Isothiocyanates: cancer chemopreventive agent

Mrunmayee P. Toraskar*, Vithal M. Kulkarni, Shweta T. Dhanashire, Vilasarao J. Kadam
Department of Pharmaceutical chemistry, Bharati Vidyapeeth’s College of Pharmacy, Sector 08, CBD Belapur, Navi-Mumbai – 400614, India.

Received on: 20-05-2009; Accepted on:15-07-2009

ABSTRACT

Isothiocyanates- plant derived dietary compounds, are promising chemopreventive agents generally non-toxic substances that interfere with the process of cancer development. Several natural and synthetic isothiocyanates have demonstrated cancer-preventive properties in animals treated with chemical carcinogens, including polycyclic aromatic hydrocarbon nitrosamines. Some of these compounds, the isothiocyanates – particularly allyl isothiocyanate and phenethyl isothiocyanate, have anticancer activity. Epidemiologic and clinical studies indicate a positive correlation between the general consumption of cruciferous vegetables and the decreased incidence of some cancers including non-Hodgkin’s lymphoma, liver, prostate, cervical, ovarian, lung, gastrointestinal tract. Natural isothiocyanates such as sulforaphane and phenethyl isothiocyanate possess strong antitumor activities both in vitro and in vivo. It exerts strong anticarcinogenic effects in a number of animal models of cancer, presumably by modulation of xenobiotic-metabolizing enzymes, such as by inhibition of cytochrome P-450 and/or by induction of phase II detoxifying enzymes.

Keywords: Isothiocyanates, Cancer chemopreventive agent, Apoptosis

INTRODUCTION

Chemopreventive isothiocyanates

Isothiocyanates acts as chemopreventive by inducing apoptosis process i.e cell death The mechanism by which Phenyl ethyl isothiocyanates inhibits chemically induced cancer involves inhibition of carcinogen activation due to inhibition of cytochrome P-450 dependent mono-oxygenases and increased carcinogen inactivation due to induction of phase II enzymes, including glutathione transferase. Cytotoxicity was associated with an initial decrease in GSH and GSSG, with a concomitant formation of the GSH adduct S-(N-phenethylthiocarbamoyl) glutathione inside cells which was then exported from cell. Then S-(N-phenethyl thiocarbamoyl) glutathione spontaneously fragmented to GSH and phenethyl isothiocyanate. GSH oxidized to GSSG and glutathionyl-protein disulphides and phenethyl isothiocyanate hydrolyzed to phenylethylamine. Induction of apoptosis by dietary isothiocyanates due to activation of the stress-activated protein kinase JNK1 (Junction N-terminal Kinase) with cleavage of p22 BID protein to p15, p13 and p11 fragments.

MECHANISM OF ACTION OF ISOTHIOCYANATES

Isothiocyanates acts as chemopreventive by inducing apoptosis process i.e cell death The mechanism by which Phenyl ethyl isothiocyanates inhibits chemically induced cancer involves inhibition of carcinogen activation due to inhibition of cytochrome P-450 dependent mono-oxygenases and increased carcinogen inactivation due to induction of phase II enzymes, including glutathione transferase. Cytotoxicity was associated with an initial decrease in GSH and GSSG, with a concomitant formation of the GSH adduct S-(N-phenethylthiocarbamoyl) glutathione inside cells which was then exported from cell. Then S-(N-phenethyl thiocarbamoyl) glutathione spontaneously fragmented to GSH and phenethyl isothiocyanate. GSH oxidized to GSSG and glutathionyl-protein disulphides and phenethyl isothiocyanate hydrolyzed to phenylethylamine. Induction of apoptosis by dietary isothiocyanates due to activation of the stress-activated protein kinase JNK1 (Junction N-terminal Kinase) with cleavage of p22 BID protein to p15, p13 and p11 fragments.
Mechanism of induction of tumour cell apoptosis by dietary isothiocyanates

The mechanism of induction of apoptosis involves the entry of the isothiocyanate into cells and formation of the GSH conjugate, S-(N-alkyl or arylalkyl thiocarbamoyl) glutathione. Concurrently, there is a slow inactivation of phenyl ethyl isothiocyanates by spontaneous hydrolysis then expulsion of the GSH conjugate via the MRP protein channel, with a consequent depletion of cellular GSH. There is a temporary, reversible modification of cellular protein thiols – protein thiocarbamoylation.

Event 3 triggers recruitment of adaptor protein FADD to apoptosis-mediating cell death receptors (tumour necrosis receptor 1 and/or the fas receptor) and activates caspase-8. After this there is cleavage of mitochondrial protein BID, release of cytochrome c from mitochondria and activation of caspase-3. Activation of the stress activation protein kinase JNK (which induces increased expression of the fas receptor ligand – further potentiating apoptosis) via activation of the mitogen activated kinase MEKK1.

