NUTRITIONAL MEDICINE REVIEWS

Sulforaphane, 3,3'-Diindolylmethane and Indole-3-Carbinol: A Review of Clinical Use and Efficacy

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ABSTRACT

Sulforaphane (SFN), 3,3'-diindolylmethane (DIM) and indole-3-carbinol (I3C, a precursor of DIM) are compounds that are obtained through eating cruciferous vegetables, which have been shown in epidemiological studies to have health benefits. SFN and DIM have been shown to have antioxidant, anti-inflammatory and anti-cancer effects, as well as playing important roles in cellular detoxification of xenobiotics through their effects on nuclear factor-kappa B (NF-kB) and nuclear factor erythroid 2-related factor 2 (Nrf2). DIM and I3C have also been shown to affect oestrogen metabolism. In view of these properties, SFN, DIM and I3C have been studied in clinical trials and, although clinical research is still limited, promising results have been seen in a number of health conditions.

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INTRODUCTION

Epidemiological studies have shown that cruciferous vegetables, such as broccoli, kale, cabbage, bok choy, cauliflower, collards, kohlrabi and Brussels sprouts, may have a protective effect, for example, with regards to certain cancers and cardiovascular disease (CVD), although associations are weak and not always consistent.[1,2] These vegetables contain glucosinolates, including glucoraphanin (GPN). Glucosinolates are broken down by the enzyme myrosinase during storage, food preparation and/or chewing, yielding a variety of isothiocyanates, such as sulforaphane (SFN), and indoles, such as indole-3-carbinol (I3C).[1,3] I3C is unstable and is converted to other compounds, including 3,3'-diindolylmethane (DIM; about 60% of I3C is converted to DIM).[1]

The enzyme myrosinase is present in cruciferous vegetables, which also include watercress, radishes, rocket and mustard seeds, and starts acting on glucosinolates during chopping or chewing the vegetables. Whilst humans do not produce the enzyme, some of our gut bacteria do; therefore, the composition of the microbiome and exposure to antibiotics can influence bioavailability.[2,3] Conversion of I3C to DIM is also dependent on the acidic environment of the stomach, so increasing the gastric pH, for example through acid-lowering medication, may also affect bioavailability.[1]

After ingestion, the highest concentration of DIM is found in the liver, with lower concentrations in the kidneys, lungs, heart, plasma and brain. Metabolites of DIM can be found in both serum and urine, but there is significant clearance within 24 hours.[1] Similarly, SFN reaches peak concentrations within 1–3 hours after ingestion, with levels reverting to baseline after 72 hours.[3]

The amounts of SFN, I3C and DIM available from foods vary depending on cultivation of vegetables and food preparation, with broccoli sprouts having the highest content. All three compounds have been used in both preclinical and clinical trials in a variety of conditions and, below, human clinical studies will be explored by condition rather than compound used.

GENERAL EFFECTS

Anti-inflammatory and antioxidant effects

Nuclear factor-kappa B (NF-kB) is a protein complex regulating the expression of genes involved in various inflammatory processes. By supressing NF-kB signalling pathways, SFN can attenuate a number of inflammatory mediators and enzymes.[2] DIM has also been shown to reduce inflammation by reducing inflammatory enzymes and signalling.[1] Both SFN and DIM can also induce various antioxidant enzymes via nuclear factor erythroid 2-related factor 2 (Nrf2), an important regulator of expression of antioxidant enzymes.[1,2]

Preclinical findings are backed up by human intervention trials. For example, a study in 40 overweight but otherwise healthy individuals showed that 30 g of broccoli sprouts, a source rich in glucosinolates, per day for 10 weeks significantly reduced the inflammatory markers interleukin-6 (IL-6) and C-reactive protein (CRP). Whilst CRP reverted to baseline value in the 10-week follow-up phase, IL-6 remained lower than baseline.[4]

Further studies evaluating anti-inflammatory and antioxidant biomarkers with varying results are discussed under several conditions, see below.

