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Molecular nutrition with
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[ANTI-CANCER EFFECT OF SULFORAPHANE]

Abstract:

Broccoli, a vegetable from the cruciferous family, has a high content of the glucosinolate glucoraphanin. Glucoraphanin is the precursor of the bioactive isothiocyanate sulforaphane. There is evidence for several health benefits with dietary sulforaphane, such as anti-cancer effects and antioxidation capacity. This review explains the metabolism and some of the anti-cancer effects of sulforaphane. Sulforaphane is metabolized by the mercapturic acid pathway and excreted in urin. Studies have shown that sulforaphane is efficient both in prevention of cancer and in treatment of cancer. Some of the anti-cancer effects are; inhibition of the cell cycle, anti-angiogenesis, anti-metastasis and induced apoptosis. The effects are often results of the generegulatory capacity of sulforaphane. This review indicates that sulforaphane can be a good and safe complement to other cancer treatment, but more in vivo studies on humans is needed to evaluate the effects.

Introduction

Vegetables from the cruciferous family (such as broccoli, cauliflower and cabbage) are well known for their content of glucosinolates. Glucosinolates are precursors for isothiocyanates (*R*–N=C=S), which are bioactive compounds and considered to have many health benefits. There is evidence that isothiocyanates for example decrease cancer risk and have anti-inflammatory effects (1).
There have been found over 120 different types of glucosinolates in cruciferous vegetables. The variation is large and differs between species but also within them, depending on growth conditions and genotype (2).

Formation and uptake of Sulfprahane

One interesting glucosinolate is glucoraphanin (**figure 1A**), found in broccoli. The mean glucoraphanin content of broccoli is in one study determined to 0,48 ± 0,23 µmol/g (3). Glucoraphanin is hydrolized into sulforaphane (**figure 1B)** in the gut by the enzyme myrosinase or by the gut microbial flora. Myrosinase is released from the plant cells when they are crushed as in chopping or chewing. Human cells have no ability to hydrolyze glucoraphanin on their own (2, 4)**.**

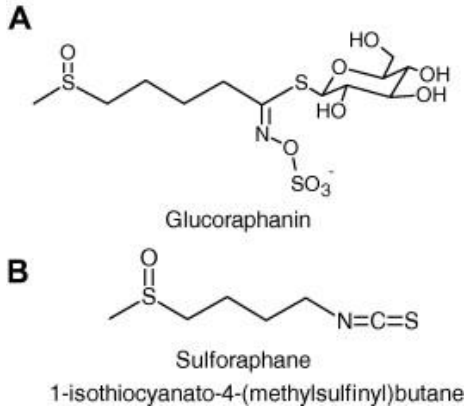

Figure 1. **Glucoraphanin (A) is transformed in to the isothiocyanate Sulforaphane (B) by myrosinase.**
(*Clarke JD, Dashwood RH, Ho E. Multi-targeted prevention of cancer by sulforaphane. Cancer Lett. 269 (2008) 291-304*)

Myrosinase cleaves glucoraphanin to glucose, hydrogen sulfate and sulforaphane (2). The myrosinase activity is described in **figure 2**.
Myrosinase is inactivated by heat and the uptake of sulforaphane is about three times higher when the broccoli is eaten raw instead of steamed (3).
The suggested absorption mechanism is described in **Figure 3**. After cleavage, sulforaphane is easily absorbed by passive transport and distributed in the body (2). One study indicates that 74 ± 29 % of the sulforaphane content in jejunum is absorbed, but parts will be transported back to the lumen after conjugation (see *Sulforaphane metabolism*), possibly by the Pgp-transporter protein. Sulforaphane is transported from the epithelial cell into the blood

by MRP1. Blood levels will reach µM levels of sulforaphane after normal intake, which is enough for most of the bioactivity effects (5).