All these events induce apoptosis, DNA fragmentation and cell death. Where the isothiocyanate (RN=C=S) is administered as the corresponding thiol conjugate (RNH(C=S)-SR''), this sequence of events is preceded by fragmentation of the thiol conjugate to the free isothiocyanate.

EXAMPLES OF ISOTHIOCYANATE

1. Benzyl Isothiocyanate

2. Phenethyl Isothiocyanate

3. Phenyl Methyl Isothiocyanate

4. Phenylhexyl Isothiocyanate

5. Sulphoraphane

Metabolism and Bioavailability:

Myrosinase a class of enzymes that catalyzes the hydrolysis (breakdown) of glucosinolates, is physically separated from glucosinolates when plant cells are intact. When cruciferous vegetables are chopped or chewed, myrosinase can interact with glucosinolates and release isothiocyanates from their precursors. During metabolism, isothiocyanates are conjugated (bound) to glutathione, an activity that is promoted by a family of enzymes called glutathione-S-transferases (GSTs), and further metabolized to mercapturic acids. These isothiocyanate metabolites can be measured in the urine, and are highly correlated with dietary intake of cruciferous vegetables.

BIOLOGICAL ACTIVITIES

Effects on Biotransformation Enzymes Involved in Carcinogen Metabolism

Biotransformation enzymes play important roles in the metabolism and elimination of a variety of chemicals, including drugs, toxins and carcinogens. In general, phase -I biotransformation enzymes catalyze reactions that increase the reactivity of hydrophobic (fat-soluble) compounds, preparing them for reactions catalyzed by phase -II biotransformation enzymes.

Inhibition of Phase - I Biotransformation Enzymes

Some procarcinogens (carcinogen precursors) require biotransformation by phase-I enzymes, such as those of the cytochrome P450 (CYP) family, in order to become active carcinogens that are capable of binding DNA and inducing mutations. Inhibition of specific CYP enzymes involved in carcinogen activation inhibits the
development of cancer in animal models. Isothiocyanates, including PEITC and BITC (benzyl isothiocyanates), have been found to inhibit carcinogen activation by CYP enzymes.

**Induction of Phase -II Biotransformation Enzymes**

Many isothiocyanates, particularly SFN (Sulforaphane), are potent inducers of phase -II enzymes in cultured human cells. Phase -II enzymes, including GSTs, UDP-glucuronosyl transferases (UGTs), quinone reductase and glutamate cysteine ligase, plays important roles in protecting cells from DNA damage by carcinogens and reactive oxygen species.

**Anti-inflammatory Activity**

Inflammation promotes cellular proliferation and inhibits apoptosis, increasing the risk of developing cancer. Isothiocyanates have been found to decrease the secretion of inflammatory signaling molecules by white blood cells and to decrease DNA binding of NF-kappaB, a pro-inflammatory transcription factor.

**Preservation Of Normal Cell Cycle Regulation**

After cell divides it passes through a sequence of stages known as the cell cycle before dividing again. Following cell damage the cell cycle can be transiently arrested to allow for DNA repair or activation of pathways leading to cell death (apoptosis) if the damage cannot be repaired. Defective cell cycle regulation may result in the propagation of mutations that contribute to the development of cancer. A number of isothiocyanates, including AITC, BITC, PEITC and SFN, have been found to induce cell cycle arrest in cultured cells.

**ROLE OF ISOTHIOCYANATES IN VARIOUS CANCER TYPES**

1. **Lung Cancer**

Phenyl isothiocyanate and benzyl isothiocyanate: -

These compounds inhibited the metabolic activation of nicotine-derived nitrosamine ketone (NNK), a powerful pulmonary carcinogen found in cigarette smoke, capable of inducing lung tumours in animals whatever the route of administration. With regard to isothiocyanates, much research, in particular by Hecht and colleagues, has focused on two compounds: phenethyl isothiocyanate and benzyl isothiocyanate.

2. **Prostate, Liver, Ovarian, And Colon Cancer**

Sulforaphane :-

Isothiocyanates are components of certain plants and vegetables that have selective biological activities and functions against carcinogenesis. Sulforaphane, a potent cancer preventive agent, is a dietary isothiocyanate compound found as a precursor glucosinolate in cruciferous vegetables such as cauliflower, broccoli, and Brussels sprouts. Epidemiologic and clinical studies reviewed by Murillo and colleagues indicate a positive correlation between the general consumption of cruciferous vegetables and breast cancer risk.

Clinically, it is usually diagnosed in patients over 50 years old. Thus, there could be opportunity for intervention using cancer chemopreventive compounds that prevent or slow the progression of this disease. Recent studies have indicated that using dietary chemopreventive compounds, such as isothiocyanates, might be a promising strategy to decrease the incidence of prostate cancer. Some isothiocyanates derived from cruciferous vegetables, such as sulforaphane (SFN), are highly effective in preventing or reducing the risk of cancer.