Effects on detoxification

Nrf2 is not only a major regulator of antioxidant enzymes, but also of cellular detoxification through regulating gene expression. SFN can affect Nrf2 activity and thus induce enzymes of Phase II detoxification. Detoxification, or better biotransformation, of toxins, as well as metabolic end-products, excess or old hormones, happens in a two-step process. In Phase I, the usually fat-soluble compound is converted into a more reactive compound, which can then be conjugated to one of a number of amino acids by specific enzymes (Phase II) to make them water soluble and ready for excretion via bile or urine. The intermediates created in Phase I are generally more reactive and dangerous than the initial toxin.[2] Like SFN, DIM has been shown to stimulate cellular detoxification by affecting Nrf2 signalling, as well as by promoting the expression of the cytochrome P450 (CYP) family of enzymes (Phase I).[1]

In addition to a large body of preclinical evidence, a number of small, human studies have found that consuming broccoli in large amounts or broccoli high in glucosinolates can induce a number of enzymes involved in the detoxification of xenobiotics (substances that are not naturally present in our bodies).[5,6]

During detoxification, free radicals and reactive oxygen and nitrogen species are produced, and these can cause oxidative damage. As discussed above, SFN and DIM can induce antioxidant enzymes, thus also supporting this aspect of detoxification.

Detoxification of air pollutants

Four randomised-controlled trials (RCTs) from Qidong, China, a region with high levels of air pollution and a high incidence of liver cancer due to a high level of aflatoxin consumption, have evaluated the potential of SFN and GPN to increase detoxification of air pollutants.[7,8,9,10] They found that broccoli sprout drinks significantly increase urinary excretion of benzene and acrolein, two air pollutants.[8] Drinks with different levels of SFN and GPN were used, and both SFN and GPN were found to be effective on their own.[9] A combination of 600 µmol GPN and 40 µmol SFN increased benzene excretion by 63%, whilst drinks with lower levels of active ingredients failed to increase benzene excretion statistically significantly more than placebo.[7]

Effects on oestrogen metabolism

Uncharacteristically for a hormone, oestrogen is not just one specific hormone, but actually a group of interchangeable hormones, oestrone (E1), oestradiol (E2) and oestriol (E3), with varying biochemical and physiological characteristics. Oestrogens need to be metabolised for excretion and, therefore, biotransformation (or detoxification) of oestrogen plays an important role in regulating hormone metabolism.

DIM can affect the CYP enzymes, including CYP1A1, CYP1A2 and CYP3A4, involved in this biotransformation and therefore the balance of certain oestrogen metabolites, in particular 2-hydroxyestrone (2OHE1), 4-hydroxyestrone (4OHE1) and 16-hydroxyesterone (16αOHE1) and the 2OHE1:16αOHE1 ratio, which have been implicated in hormone-related cancers, including breast and ovarian, as well as other hormone-related disorders. Whilst 4OHE1 and 16αOHE1 have been associated with an increased risk of breast cancer and 2OHE1 has been suggested to have protective effects against breast cancer, it is important to note that they need to be in balance for good health.[1] Due to the fact that DIM can strongly affect that balance, it would be prudent in clinical practice to check levels before initiating supplementation.

These metabolites have been used as biomarkers for breast cancer risk and other conditions, which are discussed below.

Anti-cancer effects

The initiation and progression of cancer involves both genetic and epigenetic changes, which lead to a dysregulation of gene expression. Whilst genetic mutations are irreversible, epigenetic alterations can be affected by diet, nutraceuticals and lifestyle.[3]

All of the factors mentioned above, antioxidant and anti-inflammatory effects, detoxification of carcinogenic substances and regulation of hormone balance, contribute to the anti-cancer effects of SFN and related compounds.[2] Further anti-cancer effects of SFN and/or DIM include their promotion of apoptosis (programmed cell death, which is aberrant in cancer cells), inducing cell cycle arrest (stopping cancer cells replicating), inhibiting angiogenesis (the formation of blood vessels to feed the tumour), and reducing invasion of tissues and formation of metastases.[1,3] A number of studies have shown large amounts of broccoli to protect cells of smokers from DNA damage.[11,12]

Human clinical studies looking at the impact of SFN, DIM and I3C on cancer biomarkers are discussed below in the section 'Clinical uses' under the respective cancer.

CLINICAL USES

Asthma

Oxidative stress appears to play an important role in asthma, and SFN has been investigated in asthma and airway inflammation for its potential to alleviate oxidative stress through its ability to induce Phase II antioxidant enzymes.[13]

Most studies have looked at either induction of Phase II enzymes or markers of inflammation in the airways after short-term supplementation

(3–4 days) in either healthy individuals or people with asthma or other allergies. Two studies found no effects of SFN (100 g or 200 g broccoli sprouts) on Phase II enzymes or other antioxidant and/or anti-inflammatory markers.[14,15] Two other studies, on the other hand, found benefits in terms of reduction of inflammatory response[16] and induction of Phase II enzymes.[17] The latter study was a dose-escalation study and found a dose-dependent response with dosages of 100 g or more of a broccoli sprout homogenate necessary to see benefits. These four studies involved healthy volunteers rather than people with asthma.