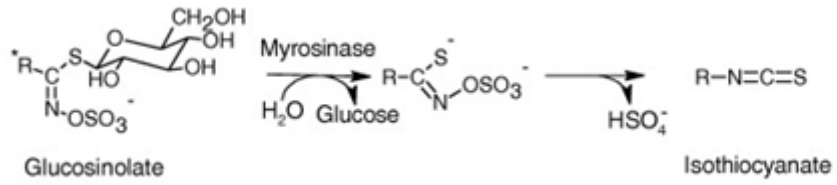

Figure 2. The enzyme myrosinase transformes a glucosinolate into a isothiocyanate. (*Clarke JD, Dashwood RH, Ho E. Multi-targeted prevention of cancer by sulforaphane. Cancer Lett. 269 (2008) 291-304*)

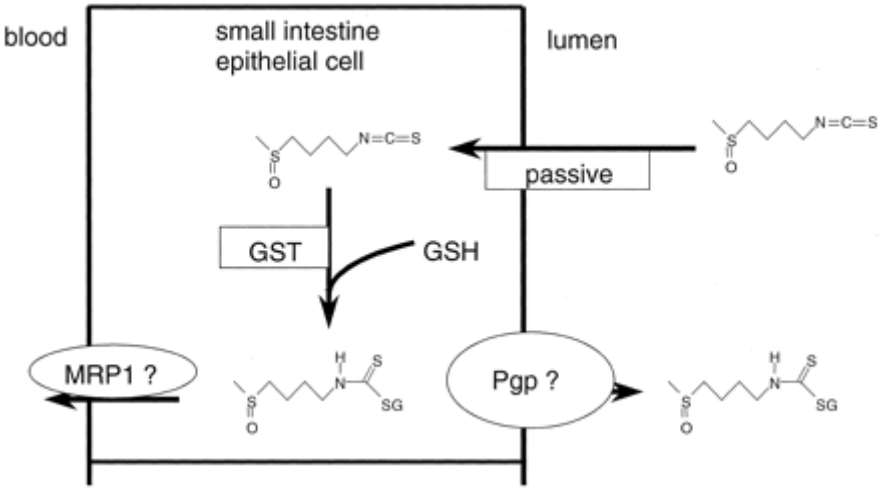

Figure 3. Suggested mechanism for sulforaphane absorption with possible transporters.
(Petri N, Tannergren C, Holst B, Mellon FA. Absorption/metabolism of sulforaphane and quercetin, and regulation of phase II enzymes, in human jejunum in vivo. Drug metabolism and dispotsition 31 (2003) 805-813)

Sulforaphane metabolism

Sulforaphane is metabolized by the mercapturic pathway. First step is conjugating between the electrophilic carbon of the isothiocyante-group and glutathione, this reaction is catalyzed by glutathione S-transferase (Phase 2 metabolism). The conjugate is further transformed by the enzymes gamma-glutamyltranspeptidase, cysteinylglycinase and histonacetyltransferas, and is finally excreted as *N*-acetylcystein-sulforaphane in urine, see **figure 4** (2, 4). Polymorphism in phase II sulforaphane metabolizing genes influences individuals' sulforaphane metabolism and excretion time. (2), but a single dose of sulforaphane (200 µmol) has been excreted in 24 hours in most individuals (6).

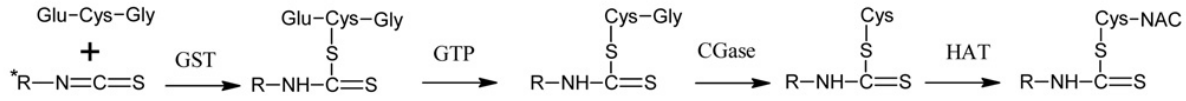

Figure 4. Mercapturic pathway. Sulforaphane is conjugated with glutathione by glutathione S-transferas (GST) and further transformed by gamma-glutamyltranspeptidas (GTP), cysteinglycinase (CGase) and histonacetyltransferas (HAT).
Clarke JD, Dashwood RH, Ho E. Multi-targeted prevention of cancer by sulforaphane. Cancer Lett. 269 (2008) 291-304)

Sulforaphane toxicities

Sulforaphane is believed to have no toxic effect on humans and at least one study confirms the safety of dietary sulforaphane (7).

Isothiocyantates is commonly known as healthy dietary components, but one study shows that a very high dietary dose of benzyl isothiocyanate (found in cabbage and garden cress) can enhance urinary bladder carcinogenesis in rats. A normal dietary dose of isothiocyanates from cruciferous vegetables is about 0.2 mg/kg body weight/day in humans. The dose given in this study was 5 and 50 mg/kg/day, which represent 25-250 times more than in normal diet (8).