**Curcumin** (from turmeric) is one of the most well known naturally occurring compounds. It has cancer chemopreventive potential against many types of cancerous cells, including prostate cancer. It is a potent nuclear factor-kappaB (NF-kB) inhibitor as well as an inducer's of apoptosis.

4. **Glioblastoma Cancer**

**Dietary Iberin isothiocyanate:**

Glioblastoma is the most common malignant tumor of the adult central nervous system and is hallmark by high proliferation of tumor cells with increased cellularity and necrosis. Recent studies indicate that natural isothiocyanates such as sulforaphane and phenethyl isothiocyanate possess strong antitumor activities both in vitro and in vivo.

5. **Breast Cancer Risk In Premenopausal Women**

Isothiocyanates, formed from glucosinolates in cruciferous vegetables, could also be responsible for inverse associations between cruciferous vegetable consumption and breast cancer risk. The anti carcinogenic effects of cruciferous vegetables may derive from the glucosinolates they contain; these are degraded into isothiocyanates derived from glucosinolates in cruciferous vegetables, such as sulforaphane (SFN), are highly effective in preventing or reducing the risk of cancer.

**Food Sources**

**Cruciferous Vegetables**

Cruciferous vegetables, such as bok choy, broccoli, Brussels sprouts, cabbage, cauliflower, kale, kohlrabi, mustard, radish, rutabaga, turnip and watercress contain a series of relatively unique secondary metabolites of amino acids, called glucosinolates. Sinigrin, the predominant aliphatic glucosinolate in cruciferous vegetables, is hydrolyzed to yield allyl isothiocyanate (AITC), which, after absorption and metabolism in humans, is excreted in the urine as an N-acetylcysteine (NAC) conjugate. Unlike some other phytochemicals, glucosinolates are present in relatively high concentrations in commonly consumed portions of cruciferous vegetables. For example 1/2 cup of raw broccoli might provide more than 25 mg of total glucosinolates. Total glucosinolate contents of selected cruciferous vegetables are presented in Table 1. Some cruciferous vegetables are better sources of specific glucosinolates (and isothiocyanates) than others. Vegetables of relatively good sources of some isothiocyanates currently under study for their cancer-preventive properties are listed in Table 2.
involved in the activation of a variety of carcinogens. Recently, it has been shown that isothiocyanates can inhibit at least one cytochrome P-450 (CYP2E1) activity, and it has been reported that Pacific blue colon cancer patients who consumed 170 g/d (6 oz/d) of watercress, urinary excretion of phenethyl isothiocyanate increased significantly, Brussels sprouts are rich in a number of glucosinolates, including precursors of Allyl isothiocyanate and Sulforaphane. Consumption of 300 g/d (11 oz/d) of Brussels sprouts for a week significantly increased plasma and intestinal GST (glutathione S-transferase) levels in nonsmoking.

From these studies it was confirmed that isothiocyanate acts as a cancer chemopreventive agent. CONCLUSION By consuming cruciferous vegetables containing isothiocyanates we may prevent cancer or may prolong it. Intervention with chemopreventive agents such as isothiocyanate at an earliest stage in cancer is more attractive than attempting to eradicate fully developed tumours with chemotherapeutic drugs.

REFERENCES:
1. S.V. Bhide & G. B. Maru, Chemoprevention of cancer, Omega Scientific publisher, 1998, 12,13,120,121
3. http://www2.warwick.ac.uk/fac/med/research/csri/proteindamage/researchinterest/isothiocyanates
4. Dong Xiao, Yan Zeng,. Caspase-Dependent Apoptosis Induction by Phenethyl Isothiocyanate, a Cruciferous Vegetable-Derived Cancer Chemopreventive Agent, Is Mediated by Bak and Bax, clin cancer res.2003:1197) April11, 2005
8. Jane Higdon, Ph.D, Linus Pauling Institute,Oregon,state University,September 9,2005
10. Laurence Gamet-Payrastre, Sulforaphane, a Naturally Occurring Isothiocyanate, Induces Cell Cycle Arrest and Apoptosis in HT29 Human Colon Cancer Cells, Cancer Research 60, 1426–1433, March 1, 2000
11. Jung-Hwan Kim1, Changjiang Xu, Young-Sam, Inhibition of EGFR signaling in human prostate cancer PC-3 cells by combination, treatment with b-phenethyl isothiocyanate and curcumin, Carcinogenesis vol.27 no.3 pp.437–445
12. Changjiang Xu, Xuoxiang Shao, ERK and JNK signaling pathways are involved in the regulation of activator protein 1 and cell death elicited by three isothiocyanates in human prostate cancer PC-3 cells, Carcinogenesis vol.27 no.3 pp.437–445
14. Christine B. Ambrosone, Susan E. McCann, Breast Cancer Risk in Premenopausal Women Is Inversely Associated with Consumption of Broccoli, a Source of Isothiocyanates, but Is Not Modified by GST Polymorphism, Jama, June 28, 2005