One study, involving 44 people with moderate asthma, supplemented 100 µmol SFN for 14 days.[13] Interestingly, the results showed a significant inter-individual heterogeneity amongst the response to SFN, with 60% of patients experiencing an improvement with SFN in a challenge experiment, whilst 20% had a worsening, and the remaining 20% no change.

Whilst some of these results are promising, at this point there is not enough evidence to recommend SFN to people with asthma, as clinical trials evaluating potential benefits have given contradictory results and studies on longer-term supplementation are lacking.

Autism

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterised by impaired social communication and function, and affected individuals often display stereotypical interests and restrictive and repetitive behaviours. In the USA in 2018, the rate of ASD was thought to be 1 in 40, with boys four times more likely to be affected than girls.[18]

Abnormalities in glutathione (GSH) metabolism are thought to play an important role in ASD as GSH affects redox metabolism, oxidative stress, mitochondrial function, immune function, neuroinflammation and cell signalling.[18,19]

A 2020 review reported that all five RCTs included found benefits in a variety of outcome measures of broccoli sprouts or seeds in children with ASD.[18] Dosages ranged from about 1 to 2.5 µmol per kg body weight (µmol/kg) SFN, and duration of the studies ranged from 12 to 36 weeks. A follow-up 3 or more years after completion of one of the studies discussed in the review[20] found that out of 16 families who responded, nine were still supplementing their child with SFN with sustained improvements, one had discontinued SFN with lasting improvements, three felt that their child had not responded to the initial treatment, two were unsure as to whether to continue supplementation and one was planning on continuing, suggesting that for a substantial proportion of children, benefits were lasting for several years.[21]

A more recent double-blind, placebo-controlled trial of SFN versus placebo alongside risperidone (an anti-psychotic medication used in autism) in 60 children with ASD, aged 4–12 years, found that patients receiving SFN improved significantly more in terms of irritability and hyperactivity/non-compliance, but not other scores, than children in the placebo group.[22] Dosages were 50 µmol and 100 µmol for children weighing up to 45 kg or 45–90 kg, respectively, and the duration of intervention was 10 weeks.

Based on these study results, SFN appears to be a promising, safe support for children with ASD, with the most commonly used doses being 1–2.5 µmol/kg per day. Duration should be at least 10 weeks, with longer-term supplementation potentially maintaining benefits for years.

It is thought that the benefits of SFN are due to its ability to increase GSH levels, and as such protecting cells from oxidative damage and/or mitochondrial dysfunction and/or supporting excretion of toxic metals.[18]

Cancer

Breast cancer prevention

A number of genes have been shown to increase breast cancer risk. One of these is BRCA1, a tumour suppressor gene responsible for repairing DNA, which, when mutated, confers a very high risk of getting breast cancer (80% lifetime chance) and ovarian cancer (40%).[23] A number of studies have looked at the effect of DIM on biomarkers or other predictive factors

for developing breast cancer in healthy women with this mutation. One study in 23 women taking 100 mg DIM per day for 1 year found a reduction in breast tissue density, a recognised predictive factor of breast cancer risk in seven of the women, whilst one had an increase and the remaining 15 no change.[23] Levels of oestrogen and testosterone also reduced in this study, whilst other hormones remained unchanged. Another study in 15 BRCA1 mutation carriers found that 300 mg DIM per day for 4–6 weeks increased BRCA1 gene expression by 34%, although this did not reach statistical significance possibly due to the small numbers involved.[24] There was substantial heterogeneity between individuals, with an increase in 10 participants, a decrease in two and no change in one. The same study also looked at the 2OHE1:16αOHE1 ratio and found that there was no average change in the 10 pre-menopausal women, whilst the ratio approximately tripled in the three post-menopausal women.[25] Again this failed to reach statistical significance possibly due to the small numbers. Several other trials looked at the effects of either DIM or I3C on the 2OHE1:16αOHE1 ratio, which has been associated with breast cancer risk,[26] in women at high risk of developing breast cancer but not specifically carrying the BRCA1 mutation.

