MINI-REVIEW

Isothiocyanates in Brassica: Potential Anti Cancer Agents

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Abstract

Isothiocyanates are naturally occurring small molecules that are formed from glucosinolate precursors of cruciferous vegetables. Many isothiocyanates, both natural and synthetic, display anti-carcinogenic activity because they reduce activation of carcinogens and increase their detoxification. This minireview summarizes the current knowledge on isothiocyanates and focuses on their role as potential anti-cancer agents.

Keywords: Isothiocyanates - cancer chemopreventive agents - detoxification - carcinogen activation

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Introduction

Members of family cruciferae contain many health promoting and potentially protective phytochemicals including folic acid, phenolics, carotenoids, selenium, glucosinolates and ascorbic acids (Ballesta et al., 2015). These bioactive compounds offers powerful, broad-spectrum support for protecting against the ubiquitous cancer provoking agent encountered every day in our environment (Cartea et al., 2010). This fact is due to the presence of a type of bioactive components: Isothiocyanates.

Isothiocyanate is the chemical group containing --N=C=S functionality (Figure1), formed by substituting the oxygen in the isocyanate group with a sulfur (Bedane et al., 2015). In the isothiocyanate structure R-N=C=S, R is an alkyl or aryl group. Many natural isothiocyanates from plants are produced by enzymatic conversion of metabolites called glucosinolates.

Isothiocyanates are reactive compounds containing --N=C=S functionality and performs nucleophilic attack at the electron-deficient central carbon atom. In this reaction, attack by different chemical groups' forms different unstable compounds. For example nucleophilic attack of isothiocyanates by thiol group forms dithiocarbamates R-N (=S)-SR' and the reaction is called as thiocarbamoylation (Wu et al., 2009). These are spontaneous reactions and under physiological conditions these compounds are unstable and readily undergo a reverse reaction resulting establishment of equilibrium:

R-N=C=S + R'SH ⇆RNHC (=S)-SR'

Similarly nucleophilic attack by amino groups forms thiourea derivatives whereas attack by hydroxide ion forms monothiocarbamate derivatives. These unstable compounds eliminate carbonyl sulfide COS, which produces the corresponding amine derivative RNH2 (Wu et al., 2009).

Isothiocyanates synthesis has drawn attention of chemists' worldwide (Zhong et al., 2006). This is very useful for the synthesis of heterocycles e.g. triazoles, thiazoles, oxazines, benzimidazole etc. (Trofimov, 1999; Sommen, 2004). Some isothiocyanates are volatile and will be lost either by vaporization or evaporation at the boiling point or sometimes below the boiling point; for example, loss of allyl isothiocyanate occurs at a boiling point of 88 °C (Shapiro et al., 1998).

Discussion

Isothiocyanates occur naturally as glucosinolate conjugates in cruciferous vegetables. These compounds are found in cruciferous vegetables such as broccoli, cauliflower, kale, turnips, collards, Brussels sprouts, cabbage, radish, turnip and watercress (Hecht et al., 2004; Zhang et al., 2004). Phenylethylisothiocyanate (PEITC), benzylisothiocyanate (BITC) and 3-phenylpropylisothiocyanate and sulforaphane (SFN), allylyisothiocyanate (AITC) are some of the examples of isothiocyanates with strongest anticancer effects (Zubía, et al., 2008; Singh, et al., 2012). Recently, the isothiocyanate sesquiterpenes have been isolated from a sponge of the genus Axinyssa (Zubia et al., 2008). These compounds are responsible for the typical flavour of the vegetables also (Sconhof et al., 2004; Padilla et al., 2007).

Biological activity

Glucosinolates are precursors of isothiocyanates. Glucosinolates are sulfur-enriched, anionic secondary metabolites of plants synthesized from amino acids and sugars. They are synthesized in all vegetables and oilseed plants of the order Brassicales (Halkier and Gershenzon, 2006). All known glucosinolate producing plants have at least one β-thioglucosidase often named myrosinase (thioglucoside glycohydrolase, EC 3.2.3.1) (Bones and Rossiter, 2006). Myrosinases hydrolyses glucosinolates

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into several potentially toxic compounds dependent on the reaction conditions and the presence of specifier proteins (Kong et al., 2012).