Aim

The aim of this review is to summarize parts of the anti-cancer effect of sulforaphane.

Anti-cancer effects of Sulforaphane

Several studies have proved many different anti-cancer effects of sulforaphane. Sulforaphane seems to be efficient both in prevention of cancer and in treatment (2, 6, 9). Some of the effects are summarized in **Table I**.

Table I. Anti-cancer effects of sulforaphane

(Zhang Y, Tang L. Discovery and development of sulforaphane as a cancer chemopreventive phytochemical. Acta Pharmacol Sin 2007;28(09, 1343-1354)

Biological changes	**Molecular targets**
Induction of phase 2 genes	Keap1-Nrf2 complex
Programmed cell death -Mitochondria-mediated apoptosis -Death receptor-mediated apoptosis -Autophagic cell death	Bcl-2 proteins, death receptor, MAPK, mitochondria-associated apoptotic protein
Cell cycle arrest -Mainly G2/M, but also targets G1 and S in certain cells	Cyclins, Cdks, Cdc25C, HDAC, p21, microtubule
Anti-angiogenesis	VEGF and its receptor
Inhibition of invasion and metastasis	MMP-2, MMP-9
Other effects	*H.pylori*, MGMT, MRP2, NFκB, ODC

Modulating phase 1 metabolism and induction of phase 2 metabolism genes

Cytochrome P_{450}-enzymes (CYP) is a large family of phase 1 enzymes that plays an important part of metabolizing xenobiotic substances, but they are also transforming some procarcinogens into carcinogens. By inhibition of CYP sulforaphane protects the cells from these carcinogens. Sulforaphane interacts with CYP which leads to a more inactive enzyme.

There is also some evidence that sulforaphane regulate CYP gene transcription but the mechanism is not known (2, 6, 10).

Sulforaphane is not only suppressing phase 1 enzymes, it is also inducing phase 2 enzymes. Phase 2 enzymes detoxify xenobiotics and make them more easily excreted (4).
The antioxidant response element (ARE) is confirmed to regulate many of phase 2 genes with products protecting cells from carcinogens, toxic substances and oxidants (2, 4, 11). Some of the products from ARE-regulated genes are; epoxide hydrolase, ferritin, glutamate cystein synthetase, glutathione peroxidase, glutathione reductase and glutathione S-transferase (4).
The key activator of ARE is the nuclear factor erythroid 2-related factor 2 (Nrf2). As inactive, Nrf2 interacts with its repressor Kelch-like ECH-associated protein (Keap1) in the cytosol. Sulforaphane has the ability to react with thiol-groups in Keap1, which promotes dissociation from Nrf2. After dissociation Nrf2 is translocated to nucleus and binds to ARE together with some other nuclear factors, which will activate the gene (**figure 5**) (2, 6, 11).

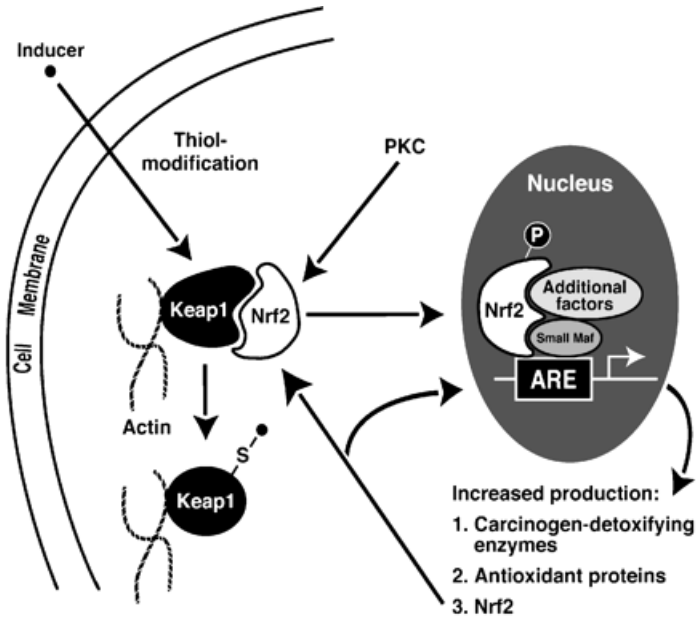

Figure 5. **Activation of ARE-regulated genes.** *(Zhang Y, Gordon GB. A strategy for cancer prevention: stimulation of the Nrf2-ARE signaling pathway. Molecular Cancer Therapeutics, 2004;3 (7)885-93)*