An RCT of 60 women comparing 400 mg I3C per day versus a fibre supplement versus placebo for 3 months found that there was a significant mean increase of the 2OHE1:16αOHE1 ratio from 0.72 to 1.17 in the I3C group after 1 month, which was maintained over the 3-month study period.[27] No change was observed in three of the 20 women receiving I3C, suggesting inter-individual differences in response. Similar results were seen in five obese but otherwise healthy women with 300 mg I3C per day for 5 months,[28] and with 400 mg I3C per day for 4 weeks in 17 healthy high-risk women.[29] The latter study also found corresponding increases in CYP1A2 and no further benefits from taking 800 mg I3C per day. An early study looked at the best dose of I3C for improving the 2OHE1:16αOHE1 ratio in at-risk women, and found 300 mg per day to be the minimum effective dose.[30]

Two RCTs evaluated the effects of DIM on oestrogen metabolism, and found increases in the 2OHE1:16αOHE1 ratio and sex hormone binding globulin with 300 mg per day for 12 months alongside tamoxifen, and a significant increase in 2OHE1 and a non-significant increase in 2OHE1:16αOHE1 ratio with 108 mg per day (the equivalent of 400 mg I3C) for 30 days, respectively.[26,31] Benefits have also been observed in peripheral blood mononuclear cell histone deacetylase (HDAC) activity, but not in other biomarkers (H3K18ac, H3K9ac, HDAC3, HDAC6, Ki-67, p21), with a broccoli seed extract providing 180 mg GPN per day for 2–8 weeks.[32]

It is important to note that none of the studies used breast cancer incidence as an outcome and that numbers of study participants were small. Whilst the evidence suggests that I3C and DIM can have positive effects on oestrogen metabolism and other breast cancer risk markers in women at high risk of developing breast cancer, at this point there is no evidence that DIM reduces breast cancer incidence in women. It also needs to be pointed out that not all breast cancers are oestrogen sensitive. There appears to be significant inter-individual variability in response to I3C and DIM. Functional testing, for example of the 2OHE1:16αOHE1 ratio, before and after 1 month of supplementation may therefore be a good strategy to advise whether these supplements are likely to be of benefit to the individual.

Cervical and other female cancers

Cervical intraepithelial neoplasia (CIN) is an abnormal, precancerous growth of cells on the cervix that can develop into cervical cancer and is usually associated with human papillomavirus (HPV). Surgical treatment of CIN, which requires local destruction of the cervix, can affect fertility and, as CIN commonly affects women of reproductive age, alternative therapies that do not affect fertility are being sought.[33]

Three studies looked at the potential benefits of DIM for CIN. In a study of 78 women with CIN, using DIM intravaginally, 100% of patients on 200 mg DIM and 90.5% of women on 100 mg DIM had complete regression of CIN after 180 days (baseline CIN I–II), which was significantly superior to placebo (61.1%).[34] However, two other studies looking at oral supplementation did not find DIM to be more effective than placebo in improving CIN or HPV status at dosages of 150 mg per day for 6 months and 2 mg/kg body weight for 12 weeks, respectively.[33,35]

A small trial of 27 patients with CIN II–III found that four of eight patients on 200 mg I3C per day and four of nine on 400 mg I3C per day had complete regression after 12 weeks, compared with none of the 10 patients on placebo.[36] The mean change in CIN grade showed a linear dose–response relationship. The 2OHE1:16αOHE1 ratio increased in both I3C groups and decreased in the placebo group, whilst there was no difference between I3C and placebo in HPV status, suggesting that the benefits are independent of HPV.

Beneficial effects of I3C have also been seen in a small uncontrolled trial in 12 women with high-grade vulvar intraepithelial neoplasia (VIN; a precancerous condition), with both 200 mg and 400 mg resulting in a significant improvement in symptoms (pain, itch), severity and size of lesions, although tissue biopsy from the worst-affected vulval areas revealed no improvement.[37] Results did not differ between the 200-mg and 400-mg groups.

One long-term study on I3C, 400 mg per day, in women with stage III–IV ovarian cancer showed significant benefits alongside standard therapy with and without additional epigallocatechin-3-gallate (EGCG; 400 mg per day). Overall survival was 60 months in the I3C and I3C + EGCG groups, compared with 44 months on standard therapy alone, and progression-free intervals were 39.5 months, 42.5 months and 22 months, respectively.[38]

Limited evidence suggests that oral DIM does not improve outcomes of CIN; however, there is limited evidence for the benefits of I3C in CIN, VIN and ovarian cancer at a dose of 200–400 mg per day for at least 12 weeks.