Table 1. Glucosinolate Content of Selected Cruciferous Vegetables

<table>
<thead>
<tr>
<th>Food (raw)</th>
<th>Serving</th>
<th>Total Glucosinolates (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brussels sprouts</td>
<td>½ cup (44 g)</td>
<td>104</td>
</tr>
<tr>
<td>Garden cress</td>
<td>½ cup (25 g)</td>
<td>98</td>
</tr>
<tr>
<td>Mustard greens</td>
<td>½ cup, chopped (28 g)</td>
<td>79</td>
</tr>
<tr>
<td>Turnip</td>
<td>½ cup, cubes (65 g)</td>
<td>60</td>
</tr>
<tr>
<td>Cabbage, Savoy</td>
<td>½ cup, chopped (45 g)</td>
<td>35</td>
</tr>
<tr>
<td>Kale</td>
<td>1 cup, chopped (67 g)</td>
<td>67</td>
</tr>
<tr>
<td>Watercress</td>
<td>1 cup, chopped (34 g)</td>
<td>32</td>
</tr>
<tr>
<td>Cabbage, red</td>
<td>½ cup, chopped (45 g)</td>
<td>29</td>
</tr>
<tr>
<td>Broccoli</td>
<td>½ cup, chopped (44 g)</td>
<td>27</td>
</tr>
<tr>
<td>Horseradish</td>
<td>1 tablespoon (15 g)</td>
<td>24</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>½ cup, chopped (50 g)</td>
<td>22</td>
</tr>
</tbody>
</table>

Table 2. Food Sources of Selected Isothiocyanates and Their Glucosinolate Precursors

<table>
<thead>
<tr>
<th>Isothiocyanate</th>
<th>Glucosinolate Food Sources (precursor)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allyl</td>
<td>Sinigrin, Broccoli, Brussels sprouts, cabbage,</td>
</tr>
<tr>
<td>Isothiocyanate (AITC)</td>
<td>horseradish, mustard, radish</td>
</tr>
<tr>
<td>Benzyl</td>
<td>Glucoraphanin, Cabbage, garden cress, Indian cress</td>
</tr>
<tr>
<td>Isothiocyanate (BITC)</td>
<td>Glucoraphanin, Watercress</td>
</tr>
<tr>
<td>Phenethyl-</td>
<td>Glucoraphanin, Watercress</td>
</tr>
<tr>
<td>Isothiocyanate (PETIC)</td>
<td>Sulforaphane (SFN)</td>
</tr>
</tbody>
</table>

RESULT AND DISCUSSION
Sulforaphane is an isothiocyanate that has been isolated from SAGA broccoli. Laurence Gamet-Payrastre, Pengfei and Li, SolangeLeumeul et al evaluated the effect of sulforaphane on the growth and viability of HT29 cells during their exponentially growing phase. This compound has been shown to block the formation of tumors initiated by chemicals in the rat. It is a very potent monofunctional inducer of phase II enzymes in both cultured cells and mouse tissues; and it has recently been shown to inhibit at least one cytochrome P-450 (CYP2E1) involved in the activation of a variety of carcinogens.

Review article by Kuper et al. regarding tobacco use and carcinogenesis. Studies show that vegetable consumption decreases the risk of lung cancer. In studies to date, much work has been focused on isothiocyanates. These compounds inhibited the metabolic activation of nicotine-derived nitrosamimoketone (NNK), a powerful pulmonary carcinogen found in cigarette smoke, capable of inducing lung tumors in animals whatever the route of administration. It has shown that isothiocyanates are effective inhibitors of tumour development in carcinogen-treated rodents. Isothiocyanate and benzyl isothiocyanate in combination may be chemo preventive for lung cancer in smokers. Indeed, a mixture of these agents given in the diet inhibits lung-tumour induction in mice treated with NNK and benz[a]pyrene.

Limited data from clinical trials suggests that glucosinolate-rich foods increases phase II enzyme activity in humans. When smokers consumed 170g/d (6 oz/d) of watercress, urinary excretion of glucuronidated nicotine metabolites increased significantly, Brussels sprouts are rich in a number of glucosinolates, including precursors of Allyl isothiocyanate and Sulforaphane. Consumption of 300 g/d (11 oz/d) of Brussels sprouts for a week significantly increased plasma and intestinal GST (glutathione S-transferase) levels in nonsmoking.