Glucosinolate degradation products contribute to the distinct taste and flavor of cruciferous vegetables such as broccoli, mustard and wasabi and they constitute a potent defense system against herbivores and pathogens (Wittstock and Burow, 2010; Razis et al., 2013).

Myrosinase is stored separately from its substrates in specialized cells called myrosin cells (Kissen et al., 2009b) to prevent the potential damage to the plant cell. The hydrolysis products (Figure 2) are produced upon attack by herbivores or pathogens when damage to the plant tissue and disruption of the cells causes myrosinase to come into contact with glucosinolates (Overby et al., 2015). The small sulfur-containing isothiocyanates (ITCs) are among the biodegradation products of glucosinolates. Due to their anticancer and chemopreventive properties, the ITCs have been the target of substantial research efforts over the last years (Navarro et al., 2011).

Many isothiocyanates, both natural and synthetic, display anticarcinogenic activity because they reduce activation of carcinogens and increase their detoxification. Recent studies show that they exhibit anti-tumor activity by affecting multiple pathways including apoptosis, MAPK signaling, oxidative stress, and cell cycle progression (Hu et al., 2003; Satyan et al., 2006; Wu et al., 2009).

ITCs occur primarily in cruciferous vegetables, many of which show significant cancer chemopreventive activities, and therefore are widely suspected to account in part for the cancer preventive activities of these vegetables in humans. Sulforaphane is perhaps the most widely known crucifer-derived cancer chemopreventive ITC (Fimognari et al., 2007; Zhang et al., 2007). In addition to their anti-carcinogenic properties in the animals that consume them, it has been reported that glucosinolates are anti-oxidative (Halkier et al., 2006) and protects against herbivores and microbes (Fahey et al., 2002; Kusznierewicz et al., 2008; Yi et al., 2015). Calms et al., (2015) reported that Isothiocyanates (ITCs) have negative effects on the growth of various fungal species. In line with this, it has been shown that following exposure to ITC, fungal cells displayed biological stress with over-expression of several genes involved mainly in cell protection against oxidative damage (Sellam et al., 2007b; Wu et al., 2009; Calmes et al., 2015).

The cell toxicity of ITCs has been mainly studied on mammal cells due to their antitumor activity (Clay et al., 2009; Stotz et al., 2011; Calmes et al., 2015). Boreddy et al. (2011) reported that ITCs have the capacity to inhibit the growth of several types of cancer cells by causing apoptotic and autophagic cell death. However, the mechanism by which ITCs cause cell death is not yet fully understood but it has been mentioned by the scientists that they may react via direct protein modification or indirectly by disruption of redox homeostasis and increased thiol oxidation (Brown and Hampton, 2011).

Once isothiocyanates are ingested or formed in the lumen of the gastrointestinal tract, they cross the gastrointestinal epithelium and the capillary endothelium by passive diffusion (Wu et al., 2009). They bind rapidly and reversibly to thiols of plasma protein and cross the plasma membrane into cells of tissues.

Inside cells, isothiocyanates react with glutathione to form the glutathione conjugate, which is expelled from cells by transporter proteins and further metabolized to mercapturic acids

(Chung et al., 1998; Mennicke et al., 1987). These isothiocyanate metabolites can be measured in the urine and are highly correlated with dietary intake of cruciferous vegetables. There is also some evidence that isothiocyanate metabolites contribute to the biological activity of isothiocyanates (Hecht et al., 2004).

Health Benefits of Isothiocyanates

Studies have shown that isothiocyanates help to prevent various types of cancers mainly lung cancer and esophageal cancer. Isothiocyanates can also lowers the risk of other cancers, including gastrointestinal cancer (Cuddihy et al., 2008; Mi et al., 2008; Boreddy et al., 2011). Researchers at the Johns Hopkins University School of Medicine in Baltimore studied the metabolism of isothiocyanates and found that isothiocyanates were about six times more bioavailable than glucosinolates.

Isothiocyanates works in different ways e.g. they don't allow carcinogens to be activated or they counteract the poisonous effects of carcinogens that have been activated & they can also speed up their removal those carcinogens from the body.