Prostate cancer

Prostate cancer is the second most common cause of cancer-related deaths in men in the USA, and is associated with deregulated androgen receptor (AR) signalling.[39] Prostate-specific antigen (PSA) is a protein produced in the prostate and, although not specific for prostate cancer, is used as a screening and monitoring tool in prostate cancer, with increasing PSA levels after radical prostatectomy being indicative of cancer progression.[40] Apart from the various anti-cancer effects of DIM observed in preclinical research, DIM is also thought to act as an AR antagonist leading to a decline in PSA.[41]

Two studies looked at the effects of DIM on prostate cancer biomarkers prior to prostatectomy. One study found that in 96% of patients there was a favourable effect on AR activity, and a decline in PSA was seen in 70% of the 28 patients on DIM, 450 mg per day, for 14–72 days.[41] The other study found a significant increase in the 2OHE1:16αOHE1 ratio with 400 mg DIM per day versus placebo for 21–28 days, but changes in other biomarkers failed to reach statistical significance.[42] DIM has also been investigated in prostatic intraepithelial neoplasia (PIN; a precancerous condition), and it was found that 900 mg DIM for 1 year improved the morphological index whilst this deteriorated in the placebo group, and led to a complete remission in 45% of patients whilst no complete regressions were seen in the placebo group.[43] Changes in a variety of clinical symptoms failed to reach statistical significance in this study.

Two studies evaluated the safety and tolerability of DIM in patients with prostate cancer or PIN. One dose-escalation study established the maximal tolerated dose to be 600 mg per day for up to over 1 year,[44] whilst the other study found 900 mg per day for 3 months to be well tolerated.[45] The former study also found a decrease or stabilisation of PSA in two of the 12 participants, whilst the other 10 experienced a slowing in PSA increase during supplementation.

A number of studies looked at SFN in the form of SFN-rich broccoli foods or extracts. Beneficial effects of SFN were seen on PSA in patients following prostatectomy[40] or with recurrent prostate cancer.[46] Dosages were 60 mg (339 µmol) SFN per day for 6 months and 200 µmol SFN per day for 20 weeks, respectively, with benefits seen from 3 months of supplementation. Another study found no benefit in various genetic/epigenetic biomarkers with SFN, 200 µmol per day; however, the duration of supplementation was only 4–8 weeks so may have been too short to find statistically significant improvements.[47]

One study found benefits of GPN-rich broccoli soups in patients at risk of prostate cancer

on active surveillance, with changes in gene expression and associated oncogenic pathways improving in a dose-dependent manner.[48]

Overall, the evidence suggests that DIM, SFN and GPN can have a beneficial effect on biomarkers for prostate cancer, with dosages of at least 450 mg DIM or 200 µmol SFN per day, and durations of at least 4 weeks for DIM and 3 months for SFN.

Other cancers

SFN, DIM and I3C have also been tested in a number of other cancer types, with results warranting further research.

Two studies have investigated long-term supplementation with I3C in recurrent respiratory papillomatosis (RRP), a benign disease associated with HPV but with the potential to become malignant and cause serious problems.[49,50] Standard treatment of this condition is surgical removal of papillomas as necessary. Both studies found that about one-third of patients achieved a full response, i.e. did not need any surgery during supplementation, about one-third had a partial response, i.e. needed surgery less frequently than before, and the remaining patients showed no response. No participant had a worsening of disease. The studies involved both adults and children from the age of 2 years. The adult dose was 400 mg I3C per day, whilst the dose was weight-adjusted for children. In one study the minimum length of supplementation was 8 months, the mean duration in the other was 4.8 years.

An RCT in patients with melanoma showed a dose-dependent increase in SFN in both plasma and skin, with dosages ranging from 50 to 200 µmol per day for 4 weeks, and significant decreases in pro-inflammatory cytokines and an increase in the tumour suppressor compound decorin.[51]

In a study in patients with pancreatic cancer who received palliative chemotherapy, the mean death rate was lower during the first 6 months of intake in the group receiving additional pulverised broccoli sprouts containing 508 µmol SFN and 411 µmol GPN per day, compared with those receiving placebo.[52]

Thyroid proliferative disease (TPD), which comprises thyroid cancer and goitre, is four–five times more common in women than men, and oestrogen is thought to play a role in its development. In a small, pilot study, seven patients with TPD were given 300 mg DIM per day for 2 weeks prior to total or partial thyroidectomy.[53] DIM was detected in thyroid tissue, blood and urine, and the ratio of 2OHE1:16αOHE1 increased, suggesting a positive effect on oestrogen metabolism in these patients, but no other outcomes were evaluated.