Programmed cell death (apoptosis)

Sulforaphane induces cell death in cancer cells from various types of cancer, shown both in vitro and in vivo, which is positive in both preventing carcinogenesis and in cancer therapy. There are several mechanisms for apoptosis and sulforaphane activates both death-receptor mediated apoptosis and mitochondria mediated apoptosis (**figure 6**) by inducing activity of caspases and regulate Bcl-2 protein levels. There is also evidence that sulforaphane has effect on autophagic cell death (2, 6).

Cell cycle arrest

Sulforaphane has been shown to arrest cells in different phases in the cell cycle, by regulate several cell cycle factors, for example; p21 (cell cycle inhibitor) and Cdc25c (cell cycle

activating phosphatase) (**figure 6)** (2).

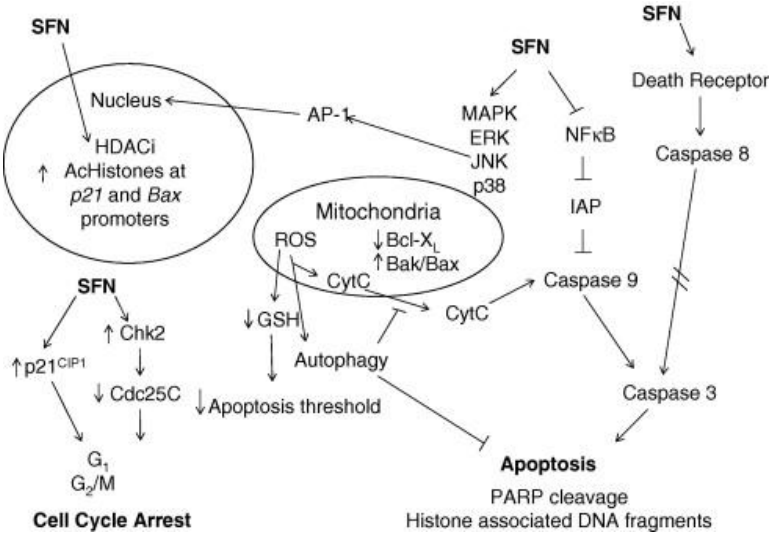

Figure 6. Suggested mechanisms for inducing apoptosis and cell cycle arrest.
(*Clarke JD, Dashwood RH, Ho E. Multi-targeted prevention of cancer by sulforaphane. Cancer Lett. 269 (2008) 291-304*)

Anti-angiogenesis

Angiogenesis, the formation of new blood vessels, is important for the development and survival of a cancer tumor. By inhibit the angiogenesis sulforaphane also inhibit the development of cancer. The mechanism of the antiangiogenesis effect of sulforaphane is associated with downregulation of transcriptional factors important for angiogenesis, for example vascular endothelial growth factor (VEGF) and its receptor (6).

Inhibition of invasion and metastasis

Sulforaphane inhibit invasion of cancer cells and metastasis by transcriptional downregulation of MMP-2 and MMP-9 genes (matrix metalloproteinase) (6). MMP-2 and MMP-9 are involved in degradation of extracellular matrix and basement membranes, which is necessary for tumor invasion and metastasis. MMP genes are normally regulated by several cytokines and growth factors (12).

Other effects

There is evidence that sulforaphane has an antibacterial effect against *Helicobacter pylori*. *H. pylori* in the gut microbial flora is a risk factor for gastronomical cancer (13).

Sulforaphane has been shown to inhibit histone deacetylase (HDAC) which often has increased activity in cancer cells. Increased activity of HDAC can result in less transcription of cell-regulating proteins, which can lead to uncontrolled cell cycle, differentiation and apoptotic mechansims (2).

Sulforaphane has also been showed to maintain the antioxidant activity of vitamins A, C and E (4).

Conclusion

Sulforaphane has many shown anti-cancer effects and seems to be a safe complement to cancer treatment. There will be necessary to evaluate the effects further in vivo in humans before sulforaphane is used in medical care. More toxicities studies, with higher doses, are also desirable.

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