil treatment reduces of the viability of the cells. The sequential SFN and 5-fluorouracil treatment and SFN alone was observed has decreased the thymidylate synthetase level. SFN and 5-fluorouracil also act synergistically in induced autophagy of cell death and premature senescence.[34]

3.6. Anticancer: SFN has proven to be a potential and effective chemoprotective agent in various cell culture, i.e. breast cancer stem cells and lymphoblastic leukemia cells.[35,36] SFN promoted potent cytostatic and cytotoxic effects by the modulation of different molecular targets. Cell vulnerability to SFN-mediated apoptosis was subject to regulation by cell-cycle-dependent mechanisms but was independent of a mutated p53 status. Combination of SFN with cytotoxic therapy potentially give the cytotoxic effect intermediated by chemotherapy *in vitro* and suggest that in clinical settings the combination give potential therapeutic benefit. Moreover, SFN becomes an effective and harmless chemopreventive agent to fight cancer.[37]

Combination of withaferin A (WA) and SFN regulates key epigenetic modifiers and promotes cancer cell death in MDA-MB-231 and MCF-7 cell lines. The synergistic inhibition and a greater induction of apoptosis showed in cellular viability of MCF-7 cells. SFN-WA down-regulated HDAC expression at multiple levels, affected the pro-apoptotic BAX and anti-apoptotic BCL-2, decreased BCL-2 and increased BAX.[38] SFN and the combination of SFN treatment may be a promising strategy for breast cancer control as a cytoprotective role of autophagy in apoptosis and autophagy inhibition.[39]

In cell survival, SFN induced both time- and dose-dependent, decline cell cycle arrest, and apoptosis. The expression of multidrug resistance protein, reduced drug efflux was suppressed by treatment with SFN. SFN improved anticancer activity of other antiproliferative agents including paclitaxel. SFN showed a significant reduction in tumor volume of Barrett esophageal adenocarcinoma (BEAC). The anticancer activity could be attributed to the induction of caspase-8 and p21 and down-regulation of hsp90, a molecular chaperone required for activity of several proliferation-associated proteins.[40]

Recent *in vivo* study evidence that SFN reduced the antineoplastic effects by the simultaneous presence of other biological elements such as nerve growth factor. SFN contributes to the real *in vivo* potentiality as antineoplastic candidate.[41]

SFN selectively decreased HDAC activity, Class I and II HDAC proteins, induced cell cycle arrest and apoptosis in BPH1, LnCap, and PC3 cells but not PrEC cells. SFN also increased acetylated histone H3 at the promoter for P21, induced p21 expression and increased tubulin acetylation in prostate cancer cells. HDAC6 over-expression was able to reverse SFN-induced cytotoxicity. In PrEC cells, SFN caused only a transient reduction in HDAC activity with no change in any other endpoints tested.[42]

Combination of SFN and selenium in selenium-enriched broccoli sprouts showed superior in inducing apoptosis, inhibiting cell proliferation, and decreasing prostate-specific antigen secretion of prostate cancer cells compared to normal broccoli sprouts.[43] This combination reduces oxidative damage in colonic CCD841 cells.[44] Furthermore, selenium-enriched broccoli sprouts induced a downregulation of the survival Akt/mTOR pathway.[44]

SFN produced *in situ* could trigger apoptotic death (and even necrosis at very high doses) and exert G2/M phase cell-cycle arrest of Jurkat tumor cells. These effects can inhibit the leukemic cell growth potentially.[45]

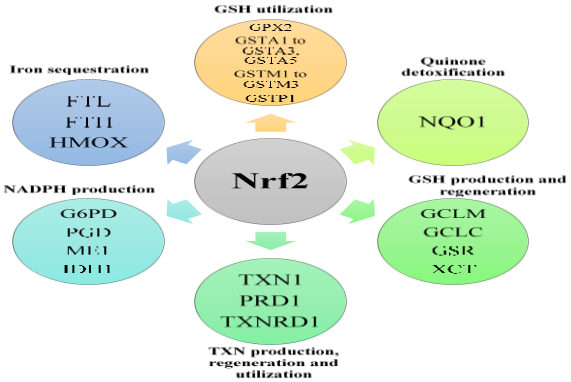

Figure. 2.

Figure 2. Nrf2 controls several different antioxidant pathways: (1) Glutathione (GSH) production and regeneration, which is regulated by the glutamate-cysteine ligase modifier complex (GCLM), the GCL catalytic subunit (GCLC), the cystine/glutamate transporter XCT, and glutathione reductase (GSR); (2) Glutathione utilization, which is regulated by glutathione S-transferases (GSTA1, GSTA2, GSTA3, GSTA5, GSTM1, GSTM2, GSTM3, and GSTP1) and glutathione peroxidase 2 (GPX2); (3) Thioredoxin (TXN) production, regeneration and utilization which is regulated by TXN1, thioredoxin reductase 1(TXNRD1) and peroxiredoxin 1 (PRDX1); (4) NADPH production, which is controlled by glucose-6-phosphate dehydrogenase (G6PDH), phosphoglycerate dehydrogenase (PHGDH), malic enzyme 1 (ME1) and isocitrate dehydrogenase 1 (IDH1). Both GSH and TXN require NADPH in order to regenerate once they have reduced reactive oxygen species. These four groups of

antioxidant genes,—which are all upregulated by NRF2—have both complimentary and overlapping functions. Additional antioxidants that are controlled by NRF2 include NAD(P)H:quinone oxidoreductase 1 (NQO1) and enzymes regulating iron sequestration, such as heme oxygenase (HMOX1), ferritin heavy chain (FTH) and ferritin light chain (FTL).[46]

4. FUTURE DIRECTIONS

SFN is interesting to be further explored for its chemoprevention activity and/or its potency as adjuvant therapy for cancer. This compound could be combined with other drugs or chemicals to obtain optimum chemoprevention activity.

5. CONCLUSIONS

Various pharmacology properties of SFN shows that this compound is potential for future phytomedicine. Moreover, SFN involves in many enzymatic and signaling pathways that eventually could protect, inhibit, or activate several mechanisms as a defense against cancer growth.

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