Cognitive function

Two human studies have looked at cognitive function. One double-blind, placebo-controlled trial found that GPN, 30 mg per day for 12 weeks, led to a significant improvement in cognitive performance in older adults.[54] A small open-label pilot study with seven patients with schizophrenia found limited evidence for the benefits of SFN, 30 mg per day for 8 weeks.[55] Whilst there was a statistically significant improvement in one cognitive test parameter, all other measures remained the same. In animal models, SFN has had benefits in traumatic brain injury, but human trials are outstanding.[56]

Overall, evidence for the benefits of SFN/GPN for cognitive function is limited but promising.

The mechanisms by which SFN exerts its benefits with regards to cognitive function are thought to be due to its antioxidant and anti-inflammatory effects.[54]

Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is associated with pulmonary and systemic oxidative stress, which correlate with severity of disease. Due to its antioxidant and anti-inflammatory benefits, SFN or its metabolites could be thought of as a potential supportive supplement.[57]

However, one double-blind, placebo-controlled trial of 85 patients with COPD found no significant change in Nrf2 expression, other inflammatory or antioxidant markers or pulmonary function with 25 µmol or 150 µmol SFN per day for 4 weeks.[57] Further research is needed to investigate higher dosages and/or longer durations of supplementation.

Cardiovascular risk factors

CVD is one of the main causes of death in Western countries and usually develops over many years. Risk factors include abnormal blood lipids, diabetes, hypertension and poor diet.

Low-density lipoprotein cholesterol

Elevated low-density lipoprotein cholesterol (LDL-C) levels are considered an important risk factor for CVD, and it has been estimated that, at a population level, a 1% reduction in LDL-C is associated with a 1–2% reduction in risk of coronary artery disease.[58]

Two independent studies conducted by the same team showed that consuming 400 g of broccoli high in GPN (approximately 3.5 times as much as standard broccoli) for 4 weeks decreased LDL-C significantly more than eating the same amount of standard broccoli, with a decrease of 5.8% in LDL-C averaged over both trials (improvements in the individual trials failed to reach statistical significance, probably due to small numbers).[58]

A small, pilot study in 12 healthy volunteers also showed a significant beneficial effect on total, LDL-C and high-density lipoprotein cholesterol (HDL-C) after just 1 week of consumption of 100 g fresh broccoli sprouts per day.[59]

The benefit of SFN is thought to be mediated through expression of Nrf2, which is associated with modulation of lipid synthesis and mitochondrial fatty acid oxidation.[58]

Diabetes

Low-grade inflammation is associated with both the development of type 2 diabetes mellitus (T2DM) and with its complications.[60]

One double-blind, placebo-controlled study looked at the effects of SFN on a large number of biochemical parameters in 63 patients with T2DM, comparing two dosages of a broccoli sprout powder, providing either 225 µmol or 112.5 µmol SFN per day for 4 weeks, with placebo. Statistically significant benefits of the high-dose supplement compared with placebo were seen in serum insulin, HOMA-IR (homeostasis model assessment of insulin resistance), the inflammatory markers CRP and IL-6, triglycerides, oxidated LDL-C, HDL-C and atherogenic index of plasma.[60,61,62] Oxidative stress markers improved in both SFN groups compared with placebo.[63] The authors of the study considered the antioxidant effects of SFN to mediate the benefits seen in diabetic patients.

Endothelial function and hypertension

A small RCT evaluated the effects of 10 g dried broccoli sprouts per day (equivalent to 100 g fresh sprouts, providing approximately 259 µmol GPN) for 4 weeks on blood pressure and endothelial function in 40 hypertensive individuals without diabetes and with normal levels of cholesterol.[64] Changes in endothelial function and blood pressure failed to reach statistical significance compared with control patients who did not receive the preparation.

Whilst evidence is limited to a few studies, it appears that people with diabetes and elevated cholesterol levels may benefit from supplementation with SFN/GPN. Due to the fact that CVD is strongly associated with inflammation and oxidative stress, supplementing SFN or related compounds may offer benefits for people with or at increased risk of CVD, although there are insufficient data to suggest a particular dosing regimen.

Endometriosis

Endometriosis is a common condition where endometrial tissue starts to grow in places outside the uterus, such as the ovaries and fallopian tubes, causing symptoms including pelvic pain, heavy periods and subfertility. It has been associated with higher levels of 4OHE1, which is thought to have pro-proliferative effects.[65]

One small RCT in eight women with endometriosis found that DIM, 300 mg per day for 3 months, alongside standard treatment with Dienogest (a progesterone analogue) significantly improved pelvic pain and bleeding patterns in the four women receiving DIM compared with those who only took Dienogest.[66] An accompanying tissue study showed that viability and oestrogen secretion of endometriotic but not normal endometrial cells was reduced through the use of DIM, suggesting that the effects of DIM on oestrogen metabolism and its antiproliferative properties may explain the clinical benefits.[66]

Whilst the evidence is very limited, theoretical considerations regarding the effect of DIM on oestrogen metabolism and these clinical results suggest that DIM may have potential, but requires more research.