Isothiocyanates directly influences the biotransformation enzymes and therefore modifies the metabolism of carcinogenic compounds (Lawson et al., 2015). They can also act by inhibition of cell proliferation and induction of apoptosis (Brown and Hampton, 2011). For example, in mice, signaling network of a protein called Akt3 which plays a key role in melanoma development can be blocked by a combination of isothiocyanates and selenium (Dinkova et al., 2012). Major isothiocyanates with the strongest anticancer effects are phenylethylisothiocyanate, benzylisothiocyanate and

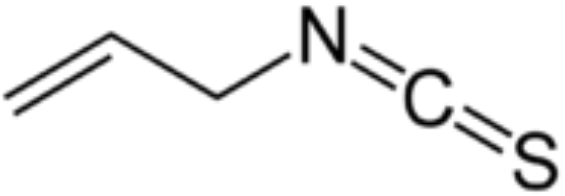

Figure 1. Isothiocyanates (Source: www.wikiwand.com)

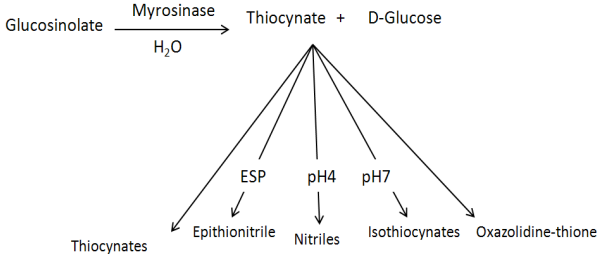

Figure 2. Glucosinolates and Products at Different pH Values. Epithiospecifier protein (ESP), pH are few critical parameters to determine the product

3-phenylpropylisothiocyanate.

Many isothiocyanates, particularly SFN, are potent inducers of phase II enzymes in cultured human cells (Fimognari et al., 2007). Phase II enzymes, including glutathione S-transferases (GSTs) , UDP-glucuronosyl transferases (UGTs), quinone reductase, and glutamate cysteine ligase, protect cells from DNA damage by carcinogens and reactive oxygen species (Kensler et al., 2004).

Isothiocyanates can also induce cellular oxidative stress by rapidly conjugating with cells of GSH in leukemia cells (Xu et al., 2001) and therefore reducing the glutathione (GSH) level. However drug resistant cancer cells can be treated by oxidative stress caused by isothiocyanates (Trachootham et al., 2008). Zhang et al. (2004) and Lawson et al. (2015) reported that a number of isothiocyanates e.g. BITC, PEITC, and SFN induces cell cycle arrest in cultured cells. This protective effect may be due to improved phytochemicals with antioxidant status (de Figueiredo et al., 2013). Phytochemicals are the most important compounds because of their nutraceutical potentials such as antioxidant, anti-inflammatory, anti-carcinogenic, antimicrobial, anti-diarrheal, antiulcer, and anti-cardiovascular properties (Sharma et al., 2016).

Food Sources

Isothiocyanates can be found in cruciferous or "cabbage family" vegetables such as broccoli, cauliflower, kale, turnips, collards, Brussels sprouts, cabbage, kohlrabi, rutabaga, Chinese cabbage, bok choy, horseradish, radish, and watercress. These vegetables add crunch or flavor to many of our familiar dishes, such as coleslaw, vegetable stir-fry, collard greens, and salads. Zubia et al. (2008) isolated isothiocyanate sesquiterpenes from a sponge of the genus Axinyssa. Significant losses of isothiocyanates are expected during food processing. Some isothiocyanates are volatile and will be lost to the atmosphere by vaporization at the boiling point and evaporation at temperatures below the boiling point for example, loss of allyl isothiocyanate occurs at a boiling point of 88°C. Isothiocyanates also are hydrolyzed at physiological temperatures, a process that becomes more rapid at higher cooking temperatures (Wu et al., 2009).

Different cooking methods that uses less water such as steaming, microwaving will increase the bioavalability of isothiocyanates (Song et al., 2007). However there are some other cooking practices also which may reduce enzyme myrosinase such as microwaving at high power (>750 watts) (Verkerk et al., 2004), boiling (Shapiro et al., 2001) and steaming at high temperature (Rungapamestry et al., 2006) thereby reduce the isothiocyanate concentrations.

In conclusion, it can be concluded that isothiocyanates are chemopreventive agents and also exhibit antitumor activity. Their tumorigenesis ability depends on the structure of the isothiocyanates, the animal species, target tissues, and the specific carcinogen employed. The MAPK pathway, oxidative stress, and the cell cycle machinery etc are the main pathways which are targeted by isothiocyanates. The mechanism of these activities is not fully understood.

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