Helicobacter pylori and gastric inflammation

Helicobacter pylori (H. pylori) is a common cause of gastritis, gastric and duodenal ulcers, and gastric cancer.[67] In preclinical studies, SFN has shown antimicrobial effects against *H. pylori.*

Three RCTs have evaluated the effects of SFN on *H. pylori.* Whilst two of these studies found some short-term benefit with regards to *H. pylori*,[68,69] the third one did not.[67] However, two of these studies also looked at markers for gastric inflammation and found significant reductions in those markers.[67,68] The studies that saw an improvement in gastric inflammation used broccoli sprouts or broccoli sprout extracts at dosages of 2 mg SFN (equivalent to 11 µmol) for 4 weeks and 420 µmol of SFN precursor for 8 weeks, respectively.

Whilst the evidence is limited, SFN appears to reduce gastric inflammation. This is unlikely to be due to any antimicrobial effects against *H. pylori* as such, but SFN is thought to protect the gastric mucosa against oxidative stress induced by *H. pylori*.[68] Due to the difference in preparations and dosages used in the trials it is difficult to suggest a dosage, but a duration of at least 4 weeks seems reasonable.

Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is a common disorder caused by fat deposits within liver cells, and is associated with obesity/overweight, metabolic syndrome and T2DM.

One double-blind, placebo-controlled study investigated the effects of a broccoli sprout extract (providing 69 µmol GPN per day) for 2 months in 52 men with NAFLD, and found a significant lowering of liver enzymes (a marker of liver health) and a marker of oxidative stress in the GPN but not the placebo group.[70] GPN is a precursor to SFN, which has cell-protective effects, including induction of detoxification and antioxidant enzymes, and is thought to mediate its benefits in NAFLD.[70]

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is an autoimmune condition that causes inflammation in various tissues, commonly affecting skin and joints, but may also involve internal organs such as the heart and kidneys. SLE is more common in women than men, and oestrogen imbalances are thought to promote disease activity.[71]

One open-label study comprising a 1-week metabolic study (17 patients) and a 3-month disease activity observation (12 patients) found a statistically significant increase of 2OHE1:16αOHE1 ratio from 1.84 to 3.15 after 1 week of supplementation with I3C, 375 mg per day.[71] SLE Disease Activity Index scores were 10.0 at baseline (scale 1–105), 6.25 following 3 months on I3C, and 8.8 at 3 months after I3C cessation, although these changes were not statistically significant. The authors concluded that whilst I3C can improve oestrogen metabolism in women with SLE, no substantial effects on disease activity during 3 months of supplementation were observed.[71]

Pre-eclampsia

Pre-eclampsia is a pregnancy-related condition, characterised by hypertension, proteinuria (protein in urine) and oedema, and is potentially life-threatening to both mother and baby. The condition is associated with autoimmune conditions such as SLE and antiphospholipid syndrome, diabetes type 1 as well as a history of high blood pressure, and oxidative stress is considered to be one of the underlying mechanisms.

One small trial evaluating the pharmacokinetics of SFN compared a single dose of a myrosinase-activated with a non-activated broccoli sprout extract containing 32 mg SFN in six non-pregnant women, and found the activated extract to have higher bioavailability.[72] The investigators then evaluated a single dose of the activated extract, either 32 mg or 64 mg, in six women each with pregnancy-related hypertension, and found blood levels of SFN and metabolites to be significantly lower compared with the non-pregnant women. A statistically significant 10% reduction in diastolic but not systolic blood pressure was seen, with a greater reduction with the higher dose, although the difference between the dosages was not

statistically significant. A significant decrease in soluble fms-like tyrosine kinase 1 (sFlt-1; an anti-angiogenic protein implicated in the development of pre-eclampsia) was also seen, irrespective of dose.

Whilst this study is promising, with no further longer-term studies there is no human clinical evidence for the benefits of SFN in preventing or managing pre-eclampsia.

Sickle cell disease

Sickle cell disease is an inherited disorder that affects red blood cells, causing their deformation and breakdown. The red blood cells have a reduced oxidative stress capacity, which is thought to be caused by decreased expression of Nrf2. Preclinical evidence suggests that SFN can activate Nrf2, which in turn increases levels of foetal haemoglobin, which reduces disease severity.[73] A small open-label, dose-escalation study found that SFN significantly increased the expression of heme oxygenase-1 (HMOX1), an antioxidant enzyme, but increases in gene expression for foetal haemoglobin failed to reach statistical significance.[73]

SAFETY

SFN, DIM and I3C are all generally well tolerated.[74,75,76]

Some clinical trials with SFN have reported digestive complaints, including bowel discomfort, gastrointestinal upset, heartburn and vomiting.[74] Two patients with autism and a history of seizures experienced seizures after intake of SFN, although it is unclear whether SFN increases the risk of seizure in susceptible individuals.[20]

Adverse events (AEs) reported in clinical trials with DIM include nausea,[31,33,77] rash,[31] arthralgia[31] and headaches,[77] although causality was not established. In a dose-escalation study, asymptomatic hyponatraemia was observed at 600 mg DIM per day for 28 days.[44] Hyperglycaemia, digestive complaints, fatigue, pruritus, anaemia, increased creatinine, urinary frequency and incontinence were also reported in this trial (causality unknown). One study of 600 women (400 on DIM, 200 on placebo) provided a detailed analysis of AEs, including some serious ones (requiring hospitalisation; 1% in DIM group, 3% in placebo group).[35] The only AE that was more common in women on DIM compared with placebo was a darkening of urine.[35]

AEs reported in clinical trials with I3C include rash,[29,71] musculoskeletal complaints,[29] headaches,[29] gastrointestinal complaints[29] and upper respiratory infections.[29] I3C has also been associated with unsteadiness and imbalance at higher doses (800 mg per day), which resolved on return to a lower dose.[50]

There have been concerns about SFN and thyroid function and thyroid autoimmune disease that were addressed in a recent RCT,[78] which is a subset of another study discussed under the section 'Detoxification of air pollutants'.[8] Thyroid hormones and antibodies were evaluated for 45 women before and after 84 days of consumption of a daily broccoli sprout drink containing 600 µmol GPN and 40 µmol SFN or placebo. There were no significant differences in thyroid hormone levels between the broccoli sprout and the placebo groups, and a non-significant decrease in numbers of participants with subclinical hypothyroidism and positive thyroid antibodies in the broccoli sprout group compared with the placebo group, suggesting that GPN and SFN are not associated with an increased risk of thyroid disease, at least in the short term.

DRUG INTERACTIONS/CAUTIONS

Due to the fact that SFN, DIM and I3C can affect the cytochrome P450 pathways, care should be taken with concomitant medications that are metabolised through these pathways, especially CYP1A2.[74,75,76]

In view of the potential of DIM to cause hyponatraemia, care should be taken with diuretic drugs.

DIM and I3C affect oestrogen metabolism, which needs to be taken into account when being used alongside oestrogen treatments, such as the contraceptive pill or hormone replacement therapy.

In animal models, I3C has been shown to have anti-thrombotic activity,[79] and may therefore potentially interact with anticoagulant and antiplatelet medications.

PREGNANCY AND BREASTFEEDING

Whilst SFN, DIM and I3C are considered safe in amounts typically present in the diet, there is no information on safety at higher doses.[74,75,76]

AGE LIMITS/MINIMUM AGE

I3C has been used in children with RRP from the age of 2 years long term, with a weight-adjusted dose, and no immediate or long-term side-effects were noted.[49,50] Two children who accidentally took triple their usual dose experienced unsteadiness, which resolved completely on return to their normal dose.[50]

In children with autism, SFN has been used from the age of 3 years. A number of side-effects were reported, including insomnia, flatulence, constipation, weight gain, vomiting, diarrhoea, increased aggression and exacerbation of seasonal allergies, with incidences ranging from 12% to 19%.[18] As mentioned above, two children with a history of seizures had seizures following SFN supplementation, although it is unclear whether SFN may increase seizure risk in at-risk children.[18]

CONCLUSION

There are promising results from clinical trials in a wide range of conditions, including cognition, autism, gastric inflammation, cardiometabolic and cancerous conditions, although the latter are mostly based on biomarker studies. Dosages have varied widely, making it difficult to suggest specific dosages. A number of studies have shown wide inter-individual variability of response with regards to effects on oestrogen metabolism, it would therefore be well advised to test oestrogen/oestrogen metabolite levels prior to supplementation.

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